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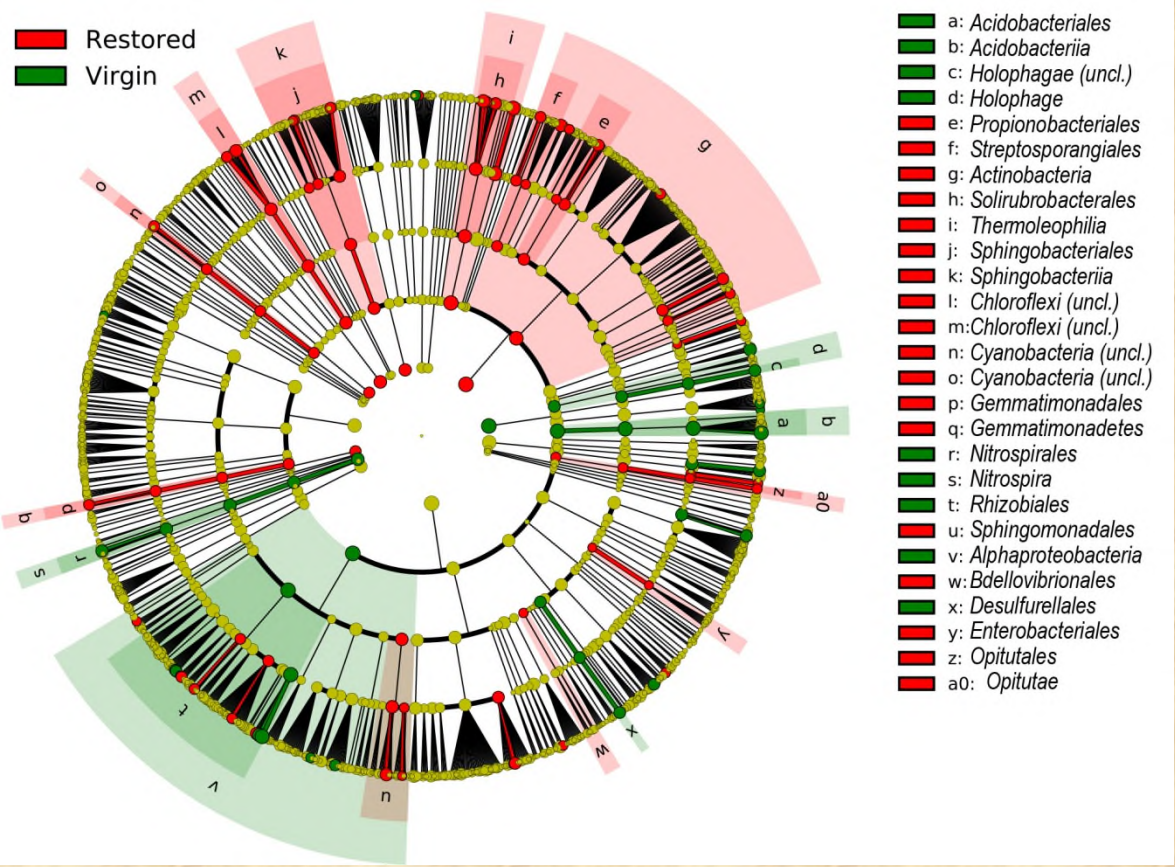

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COVER: Cladogram representing LEfSe (Linear Discriminant Analysis Effect Size) results featuring bacterial taxa most likely to explain differences between Ben Geren Park and Massard Prairie soil samples. *From:* Massard Prairie Restoration and Soil Microbiome Succession. J.M. Shaver, *et al*., pp7-11 (Manuscript 3381)

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NO MEETING WAS HELD IN THIS YEAR DUE TO THE WORLDWIDE COVID-19 PANDEMIC

Vertebrate Natural History Notes from Arkansas, 2020

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Running Title: Vertebrate Natural History Notes, 2020

Abstract

Smaller details of natural history often go undocumented to science if those details are not parts of larger studies, but small details can provide insights that lead to interesting questions about ecological relationships or environmental change. We have compiled recent important observations of distribution and reproduction of fishes and mammals. Included are new distributional records of mammals, and observations of reproduction in several mammals for which few data exist in Arkansas. A rare record of the long-tailed weasel, a species of special concern in Arkansas, is documented from Newton Co. We also provide evidence that Seminole bats likely reproduce in Arkansas.

Introduction

The constantly changing venues of human-altered environments provide field biologists opportunities to observe adjustments in natural history parameters and relationships among organisms. Although knowledge of distribution and natural history of many species within Arkansas is becoming better documented, much remains to be discovered and reported. We continue to update the state of knowledge of vertebrates of Arkansas vertebrates (see Tumlison *et al*. 2017, references therein, and yearly updates provided in this journal). Here, we include previously unreported records of distribution and reproduction in vertebrates from Arkansas.

Methods

Fishes were collected with 3.1 × 1.4 m, 3.1 × 1.8 m, and 6.1 × 1.8 m seines (all 3.175 mm mesh), or by hook and line. Fish specimens were documented either by a photo voucher or by specimens housed in the vertebrate collections at the Southern Arkansas University Vertebrate Collection (SAU) in Magnolia, AR. Voucher specimens of fishes were fixed in 10% formalin and preserved in 50% isopropanol. Museum numbers of voucher specimens are reported where available. Localities are reported as GPS (latitude and longitude) coordinates where available, except in the case of new records of bats, for which section, township, and range are reported to protect sensitive specific locality data.

Measurements such as total length (TL) are reported as initially recorded, if they were not taken originally in metric units. This is to avoid distortion by conversion from imprecise to what would appear to be precise distances. Bat records were based on catch/release surveys by expert chiropterologists, or from specimens sent to the Arkansas Department of Health to be tested for rabies.

An internet search through the VertNet Portal produced the reproductive data from the Sam Noble Oklahoma Museum of Natural History.

Results and Discussion

CLASS ACTINOPTERYGII

Hiodontidae – Mooneyes and Goldeyes

***Hiodon alosoides* (Rafinesque) – Goldeye**. In Arkansas, the Goldeye is restricted to large rivers, particularly the lower White River and the Mississippi River, although it occurs sporadically in the Red River and in the lower Arkansas River (Robison and Buchanan 2020). Boschung and Mayden (2004) reported that impoundments on large rivers have jeopardized the Goldeye throughout much of its range. On 10 October 1984, a single Goldeye (137 mm TL) was collected from the Red River about 8 km (6 mi). S of Garland, Miller Co., AR by E. J. Satterwhite. This represents only the fourth record of the Goldeye from the Red River in Arkansas (Robison and Buchanan 2020) and fills in a gap in its known distribution in the Red River.

Percidae - Perches

***Perca flavescens* (Mitchell) – Yellow Perch**. The Yellow Perch is native to northern North America, east of the Continental Divide (Robison and Buchanan 2020), and occurs in the Atlantic, Arctic, Great Lakes, and Mississippi River drainages south to Nebraska, Illinois, Ohio, and South Carolina (Page and Burr 2011). It has been widely introduced throughout the United States. Buchanan *et al*. (2000) reported a single specimen of *P. flavescens* collected from the Trimble Creek arm of Bull Shoals Lake in Arkansas in 1999. Floods in 2011 appear to have allowed this fish to escape downstream of Bull Shoals Lake, as specimens were collected in 2011 from the Buffalo River in Marion Co., and the White River in Independence Co. (Connior *et al*. 2013).

On 12 October 2019, 5 specimens of *P. flavescens* (approximate total lengths of 4.5-5 in., 5 in., 6 in., 7 in., and 8 in.) were caught in the White River at "White Hole access" (GPS 36.343518, -92.527367) near Cotter, Marion Co., AR by John Aufderheide and Hamilton Bell. All fish were released after being photographed. The same anglers caught 2 additional specimens (5-6 in.) of the Yellow Perch just upstream of the bend in the White River at Denton Ferry Road, across the river from Stetson's Marina, Baxter Co., AR (GPS 36.351794, -92.534718) on the same date. Current records confirm that this non-native fish has established in the Buffalo and White River drainages in Arkansas.

CLASS MAMMALIA - reproductive data

ORDER RODENTIA

Echimyidae (former Myocastoridae) – Coypu or Nutria

***Myocastor coypus* (Molina) – Coypu**. No information exists about field ecology of coypu in Arkansas (Sealander and Heidt 1990). We visited the oxidation ponds 4 km S of Arkadelphia, Clark Co., 47 times from mid-April 2019 through 29 March 2020. Lush vegetation comprised almost entirely of Smooth Bur Marigold (*Bidens laevis*, family Asteraceae) and Floating Marsh Pennywort (*Hydrocotyle ranunculoides*, family Apiaceae) filled one pond of approximately 4.2 ha. We had observed coypu in the pond the previous winter, but we found no coypu through the spring (first trip, 14 April 2019) and summer months of 2019. During this time, we counted up to 16 alligators (*Alligator mississippiensis*), which likely would be the major predators of coypu in the ponds (5 alligators were seen on 14 April).

After onset of colder weather, we saw the first coypu on 14 October, when 16 alligators also were counted. Only 1-2 coypu were seen until 17 November, when 4 coypu and 7 alligators were found. On 27 November and thereafter, alligators were inactive (none seen) but 5 coypu were counted, and on 17 December we observed a female coypu nursing 4 offspring (Fig. 1). About a month later (19 January 2020) approximately 20 coypu were seen. Coypu are known to be nonseasonal breeders, so winter breeding is expected (Woods *et al*. 1992). Density was about 4.2 coypu per ha.

Figure 1. Coypu nursing 4 young on a platform nest in the Arkadelphia oxidation ponds, Clark Co., on 17 December 2019. Photo by RT.

Coypu rested on platform nests constructed of piles of vegetation, which permitted them to get out of the water while distancing themselves from the banks of the pond, and where they rested and fed their offspring. Though numerous offspring were present in the pond, we were able to count only 1 litter of 4 offspring associated with a female on a platform nest. However, several platform nests contained 2-3 coypu, and many of those were juveniles. During our observations, growing juveniles began to occupy their own platform nests.

Foods consumed by the coypu included both of the 2 plants noted, but especially the *H. ranunculoides* which spread across the pond during winter when the *B. laevis* had subsided. In March 2020, with the return of alligators, we noted a decrease in numbers of coypu to almost absence: we could not be sure whether they were consumed by the alligators or dispersed to avoid predation.

CLASS MAMMALIA – reproductive data

The following collects reproductive data on Arkansas mammals gleaned from specimen data in the Sam Noble Oklahoma Museum of Natural History. In some cases, the Arkansas data falls within the range of embryo counts in adjacent states, however, we report them here because no data specific to Arkansas have been reported to date.

ORDER DIDELPHIMORPHIA

Didelphidae - Opossums

***Didelphis virginiana* Kerr – Virginia opossum**. Five opossums collected in Crittenden and St. Francis cos. in early January contained an average of 9.2 young in the pouch (range 6-13). Sealander and Heidt (1990) reported an average of 7-9 young.

ORDER EULIPOTYPHLA

Soricidae - Shrews

***Blarina carolinensis* (Bachman) – Southern Short-tailed Shrew**. One female collected in Sebastian Co. on 22 Jan 1991 contained 6 embryos. Reported embryo counts from Arkansas specimens range from 2-4 (Connior *et al*. 2014a; Tumlison *et al*. 2015).

ORDER LAGOMORPHA

Leporidae - Rabbits

***Sylvilagus floridanus* (J. A. Allen) – Eastern Cottontail**. – Two cottontails collected in Sebastian Co. in early January contained an average of 3.5 embryos (range 3-4). Conaway *et al*. (1974) reported a mean embryo count of 4.1 for the first litter of the year.

ORDER RODENTIA

Geomyidae - Gophers

***Geomys breviceps* Baird – Baird's Pocket Gopher**. Three pocket gophers collected in Pulaski Co. in early January contained an average of 2.3 embryos (range 2-3). Connior *et al*. (2014b) reported an average of 1.8 embryos in northern Louisiana.

Cricetidae – New World Rats and Mice

***Oryzomys texensis* (Harlan) – Marsh rice rat**. Two marsh rice rats collected in Sebastian Co. in early January both contained 2 embryos. This embryo count falls within the range of 2-5 reported by Roehrs *et al*. (2012) from southeastern Oklahoma.

***Reithrodontomys fulvescens* J. A. Allen – Fulvous Harvest Mouse**. Three fulvous harvest mice collected in Sebastian Co. in early January contained an average of 3.7 embryos (range 2-5). Three females reported by Connior *et al*. (2017) also contained an average of 3.7 embryos.

***Peromyscus attwateri* J. A. Allen – Texas Deermouse.** A single Texas deermouse collected in Stone Co. in early January contained 3 embryos. The typical litter size is 3 (Cockrum 1952, Long 1961).

***Peromyscus leucopus* (Rafinesque) – White-footed Deermouse**. Five white-footed deermice collected in Stone Co. in early January contained an average of 2.6 embryos (range 1-4). In northern Arkansas (Marion Co.), 3 females had an average embryo count of 3.3 (Tumlison *et al*. 2015).

***Peromyscus maniculatus* (Wagner) – North American Deermouse**. Five deer mice collected in Sebastian Co. in early January contained an average of 3.8 embryos (range 2-5). Deer mice typically have a litter size of 3-4 (Svihla 1932).

***Sigmodon hispidus* Say and Ord – Hispid Cotton Rat**. Two hispid cotton rats collected in Sebastian Co. in early January contained an average of 4.5 embryos (range 3-6). In northwestern Arkansas (Washington Co.), litter size ranged from 4 to 10, with a mean of 6.6 (Sealander and Walker 1955).

***Neotoma floridana* (Ord) – Eastern Woodrat**. Two woodrats collected in Sebastian Co. in January both contained 2 embryos. Mean litter size is about 3 with a range of 1-7 (Goertz 1970).

***Microtus pinetorum* (Le Conte) – Woodland Vole**. A single female contained 2 embryos in early January in Sebastian Co. In Kansas, 3 females each had 2 embryos (Cockrum 1952).

ORDER CHIROPTERA

Vespertilionidae – Vesper Bats

***Lasiurus seminolus* (Rhoads) – Seminole Bat**. A female captured on 27 May 2016 in Sec. 7, T8N, R17W, Conway Co., was pregnant. Reproduction by Seminole bats in Arkansas was first inferred based on a recently volant specimen collected 26 July 2011 in Garland Co. (Tumlison *et al*. 2002), and supported by capture of a post-lactating adult female on 24 July 2018 in Hempstead Co. (Tumlison *et al*. 2019). The pregnant specimen herein reported further supports the idea of a reproductive population occurring in Arkansas.

CLASS MAMMALIA – distributional records

ORDER CHIROPTERA

Unless otherwise indicated, all records of bats are new county records for the species in Arkansas.

Vespertilionidae – Vesper Bats

***Myotis austroriparius* (Rhoads) – Southeastern Myotis**. On 18 August 2018, RR captured an adult male southeastern bat in a mist net placed over a trail in Sec. 6, T6S, R5W, in Jefferson Co. On 1 August 2018, RR captured 4 adult females in a mist net placed on a trail in Sec. 17, T19N, R3E, in Randolph Co.

***Myotis grisescens* A. H. Howell – Gray Myotis**. On 10 September 2019 a male from Maumelle, Pulaski Co., submitted to the Arkansas Department of Health was found to be negative for rabies. This southern extralimital observation is only the second record of this species in Pulaski Co. (Tumlison *et al*. 2016) and like the previous record, was a male found in the late fall and could represent a vagrant migration incident.

***Myotis lucifugus* (Le Conte) – Little Brown Myotis**. On 25 June 2019 a male little brown myotis from Little Rock in Pulaski Co. was submitted to the Arkansas Department of Health and was found to be negative for rabies. While Sealander and Heidt (1990) indicated that a museum specimen was available for this county, no actual record of this specimen or other observations of this species in Pulaski Co. are known to the authors.

***Lasionycteris noctivagans* (Le Conte) – Silver-haired Bat**. On 20 May 2016 LKB captured an adult male in a mist net in Sec. 23, T9N, R19W, in Pope Co. Also on 20 May 2016, DC captured 4 adult males in a mist net set over a stream in Sec. 9, T8N R17W, in Conway Co. On 9 November 2010, RWP captured an adult male in a mist net placed over a pond in Perry Co., Sec. 36, T2N, R20W.

***Perimyotis subflavus* (F. Cuvier) – Tri-colored Bat**. On 21 July 2016 TI captured an adult male in a mist net placed on a trail in Sec. 12, T10S, R32W in Sevier Co. On 21 April 2019, MBC found a single tri-colored bat roosting during the day on a concrete underpass of AR Hwy 72 (Fig. 2) in Bentonville (Benton Co.). Although tri-colored bats are known to occupy bridges and culverts, Keeley and Tuttle (1999) reported this species to comprise only 1% of the total number of bats that occupied structures. Because this bat is believed to be very susceptible to white-nose syndrome, records of its roosting patterns are important to understanding the spread and effect of the fungal disease.

***Aeorestes cinereus* (Palisot de Beauvois) – North American Hoary Bat**. On 10 August 2016 ZB captured a juvenile female in a mist net set over a gravel road in Sec. 13, T10N, R23W, in Johnson Co.

On 21 August 2017, RR captured an adult female in a mist net placed over a stream in Sec. 23, T21N, R28W, in Benton Co.

***Lasiurus seminolus* (Rhoads) – Seminole Bat**. On 26 and 27 May 2016, RS captured a single adult female Seminole bat each night in a mist net set over a dirt road in Sec. 7, T8N, R17W, in Conway Co. On 9 November 2010, RWP captured an adult male Seminole bat in a mist net placed over a pond in Perry Co., Sec. 36, T2N, R20W.

Mollossidae – Free-tailed Bats

***Tadarida brasiliensis* (I. Geoffroy) – Brazilian Free-tailed Bat**. On 16 August 2018, RR captured an adult male in a mist net placed over a stream in Sec. 7, T5S, R6W, in Arkansas Co. On 15 May 2019, about 200 Brazilian free-tailed bats were discovered by TI and LL roosting under a joint of the Old Clarendon Bridge over the White River in Monroe Co.

Figure 2. Tri-colored Bat (*Perimyotis subflavus*) roosting singly under a concrete overpass, Benton Co. Photo by MBC.

ORDER RODENTIA

Cricetidae – New World Rats and Mice

***Oryzomys palustris* (Harlan) – Marsh Oryzomys**. On 5 June 2017, a marsh oryzomys was collected 1.5 km N Morrilton, Conway Co. (35.19307N, 92.7125471W, WGS 84). The specimen (Arkansas State University Museum of Zoology, ASUMZ mammal catalog 28540) was collected incidentally, crushed within the GI tract of a small western ratsnake (*Pantherophis obsoletus*; ASUMZ herp catalog 33752) that was DOR. This is a new county record for this rodent (Sealander and Heidt 1990).

ORDER CARNIVORA

Mustelidae – mustelids

***Mustela frenata* Lichtenstein – Long-tailed Weasel**. This carnivorous mustelid is considered rare at local and regional scales, and is classified by the Arkansas Game and Fish Commission as a species of greatest conservation need (Fowler 2015). A recent statewide survey produced a single observation at a heavily sampled site (Johnston *et al*. 2019). As part of a large-scale field research effort on plains spotted skunk (*Spilogale putorius interrupta*), SDH conducted a baited camera trap survey (Higdon and Gompper 2020) in mixed oak-hickory and oak-pine forests in the Ozark National Forest and Gene Rush Wildlife Management Area. During 8,119 trap nights, we photo-captured one long-tailed weasel (Fig. 3) on 9 April 2017 at 0217 hr, resulting in a capture success rate of 0.01%. The site in Newton County (GPS 35.85525, -92.94611) had canopy cover and low-lying understory cover of 62.75% and 96%, respectively. Low rate of capture of long-tailed weasel in our survey reiterates the rarity of the species in the Ozark ecoregion of Arkansas.

Figure 3. Long-tailed Weasel (*Mustela frenata*) photo-captured in Newton Co. on 9 April 2017.

Acknowledgments

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Massard Prairie Restoration and Soil Microbiome Succession

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Running title: Massard Prairie Restoration and Soil Microbiome Succession

Abstract

We have initially sequenced soil microbial DNA from 4 restored and 3 virgin tallgrass prairie soil samples from Ben Geren Park and Massard Prairie (Fort Smith, AR), respectively. As expected, the soil microbiomes are distinct, with several lineages of nitrogen-fixing bacteria more common in virgin tallgrass prairie. However, we predict that as restoration of tallgrass prairie in Ben Geren Park progresses, the soil microbiome of restored prairie will more closely mirror those of the virgin prairie.

Introduction

An ongoing project at Ben Geren Park (Fort Smith, AR) is the re-establishment of Massard Prairie, which once existed on the current site. Botanist Thomas Nuttall gave the first descriptive account of Massard Prairie in 1819, while visiting the newly established Fort Smith (Nuttall 1821). In Arkansas, tallgrass prairie once covered over 700,000 acres, but less than 0.5 percent remain today (Barone 2018). This makes tallgrass prairie one of the most rare and threatened ecosystems in the state. Prior to European settlement, North American prairie covered about 3.6 million-km^2 (Mackelprang *et al.* 2018). Conversion to row crop agriculture and urban development has reduced North American prairies by 99.9% (Samson and Knopf 1994). This is of immediate concern, because prairie soils contain over 35% of soil carbon in the continental United States, making them some of the most productive and fertile in the world (Mackelprang *et al.* 2018).

The restoration areas in Ben Geren Park likely have altered microbial diversity and composition in the soil due to past cattle operations and maintenance practices involved in maintaining a golf course. The goal of this project is to establish a baseline for understanding the impacts of past land management and future prairie maintenance, development and restoration on soil microbial communities. In this preliminary study, 4 soil samples from developed prairie undergoing restoration that was initiated in 2016-2017, within Ben Geren Park, were compared to 3 virgin prairie samples obtained from Massard Prairie. The primary method we used for this study is 16S rRNA gene sequencing. LoopSeq synthetic long read sequencing, covering all 9 variable regions, facilitated species-level identification of soil bacteria discriminating virgin and restored tallgrass prairie soil samples.

Materials and Methods

Four soil samples from restored prairie locations in Ben Geren Park and 3 soil samples from Massard Prairie were collected on February 10, 2019. Within each distinct sampling location, different soil types were taken into account when sample sites were identified, and soil temperatures and pH were also recorded, with GPS coordinates, as samples were collected (Table 1). All samples were taken at each of the sites with a 2.54-cm diameter soil corer to a depth of 10 cm. Each core sample was initially placed in a sterile plastic bucket and homogenized by hand. Buckets were sterilized by washing with soap and rinsed with isopropyl alcohol. After homogenization, a portion of the sample was placed in a sterile 50-ml plastic falcon tube and immediately placed on ice, and the bulk of the sample was placed in a thick paper bag for soil analysis. After all samples were collected, all samples on ice in 50-ml plastic falcon tubes were kept frozen in the lab at UA Fort Smith until DNA was extracted. A subsample of approximately 250 mg from each soil sample was used for DNA extraction and 16S

Table 1. Soil sample collection information, including GPS coordinates location, pH, temperature, and soil description. Restoration efforts for sample areas began in 2016 (Sample 1) and 2017 (Samples 2-4).

Sample	GPS	Prairie Location	pH	Temp (°C)	Soil Description
1 (A3)	N 35.314230 W 94.362009	Restored	5.29	5.5	Wt - Wrightsville Messer complex
2 (B3)	N 35.313273 W 94.361.789	Restored	5.00	5.0	Wt - Wrightsville Messer complex
3 (C3)	N 35.315214 W 94.358842	Restored	4.88	4.4	Wt - Wrightsville Messer complex
4 (D3)	N 35.317022 W 94.359211	Restored	4.96	4.9	WsA - Wrightsville complex
5 (E3)	N 35.294147 W 94.380950	Virgin	5.59	5.2	LeB - Leadvale Silt Loam
6 (F3)	N 35.298083 W 94.381193	Virgin	5.28	5.6	WsA - Wrightsville complex
7 (G3)	N 35.297956 W 94.384681	Virgin	5.13	6.0	MID - Montevallo gravelly loam

rRNA gene sequencing.

DNA was extracted from 250 mg soil portions using the ZymoBIOMICS DNA Miniprep Kit (Cat # D4300) according to the manufacturer's protocol (Zymo Research, Irvine, CA, United States). The V1-V9 region of the small subunit (SSU) rRNA gene was amplified using the LoopSeq™ 16S Long Read Kit according to the manufacturer's protocol (Loop Genomics, San Jose, CA, United States). Sequencing of amplicons was performed at the Arkansas Childrens Research Institute (Little Rock, AR) using an Illumina sequencer following manufacturer's instructions. For more information on 16S Long Read technology, see: *https://www.loopgenomics.com/16s.*

We used the linear discriminant analysis (LDA) effect size method (LEfSe) to identify particular microbial taxa associated with different soil types (Segata 2011). Using tabular data describing the relative abundance of bacterial 'biomarkers' in different samples, LEfSe identifies the taxa that most strongly differentiate two or more classes of samples. In this case, we used LEfSe to identify taxa that most strongly differentiated microbial communities from virgin and restored prairie soil samples.

We used the Galaxy implementation of LEfSe, with per-sample normalization to 1 M and default parameters, available at *https://huttenhower.sph.harvard.edu/galaxy.* Virgin and restored tallgrass prairie samples were also compared by Pearson's Chi-square test using phylum counts and Principal Components Analysis (PCA) based on unscaled taxon abundances.

Results

The proportion of 16S rRNA sequence counts for each bacterial phylum from the virgin and restored tallgrass prairie samples were compared (Fig. 1). Nine phyla compared represent at least 97% of the sequence counts. Virgin and restored tallgrass prairie samples were compared by Pearson's Chi-square test using phylum counts. The P-value (0.000) that resulted from the comparison of soil microbiome of virgin to restored tallgrass prairie soil samples was less than a significance level of 5% (8 degrees of freedom). Virgin and restored samples also clustered separately along the first principal component dimension, which explained 64.2% of total variation in taxon abundances among samples (Fig. 2).

Eighty-six lineages of bacteria were significantly more common in virgin prairie samples compared to the restored prairie samples (Fig. 3). Four classes overall were more abundant in virgin prairie: *Alphaproteobacteria*, *Acidobacteriia*, *Holophagae*, and *Nitrospira*. The higher abundance of *Alphaproteobacteria* in virgin prairie soils was driven by several lineages of *Rhizobiales*, including members of the *Xanthobacteraceae*, *Methylobacteriaceae*, *Hyphomicrobiaceae*, and *Bradyrhizobiaceae*. In contrast, 123 lineages were more common in restored prairie samples. Seven classes were more abundant in restored prairie samples, including *Actinobacteria* (particularly the families *Propionibacteriales* and *Streptosporangiales*), *Gemmatimonadetes*, *Opitutae*, *Sphingobacteriia*, *Thermoleophilia*, and unclassified members of *Cyanobacteria* and *Chloroflexi*.

Discussion

Even with a small sample size, we identified several bacterial taxa that have previously been suggested as bioindicators of healthy prairie soils. In our study, one of the most strongly differentiating families was the *Xanthobacteraceae* within *Rhizobiales*; several particular lineages within three

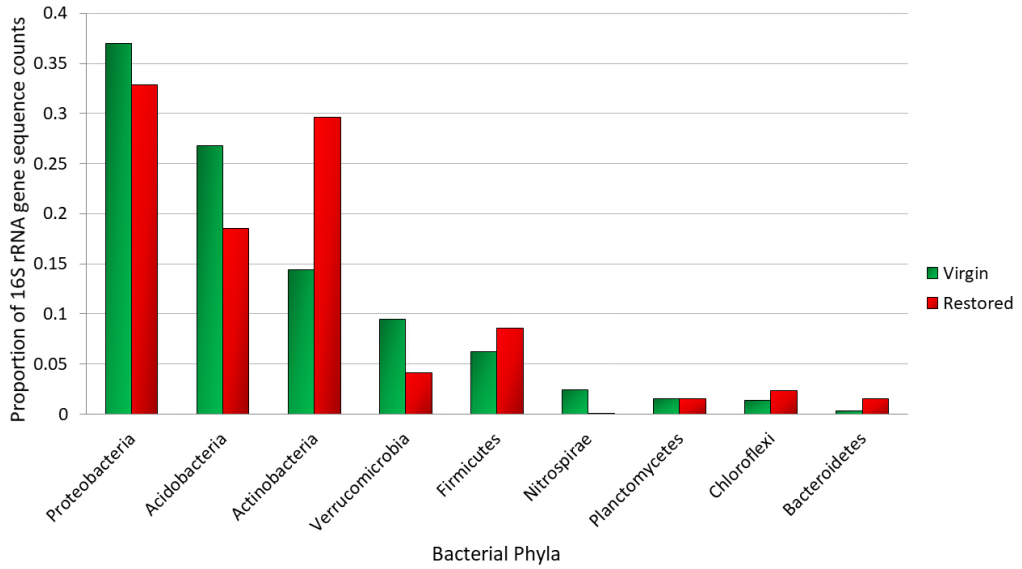

Figure 1. Proportion of 16S rRNA gene sequence counts for each bacterial phylum from the virgin and restored tallgrass prairie samples. The nine phyla included represent at least 97% of sequence counts. Samples were compared by Pearson's Chi-square test using phylum counts. The P-value that resulted from the comparison of soil microbiome of virgin to restored tallgrass prairie soil samples was less than a significance level of 5%.

other families of *Rhizobiales* were also more significantly abundant in virgin prairie. Multiple families of *Rhizobiales* were more commonly associated with prairie soils compared to soils under corn cultivation (Mackelprang *et al.* 2018). Many rhizosphere-associated taxa, including most lineages of symbiotic nitrogen-fixing rhizobia, are classified within families of the *Rhizobiales*.

Within the *Verrucomicrobia*, the *Spartobacteria* are a taxon of interest for tallgrass prairie restoration efforts, given their likely importance for carbon dynamics (Fierer *et al.* 2013) and abundance in older, 10+ year-old restorations and virgin prairie (Barber *et al.* 2017). Of the *Spartobacteria* detected in our study, members of the *Xiphinematobacteraceae* and an unclassified family within the *Chthoniobacterales* were more abundant in virgin soils compared to restored prairie soils. The *Xiphinematobacteraceae* lineages detected were all most similar to '*Candidatus Xiphinematobacter*', endosymbionts of soil nematodes (Brown *et al.* 2015). Another verrucomicrobial taxon, the *Opitutae*, were slightly more abundant in restorations in our study compared to virgin prairie, consistent with their higher abundance in early restorations and decrease over time in older restorations (Barber *et al.* 2017).

Surprisingly, we found that *Nitrospirales* were more common in virgin soils than restored prairie soils. *Nitrospirales*, involved in nitrification, were more abundant under corn cultivation than in virgin prairie in a previous study (Mackelprang *et al.* 2018).

For future study, we will sequence bacterial and fungal DNA from 12 new soil samples taken in March 2020 from locations in Ben Geren Park and Massard Prairie, and sequence fungal DNA from 12 soil samples collected from the same locations in January 2019. LoopSeq synthetic long read sequencing (generating ~2.5 kb contigs covering the 18S-ITS1-ITS2 region) will facilitate species level identification of soil fungi present in these 24 samples We will explore the use of multilayer networks for analysis of

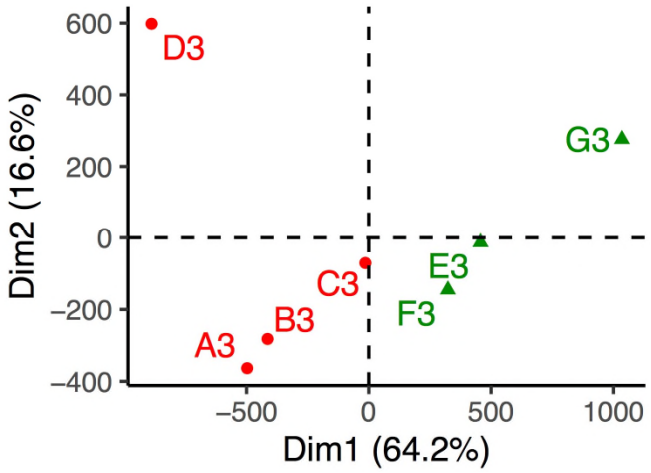

Figure 2. Principal Component Analysis (PCA) based on unscaled bacterial taxon abundance in virgin and restored prairie samples. Restored tallgrass prairie soil samples shown as red circles. Virgin tallgrass prairie soil samples shown as green triangles.

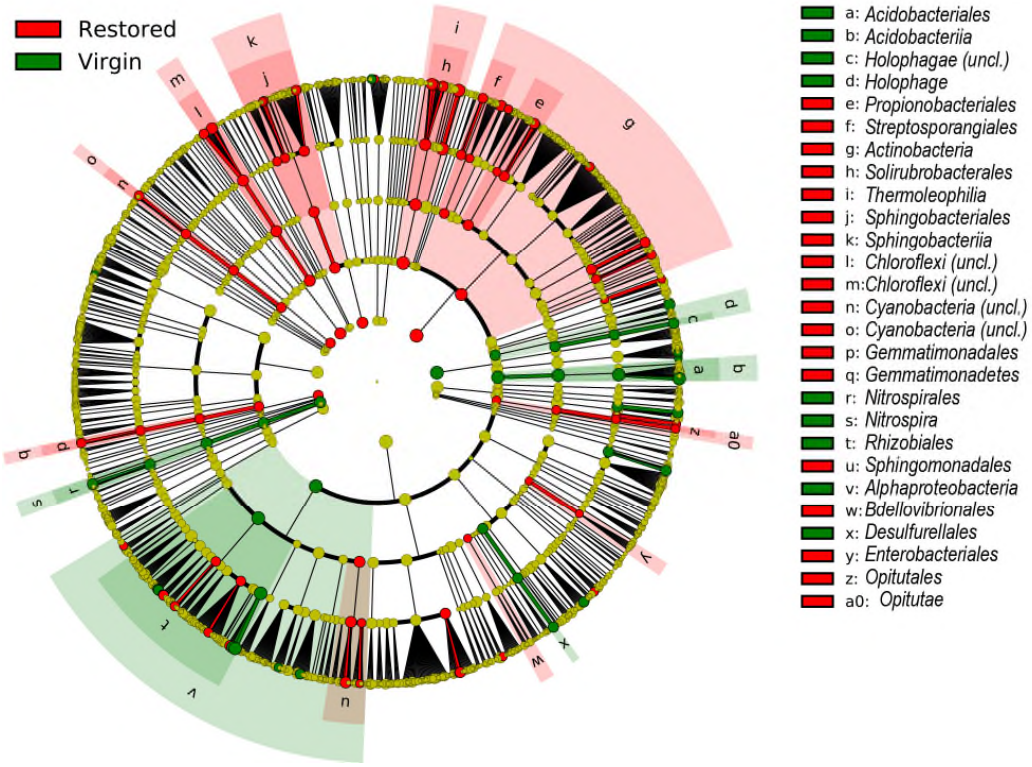

Figure 3. Cladogram representing LEfSe (Linear Discriminant Analysis Effect Size) results featuring bacterial taxa most likely to explain differences between Ben Geren Park and Massard Prairie soil samples. Each 'ring' in the figure represents a different hierarchical level of classification, with nodes in the innermost ring representing different bacterial phyla. Nodes are colored if they represent a lineage significantly more abundant in restored (red) or virgin (green) tallgrass prairie samples at each level of taxonomic classification. Bacterial classes (second ring) and orders (third ring) that are significantly differentiated between restored and virgin samples are shaded on the cladogram and given an abbreviated label (see legend).

co-occurrence data, wherein each layer represents a different sampling date, and inter-layer edges represent temporal change in abundance of a particular taxon. We hypothesize higher fungal taxonomic diversity in the virgin soils and an increase in fungal diversity over time under restorations.

Acknowledgements

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Distal Urogenital Anatomy of Male Prairie Racerunners, *Aspidoscelis sexlineatus viridis* (Reptilia: Sauria: Teiidae)

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Running Title: Distal Urogenital Anatomy in Male *Aspidoscelis sexlineatus viridis*

Abstract

I examined histologically the distal urogenital anatomy in male Prairie Racerunners (*Aspidoscelis sexlineatus viridis*) from a small seasonal sample of individuals collected in Arkansas in order to provide additional information regarding squamate urogenital anatomy. Specifically, I focused on the basic anatomy and positioning of posterior ducts and associated structures in this teiid lizard. The anatomical structures included the ductus deferens, ampulla ductus deferens, ampulla urogenital papilla (Aup), ureter, inner core tissue mass, urodaeum, and the urogenital papilla. The two Aup, which are small complimentary blind pouches representing the terminal repositories for products released by urogenital ducts, are striking anatomical features of the distal urogenital anatomy in this lizard. Interestingly, the Aup, which are characteristic anatomical structures present in very few additional lizard species (e.g., in some members of families Gerrhosauridae, Gymnophthalmidae, and Varanidae) are also present in colubrid snakes and not in crotalid snakes.

Introduction

Interest in the reproductive anatomy of reptiles has increased in recent years and has revealed new information on squamate (lizards and snakes) urogenital systems. Major investigations specifically involving distal urogenital structures, which are supplemented with literature summaries, include Trauth and Sever (2011), Rheubert *et al*. (2015), Pewhom and Srakaew (2018), and Trauth (2018).

Early macroscopic descriptions and illustrations of distal urogenital morphologies in male squamates provided valuable resources for today's anatomical studies. The pioneer works by Martin Saint Ange (1854), Brooks (1906), and Volsøe 1944) laid the groundwork for more rigorous histological studies as reported by Gabe and Saint Girons (1965) and Fox (1977). The above studies, as a whole, reported on various aspects of male urogenital anatomy, and more recent studies into this anatomical region described several new caudal micro-anatomical structures (Gribbins and Rheubert, 2011; Trauth and Sever, 2011; Rheubert *et al*., 2015). One of these anatomical structures, the ampulla urogenital papilla, was named by Trauth and Sever (2011) in North American male colubrid snakes and was also referenced in Siegel *et al*. (2011). Although these paired structures were originally illustrated by Martin Saint Ange (1854), Trauth and Sever (2011) described them in histological detail. These two complementary blind pouches reside in the anterior extent of the cloacal cavity. The pouches represent the terminal repositories of products released from the urogenital tracts. Brooks (1906) referred to these pouches as seminal vesicles in the Texas Spotted Whiptail (*Aspidoscelis gularis*), whereas Rheubert *et al*. (2015) more thoroughly examined these paired pouches histologically in this species.

The family Teiidae is a relatively large, New World assemblage of small-to-large lizards containing over 130 species (Vitt and Caldwell, 2014). One species of teiid lizard, the Prairie Racerunner (*Aspidoscelis sexlineatus viridis*), is widespread from the high plains fringing the Rocky Mountains eastward and southward to the eastern coast of the United States (Powell *et al*., 2016); this species is commonly found in Arkansas (Trauth and McAllister 1996; Trauth 1980; Trauth 1983; Trauth *et al*., 2004). Rheubert *et al*. (2015) briefly described aspects of the male urogenital anatomy in the Prairie Racerunner and included comments regarding reproductive macro-anatomy and scanning electron micrographs of its urogenital papilla.

In the present study, I provide a more intensive examination of the distal urogenital anatomy of male Prairie Racerunners using seasonal histology. I also discuss these anatomical findings by comparing them with those published on the Texas Spotted Whiptail reported as in Rheubert *et al*. (2015) and on colubrid snakes as reported by Trauth and Sever (2011).

Materials and Methods

I examined the distal urogenital anatomy of adult male Prairie Racerunners currently retained in my personal collection of these lizards (SET tag numbers with date of collection are below); each, however, has been reassigned a permanent museum tag (Arkansas State University Museum of Herpetology—ASUMZ 32724, 12 June 2013; 34075 [SET 975, 6 October 1974]; 34076 [SET 1583, 12 April 1975]; 34078 [SET 2756, 9 June 1978]; 34080 [SET 1662, 3 May 1975]; 34081 [SET 1227, 23 December 1974]; 34082 [SET 1515, 5 April 1975], and 34090 [SET 1213, 21 December 1974). All lizards, except ASUMZ 32724, 34076, 34078, and 34080 were excavated from hibernation burrows. Specimens will be permanently housed in the herpetological collection in the Arkansas Center for Biodiversity Collections at Arkansas State University. Most of the lizards were collected in Arkansas in 1974 and 1975 during a multi-year study of the species (Trauth 1980). All 8 specimens were euthanized with an intra-pleuroperitoneal injection of sodium pentobarbital following current IACUC protocol guidelines; the urogenital organs from 7 lizards were then fixed using abdominal injections of 10% formalin. The specimens were eventually preserved in 70% ethanol.

I removed segments of distal urogenital anatomy (approximately 5 mm in length; see Fig. 1) and placed the tissues temporarily into vials of 70% ethanol. Then, I followed standard histological procedures to prepare tissues for light microscopy following the paraffin embedding techniques described in Presnell and Schreibman (1997) and Trauth (2018). Briefly, these steps included tissue dehydration in ethanol solutions (70 to 100%), clearing in 100% xylene, infiltration overnight in a paraffin oven (56°C), embedding in paraffin using plastic molds (tissue positioned in a cranial-to-caudal axis), sectioning with a rotary microtome into 8 or 10 µm serial strips (affixed onto glass microscope slides coated with Haupt's adhesive prior to floating strips in 2% formalin on a slide warmer), and staining using either hematoxylin/eosin (H&E) to reveal general cytology or Pollak trichrome stain (Pollak) for the enhancement of connective tissues and muscle. Cover slips were then adhered to microscope slides with Permount© (Fisher Scientific Products).

For slide photomicroscopy, I used a Leica MC 120 HD camera atop a Leica DM 2000 LED compound light microscope. For macrophotography, I used a Canon T4i digital single lens reflex camera fitted with a 50 mm macro lens.

All descriptions of urogenital structures follow the terminology found in Trauth and Sever (2011),

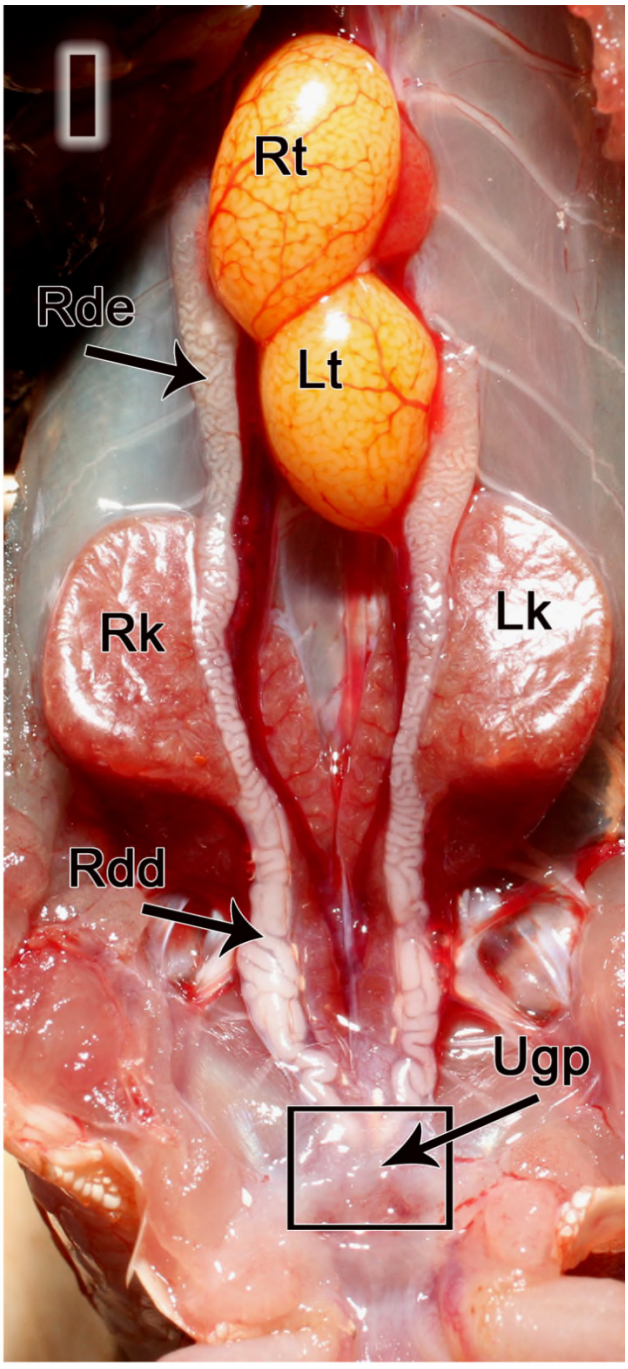

Figure 1. Macroscopic view of the urogenital system of a recently sacrificed reproductively active *Aspidoscelis sexlineatus viridis* (ASUMZ 32724) as adapted with modifications from Rheubert *et al.* (2015). Graphic box delimits region where tissues were histologically examined and represents the distal urogenital complex. Lt, left testis; Lk, left kidney; Rt, right testis; Rk, right kidney; Rde, right ductus epididymis; Rdd, right ductus deferens; Ugp, urogenital papilla. Scale bar at upper left = 5 mm.

Rheubert *et al.* (2015), and Trauth (2018). Microscope slides are currently in my possession and will be deposited in the herpetological collection in the Arkansas Center for Biodiversity Collections.

Results

Gross morphology

A gross morphological aspect of the urogenital anatomy (ventral view) of a reproductively active male is shown in Figure 1, and a brief description of the structural morphology is provided here. The paired testes appear yellow in color with the right testis lying more cranial compared to the left. Each testis is flanked laterally by a highly looped ductal complex, the ductus epididymis and other efferent ductules. The ductus deferens extends caudally from this ductal complex. The ductus deferens lies along the ventromedial surface of the primary lobe of each kidney. The ductus deferens also continues as a slightly looped duct on both sides as each enters the distal urogenital complex (graphic box in Fig. 1). Not seen in Figure 1 are the ureters, which lie dorsomedial to the ductus deferens. A urinary bladder is absent in this species.

Light microscopy

The highly looped, posterior segment of each ductus deferens (Pdd in Fig. 2) resides immediately anterior to the ampulla ductus deferens (Add) of the distal urogenital complex. It is lined with a low simple columnar epithelium and is packed with sperm (Sp) in this lizard collected on 9 June 1978. Each Pdd becomes abruptly constricted caudally into a narrow, straight duct, the Add. The ductal epithelium is now lined with pseudostratified columnar epithelium. The ampulla urogenital papilla (Aup) is also revealed as a conspicuous lateral sac on either side of the midline in Figure 2. Not seen in Figure 2 are the ureters (Ur), which lie dorsomedial to the Pdd and Add.

For most of its length, the Add is a circular-to-oblong linear duct within the distal urogenital complex and is uniformly lined with either a short or tall pseudostratified columnar epithelium depending upon the season (Fig. 3). Closely associated with each Add is a ureter (Ur), which exhibits a transitional epithelium, also known as the urothelium. In addition, depending upon the season, the Add is encompassed by thin-to-relatively thick layers of smooth muscle, the muscularis (Fig. 3). As both Add and Ur extend caudally, each duct becomes displaced slightly to lie in a more medial position (Figs. 4A – C; 6A – E). Near the cloacal region and more specifically, the urodaeum (Uro), these ducts independently dump their contents into each Aup, which becomes apparent as progressively enlarging pouches lateral to the Add and Ur (Figs. 4 – 7).

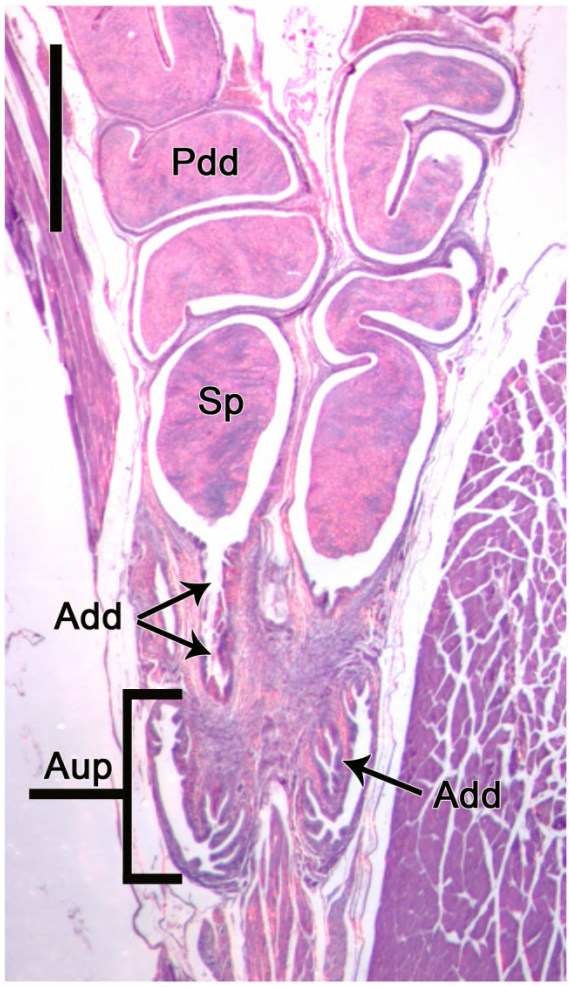

Figure 2. Light micrograph of a bilateral longitudinal section through the urogenital structures of a reproductively active *Aspidoscelis sexlineatus viridis* (ASUMZ 34078) collected on 9 June 1978. The ampulla ductus deferens (Add) is a narrow, constricted duct compared to the expansive, highly looped posterior portion of the ductus deferens (Pdd), which is packed with sperm (Sp). Both ampullae urogenital papillae (Aup) are clearly visible in this image. Scale bar = 2 mm at upper left. H&E, 10 µm.

Seasonal differences between the urothelial lining of the Ur and the pseudostratified columnar epithelium of the Add are revealed in Figures 3A and 4. In the inactive lizard (Fig. 3A), the urothelium is thin, irregular, and much reduced in height, whereas in the active lizard (Fig. 3B), the urothelium is much thicker and relatively unpleated. In comparison, the epithelial

thickness of the Add is basically similar (in both Fig. 3A, B); however, the muscularis layer (M) in the wall of the Add in the inactive lizard is thin, whereas it is much thicker in the active lizard.

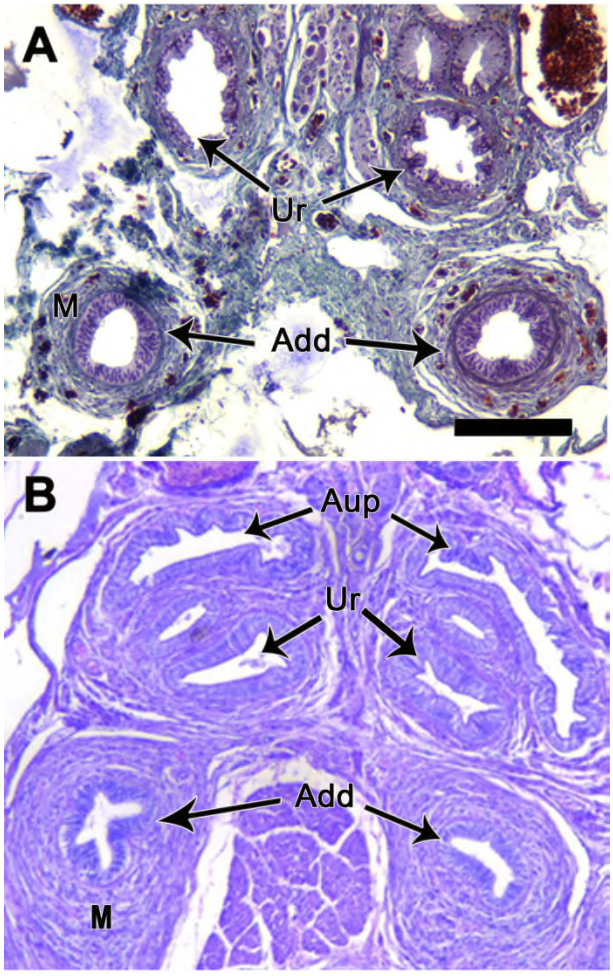

Figure 3. Seasonal variation in the Ur and Add in the anterior region of the distal urogenital complex in *Aspidoscelis sexlineatus viridis* as revealed by transverse sections. A. ASUMZ 34075 collected on 6 October 1974. B. ASUMZ 34076 collected on 12 April 1975. Aup, ampulla urogenital papilla; M, muscularis. See text for explanation of ductal morphology. Scale bar = 200 µm for A and B. A, Pollak, 8 µm; B, H&E, 10 µm.

The expansion of each Aup is characterized by a circumferential movement of these pouches (Figs. 4 – 7). Both Aup increase in length and diameter as they completely surround the inner core of connective and muscular tissues (hereafter called the inner core tissue mass, ICTM) supporting the paired Add and Ur. I observed that the positioning of the Ur varied among the lizards examined. For example, in Figures 4 and 5, the Ur elongates as each moves to take a dorsal position above the Add (Figs. 4E, 5A) within the ICTM. (See Fig. 7 for a more complete explanation of the ICTM complex.) The left Ur elongates (Fig. 4F) in a ventral direction and opens into the left Aup. Each Add is now embedded in the ICTM at a point ventral to the Ur. The left Add (Fig 4E) exhibits a ventral expansion reminiscent of the left Ur as viewed in Figure 4F. The urogenital papilla (Ugp), shown in Figure 5A, begins to appear approximately 20 µm posterior to Figure 4F. The Aup extend ventrally into the tip of the Ugp. Both pouches will eventually open into the Uro. These orifices of the Aup are shown in Figures 5B and 7B.

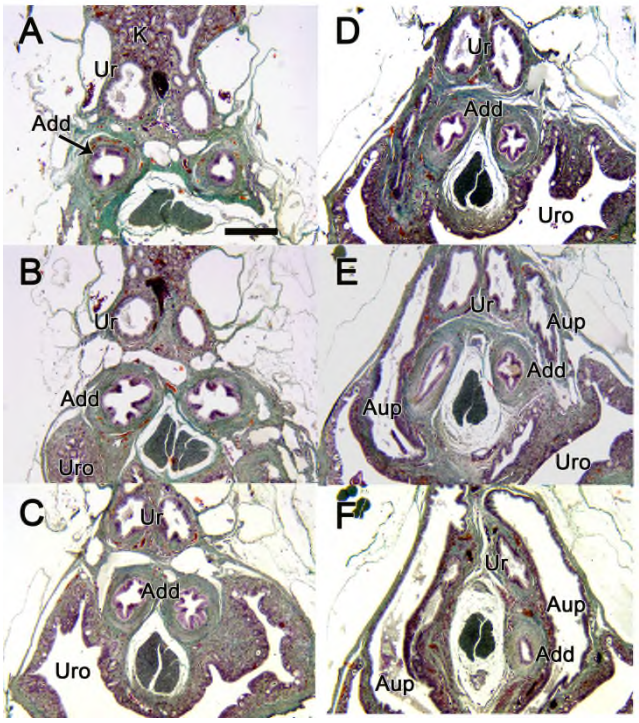

Figure 4. Light micrographs of the distal urogenital anatomy in an inactive *Aspidoscelis sexlineatus viridis* (ASUMZ 34075) as revealed by a cranial-to-caudal series of transverse sections. A. Section through the anterior region of the urogenital complex at the level of the Add and the Ur. B. Section showing movement ventrally by the Ur and medially by each Add as they near the urodaeum (Uro). C. Section showing all structures in B, but revealing more of the Uro. D. Section showing the ventral elongation of the Ur. E . Section revealing the Aup lateral to the Add. F. Section showing the expansion of the Aup and the orifice of the left Ur (arrow) opening into the Aup. K, kidney. Scale bar = 500 µm for A – F; Pollak, 8 µm.

The relationship between the distal urogenital complex and the alimentary tract is shown in Figures 6 and 7. At the anteriormost level of this complex (Fig.

6A), a collecting duct (Cd) extends ventrally from the posterior lobe of the left kidney and leads into the left Ur. Both Ur and Add are situated dorsal to the large intestine (In). As the arms of the Uro move lateral to the Add (Fig. 6B), the In can be seen constricting to become a much narrower passageway, termed the anterior sphincter of the coprodaeum (Sph), which marks the terminal point of the In (Fig. 6C). Just caudal to this juncture, the Uro spreads both laterally and ventrally to encompass the emerging ICTM (Fig. 6D, E). The ICTM is characterized by a central-located pair of striated muscle masses (see Figs. 6 – 8). The appearance of the pouches of the Aup (Fig. 6D) and their further enlargement (Fig. 6F) displace the lateral arms of the Uro which had previously surrounded the ICTM. Eventually, the Aup elongate further to become narrow passageways within the Ugp (Figs. 5A, 7B). These passageways eventually open into the Uro of the cloaca.

A comparison of the epithelial linings of the Ur, Add, and Aup is shown in Figure 8. The lining of the Aup, not mentioned previously, appears as a low columnar epithelium.

Discussion

The primary morphological structures associated with the distal urogenital system in *Aspidoscelis sexlineatus viridis* are as follows: 1) ductus deferens, 2) ureter, 3) urodaeum, 4) inner core tissue mass, and 5) the urogenital papilla. The following discussion pertains to these anatomical structures by separately comparing each with what has been published about them in the most recent literature.

Ductus deferens

Pewhom and Srakaew (2018) mentioned two segments of the ductus deferens in the Butterfly Lizard (*Leiolepis ocellata*): a ductal portion and an ampullary portion. The ductal portion is further subdivided into the proximal and distal ductal regions based on the type of epithelium present. The posterior segment of the ductus deferens in *Aspidoscelis sexlineatus viridis* (i.e., the Pdd in Fig. 2) equates closely to the distal ductal portion mentioned in *L. ocellata* based on epithelial type, which these authors describe as simple cuboidal. In contrast, this epithelium in *A. s. viridis* more closely resembles a low simple columnar epithelium. Also, Pewhom and Srakaew (2018) defined the ampulla ductus deferens as the straight terminal segment of the testicular ducts; however, they did not thoroughly examine the Add within the distal

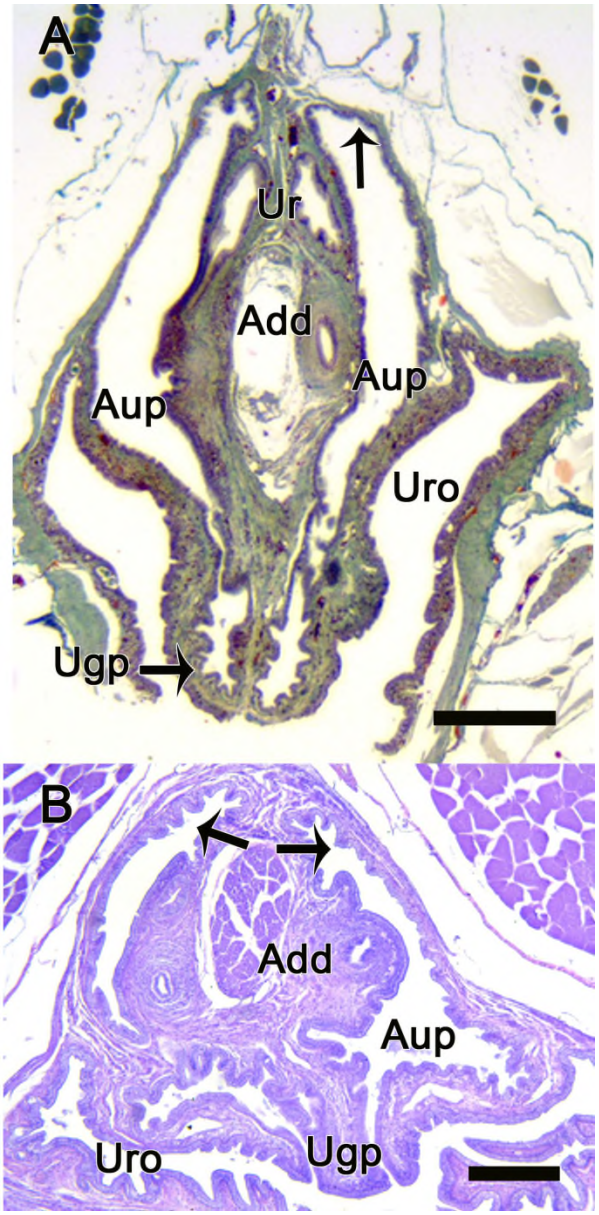

Figure 5. Light micrographs comparing the urogenital papilla (Ugp) of an inactive and an active *Aspidoscelis sexlineatus viridis*. A. Section through the Ugp of an inactive lizard (ASUMZ 34075). Arrow points to thin epithelial lining of the Aup. B. Section through the Ugp of an active lizard (ASUMZ 34076). Arrows point to thick folded epithelial lining of the Aup. Abbreviations the same as in previous figures. Scale bar = 200 µm for A and B. A, Pollak, 8 µm; B, H&E, 10 µm.

urogenital complex as described herein. Consequently, no comparison with the Add of *A. s. viridis* is available for that region. Although *L. ocellata* does possess a deeply folded modification of the ampullary portion of the ductus deferens, a structure termed the ampulla ductus deferentis (Akbarsha *et al.* 2005), *A. s. viridis* does not possess this modification of the Add.

Rheubert *et al.* (2015) found the ampulla ductus deferentis in 5 lizard species (i.e., in one scincid-*Scincella lateralis* and 4 phrynosomatids–*Cophosaurus texanus*, *Holbrookia propinqua*, *Phrynosoma cornutum*, and *Sceloporus consobrinus*), and Trauth (2018) also found an ampulla ductus deferentis in

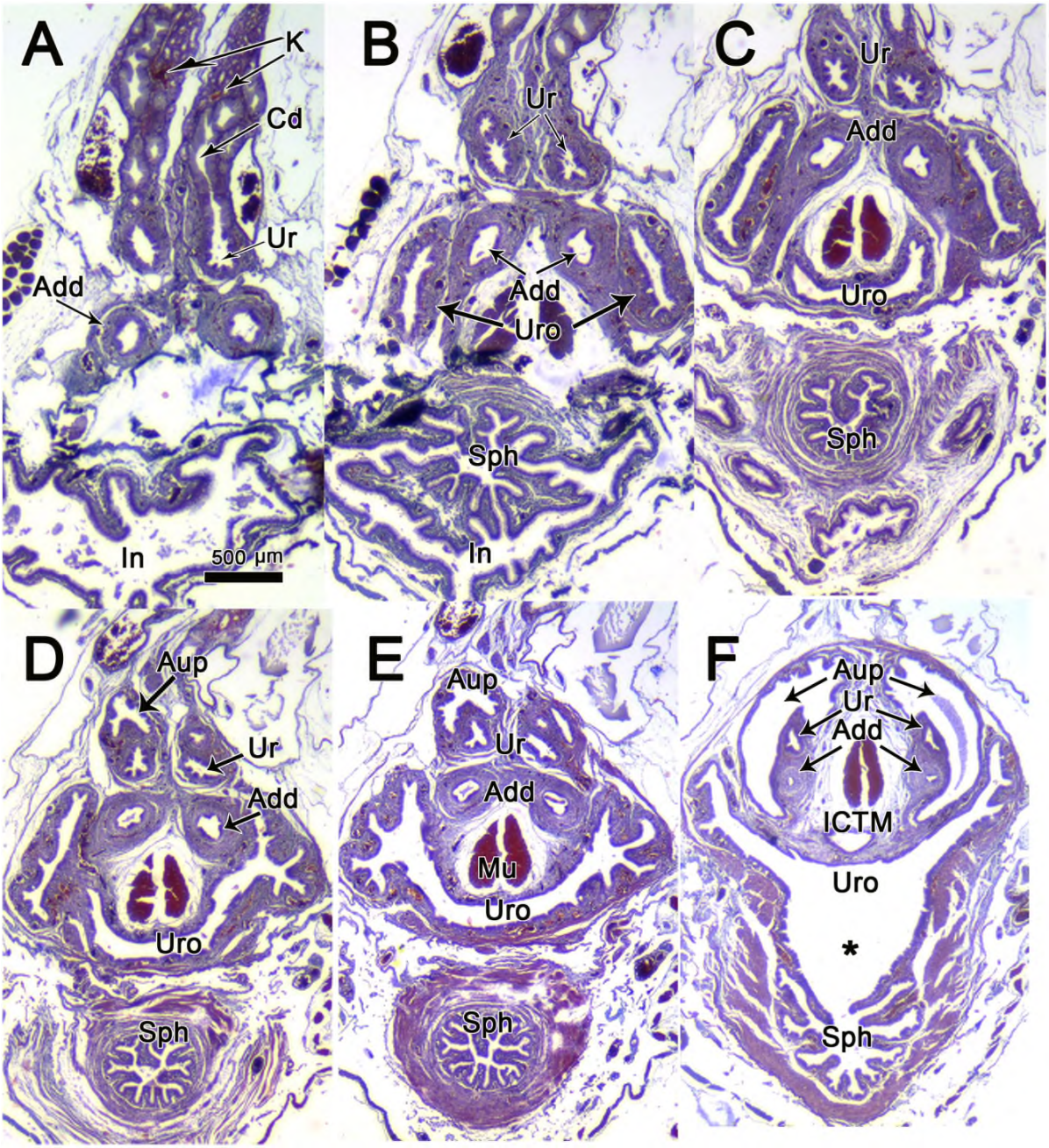

Figure 6. Light micrographs of the distal urogenital complex in *Aspidoscelis sexlineatus viridis* (ASUMZ 34090) as revealed by a cranial-to-caudal series of transverse sections. A. Section through the complex at the level of the Add and the Ur. A collecting duct (Cd) from the kidney is merging with the right Ur. B. Section showing movement ventrally by the Ur and medially by the Add as they near the region of the Uro. C. Section showing all structures in B, but revealing enlargement of the anterior arms as well as the ventral extension of the Uro. D. Section showing the elongation of the Ur and the first appearance of the Aup dorsolateral to the Ur. E. Section revealing the complete isolation of the inner core tissue mass (ICTM as labeled in F) by the Uro; striated muscle masses (Mu) are prominent. The Aup begin to increase in size lying dorsolateral to the Ur. F. Section showing the greatly expanded Aup surrounding the lateral protuberances of the ICTM containing the Ur and Add. The merging of the Uro with the coprodaeum (asterisk). Scale bar = 500 µm for A – F. Sph, anterior sphincter of the coprodaeum. Abbreviations as in previous figures; Pollak, 8 µm.

another scincid, *Plestiodon anthracinus pluvialis.* Also, Rheubert *et al.* (2015) did not mention this modification in *Aspidoscelis gularis*. Trauth and Sever (2011) provided numerous light micrographs of the ampulla ductus deferentis in a number of North American colubrid snakes. Akbarsha *et al.* (2005) suggested that the ampulla ductus deferentis in some agamid lizards functions like seminal vesicles found in mammals.

Ureter

Rheubert *et al*. (2015) examined the Ur of 7 species of lizards. The urothelium of the Ur in *Aspidoscelis sexlineatus viridis*, as reported in the present study, is similar to the urothelia found in these other species. However, only one of these species examined (*Aspidoscelis gularis*) possesses an Aup, and, as expected, the pouch structure is very similar to that found in *A. s. viridis.* Consequently, the terminal release point of urinary products in most lizards differs structurally (i.e., materials go directly from the Add into a Ugp or directly into the Uro) compared to the situation found in teiid lizards.

Urodaeum

The morphology of the Uro in *Aspidoscelis sexlineatus viridis* differs slightly from that of *Aspidoscelis gularis* as illustrated by Rheubert *et al.* (2015). In *A. gularis*, the Uro advances beneath the ICTM as a pair of ventrolateral blind pockets. More caudally these two pockets eventually merge ventrally into a broad and somewhat flattened space, termed the anterior dorsal recess (Adr) of the Uro and remain ventral in position to the developing ICTM. In contrast, *A. s. viridis* exhibits a Uro with anterior lateral arms as well as a mid-ventral pouch. These three branches eventually merge and expand posteriorly to surround the ICTM (Fig. 6C – E). Consequently, the Uro of *A. s. viridis* has structural differences from the Adr of *A. gularis*. The Adr was first described in both colubrid and crotalid snakes as anterior projecting cavities of the Uro (Trauth and Sever, 2011).

The internal lining of the Uro in the two teiid lizards mentioned above shows some structural similarity to that found in scincid lizards as illustrated in Rheubert *et al.* (2015) and Trauth (2018). For example, in the skinks (*Plestiodon fasciatus*–former study and *Plestiodon anthracinus pluvialis*–latter study), the epithelium of the Adr is highly convoluted and contains numerous primary and secondary crypts. Electron microsocopic analysis of these crypts revealed in *P. fasciatus* an orderly arrangement of sperm clustered within these spaces. In *A. s. viridis*, dorsolateral crypts are evident (Figs. 4C, 5B, 6F, 7) and are lined with a bistratified columnar epithelium.

Inner core tissue mass

The tissues of the ICTM, a previously undefined region of the distal urogenital complex in lizards, support the Ur and Add as these ducts descend ventrally into their final anatomical positions within the distal urogenital complex. Centrally located inside this tissue mass in *Aspidoscelis sexlineatus viridis* are a bilateral pair of longitudinal skeletal muscle masses (Figs. 4, 6, 7). In *A. gularis*, the muscle core appears as a single mass comprised on multiple bands of skeletal muscle (Rheubert *et al.* 2015). Immediately surrounding this inner muscle core in both species, though, is an area of loose connective tissue and blood vessels. More lateral to this layer of loose connective tissue are tightly arranged bundles of dense irregular connective tissue and elastic fibers which support the ducts. The muscularis layer of the Add supports the Add within its position in the ICTM (Figs. 7 – 8). Near their termination, orifices of both the Ur and Add are projecting outward into each Aup from the ICTM and are supported by tissue prominences (Fig. 7), although these masses were not as apparent in specimen ASUMZ 34075 (Figs. 4, 5A). The muscular core of the ICTM undoubtedly helps maintain some stability in the final positioning of these distal ducts, although their precise function remains unclear.

Ampulla urogenital papilla

The first illustration of these pouches was provided by Martin Saint Ange (1854) as drawn from the reproductive anatomy of the Grass Snake (*Natrix natrix*), a European colubrid snake. Trauth and Sever (2011) named these pouches, noting their presence in North American colubrid snakes and their absence in North American crotalid snakes. These urogenital pouches are homologous to the ampullae uriniferous papillae of female colubrid snakes (Siegel *et al.* 2011).

The Aup are striking features of the distal urogenital complex of *Aspidoscelis sexlineatus viridis* and represent a diagnostic feature found in teiid lizards (Figs. 2, 3B, 4 – 8); they are also illustrated in its close relative, *Aspidoscelis gularis* (Rheubert *et al.* 2015). These pouches receive sperm and urinary products and then immediately redirect all materials emanating from these distal urogenital ducts into the cloaca through the orifices of a urogenital papilla (Fig. 7A). A moderate degree of folding was observed within the lining of the

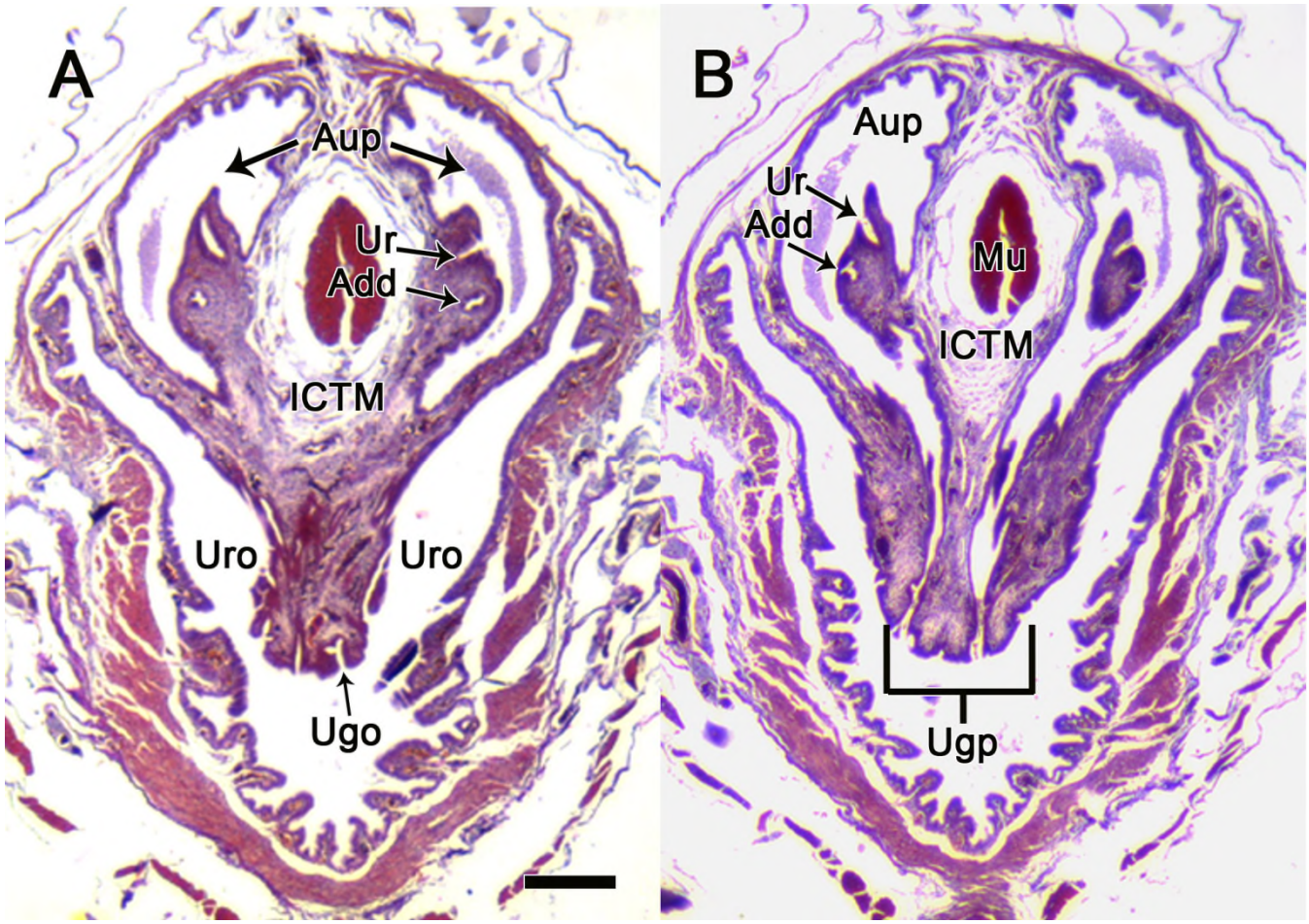

Figure 7. Continuation of Fig. 6. Formation and structure of the Ugp in *Aspidoscelis sexlineatus viridis*. A. The Ugp projects ventrally from the roof of the Uro. Both the Ur and the Add are held within fleshy masses projecting outward from medial surfaces of the ICTM. The ICTM exhibits a central pair of longitudinal masses of striated muscle (Mu) at its core that are surrounded by loose connective tissue. (See text for further explanation.) These muscle masses are present in Figs. 4; 6 – 8). B. The Ur and Add are adjoining the Aup, and urogenital orifices (Ugo) of the Ugp are present. The Ugp projects ventrally into the coprodaeum. Scale bar = 200 µm for A and B; Pollak, 8 µm.

Aup (e.g., Figs. 5B, 8A). The folding may allow for some expansion of these sacs upon the delivery of sperm. Another possible function could be temporary housing for sperm, as was observed in the Adr of *A. gularis*. At present, the function of these folds remains unclear. The internal lining of the Aup appears to be a low-to-bistratified columnar epithelium (Fig. 8).

Urogenital papilla

The termination release structure for all ductal materials in male squamates is the Ugp, which may be either a single medial structure or paired bilateral structures hanging from the dorsal wall of the urodaeum of the cloaca (Trauth and Sever, 2011; Rheubert *et al*., 2015). A detailed description and illustration of a generalized Ugp morphology (and its surrounding tissues) was provided by Trauth and Sever (2011). In addition, the highly variable external micro-anatomy of the Ugp in squamates is best viewed using scanning electron microscopy as depicted in Trauth and Sever (2011) for snakes and Rheubert *et al.* (2015) for lizards.

In *Aspidoscelis sexlineatus viridis*, the Ugp develops as a ventral extension of the ICTM (Fig. 7) and drops into a transitional space between the Uro and the coprodaeum. A thin tissue barrier separates the two Aup within the neck of the Ugp (Fig. 7B).

Only a single Ugp was present in the *A. s. viridis* examined in the present study. Variation does exit, however, with respect to Ugp structure in teiid lizards. Rheubert *et al.* (2015) showed SEM images of paired papillary mounds and paired Ugp in this species. The Ugp of other teiid lizards is found in Rheubert *et al.* (2015).

Ampulla urogenital papilla in lizards and snakes

There are 43 lizard families according to Vitt and Caldwell (2014), and I have histologically examined the distal urogenital complex in 35 species from 17 of these families. At present, only members of the Gerrhosauridae (1), Gymnophthalmidae (2), Teiidae (5), and Varanidae (1) exhibit Aup. In snakes, all members of North American colubrid snakes possess Aup, whereas no North American crotalid snakes have them (Trauth and Sever 2011). Future research in other squamates may reveal interesting relationships expressed by the presence or absence of these pouches.

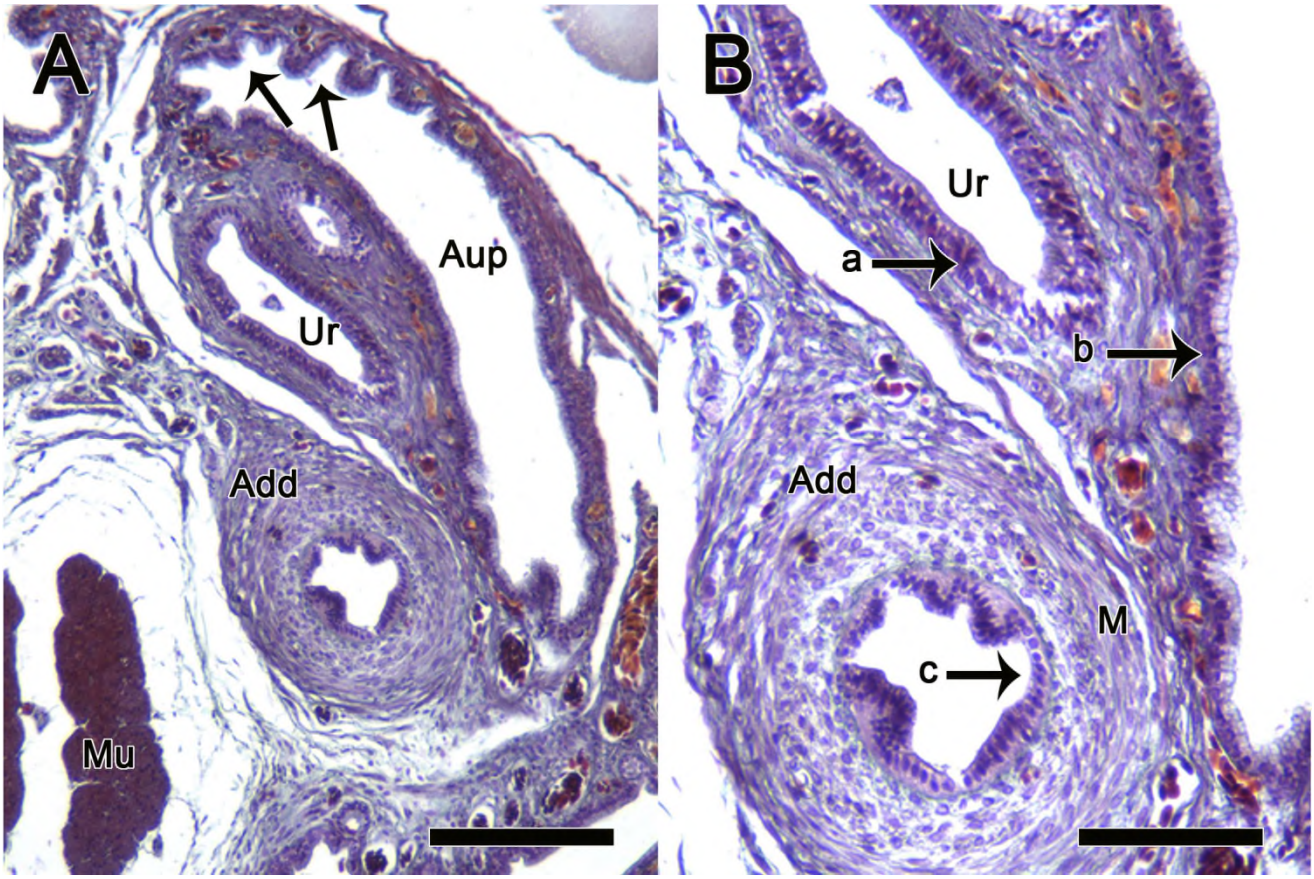

Figure 8. Light micrographs showing the urogenital epithelial types in *Aspidoscelis sexlineatus viridis* in an inactive lizard (ASUMZ 34090). A. Image of the Aup lying lateral to the Ur and Add. Arrows point to dorsal folding. B. Magnification of A. Urogenital epithelial linings: a–urothelium; b–low columnar epithelium; c–pseudostratified columnar epithelium. Mu = striated muscle mass of ICTM. Abbreviations the same as in previous figures. Scale bar = 200 µm for A; 100 µm for B. Pollak, 8 µm.

Acknowledgments

I thank the Arkansas Game and Fish Commission for authorizing scientific collection permits for me for the past 35+ yr. Also, I dedicate this paper to the late Dr. D. M. Sever (1948-2019), a longtime friend, academic colleague and the most productive and articulate histo-herpetologist of all time.

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The Common Feeder Cockroach *Blaptica dubia* Shows Increased Transmission Distance Based on Mode of Acquisition of Environmental Bacteria

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Running Title: *B. Dubia* Microbial Transmission Distance by Mode of Acquisition

Abstract

Although some researchers claim that cockroaches are masters of disease transmission, these claims have little to no scientific support. Most studies concerning cockroaches as a vector of disease only focus on the bacteria found on the body surface, not on whether cockroaches have actually transferred pathogenic bacteria via surface contact. We set out to determine if cockroaches would act as a mechanical vector for the transfer of the opportunistic pathogen, *E. coli*. Roaches were contaminated with Green fluorescent protein expressing *E. coli* (GFP-E. coli) broth by either walking the roach through a broth culture or by complete immersion in the culture. We then ran the roaches down a sterile agar track and measured the length of the glowing trail. Roaches were able to transmit *E.coli*, but only for a continuous distance of less than 50 cm, with the occasional sporadic colony growing after that. Roaches that were immersed in bacterial broth tracked the bacterium further than those that only walked through the solution. This suggests that while cockroaches are capable of acting as a mechanical vector, they are not capable of transporting transient flora over long distances. Future studies should explore this mechanism.

Introduction

The world we live in is full of pathogens. Our modern Western culture encourages sanitizing everything, yet we are not able to fully separate ourselves from all potential health threats. We still do not fully understand how and to what extent organisms that enter our homes bring microbial life with them. Humans are known to harbor the occasional pathogenic bacteria on our body surfaces (Chiller *et al.* 2001). *Vectors* are any organism that transmit bacteria, viruses or parasites from one organism to another. Vectors can transmit potential pathogens into our environments or even into our bodies in multiple ways. *Transmission* is the passing of a pathogen by direct contact to a new host (Tan *et al.* 1997). Transmission is *biological* if the pathogen reproduces or develops within a potential vector. Transmission is *mechanical* only if there is no reproduction or development of the pathogen in the vector (Mullen and Durden 2009).

Arthropods are regular, oftentimes, unknown visitors to our living spaces, and some are potential vectors of disease transmission. Cockroaches are common arthropods that have been associated with disease for generations, and are commonly found in and around dwelling places. (Moges *et al.* 2016). Because of their association with disease, people often assume that roaches will bring pathogens into these spaces biologically or mechanically (EL-Sherbini and Gneidy 2012).

The American cockroach *Periplaneta americana* and German cockroach *Blattella germanica,* common pest species of roaches, are both members of what the FDA deems the Dirty 22, which consists of the species most commonly associated with the spread of foodborne pathogens (Jones *et al.* 2013). Numerous studies have shown that wild-caught cockroaches do in fact carry various pathogenic bacteria such as *E. coli*, *Salmonella sp*, *P. aeruginosa*, etc., (Tatfeng *et al.* 2005; Fotetar *et al.* 2009; Hamu *et al.* 2014; Xue *et al.* 2009; EL-Sherbini and Gneidy 2012; Moges *et al.* 2016; Mpuchane *et al.* 2006).

Although many pathogens have been recovered from the bodies of natural populations of cockroaches, this does not necessarily mean that cockroaches serve as vectors for these pathogens. Isolation of pathogens from cockroaches may simply indicate the natural microbial fauna and flora of the domestic environment in which they were found (Mullen and Durden 2009). Cockroaches tend to live in dark, damp conditions, such as municipal sewer systems or septic tanks, and this can be a cause for concern because they also are commonly found in living spaces such as pantries and bathrooms. Yet, there have been few studies of the actual transmission of pathogenic bacteria by

cockroaches, or any arthropods. Transmission can be accomplished by behaviors that include walking or landing on a surface, feeding on a substrate, regurgitating on a surface, or defecating on a surface that will then be contacted by another organism such as a human (Foil and Gorham 2004). Few studies directly test the ability of cockroaches to transmit potential pathogens directly. While cockroaches may have been found to have pathogenic strains of bacteria on their surfaces, if they are not able to transmit a meaningful quantity of bacteria by walking across a surface, there would be no reason to see them as a significant vector of disease transmission.

In addition to a lack of direct implication of roaches as vectors, some suggest that chitin and chitosan found in roach bodies have some antimicrobial properties; this could possibly affect the potential transmission of bacteria (Basseri *et al.* 2019). Even knowing this, we hypothesized that the roaches would likely transmit some bacteria mechanically, based on research done on *Musca domestica* and their transmission of Rotavirus from their legs and wings (Tan *et al.* 1997).

In the process of determining if roaches could physically transmit bacteria, we also wanted to determine whether the mechanism of roach contamination would affect the distance that the bacteria would be tracked. Some species of roaches are capable of crawling through plumbing, having been immersed in potentially pathogen-filled fluids, while others may enter homes and merely walk across trash or contaminated surfaces. So, we asked if the mechanism of contamination would affect the ability and efficiency of physical transmission of bacteria.

The species we chose to use is *Blaptica dubia*, a common feeder roach that was readily available in the laboratory. In addition, we chose to use a non-pathogenic strain of *E. coli* (strain HB101 transformed with the pGLO plasmid) to reduce the danger of infection, while still using a microbe very similar to a common pathogen that might be encountered by both an escaping feeder roach or a home invader.

When determining the best course of action in testing our questions, we found there was no standard method of testing the physical transmission of pathogenic bacteria by arthropods. This study will be both a first test of direct physical transmission by a cockroach and also an introduction to a preliminary set of methods that can be altered and improved for future use. With this project, we can begin directly testing long-held assumptions about pathogens and arthropods that we encounter in our daily lives.

Materials and Methods

All animals that we used came from a well-maintained colony of *B. dubia* housed at Harding University. Roaches were not used more than once and were euthanized after exposure to bacterial contaminants.

In order to determine the length of track that we would need, roaches were run down a track made of a 1 m long piece of wood painted black with sides of aluminum flashing 8.89 cm tall. The roaches were placed in a dish of neon orange chalk in which they could walk around before moving down the long track. A dark hiding place was located at the end of the board to encourage the roach to move from the bright lights of the lab to the end of the track. A clear plastic sheet with 5 mm squares printed on it was laid over the board and the number of squares with chalk in them and the length of the trails was measured and recorded. The number of squares with chalk did not turn out to be as useful in analysis so the length of the trails in 5 mm square units was used as the dependent variable for future tests. This test demonstrated that a couple of roaches did track chalk beyond a 0.5 m distance and most chalk was deposited very close to the origin so a 1m track was used to both conserve agarose gel and provide an adequate distance to test for transmission.

Preparation of the Agar track and Bacteria

Aluminum flashing was wrapped in foil and autoclaved at 121 ℃ for 20 minutes. Next, 600 mL of LB agar with 10 % w/v arabinose sugar was poured into the base of the 1 m track made of the pre-sterilized aluminum flashing. This produced a single unbroken sheet of agarose. The arabinose added to the agar was necessary to activate the arabinose operon in the GFP-producing *E. coli.* Colonies that grew would glow under UV light. Plastic wrap was used to cover the opening at the top of the track. This allowed us to both see the roach running and allow light in to motivate roach movement, while limiting airborne bacterial contamination. A new batch of fresh liquid GFP *E. coli* was made up for every trial of the experiment. One hundred ml of *E. coli* was added to 250 ml of broth with 60 ml of arabinose and incubated overnight at 37 ℃.

Mechanical Transmission Assay

Equal numbers of both male and female roaches of at least 1.5 cm were chosen for each trial, and placed in

numbered centrifuge tubes. The roaches were then split into two groups with one group strictly walking through a small petri dish filled with 3 ml of bacterial culture broth and immediately across the agar track, and other roaches were shaken 10 times in a 50 ml conical tube containing 3 ml of bacteria broth solution. Methods were adapted from a study of bacterial sampling from roach and fly bodies in mechanical transmission of medical important parasites (EL-Sherbini & Gneidy 2012). We placed roaches at one end of the track and observed the roaches as they moved to the other end of the track. Normal room lights and a dark hiding spot provided by an egg crate at the end of the track was used to motivate animals to run the length of the track. We noted any stops and other activities of the roaches such as chewing the agar and walking in an irregular pattern. The roaches were euthanized after each trial and the tracks were incubated at room temperature for 48 hours. Next, we observed tracks under a black light and GFP *E. coli* colonies were counted using the plastic square method used in the chalk trials. We measured with a ruler and recorded any long continuous trails of *E.coli*. We also noted any other non-glowing bacterial and fungal colonies.

Data Analysis

We performed a power analysis to determine a proper sample size of 19-26, but were limited by time and resources to a sample size of 12 individual roaches, 8 in each group. We used length of trails made by roaches as the dependent variable in an ANCOVA following a test of normality that caused us to Log transform the data. Roach body length was used as the covariate and method of contamination was used as the independent variable. We used 12 total roaches, 6 shaken and 6 walk through. Roaches that did not leave trails or that did not finish running the track were removed from analysis. Descriptive statistics were graphed using Excel.

Due to our small sample size, we ran an estimate of power for the ANCOVA test of 0.4, using the equation found in McDonald (2015), we found that in order to achieve an 80% to 90% power increase we would have needed to test 19 to 26 individuals of each category of shaken and unshaken roaches. Due to time and money constraints, we were forced to stop our trials after 12 trials.

Results

We found that roaches that were shaken in tubes showed a trend of longer trails of glowing *E.coli* than roaches that walked through the bacteria (df=1; p=0.49). The power for our test was only 40%. The average length of the trail produced by the immersed group was 21.14 cm, while the average length of the walk through group was 4.25 cm (Figure 1). Occasionally isolated colonies were observed beyond the trail but were rare and most GFP *E. coli* were observed in continuous trails.

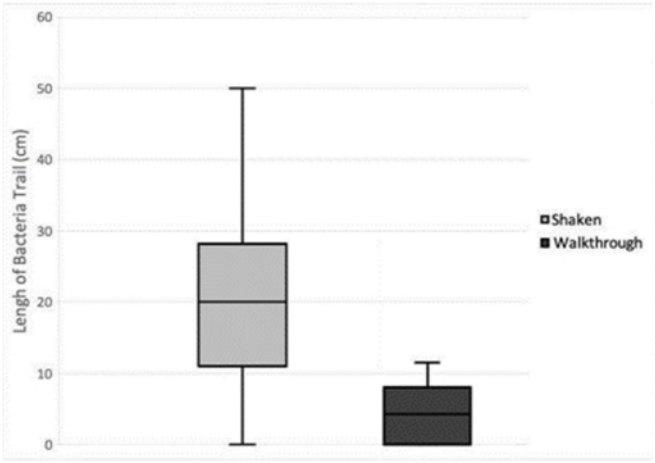

Figure 1: Transmission of GFP *E. coli* by *Blaptica dubia* by different means of exposure. Boxplot of the bacterial trail length in centimeters between *B. dubia* exposed to GFP *E.coli* by being shaken (full body exposure) and walking through a pathogenic broth

It is appropriate to note that other bacterial and fungal colonies were found to be growing on our tracks. We did not identify them at this time. It is assumed that the colony growth was transferred from the roach's body to the agar along with the *E. coli*. This was most likely due to the fact that we introduced our roaches to our E. *coli* broth, straight from their home environment in the lab. This would allow them to transmit some of their own native fauna and flora to our agar.

Discussion

Our tests indicated that roaches were capable of physically tracking *E. coli* by either walking through or by being immersed in it. The trail of bacteria in both situations was less than 0.5 m. The few isolated colonies observed, suggest the potential distance for

transmission is likely longer than our measured trails, but we did not have a large enough sample size to quantify this potential pattern. A power of 80-90% would have required a sample size of between 19-26 roaches per treatment. Despite our small sample size of 6 roaches per group Our observations suggest that roaches could be considered potential vectors, but they would not be efficient at transmission at distances greater than 0.5 m from the source of contamination. It was clear that roaches completely immersed in bacteria tracked microbes over a greater distance than those that merely walked through the petri dish of bacteria broth. Roaches tend to show positive *thigmotactic* behavior (Laurent *et al*. 2018) suggesting that they might be more likely to contact potential pathogens on multiple body surfaces in the tight places they prefer. Because of this, immersive environmental transmission might be the better approximate to a real-world transmission scenario.

Previous studies have repeatedly indicated that human habitations can have potentially-pathogenic bacteria in and around them (Tatfeng *et al.* 2005; Fotetar *et al.* 2009; Hamu *et al.* 2014; Xue *et al.* 2009; EL-Sherbini and Gneidy 2012; Moges *et al.* 2016; Mpuchane *et al.* 2006). This is the first study that has highlighted a roach directly transmitting an introduced bacterium from their body onto a surface. In this study, we did not directly test whether the transmission method was truly mechanical or biological, but due to the short period of time between infection and transmission, it can be assumed that this is an example of mechanical transmission with no pathogenic reproduction or development.

This study was also the first to use common feeder cockroaches as a model. All previous work has focused on well-known pest species, such as the American cockroach and the German cockroach that were caught in the wild, while ignoring the species that people intentionally bring into their homes. Despite many species of roach being considered major pests and health concerns, some people bring roaches into their homes to serve as food for exotic pets, or as the exotic pets themselves. There are a variety of common cockroach species that are found in the pet trade. Just because an arthropod is intentionally brought into a home, does not mean that it is not a health risk if it escapes and crawls through trash or other bacterially-infected substrates. In fact, we found that after our cockroaches were contaminated by the bacterial broth, they tracked the bacteria a relatively short distance, meaning the greatest threat is likely within centimeters of the source of contamination. Using these model organisms does limit our potential conclusions to the more common pest species; however, we have no reason to believe that roach anatomy and exoskeletal physiology differences between the species should prevent us from making tentative and testable predictions about species more relevant to public health. However, if this species can take the place of more troublesome species, then it may represent a good model for future transmission studies looking at potential contamination mechanisms. Further work is needed to support these data and to see if other species of pathogenic bacteria show different transmission patterns. Future tests should also look at whether the results seen in this species can be applied to the more pestiferous species.

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Distribution and Reproduction by the Purple Gallinule (*Porphyrio martinica*) in Arkansas

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Running Title: Purple Gallinule in Arkansas

Abstract

The Purple Gallinule (*Porphyrio martinica*) is a rare bird in Arkansas, and its populations likely have declined due to loss of marshy areas with emergent vegetation. By use of online sources for citizen science combined with a field study, we elucidate the current distribution of this bird in Arkansas, and document characteristics of reproduction and development. Purple Gallinules arrive in Arkansas as early as April and remain to late October. Nesting occurs from early May into July, and nests may represent second broods. Ontogenetic changes in in plumage and bill coloration hatchlings are described.

Introduction

Baerg (1931) observed that the Purple Gallinule (*Porphyrio martinica*) had not been reported from Arkansas, but believed it likely was a rare summer transient in Arkansas because it was apparently common in Louisiana. However, James (1974) wrote that this bird was formerly common in low wetlands from Pulaski and Lonoke counties and southward, but the species was informally listed as an endangered breeding bird in Arkansas due to limited habitat.

Larger breeding colonies of Purple Gallinules had existed in abandoned fish farms in the Grand Prairie region near Stuttgart until about 1954, when colonies declined after reclamation by agriculture (James and Neal 1986). Similarly, regular breeding by Purple Gallinules in southern Oklahoma was observed when neglected fish hatchery ponds had become chocked with vegetation, but breeding birds disappeared when the ponds were cleaned (Baumgartner and Baumgartner 1992). Because 90% of historic wetlands in Arkansas have been drained for agriculture, wetland vegetation declined, followed by declines in Arkansas populations of this bird (Budd 2007).

The most recent summary of information regarding the Purple Gallinule in Arkansas was compiled by James and Neal (1986). At that time, this marsh bird was known as a local migrant or summer resident in Chicot, Columbia, Crittenden, Hempstead, Jefferson, Lafayette, Lonoke, Pike, and Pulaski counties. Evidence of reproduction was seen in the presence of flightless young at some of these locations. The birds had been observed in cattail-lined lily-pad ponds or in flooded fields and ditches that had suitable vegetation. Because gallinules have especially long toes for walking on marsh vegetation, their habitat options are limited in Arkansas. Data gaps now exist in terms of current distribution, habitat, and reproduction.

Currently, Arkansas has a hunting season for these birds. As has been for many years, the 2019-2020 Early Migratory Bird Season for Common (*Gallinula galeata*) and Purple Gallinules was 1 September – 9 November, with daily limits of 15.

Materials and Methods

To determine distribution and dates of migration, we compiled records verified by the Arkansas Audubon Society and published on their website (http://www.arbirds.org/aas_dbase.html), the citizen science website hosted by the Cornell Lab of Ornithology (https://ebird.org/explore), and reports on the discussion list ARBIRD-L (ARBIRD-L@listserv.uark.edu) hosted at the University of Arkansas. These sources included not only records of sightings, but comments describing presence and appearance of young, indicating reproduction.

Study Site. – We discovered a population of Purple Gallinules at the oxidation ponds (part of the Arkadelphia water treatment facility) 4 km S of Arkadelphia in Clark Co., and followed their behavior and reproductive cycle through the summer of 2019. There, a rectangular pond of about 300 x 140 m (= 4.2 ha), develops a thick growth primarily of 2 plants. A tall plant reaching heights of about 1.2-1.5 m (4-5 ft.),

Smooth Bur Marigold (*Bidens laevis*, family Asteraceae) dominated the pond and provided feeding and hiding cover, and elevated perches. A shorter (< 0.3 m, or 1 ft.), trailing plant, Floating Marsh Pennywort (*Hydrocotyle ranunculoides*, family Apiaceae) grew along the banks and was scattered in a mosaic pattern among the *B. laevis* across the pond. Gallinules foraged and nested among both plants.

Adults and their young were philopatric to small territories on the pond. Over a period of several weeks, we photographed hatchlings (Nikon D7000 camera with DX 300 mm lens, distances between 10-35 m) from 2 nests at our study site, and used these images of known-aged birds to estimate ages of other young found at our study site, as well as images provided by citizens on e-bird. These data were used to estimate timing of reproduction for nests of otherwise unknown hatching dates. Detailed observation of nests and chicks was limited to those found within about 35 m of the bank.

Having estimated hatching dates of chicks based on patterns of development in size and plumage, we further back-dated to estimate the dates of nest completion. Incubation period has been measured at 18-22 d (Gross and Van Tyne 1929; Grimes 1944; Trautman and Glines 1964; Matthews 1983). We used an estimate of 20 d to determine the likely timing of nest completion and onset of incubation.

Results and Discussion

Distribution: The first Purple Gallinule recorded from Arkansas was in Hempstead Co. on 28 May 1939, followed by one in Prairie County on 25 August 1940. The earliest reported date in Arkansas was in northern Arkansas (Benton Co.) on 9 April 2010. Records of the birds across appropriate habitats in Arkansas continue almost uniformly through the spring and summer, with the last bird sighting reported on 21 October. The birds tend to become summer residents in marshland habitats near river systems (Arkansas, Ouachita, Red, and White) and their tributaries. Reported locations, including rare observations, are included in Fig. 1. As these records are composited from "citizen science", it must be remembered that the data do not represent a systematic survey, and that less accessible habitats also may support summer residents and nesting pairs.

Most sightings record only a few birds, but good habitats have produced higher counts on a given day, e.g.: Arkansas County at Arkansas Post (26); Hempstead County at Lester Sitzes III Bois d'Arc WMA (36); Howard County at Millwood Lake (10); and Pulaski County at Faulkner Lake (25). For this reason, these sites are visited often by birders wishing to see this rare species in Arkansas.

Examination of historical distribution shows the longest term of continued occurrence in the lowlands of the eastern Arkansas River area, and in southwestern Arkansas near the Red River system (Fig. 2). From 1939-1969, the species was recorded from Arkansas, Chicot, Columbia, Hempstead, Jefferson, Lincoln, Logan, Lonoke, Perry, Prairie, Pulaski, and Woodruff Cos. During the decade of 1970-1979, the bird was reported from only 4 cos. including the addition of Crittenden and Union Cos. From 1980-1989, these gallinules were reported from 5 cos. including 4 new cos. (Howard, Lafayette, Pike, and Pulaski). From 1990-1999, occurrence was reported in 4 cos. including the addition of Cleburne and Scott.

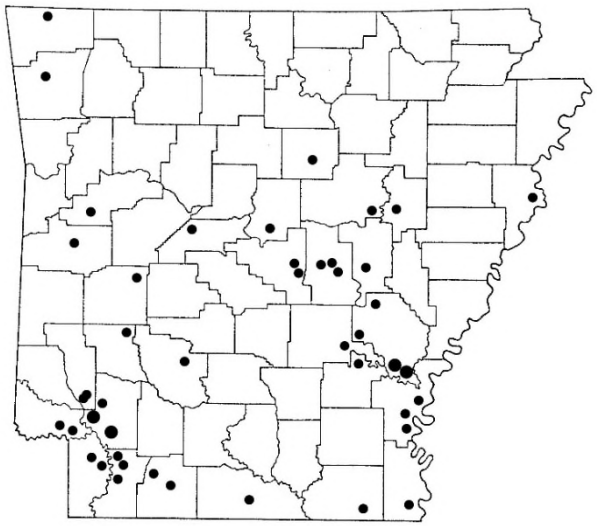

Figure 1. Distribution of Purple Gallinules (*Porphyrio martinica*) in Arkansas based on literature and records compiled in ebird.com. Dots indicate locations of observation, and larger dots (Arkansas and Hempstead Cos.) indicate "hot spots" frequented by birders due to public accessibility and expectation of seeing marsh birds.

Interest in birds and reporting of records increased after 2000. From 2000-2009, observations were reported from 10 cos., with new records for Clark, Desha, Faulkner, Little River, Miller, and White Cos. Ashley, Benton, and Montgomery Cos. were added from 2010-2019, and birds were reported from 13 cos. during the decade. To date, Purple Gallinules have been recorded in 29 counties.

Nesting, Eggs, and Hatching: Apparently, the first record of Purple Gallinules breeding in Arkansas was an observation from 1947 reported by Baerg (1951) in

Lonoke Co. James (1974) reported a second nest observed in Woodruff Co. in 1967. Although few other details of nesting have been reported in Arkansas, successful reproduction is evidenced wherever flightless young birds are seen. Birds described by citizens as chicks, poults, juveniles, immatures, young, or fledglings have been reported in Arkansas, Chicot, Clark, Hempstead, Howard, Lafayette, Lonoke, Miller, Pulaski, and Woodruff Cos. (Fig. 2). These observations reflect more recent nesting in those counties.

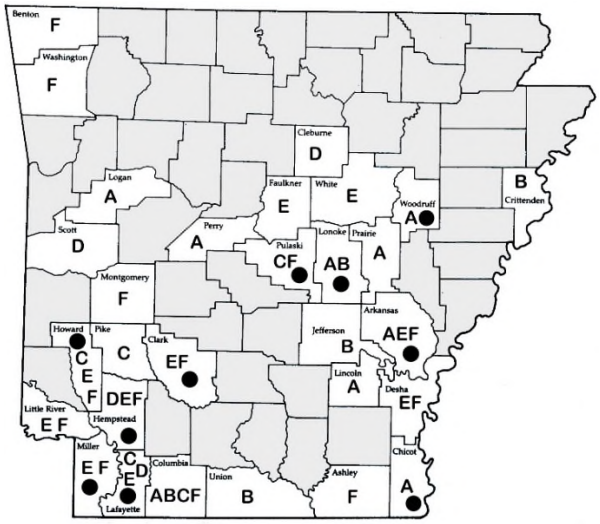

Figure 2. Historical distribution of Purple Gallinules in Arkansas. Unshaded counties have records, and lettering represents time frames for the records: A = 1939-1969, B = 1970-1979, C = 1980-1989, D = 1990-1999, E = 2000-2009, and F = 2010-2019. Dots indicate counties in which breeding has been reported.

James (1974) commented that nests often were made of cattails in shallow marshes having open water, tall weeds, and floating vegetation. Abandoned rice fields, similar to natural habitat, were thought to be suitable for nesting. Rice fields, especially those lined by ditches, support breeding Purple Gallinules in the Gulf Coastal Plain of southwestern Louisiana, where the species is common (Pierluissi *et al*. 2010). However, no breeding Purple Gallinules were detected closer to Arkansas in rice fields of the Mississippi Alluvial Valley in northeastern Louisiana (Valente *et al*. 2012). Similarly, Budd and Krementz (2011) found Purple Gallinules at only 2 sites in the Mississippi Alluvial Plain of eastern Arkansas, and no evidence of breeding except the observation of a bird at Arkansas Post National Memorial carrying nest material.

Records from eBird and ARbird web sites document nests at Joe Hogan State Fish Hatchery in Lonoke Co. from 1955-1957, on dates ranging from 4 June – 5 July. Also, in Lonoke Co., nests were observed at Anderson Minnow Farms on 7 July 1971.

We found 3 nests at the Clark Co. site. On 15 June, we located a nest with 3 newly-hatched black chicks. This nest was positioned in an open area, about 10 cm above the water and consisted of leaves of *B. laevis*, *B. laevis* pulled over the top of the nest. The female incubating the eggs sat with her wings slightly spread, presumably providing shelter against the heat. On 10 August, a third nest was found elevated about 0.5 m above the water, in *B. laevis*, and the nest composed of its leaves. These nests are consistent with the descriptions of the 3 nest types found in southern Louisiana rice fields (Helm 1982), and plant materials there included *Hydrocotyle* (Helm *et al*. 1987). Based on images of young in Arkansas, available on e-Bird, other plants used as habitat and associated with nesting populations include American Water Lotus, (*Nelumbo lutea*), Water Hyacinth, (*Eicchornia crassipes*), Alligator Weed, (*Alternanthera philoxeroides*), cattail, (*Typha* sp.), and Smartweed (*Polygonum* sp.).

In Arkansas, eggs in nests have been reported on dates ranging from 28 May – 12 June (James and Neal 1986). Consistently, more recent online reports also note nests with eggs in Lonoke and Hempstead Cos. from late May to mid-June. At our Clark Co. site, we found eggs in nests on 15 and 27 June. By use of age estimates of 14 clutches of chicks (10 at our site and 4 images posted online), we calculated (assuming a 20-day incubation period) that eggs would have started incubation on dates ranging from 3 May – 9 July. Egg dates in Texas ranged from 9 April – 12 August (Oberholser 1974).

From earlier records, James and Neal (1986) reported clutch size as 4 – 6 eggs. Our field records plus online comments show a clutch size of 3 – 6 ($\bar{X}$ = 4.6, mode = 5) based on 9 clutches of eggs. Estimated and known hatching dates together ranged from 23 May – 29 July.

Ontogeny of Young: We examined our series of images to determine changes detectable in birds of known age. Ontogenetic changes in appearance of young are illustrated in Fig. 3, and are consistent with Helm (1982). Hatchlings were black and fuzzy, and their beaks were reddish at the base, transitioning to black then whitish (which could form a band around the beak). Blackish coloration continued to near the tip of the beak, which had a white dot on top (the egg tooth, which disappears within 3 weeks). At 14 days, young were overall larger, but the neck and legs had elongated compared to the body. Otherwise, coloration

of the beak was less discrete, but the body still was uniformly black.

By 23 days, young became buffy (light brown) along the underside of the body from the face to the tail. The back was becoming lighter as well, but the back of the head and neck were black, and 2 black streaks were present from the thoracic region to the rump.

By 31 days, legs and toes were well-developed, feathers on the wings were becoming distinguishable, and only short dorsolateral streaks of black feathers were visible above and anterior to the wings. After this period, young birds became overall buffy with no black coloration, and elongation of remiges were the best indication of age. Full adult coloration did not appear in any of the immature birds we observed, though tinges of adult bluish coloration were becoming apparent on the wings as the birds were able to begin flight.

As the beaks age, the differences in color become less apparent and a dark region expands up the forehead behind the reddish base of the beak. This structure continues to expand forming a forehead shield that will become a turquoise color at maturity.

***Second Clutches and Maturation*:** Our estimated dates of hatching were bimodal, with somewhat continuous dates from 3 May to about 7 June followed by a gap of over 3 weeks, after which 5 clutches were produced in late June and the first 1.5 weeks of July. Further, we observed 2 instances in which juveniles from a territory that produced a successful nest were helping younger birds on the same territory. Those juveniles moved to cover and guide the chicks into protective vegetation upon our approach. Thus, we interpreted the chicks to be from a second clutch by the same pair of adults. A similar presumed second clutch with fledged juveniles caring for younger birds, and both responding to vocalizations of the adult, was witnessed on 15 September 2019 in Arkansas Co. Helpers in Purple Gallinules can increase reproductive success of the breeding group (Hunter 1985). Multiple clutches with juveniles as helpers are known in tropical populations (Krekorian 1978) and presumed in coastal North America (Grimes 1944, Helm 1982, West and Hess 2020).

Most adults had migrated from the Clark Co. site by 13 September, but we found a lone adult on 19 October. Several fledged, buffy juveniles remained on this date and were assumed to have migrated later. However, the smaller birds from later clutches, which had not fledged at our last observation, may have succumbed to cold weather.

***Foods and Care of Offspring*:** Adults tend to consume invertebrates (West and Hess 2020). Photos from Arkansas show dragonflies (Amberwing, *Perithemis tenera*, and a pennant *Celithemis* sp.) and an unidentified crayfish being eaten. Young forage in the territory of the parents, and respond quickly to parental vocalizations by seeking cover within vegetation (West and Hess 2020). In the oxidation ponds, dipterans are abundant and are expected to be the primary food base. We witnessed young feeding among the *H. ranunculoides*, and running for shelter when adults vocalized.

Acknowledgments

We thank the many bird enthusiasts who systematically collect and report observations of birds. We thank Dan Scheiman for his efforts in updating the eBird data base for Arkansas birds, which made this project much easier to conduct. Brett Serviss identified the plants.

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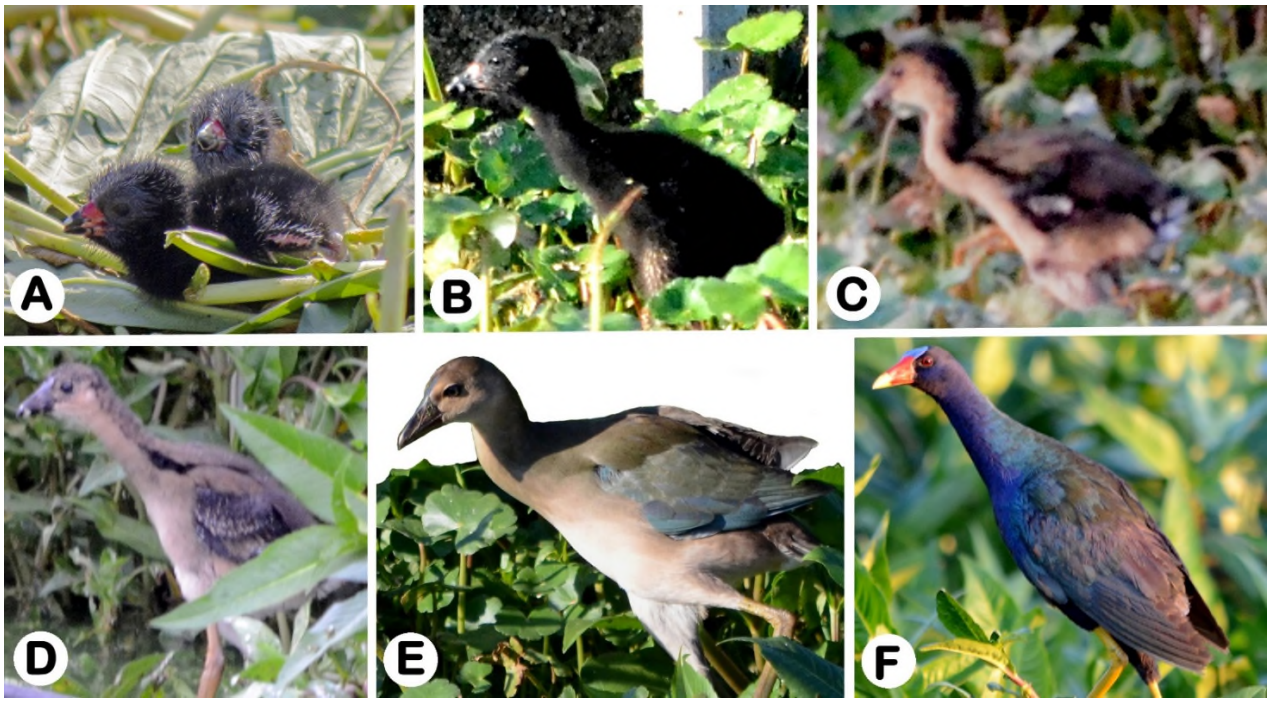

Figure 3. Ontogeny of development of chicks of Purple Gallinules in Clark Co. A: newly hatched chicks are covered in black fuzzy down. The beak is reddish at the base, transitioning to black then whitish (sometimes forming a band around the beak), then blackish continues to near the tip of the beak, which has a dorsal white dot. B: at 14 days, young were overall larger and still black but less fuzzy, the neck and legs had elongated. Reddish coloration of the beak was less discrete. C: by 23 days, young became buffy along the underside of the body from the face to the tail. The back was becoming lighter, but the crown and neck were black, and 2 dorsolateral black streaks were present from the thoracic region to the rump. Color distinction on the beak was increasingly blurred. D: by 31 days, legs and toes were well-developed, feathers on the wings were becoming distinguishable, and only short dorsolateral streaks of black feathers were visible above and anterior to the wings. Some distinction of coloration on the beak remained, but the formerly reddish region appeared reduced as the forehead shield developed up the face. E: after this period, young birds became overall buffy (darker dorsally and lighter ventrally) with no black coloration, and elongation of remiges was the best indication of relative age. The beak became more uniformly grayish, and the facial shield extends to the level of the eyes though it does not obtain adult coloration. Full adult coloration did not appear in the plumage or facial shield of any of the immature birds we observed, though tinges of adult bluish coloration were becoming apparent on the wings as the birds were able to begin flight. Images are not to the same scale.

Clinostomum marginatum (Digenea: Clinostomidae) from Fishes of Crooked Creek, Boone and Marion Counties, Arkansas

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Running Title: *Clinostomum marginatum* from Crooked Creek Fishes

Abstract

Crooked Creek is a renowned trophy (blue ribbon) Smallmouth Bass (*Micropterus dolomieu*) fishing stream. This fish, however, has been previously reported to commonly harbor some of the highest population densities of the digenean trematode parasite, *Clinostomum marginatum*, otherwise known as "yellow grub". The parasite infects the orobranchial cavity, gills, and peritoneal cavity of Smallmouth Bass. Historical studies on this fish over the last 3 decades or more from various sites on the creek have shown that *M. dolomieu* also have high mean abundances but fish from upstream sites had lower prevalence than those collected from downstream sites. Here, we survey several fishes from the creek for *C. marginatum* as well as compare our data on *M. dolomieu* to some of the previous studies conducted on the same species from the watershed. In addition, a new host record for *C. marginatum* is documented in the Ozark Bass, *Ambloplites constellatus*.

Introduction

Crooked Creek is a 129 km (80 mi.) long Ozark highland White River tributary in Boone, Marion, and Newton counties of northern Arkansas (Fig. 1). The stream's headwaters (36°06′47″N, 93°02′19″W) begins in Newton County at Sulphur Spring just south of Harrison on the north side of Sulphur Mountain and east of Marble Falls and flows north passing under State Highway (St. Hwy.) 206 just west of Elmwood (Boone County). The stream continues north traveling parallel (eastward) to St. Hwy. 7 passing through the southeastern part of Harrison and under US 65. It immediately turns eastward, beginning a long series of meanders and then turns southeast passing the communities of Pyatt and Summit (Marion County) and under US 62. It continues to flow eastward roughly paralleling US 62 (south) and crossing under St. Hwy. 14 south of Yellville. The stream continues further eastward and goes under St. Hwy. 101 near Rea Valley until entering the confluence of the White River just south of Buford Station below the town of Cotter in Baxter County (36°10′15″N, 93°06′55″W).

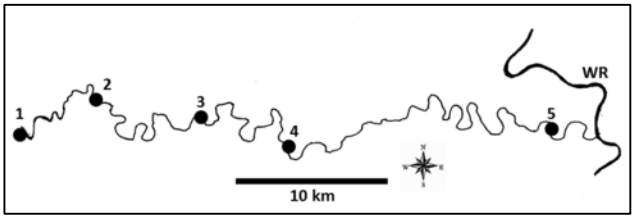

Figure 1. Crooked Creek and 5 accesses (•). 1 = Harmon; 2 = Pyatt; 3 = Snow; 4 = Kelly's Slab; 5 = Doe Bambi. Abbreviation: WR (White River).

The yellow grub, *Clinostomum marginatum* (Digenea: Clinostomidae) has a long published history of scientific documentation from basses (*Micropterus* spp.) from Crooked Creek. Studies on this trematode in mostly Smallmouth Bass (*M. dolomieu*) have originated from seminal research done by Daly and associates (Daly *et al*. 1987, 1991, 2002, 2007; Daly 2013, 2014). In addition, McAllister *et al.* (2016) reported *C. marginatum* from Northern Studfish, *Fundulus catenatus* from Crooked Creek at Kelly's Slab, Yellville, Marion County.

Clinostomum marginatum has also been found in Channel Catfish (*Ictalurus punctatus*) from a commercial pond in northwestern Arkansas (Daly and Singleton 1994). Tumlison *et al.* (2019) reported a hyperinfection of *C. marginatum* in a Black Bullhead (*Ameiurus melas*) from the upper White River, Arkansas. Other records of *C. marginatum* in Arkansas fishes include: **FUNDULIDAE:** Golden Topminnow, *Fundulus chrysotus* (McAllister et al. 2020); **ICTALURIDAE:** Ozark Madtom, *Noturus albater* and Caddo Madtom, *Noturus taylori* (McAllister *et al*. 2015); **CENTRARCHIDAE:** Bluegill, *Lepomis macrochirus*, Warmouth, *Lepomis gulosus*

(Becker and Cloutman 1975; Cloutman 1975); Spotted Bass, *Micropterus punctulatus*, Largemouth Bass, *Micropterus salmoides* (Becker and Cloutman 1975; Daly *et al*. 1999, 2002; Daly 2013).

The larval (metacercarial) stage of this trematode is commonly found in a variety of fish and Hoffman (1999) reported that it is likely capable of infecting any species of freshwater fish. The first intermediate host is a planorbid snail which has been infected from ova deposited in watersheds by definitive host fish-eating ardeid birds (herons, egrets and bitterns) (Olsen 1967). Larval forms are found in tissues of fish, amphibians, and reptiles (Bonett *et al.* 2012; Calhoun *et al*. 2019).

Robison *et al.* (2011) reported a total of 65 species of fishes distributed among 14 families in Crooked Creek. The purpose of our study was 2-fold: (1) to survey some of those fishes from the Crooked Creek watershed within Boone and Marion counties for *C. marginatum*, and (2) specifically, compare some of our data gathered from *M. dolomieu* to the historical studies involving this fish species previously done on specimens from the various parts of the creek.

Materials and Methods

During 2010 and 2011, 11 species of fish (n = 203) were collected with a boat electrofisher or traditional hook and line from 5 access sites (see Fig. 1, upstream to downstream) along Crooked Creek as follows: (1) Harmon (36°15'16.182"N, -92°57' 3.4092"W), (2) Pyatt (36°14'46.8456"N, -92°50' 4.722"W), (3) Snow (36°14'13.0164"N, -92°48' 16.7292"W), (4) Kelly's Slab (36°13'50.5596"N, -92°42'34.2216"W), and (5) Doe Bambi near White River (36°14'09.7836"N, -92°32'09.7332"W) (Fig. 1). Fish species and numbers collected and examined included: **CENTRARCHIDAE:** 99 *M. dolomieu*, 11 *M. salmoides*, 1 *M. punctulatus*, 13 Ozark Bass (*Ambloplites constellatus*), 9 Green Sunfish (*Lepomis cyanellus*), 30 Longear Sunfish (*L. megalotis*); **CATOSTOMIDAE:** 11 Golden Redhorse (*Moxostoma erythrurum*), 22 Northern Hogsucker (*Hypentelium nigricans*); **LEUCISIDAE:** 3 Southern Redbelly Dace (*Phoxinus erthyrogaster*); 3 Central Stoneroller (*Campostoma oligolepis*), 1 Striped Shiner (*Luxilus chrysocephalus*). Fish were measured for total length (TL, mm) and killed by severing their cervical vertebrae. The gills and orobranchial cavity were immediately examined macroscopically for metacercariae; the peritoneal cavity and visceral organs were not examined invasively. The location of parasites was noted and individual worms were counted. Several specimens were retained as vouchers and fixed in 70–95% DNA grade ethanol. A voucher specimen of *C. marginatum* from *M. dolomieu* was accessioned into GenBank as MF38189. Population parameters follow Bush *et al.* (1997).

Results and Discussion

Of the 203 fish examined, 43 (21%) were infected with *C. marginatum* (Fig. 2, Table 1). Hosts included: 31 of 99 (31%) *M. dolomieu*, 1 of 1 (100%) *M. punctulatus*, 4 of 11 (36%) *M. salmoides*, 2 of 13 (15%) *A. constellatus*, 3 of 9 (33%) *L. cyanellus*, and 2 of 22 (9%) *H. nigricans* (Table 1); none of the other 48 fish, including 30 *L. megalotis*, 11 *M. erythrurum*, 3 *P. erythrogaster*, 3 *C. pullum*, or 1 *L. chrysocephalus* was infected. A new host record is documented for *C. marginatum* in *A. constellatus*.

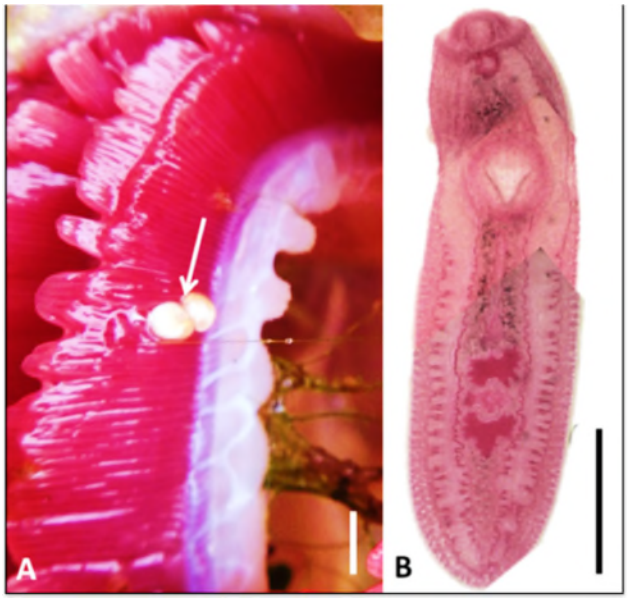

Figure 2. *Clinostomum marginatum* from *Micropterus dolomieu* from Crooked Creek. (A) Two metacercariae within capsule on gills. Scale bar = 1 mm. (B) Single stained metacercaria. Scale bar = 250 µm.

Smallmouth Bass from Crooked Creek (collected between Pyatt and Yellville) have been reported with a very high prevalence (78%) of *C. marginatum*, containing an average of 23.2 metacercariae per fish with a range from 1 to 227 worms (Daly *et al*. 1987). In a follow-up study, Daly *et al*. (2002) reported a prevalence of 72% at Pyatt, 84% at Clear Creek, 91% at Turkey, 86% at Yellville, and 70% near the White River; however, orobranchial as well as invasive soft tissue counts were included. In the present study, only 37% of *M. dolomieu* from Kelly's Slab (Access 4,

Yellville) were infected containing an average of 2 metacercariae per fish with a range from 1 to 6 worms (Table 1). When other comparative sites on the creek for *C. marginatum* in *M. dolomieu* are compared to Daly *et al.* (2007), who only used non-invasive orobranchial counts (same as present study), prevalence at Harmon (Access 1) was higher in our study (24% *vs.* 16%) but at the far downstream site near the confluence with the White River at Doe Bambi, prevalence was lower in our study (Access 5, 40% *vs.* 59%). However, samples sizes were unequal. Also, we did not find any *C. marginatum* in Smallmouth Bass at our Pyatt (Access 2) and Snow stations (Access 3), which may also be the result of smaller sample sizes (n = 2 and 6 fish, respectively).

In conclusion, Smallmouth Bass from all reaches of Crooked Creek harbor a high prevalence of *C. marginatum*. When our data is compared to previous studies of this trematode infecting *M. dolomieu* at 3 of the same stations on the creek (Daly *et al.* 2007), prevalence was lower at each station, (except Harmon site) in the present study (Table 2). In addition, however, we did discover that *M. dolomieu* from the upper reaches of the creek possessed lower prevalence compared to those of lower reaches; this is in agreement with previous historical studies. We suggest additional surveys of *M. dolomieu* for yellow grub that include 3 parameters: (1) the density of intermediate hosts, (2) the density of host fishes, and (3) the river flow at different reaches.

Acknowledgments

The Arkansas Game and Fish Commission (AG&F) provided a Scientific Collecting Permit to CTM. We thank Dr. Ronald M. Bonett (University of Tulsa) for technical assistance and several AG&F staff from the Mountain Home office who provided assistance in the collection of specimens, especially Supervisor Kenneth Shirley (retired).

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Table 1. Prevalence, mean intensity, and mean abundance of *Clinostomum marginatum* in fishes from Crooked Creek.

Species	Access	Prevalence*	Mean intensity	Abundance
Centrarchidae				
Amploblites constellatus†	Kelly's Slab	2/9 (22%)	1.5	0.33
	Harmon	0/1 (0%)	–	–
	Pyatt	0/2 (0%)	–	–
	Doe Bambi	0/1 (0%)	–	–
Lepomis cyanellus	Kelly's Slab	2/4 (50%)	1.0	0.50
	Harmon	0/1 (0%)	–	–
	Pyatt	1/4 (25%)	1.0	0.25
Lepomis megalotis‡	Kelly's Slab	0/21 (0%)	–	–
	Harmon	0/5 (0%)	–	–
	Pyatt	0/3 (0%)	–	–
	Doe Bambi	0/1 (0%)	–	–
Micropterus dolomieu	Kelly's Slab	17/46 (37%)	2.17	0.80
	Harmon	6/25 (24%)	1.67	0.40
	Pyatt	0/2 (0%)	–	–
	Snow	0/6 (0%)	–	–
	Doe Bambi	8/20 (40%)	3.75	1.50
Micropterus punctulatus	White River	1/1 (100%)	31	–
Micropterus salmoides	Kelly's Slab	1/6 (17%)	2.0	0.33
	Doe Bambi	3/5 (60%)	1.5	0.60
Catostomidae				
Hypentelium nigricans‖	Kelly's Slab	2/22 (9%)	1.0	0.09
Moxostoma erythrurum‖	Kelly's Slab	0/10 (0%)	–	–
	Pyatt	0/1 (0%)	–	–
Leucisidae				
Campostoma oligolepis‡	Kelly's Slab	0/3 (0%)	–	–
Luxilus chrysocephalus‡	Kelly's Slab	0/1 (0%)	–	–
Phoxinus erythrogaster‡	Kelly's Slab	0/3 (0%)	–	–

*Prevalence = number infected/number examined (%).
†New host record.
‡Not previously reported as a host (Hoffman 1999).
‖Previously reported as a host (Hoffman 1999).

Table 2. Comparative prevalence, mean intensity, and mean abundance of *Clinostomum marginatum* in *Micropterus dolomieu* from upstream to downstream sites on Crooked Creek.

Access	Prevalence*	Abundance	Maximum Abundance	Reference
Huzzah Creek	3/10 (30%)	2.10	10	Daly *et al*. (2002)
	1/10 (10%)	0.10	1	*Daly *et al*. (2007)
Harmon	6/25 (24%)	0.40	3	This study
	83/120 (69%)	3.0	27	Daly *et al.* (2002)
	24/120 (16%)	0.33	3	*Daly *et al*. (2007)
Pyatt	0/2 (0%)	–	–	This study
	19/27 (72%)	14.3	57	Daly *et al.* (2002)
	16/27 (59%)	1.85	10	*Daly *et al*. (2007)
Snow	0/6 (0%)	–	–	This study
Clear Creek	36/43 (84%)	19.0	92	Daly *et al.* (2002)
	21/42 (49%)	1.84	17	*Daly *et al*. (2007)
Turkey	97/107 (91%)	83.0	179	Daly *et al.* (2002)
	67/105 (64%)	3.7	25	*Daly *et al*. (2007)
George's Creek	26/31 (84%)	22.5	144	Daly *et al.* (2002)
Kelly's Slab	17/46 (37%)	0.80	6	This study
	38/44 (86%)	9.40	76	Daly *et al.* (2002)
	22/44 (49%)	1.14	11	*Daly *et al*. (2007)
Doe Bambi†	8/20 (40%)	1.50	5	This study
	36/51 (70%)	105.0	2,500	Daly *et al.* (2002)
	31/53 (59%)	23.0	400	*Daly *et al*. (2007)

*Daly *et al*. (2007) used orobranchial counts only.

†Our Doe Bambi access site equates to White River site of Daly *et al.* (2002, 2007).

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Hemoparasites (Apicomplexa: *Hepatozoon*; Kinetoplastida: *Trypanosoma*) of Green Frogs, *Rana clamitans* (Anura: Ranidae) from Arkansas

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Running Title: Hemoparasites of Green Frogs in Arkansas

Abstract

The green frog, *Rana clamitans*, has been reported as a host of several hemoparasites, including trypanosomes and *Hepatozoon* spp. In Arkansas, however, there are no reports of any hemoparasites in *R. clamitans* nor from any other anuran from the state. We collected 9 green frogs from Polk County and blood was taken from their facial musculocutaneous vein in heparinized capillary tubes. Thin blood smears were also made and stained with DipQuick stain. Seven out of 9 (78%) *R. clamitans* were infected with hematozoans. Three (33%) were infected with an unknown species of *Hepatozoon* and 4 (44%) were infected with trypanosomes of 3 distinct morphologies. Mixed infections were found in 5 (56%) of the hosts. Here, we provide the first report of hemoparasites in *R. clamitans* from Arkansas, including morphometric data and photomicrographs of the infections.

Introduction

The study of blood parasites (hematozoans) is still in its early stages in herpetofauna of Arkansas. Several studies have addressed this problem and reported hematozoan parasites from reptiles (turtles and snakes) in the state (McAllister and King 1980; McAllister *et al.* 1995, 2014, 2016; McAllister and Robison 2019). Nevertheless, a complete void remains in the study of amphibian hematozoans in Arkansas.

The green frog, *Rana clamitans* (Latreille, 1801) is a medium-sized ranid that ranges from Nova Scotia and Manitoba, Canada, south to central Florida and west to eastern Texas; it is found generally statewide in Arkansas (Powell *et al.* 2016). This frog has been previously reported as a host of several hemoparasites, including trypanosomes and *Hepatozoon* spp. (Southworth *et al.* 1968; Barta and Desser 1984; Desser *et al.* 1995; Smith 1996; Leveille *et al.* 2014). In Arkansas, however, there are no reports of any hemoparasites in *R. clamitans* nor from any other anuran from the state. Here, we provide the first report of blood parasites infecting an anuran in Arkansas.

Materials and Methods

During June 2019, 9 adult *R. clamitans* were collected by hand from a pond across from Blue Haze Vista, 4.8 km N of Mena, Polk County (34°37'40.17" N, -94°14'44.4228" W). Blood was drawn from the facial musculocutaneous vein of individuals anesthesized with tricaine methanesulfonate following the methods of Forzán *et al.* (2012) and collected in heparinized capillary tubes. Samples were centrifuged at 4,000 × g for 1 min to separate red blood cells (rbcs) from plasma and concentrating parasites in the buffy coat layer (Woo *et al.* 1969). Capillary tubes were then scored with a glass scratcher right below the buffy coat and gently snapped. Using a capillary pipette bulb, the buffy coat and plasma were dispensed from the tube onto a slide for microscopical examination for hemoparasites. In addition to buffy coat smears, thin blood smears were prepared and air-dried and stained with DipQuick J-322 stain (Jorgenson Labs, Loveland, CO). Stained slides were scanned at high power (100× objective) with an Olympus BX-51 upright research microscope for approximately 10 min to detect intracellular and extracellular parasites. When *Hepatozoon* spp. gamonts were found infecting rbcs, 10 to 20 specimens of each stage were photographed with an Olympus 5-megapixel digital camera. Gamont length and width in micrometers (μm) were measured using ImageJ (Schneider *et al.* 2012) and calibrated with a stage micrometer. Trypanosomes were also measured to the nearest 1.0 μm following Desser (2001). Parasites were identified to genus based on

previous reports of hematozoa infecting *R. clamitans* in North America (Southworth *et al.* 1968; Barta and Desser 1984; Desser *et al.* 1995; Kim *et al.* 1998; Leveille *et al.* 2014) and photovouchers were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, NE.

Results

Seven of 9 (78%) *R. clamitans* were infected with hematozoans. Three (33%) were infected with an unknown species of *Hepatozoon* (HWML 216338; Figs. 1 A–C; Table 1). Four (44%) were infected with trypanosomes of 3 distinct morphologies (Figs. 1 D–F; Table 2). Mixed infections were found in 5 (56%) of the hosts. Three frogs (33%) were infected by 2 forms of trypanosomes (HWML 216339), 2 with forms "a" and "b," and one with forms "b" and "c." One frog (11%) was infected by both *Hepatozoon* sp. and *Trypanosoma* sp. "c," and one frog (11%) had *Hepatozoon* sp. infecting erythrocytes with both fragmented and non-fragmented nuclei. Measurements for these parasites are presented in Tables 1 and 2.

Discussion

Stebbins (1903, 1905) described *Hepatozoon* (=*Haemogregarina*) *catesbianae* and *Hepatozoon* (=*Karyolysus*) *clamatae* in the blood of American bullfrogs, *Rana catesbeiana*, and *R. clamitans* in New York, respectively (see Desser *et al.* 1995; Smith 1996).

Table 1. Morphometric data (in µm) of *Hepatozoon* sp. gamonts infecting rbcs of *Rana clamitans* in Arkansas. Average ± SD*, ranges in parentheses.

Frog ID #	13 *n* = 10	14 *n* = 11	21 *n* = 11
Gamont length	26.9 ± 1.8 (25–30)	26.0 ± 1.7 (22–28)	25.2 ± 2.5 (22–31)
Gamont width	4.0 ± 0.5 (3–5)	4.5 ± 0.5 (4–5)	4.4 ± 0.5 (4–5)
Effect on host rbc nucleus	Fragmented	Fragmented	Non-Fragmented
Host rbc length	28.9 ± 2.5 (25–34)	28.5 ± 2.2 (25–33)	25.8 ± 1.8 (23–29)
Host rbc width	14.0 ± 0.8 (13–15)	14.6 ± 1.5 (11–16)	13.2 ± 0.9 (11–14)

*One standard deviation.

In addition, *H. catesbianae* and *H. clamatae* have both been reported from *R. clamitans* from Canada (Kim *et al.* 1998; Boulianne *et al.* 2007; Shutler *et al.* 2009; Trites *et al.* 2013). Traits typically used to differentiate *Hepatozoon* species, such as oocyst morphology in the mosquito vector, were indistinguishable between these 2 species (Boulianne *et al*. 2007). However, *H. clamatae* fragments the nucleus of the rbcs it infects, while *H. catesbianae* does not (Desser *et al.* 1995; Kim *et al.* 1998; Boulianne *et al.* 2007). Recent research has called this distinction into question, as analyses of genetic data do not consistently separate these species

Table 2. Morphometric data (in µm) of *Trypanosoma* spp. infecting *Rana clamitans* in Arkansas. Average ± SD*, ranges in parentheses.

	Trypanosoma sp. "a" *n* = 15	*Trypanosoma* sp. "b" *n* = 3	*Trypanosoma* sp. "c" *n* = 19
PA	55.4 ± 6.7 (40–68)	42.0 ± 5.6 (37–48)	37.5 ± 6.0 (26–54)
BW	11.8 ± 2.5 (8–17)	16.7 ± 1.5 (15–18)	31.7 ± 3.8 (23–38)
LN	4.1 ± 0.4 (4–5)	4.0 ± 0.1 (4–4)	5.0 ± 0.9 (4–7)
WN	3.4 ± 0.7 (2–4)	3.7 ± 0.6 (3–4)	4.4 ± 0.7 (3–5)
PK	7.2 ± 2.8 (3–11)	5.7 ± 4.7 (2–11)	n/a
KN	11.8 ± 2.9 (5–16)	7.7 ± 1.2 (7–9)	n/a
NA	36.9 ± 3.7 (30–45)	28.0 ± 2.6 (26–31)	n/a
PN	18.9 ± 4.2 (10–26)	13.3 ± 4.0 (11–18)	n/a
PK/PN	0.19 ± 0.07	0.20 ± 0.18	n/a
PK/PA	0.13 ± 0.04	0.13 ± 0.09	n/a
PN/PA	0.67 ± 0.06	0.67 ± 0.10	n/a
BW/PA	0.22 ± 0.05	0.40 ± 0.07	0.86 ± 0.12

Abbreviations: PA = body length, BW = maximum body width excluding undulating membrane, LN = nucleus length, WN = nucleus width, PK = distance from posterior end to kinetoplast, KN = distance from kinetoplast to center of nucleus, NA = distance from center of nucleus to anterior end, PN = distance from posterior end to center of nucleus. *One standard deviation.

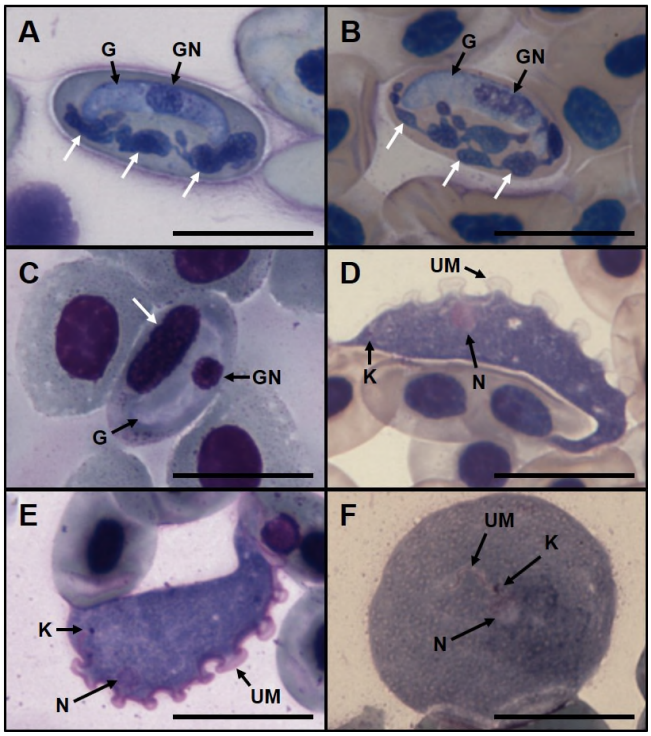

Figure 1. Photomicrographs of hematozoans infecting *Rana clamitans*. (A) *Hepatozoon* sp. infecting frog 13. Note fragmented host rbc nucleus (white arrows). (B) *Hepatozoon* sp. infecting frog 14. Note fragmented host rbc nucleus (white arrows). (C) *Hepatozoon* sp. infecting frog 21. Note host rbc nucleus displaced but not fragmented (white arrow). (D) *Trypanosoma* sp. ("a") infecting frog 16. (E) *Trypanosoma* sp. ("b") infecting frog 21. (F) *Trypanosoma* sp. ("c") infecting frog 19. Abbreviations: G = gamont, GN = gamont nucleus, K = kinetoplast, N = nucleus, UM = undulating membrane. Scale bars = 20 µm.

of *Hepatozoon* with their effect on the host rbc nucleus (Boulianne *et al.* 2007).

Both fragmented and non-fragmented types were observed in our study (Figs. 1A–C). These forms were morphologically similar to the descriptions of *H. catesbianae* and *H. clamatae* by Desser *et al.* (1995) and Kim *et al.* (1998) and were from the same host, *R. clamitans*. However, molecular data and further study of the life cycle, including examinations of stages in the definitive hosts, are needed to identify the parasites in this study (O'Donoghue 2017). Additionally, further phylogenetic analyses are warranted to determine the usefulness of the rbc nucleus fragmentation as a character to differentiate *H. catesbianae* and *H. clamatae*.

Trypanosomes have been reported previously from *R. clamitans*. Southworth *et al.* (1968) reported *Trypanosoma rotatorium* from green frogs from Louisiana. Bartlett-Healy *et al.* (2009) sequenced blood meals of *Culex territans* mosquitoes in New Jersey, and 50% of blood meals from *R. clamitans* were infected with trypanosomes. Additionally, Barta and Desser (1984) reported *T. rotatorium* and *T. ranarum* from *R. clamitans* from Ontario, Canada. In the current survey, 3 morphologies of trypanosomes were found in green frogs (Figs. 1D–F). Trypanosomes are pleomorphic, meaning they can change morphology throughout their life cycle (Desser 2001). Additionally, single amphibian hosts are often infected with multiple morphologies and it is unknown whether these forms represent different species or a single pleomorphic species. Therefore, species of trypanosomes cannot be identified based on morphology alone, and careful isolation, culturing, and experimental infections of frogs are required to describe and genuinely identify them (Desser 2001).

Additional research will be necessary to describe the species found in the present study, including molecular analyses and discovery of hematophagous arthropod definitive hosts and vectors in their life cycles.

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Energy Content of Seeds of Common Sunflowers (*Helianthus annuus*) in the Diet of Scaled Quail (*Callipepla squamata*) in Southeastern New Mexico

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Running Title: Energy Content of Seeds of Common Sunflowers in the Diet of Scaled Quail

Abstract

We analyzed the energy content of seeds of common sunflowers (*Helianthus annuus*) obtained from the crops of scaled quail (*Callipepla squamata*) collected from plains-mesa sand-scrub habitat in Eddy and Lea counties, New Mexico. Seeds were dried for 48 hours at 60°C to remove moisture and then analyzed for gross caloric value (i.e., energy content) in an oxygen bomb calorimeter. Energy content of seeds of common sunflowers from New Mexico was greater than that of many seeds previously reported from the diet of scaled quail and other granivorous birds and comparable to previous measurements of seeds of the same species made in Kansas.

Introduction

Conservation and management of birds requires knowledge of their food preferences, and of the energy content of their preferred foods. Feeding habits of scaled quail (*Callipepla squamata*) are well-studied (Lehman and Ward 1941; Schemnitz 1961; Ault 1981; Rollins 1981; Ault and Stormer 1983; Campbell-Kissock *et al*. 1985; Medina 1988), including several studies conducted in southeastern New Mexico (Davis and Banks 1973; Davis *et al.* 1975; Griffing and Davis 1976; Best and Smartt 1985; Hunt and Best 2001*b*). No study has measured the energy content of food of free-living scaled quail, although one study (Saunders and Parrish 1987) conducted in Kansas measured the assimilated energy of some potential food items by captive scaled quail. Studies have measured energy content of some known and potential food items of other birds such as mourning doves (*Zenaida macroura*—Schmid 1965; Shuman *et al.* 1988) in conjunction with determination of how well captive mourning doves metabolized various food items; some of the items measured are potential food for scaled quail. No study of energy content of foods of scaled quail has been conducted with birds from sand-scrub habitat of New Mexico.

A study of feeding habits of scaled quail in southeastern New Mexico determined that seeds of common sunflowers (*Helianthus annuus*) made up the largest portion (14.3%) of the total mass of crop contents, and were present in 36.9% of the crops of mourning doves (Hunt and Best 2001*b*). Common sunflowers are also reported to be an important food item of other birds, such as northern bobwhites (*Colinus virginianus*—Hunt and Best 2001*a*) and mourning doves (Hunt 1999). Although energy content of seeds of wild common sunflowers from Kansas have been measured (Robel and Harper 1965), no such measurements have been conducted on seeds from sand-scrub habitat of New Mexico. We used an oxygen bomb calorimeter to determine the energy content of seeds of common sunflowers from Eddy and Lea counties in New Mexico.

Methods and Materials

Scaled quail were collected at the Waste Isolation Pilot Plant site in New Mexico in conjunction with long-term studies of lead poisoning of game birds (Best *et al*. 1992*a*; 1992*b*) and studies of feeding habits of game birds in southeastern New Mexico (Hunt 1999; Hunt and Best 2001a; Hunt and Best 2001*b*). Most of the study area is in eastern Eddy County, but it also extends into western Lea County. All scaled quail were collected in uncultivated, shinnery oak-honey mesquite (*Quercus havardii-Prosopis glandulosa*) habitat, part of the plains-mesa sand-scrub vegetation type (Dick-Peddie 1993). Several studies of the feeding ecology of scaled quail have been conducted in this area (Davis and Banks 1973; Davis *et al.* 1975; Griffing and Davis 1976; Best and Smartt 1985; Hunt and Best 2001*b*). There are several human-made stock

tanks on the study site, which is heavily grazed by cattle.

In late summer and autumn 1981, 58 scaled quail were collected by shooting as encountered. Collected birds were placed on ice within 10 minutes of shooting to minimize effects of post-mortem digestion (Dillery 1965; Farner 1960; Sedinger 1986); no effect of digestion on crop contents was observed. Crops were removed, placed into plastic vials, and frozen. Contents of crops were later thawed, separated by type of food, and placed into envelopes for drying. Food items were dried for 48 hours at 60°C to remove moisture. Food items were identified by comparison with samples of plants collected at the study site, and by using identification manuals (Davis 1993; Martin and Barkley 1961). Seeds used in this analysis were unbroken and unhulled.

Samples of seeds of *Helianthus annuus* were analyzed for gross caloric value (i.e., energy content) in an oxygen bomb calorimeter (Model 1341, Parr Instrument Company, Moline, Illinois). Samples of seeds from 13 individual scaled quail with crops that contained enough seeds for analysis were selected; each sample weighed 0.5-1.0 g. Seeds were combusted in the oxygen bomb; after combustion, the bomb was washed and bomb washings were titrated with sodium carbonate to allow adjustment of results for nitrate content. Results are reported in J/kg; kcal/g are given in parentheses for comparisons with previous studies.

Results

The 13 samples analyzed contained an average of 23.8 J/kg [5.7 kcal/g] — range, 21.3-24.7 J/kg [5.1-5.9 kcal/g]; standard deviation, 0.8 J/kg [0.2 kcal/g] (Table 1). This figure is similar to that previously obtained for *Helianthus annuus* in Kansas (Robel and Harper 1965) and greater than many tested food items in the diet of other seed-eating game birds.

Table 1. Gross caloric value (energy content) of seeds of common sunflowers (*Helianthus annuus*) in the crops of scaled quail (*Callipepla squamata*) collected from Eddy and Lea counties, New Mexico, summer and autumn, 1981.

Sample No.	Energy in J/kg (kcal/g)
SQ002-81	23.8 (5.7)
SQ003-81	24.3 (5.8)
SQ008-81	24.7 (5.9)
SQ009-81	23.0 (5.5)
SQ015-81	23.4 (5.6)
SQ016-81	23.4 (5.6)
SQ017-81	23.0 (5.5)
SQ020-81	23.0 (5.5)
SQ021-81	24.7 (5.9)
SQ029-81	23.8 (5.7)
SQ031-81	21.3 (5.1)
SQ041-81	24.7 (5.9)
SQ042-81	24.7 (5.9)

Discussion

Although no study has demonstrated that scaled quail are selective in food choices, other species of granivorous birds, including quail, are known to be selective (Schmid 1965; Conley and Blem 1978; Schuman *et al*. 1988; Hayslette and Mirarchi 2001; Larson *et al.* 2012), although criteria for their selection are imperfectly understood. Among suggested criteria are shape, color, fiber content (Conley and Blem 1978), protein content (Larson *et al.* 2012), nutrient content (Hayslette and Mirarchi 2001), and energy content (Schmid 1965; Shuman *et al.* 1988). Although no study has demonstrated that scaled quail preferentially select common sunflowers over other food items, Davison (1958) categorized common sunflowers as a "choice" food plant for northern bobwhites, meaning it was digestible, nutritious, and readily eaten when encountered.

Our study demonstrates that seeds of common sunflowers have an energy content comparable to or greater than food items from previous studies. For example, Robel and Harper reported an average of 24.7 J/kg (5.9 kcal/g) for seeds of common sunflowers, and 23.0 J/kg (5.5 kcal/g) for seeds of giant ragweeds (*Ambrosia trifida*) collected in Kansas. A study of potential food items for greater prairie-chickens (*Tympanuchus cupido*—Heffron and Parrish 2005) listed 14 different commercial feeds and seeds that had a lower energy content than that which we measured for common sunflowers; the greatest energy content in that study was for hulled domestic sunflower-seed chips (23.0 J/kg [5.5 kcal/g]). A study of seeds of Texas doveweeds (*Croton texensis*) in crops of mourning doves conducted at the same study site as the current study (Hunt *et al*. 2019) reported an average energy content of 21.8 J/kg (5.2 kcal/g). In a study of 9 food items collected from crops of mourning doves in North Dakota, Schmid (1965) found only 2 that had a greater energy content—seeds of flax (*Linum usitatissimum*, 26.4 J/kg [6.3 kcal/g]) and field mustard

(*Sinapis arvensis*, 25.1 J/kg [6.0 kcal/g]). Other seeds tested from North Dakota had less energy content; examples include wild plants such as green foxtail (*Setaria viridis*, 18.4 J/kg [4.4 kcal/g]) and cultivated crops such as corn (*Zea mays*, 17.1 J/kg [4.1 kcal/g]) and wheat (*Triticum aestivum*, 16.7 J/kg [4.0 kcal/g]). Likewise, Shuman *et al*. (1988) tested 8 varieties of seeds that were considered to be potential food items for mourning doves in Kansas, and found only 1 that had greater energy content—thistle (*Cirsium*, 25.9 J/kg [6.2 kcal/g]). Other seeds analyzed in Kansas had less energy content; examples include Maximillian sunflower (*Helianthus maximiliani,* 23.4 J/kg [5.6 kcal/g]), proso millet (*Panicum millaceum*, 18.8 J/kg [4.5 kcal/g]), and timothy (*Phleum pratense*, 19.7 J/kg [4.7 kcal/g]).

Common sunflowers and other sunflowers are associated with disturbance (Whitson *et al*. 1999). Much of southeastern New Mexico is heavily grazed by cattle, and much of the landscape has been highly modified by usage for extraction of petroleum and natural gas (Hunt 2004), so that common sunflowers grow abundantly. Abundance of common sunflowers, coupled with the relatively great energy content of the seeds, helps explain their prevalence in the diet of scaled quail (Hunt and Best 2001*b*) and other granivorous birds of the area.

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Helminth Parasites of Eastern Screech Owl, *Megascops asio* (Aves: Strigiformes: Strigidae) from Arkansas

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Running Title: Helminth Parasites of *Megascops asio*

Abstract

The eastern screech owl (*Megascops asio*) is a small owl that is relatively common in eastern North America. Nothing is known of the parasites of this owl in Arkansas. Here, we document 3 helminths from a single injured *M. asio* that subsequently died and was donated by a rehabilitation center for parasitic examination. Found were 2 digenetic trematodes, *Brachylaima mcintoshi* and *Neodiplostomum americanum*, and a habronematid nematode, *Excisa excisiformis*. The former trematode represents a new host record for *M. asio*, and *B. mcintoshi* and *E. excisiformis* are reported from Arkansas for the first time.

Introduction

The eastern screech owl, *Megascops asio* (L., 1758) is a diminutive owl that occurs in eastern North America from southern Ontario, eastern Montana and the Great Lakes to the Gulf of México southward to Tamaulipas, northeastern México; in Arkansas, this owl is found statewide (Peterson 2002; Sibley 2016). This species is native to wooded environs of its distribution, and more so than any other owl in its range, has adapted well to urban development. Like many other owls, it feeds on insects and other arthropods, small mammals, birds, and other small vertebrates (König and Weick 2008).

Little is available on the parasites of *M. asio* (Kinsella *et al.* 2001; Richardson and Kinsella 2010; Woodyard *et al.* 2017; McAllister *et al.* 2019c) and nothing has been published on its parasites from Arkansas. Nevertheless, over the last few years, novel information on the parasites of owls has been gained by our research consortium from examination of salvaged road-killed specimens from Arkansas (McAllister *et al.* 2019a, 2019b) and Oklahoma (McAllister *et al.* 2017, 2018, 2019b, 2019c). In one of these studies, McAllister *et al.* (2019c) reported a nematode, *Porrocaecum depressum* from *M. asio* from Oklahoma. Here, we document, for the first time, 3 helminth parasites in *M. asio* from Arkansas.

Materials and Methods

On 7 December 2019, a single adult *M. asio* that was found to be severely injured by unknown causation at an unspecified locale in Sevier County, Arkansas, was brought to the Hochatown Rescue Center in McCurtain County, Oklahoma (34°08’22.074”N, -94°44’47.328”W), for treatment. Unfortunately, the individual died soon thereafter and was donated to the senior author for parasitological examination. Its feathers were vigorously brushed over a white enamel tray to observe possible ectoparasites. A mid-ventral incision was made from the cloaca to throat to expose the viscera and the trachea, lungs, air sacs, esophagus, proventriculus, gizzard, gallbladder, liver, kidneys, and intestine were placed in individual Petri dishes containing 0.9% saline, opened, and their contents washed. Digestive contents, as well as the mucosal surfaces, were examined at 20 to 30× under a stereomicroscope and parasites found were rinsed of mucus. Feces from the rectum was collected and placed in a vial containing 2.5% (w/v) potassium dichromate ($K_2Cr_2O_7$) and, after flotation in Sheather’s sugar solution (sp. gr. 1.30), examined for coccidians and parasite ova by brightfield microscopy. Trematodes were fixed without coverslip pressure in nearly boiling water and transferred to 95% (v/v) molecular grade ethanol. They were stained with acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted in Canada balsam. Nematodes were fixed in near boiling water and preserved in 70% (v/v) ethanol. They were later cleared and identified as temporary mounts of lacto-phenol and then returned to the preservative.

The host voucher was deposited in the EOSC

vertebrate collection, Idabel, Oklahoma. Voucher specimens of parasites were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

Results and Discussion

Two digenean trematodes and a spirurid nematode were recovered from *M. asio*. The host did not possess ectoparasites nor were coccidians being passed in its feces. Data is presented below in annotated format.

TREMATODA: DIGENEA: BRACHYLAMIDAE

***Brachylaima mcintoshi* Harkema, 1939. –** Two specimens (HWML 216252) were found in the small intestine of this host. *Brachylaima* is a large genus with well over 90 described species (Ubelaker and Dailey 1966). The terrestrial triheteroxenous life cycle includes 2 distinct or same species of land snail as first intermediate (with sporocysts) or second intermediate hosts, the latter which harbors unencysted metacercariae in their kidneys (Yamaguti 1975). Definitive hosts include humans, mice, various species of shrews, birds (especially owls) and reptiles. This digenean has been reported from barred owls (*Strix varia*) from Florida, North Carolina, Texas, and Virginia, and great horned owls, *Bubo virginianus* from Florida (Harkema 1939; Little and Hopkins 1975; Kinsella *et al.* 2001). We document *B. mcintoshi* in a screech owl and from Arkansas for the first time.

DIPLOSTOMIDAE

***Neodiplostomum americanum* Chandler and Rausch, 1947. –** Several specimens (HWML 216253) were found in the small intestine of the owl. This trematode was originally described from *B. virginianus* in Wisconsin (Chandler and Rausch 1947). It has also been reported previously from *M. asio* from Connecticut, Florida, and Mississippi (Kinsella *et al.* 2001; Richardson and Kinsella 2010; Woodyard *et al.* 2017), from *B. virginianus* from Arkansas (McAllister *et al.* 2019a) and Florida (Kinsella *et al.* 2001), and from *S. varia* from Florida (Kinsella *et al.* 2001). Other hosts include *Accipiter* spp., long-eared owl (*Asio otus*), burrowing owl (*Athene cunicularia*), *Buteo* spp., and *S. varia* from Connecticut, Florida, Louisiana, Mississippi, and Ontario, Canada (see Woodyard *et al.* 2017). In the life cycle of other *Neodiplostomum* spp., freshwater snails have been reported as first intermediate hosts (Chung *et al.* 2002) and amphibians have been implicated as second intermediate hosts (Pearson 1961). We report *N. americanum*, a relatively common helminth of *M. asio*, from Arkansas for the second time.

NEMATODA: SPIRURIDA: HABRONEMATIDAE

***Excisa excisiformis* (Yamaguti, 1935).** – a single male specimen (HWML 111455) was taken from the small intestine of the owl. This species was described from *A. otus* in Japan by Yamaguti (1935). It was reported for the first time in North America (Florida) from *S. varia*, *B. virginianus*, and *M. asio* by Kinsella *et al.* (2001) and was considered by them to be a "raptor generalist." It is a rare nematode of owls and Ferrer *et al.* (2004) and Santoro *et al.* (2012) include scops owls (*Otus scops*) from Spain, and *A. otus* from Italy, respectively, as hosts. This nematode has also been reported from the tawny frogmouth (*Podargus strigoides*) from North Queensland, Australia (Ogden 1967). Its life cycle is unknown but like other spirurid nematodes, may involve an arthropod intermediate host. It is reported from Arkansas for the first time, and more importantly, only the second locale documented from North America for *E. excisiformis*.

In conclusion, examination of road-killed or deceased hawks and owls have been shown to be noteworthy in studying their ecto- and endoparasites (see McAllister *et al.* 2017, 2018, 2019a, 2019b, 2019c; Woodyard *et al.* 2017). Future studies on rarely examined raptors in the state should provide additional new host and distributional records as well as the possibly of discovering novel parasite species.

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Helminth Parasites of the Golden Topminnow, *Fundulus chrysotus* (Cyprinidontiformes: Fundulidae) from Desha County, Arkansas

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Running Title: Helminth Parasites of Golden Topminnows

Abstract

During July 2019, 21 Golden Topminnows (*Fundulus chrysotus*) were collected from an oxbow lake in McGehee, Desha County, Arkansas, and examined for parasites. Found were 4 taxa of endohelminths, including 3 digeneans (*Clinostomum marginatum*, *Homalometron* sp., and *Posthodiplostomum minimum*) and a nematode (*Spiroxys contortus*). We document a new host record for *S. contortus* and the first report of parasites in *F. chrysotus* from the lower Mississippi River Drainage.

Introduction

The Golden Topminnow, *Fundulus chrysotus* (Günther, 1866) is a small, compressed species that ranges in the lower Mississippi River basin from Kentucky and Missouri southward through Louisiana, South Carolina, and Florida west to Oklahoma, Arkansas, and Texas (Page and Burr 2011). In Arkansas, *F. chrysotus* occurs in all major drainages of the Coastal Plain lowlands, further extending through the Arkansas River Valley (Robison and Buchanan 2020).

McAllister *et al*. (2020) recently provided information from a comprehensive parasitological survey of the Golden Topminnow (*F. chrysotus*) from 4 river drainages of Arkansas. They reported a coccidian, myxozoan, monogenean, 3 digenetic trematodes, a cestode, nematode, 3 acanthocephalans, and a crustacean from that host and localities. Here, we report a new host record as well as the first parasite survey of *F. chrysotus* from a site in the lower Mississippi River Drainage.

Materials and Methods

On 24 July 2019, 21 *F. chrysotus* (mean ± 1 SD total length = 59.7 ± 8.9 mm, range 45–79 mm) were collected with a one-man seine and dipnet from an oxbow lake off US 65 in McGehee, Desha County (33° 37'50.58"N, -91°23'42.563"W) (Figs. 1A–B). Specimens were placed in a container of aerated habitat water and processed within 24 h. We followed the methods of McAllister *et al*. (2020) for fish handling, processing, and the examination of their parasites. Tissues suspected of being infected were fixed in 10% neutral-buffered formalin, sectioned at 8 µm, and further processed following standard histological methods (Presnell and Schreibman 1997).

Parasites were either retained for future molecular studies or deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska-Lincoln, Lincoln, Nebraska. Host voucher specimens are deposited in the Henderson State University Vertebrate Collection (HSU), Arkadelphia, Arkansas. Prevalence, mean intensity ± 1SD, and range of infection are provided in accordance with terminology given in Bush *et al.* (1997).

Results and Discussion

Found were 4 taxa of parasites, including 3 digeneans (*Clinostomum marginatum*, *Homalometron* sp., and *Posthodiplostomum minimum*) and a nematode (*Spiroxys contortus*). No fish was harboring monogeneans or myxozoans on their gills. The data is presented below in annotated format.

TREMATODA: DIGENEA: APOCREADIIDAE

***Homalometron* sp. –** Ten specimens (mean intensity= 2.0 ± 0.6, range 1–3) of a *Homalometron* sp.

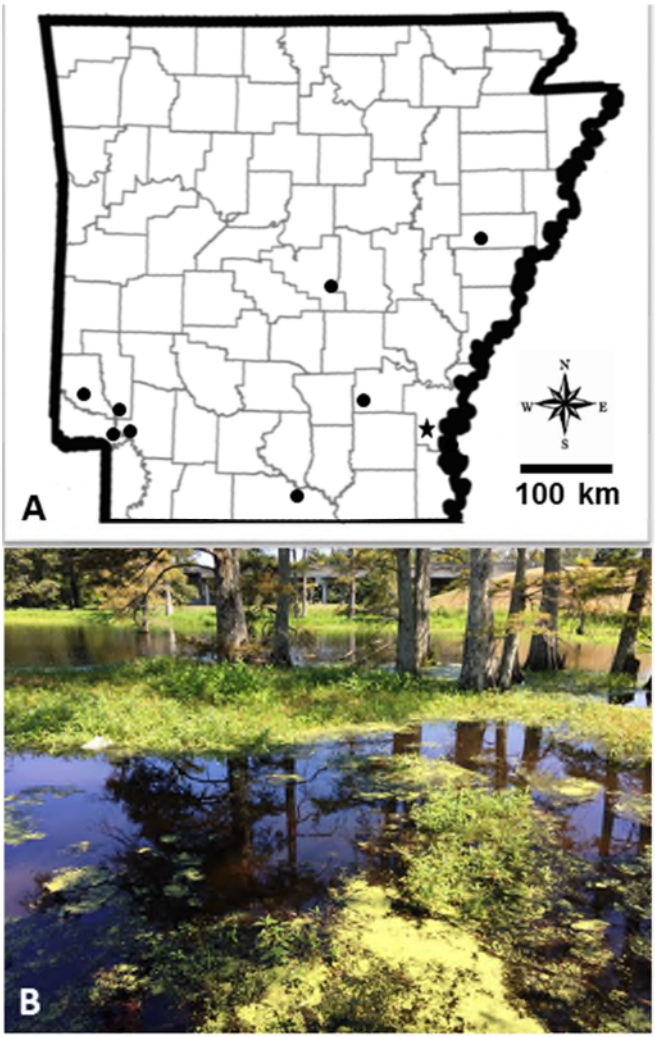

Figure 1. (A) County map of Arkansas showing approximate collecting locales (●) of McAllister *et al*. (2020) in 8 counties and present collecting site (star) in Desha County. (B) Oxbow lake collecting site in McGehee showing cypress trees and main emergent vegetation, alligator weed (*Alternanthera philoxeroides*). (Photo by H.W. Robison).

(Fig. 2A) were found in the intestinal tract of 5 (24%) *F. chrysotus*. Fayton *et al.* (2016) characterized undocumented diversity of *Homalometron* in freshwater fundulids, describing 2 new species from hosts from Oklahoma and New York, and resolving *Homalometron* specific to fundulids as a highly supported monophyletic group based on a molecular phylogeny of *28S* rDNA. Subsequently, McAllister *et al.* (2020) reported *Homalometron* sp. from *F. chrysotus* collected from sites in Pulaski and Union counties, Arkansas, with morphological deviation from the most closely allied species, *Homalometron robisoni* Fayton and McAllister, 2016 and a small number of specimens (n = 2, with only a single mature specimen) precluding a specific identification. The specimens collected in the present study, similar to those of McAllister *et al.* (2020), were morphologically similar to *H. robisoni*, which notably is the only species of *Homalometron* with the vitellarium extending anteriorly into the forebody (*Homalometron frocioneae* Fayton and Andres, 2016, the only other species of *Homalometron* from a freshwater fundulid, has a vitellarium that at most only slightly overlaps the posterior margin of the ventral sucker). Of the 3 specimens of *Homalometron* sp. available for morphological study (Table 1), the vitellarium either overlaps 80% of the length of the ventral sucker or is slightly anterior or posterior to its anterior margin. The

Table 1. Measurements (µm) of *Homalometron* sp. collected from *Fundulus chrysotus* from this study compared with most morphologically similar congeners from fundulid hosts.

Species	*Homalometron* sp. n = 3 (This study)	*Homalometron* sp. n = 1 McAllister *et al.* (2020)	*Homalometron robisoni* n = 8 Fayton *et al.* (2016)
BL	1,398–1,504	1,970	1,380–1,879
BW	326–386	544	414–467
BL to BW ratio	1:0.23–0.26	1:0.28	1:0.22–0.34
OSL as % BL	11	11	9–11*
OSW as % BW	42–48	40	34–44*
VSL as % BL	11–12	12	10–12*
VSW as % BW	47–51	43	36–46*
OS to VSW ratio	1:1.04–1.18	1:1.07	1:0.96–1.2
PL as % BL	6–7	7	5–6*
PW as % BW	25–26	25	18–24*
OL as % BL	3–6	5	4–6*

Table 1 *continued*

IB as %	22–27	24	24–29*
PS as % BL	13–17	8	12–18
F as % BL	31–33	28	27–31
ATL as % BL	8–9	8	6–9*
PTL as % BL	8–11	10	8–12*
PTS as % BL	20–22	23	20–25
SVL as % BL	7–11	8	7–10*
GP as % BL	28–31	26	26–30*
OL as % BL	7–9	8	7–9*
EL	100–114	99–107	91–111
EW	48–66	54–68	49–58
AEV as % BL	32–34	29	24–30*
EV as % BL	18–20	19	14–21

*Unpublished measurements of type material from Fayton *et al.* (2016). Abbreviations: BL = body length; BW= body width; OSL = oral sucker length; OSW = oral sucker width; VSL = ventral sucker length; VSW = ventral sucker width; PL = pharynx length; PW = pharynx width; OL = oesophagus length; IB = position of intestinal birfurcation; PS = postcaecal space; F = forebody; ATL = anterior testis length; PTL = posterior testis length; PTS = post-testicular space; SVL = seminal vesicle length; GP = genital pore position; OL = ovary length; EL = egg length; EW = egg width; AEV = anterior extent of vitellarium; EV= excretory vesicle length.

position of the anterior extent of the vitellarium is slightly more posterior compared to that of *H. robisoni* when expressed as a percent of body length. Our specimens also deviated from the type material of *H. robisoni* as follows: (1) smaller body width despite overlapping body lengths, and (2) larger ventral sucker and pharynx width expressed as a percent of body width. As to whether these 2 morphological deviations represent intraspecific or interspecific differences will have to be elucidated through molecular analysis. The site of collection for *Homalometron* from this study is notably ~ 370 km (230 mi.) east from the type locality of *H. robisoni* and in the lower Mississippi River drainage of Arkansas *vs.* the Red River drainage of Oklahoma.

To date, the only known first intermediate hosts for *Homalometron* spp. are mud snails belonging to the family Hydrobiidae. On the other hand, metacercariae are found in a much broader assemblage of second intermediate hosts including snails (Hydrobiidae and Viviparidae), veneroid bivalves (Sphaeriidae, Unionidae, and Veneridae), oligochaetes (Naididae), and captive polychaetes (Stunkard 1964; Fayton *et al.* 2016).

Voucher specimens of the *Homalometron* sp. are being retained for future DNA analyses; a photovoucher is deposited as HWML 216340.

STRIGEOIDEA: STRIGIFORMES: DIPLOSTOMIDAE

***Posthodiplostomum minimum* (MacCallum, 1921) Dubois, 1936. –** Nine (43%) *F. chrysotus* harbored a total of 38 individual (4.2 ± 2.9, range 1–10) metacercariae of *P. minimum* (HWML 216341) in their coelomic cavity and mesenteries. McAllister *et al*. (2020) previously reported specimens of *P. minimum* from *F. chrysotus* collected from sites in Howard, Lincoln, and Pulaski counties. Hoffman (1999) also previously reported *P. minimum* from *F. chrysotus*. In addition, McAllister *et al*. (2016b) reported this digenean from Northern Studfish, *Fundulus catenatus* from Arkansas. The host list and geographic range of this North American trematode (also called "white grub") includes other fundulid fishes in North America, Canada, and México (Hoffman 1999). In the life cycle, the parasite reproduces asexually in bladder snails (Physidae, *Physella gyrina* and *P. acuta*) that serve as the first intermediate host, metacercariae are found in a wide range of fishes, and fish-eating ardeid birds (bitterns, herons, and egrets) serve as definitive hosts that defecate eggs into streams (Miller 1954; Palmieri 1976).

More than 25 species of *Posthodiplostomum* have been described worldwide (Ritossa *et al.* 2013; López-Hernández *et al*. 2018). Recent ecological and molecular studies (Locke *et al.* 2010; Lane *et al.* 2015) have suggested the possibility of cryptic diversity within the subspecies, *P. minimum centrarchi*, a parasite of centrarchid fishes. Whether or not the same phenomenon exists in *P. m. minimum* is not presently known.

PLAGIORCHIIDA: CLINOSTOMIDAE

***Clinostomum marginatum* (Rudolphi, 1819).** – A

total of 28 individual (4.0 ± 4.6, range 1–15) *C. marginatum* metacercariae (HWML 216344) were found encapsulated in the dermis, musculature, and liver of 7 (33%) *F. chrysotus*. McAllister *et al*. (2020) previously reported *C. marginatum* from *F. chrysotus* collected from sites in Lincoln and Pulaski counties; all of their specimens were found in dermal tissues. However, in the present study, numerous metacercariae were found encapsulated in liver tissue (Figs. 2B,C). This digenean has been previously reported from other fundulids (Hoffman 1999), including *F. catenatus* from Arkansas (McAllister *et al*. 2016b). Hoffman (1999) reported it is likely capable of infecting any species of freshwater fish. The first and second intermediate hosts of this trematode are planorbid snails and mainly fishes, respectively, with piscivorous (ciconiiform) birds serving as the definitive host, usually herons or egrets.

Caffara *et al.* (2011), using morphological and molecular data to differentiate adult and metacercarial stages of *C. complanatum* (Rudolphi, 1814) and *C. marginatum*, concluded that *C. complanatum* is the ‘‘European’’ species and is not present in hosts of the Americas. Therefore, previous reports of *C. complanatum* from Western Hemisphere native fishes (Hoffman 1999) are not valid and represent *C. marginatum*.

NEMATODA: SPIRURIDA: GNATHOSTOMATIDAE

***Spiroxys contortus* (Rudolphi, 1819) Schneider, 1866.** – Eleven (2.8 ± 1.5, range 1–5) third-stage larval specimens of *S. contortus* (HWML 216342) were found in the intestinal tract of 4 (19%) *F. chrysotus*. Amin (1984) reported this nematode from Bayou Topminnow, *Fundulus notti* from Wisconsin. However, this is the third time *S. contortus* has been reported from any fundulid fish in Arkansas as McAllister *et al*. (2016a, 2018) reported it previously from Blackstripe Topminnow (*Fundulus notatus*) and Western Starhead Topminnow (*Fundulus blairae*), respectively. As such, *F. chrysotus* is a new host record for this nematode. This roundworm is distributed widely in various vertebrate hosts (primarily turtles) in Palearctic Eurasia, North Africa, North and South America, and the Caribbean (Ernst and Ernst 1977; Baker 1987). The life cycle involves copepods as intermediate hosts in which the third-stage larvae develop (Hedrick 1935). Some potential paratenic hosts include mollusks, dragonfly nymphs, fish, and anurans (Mascarenhas and Müller 2015).

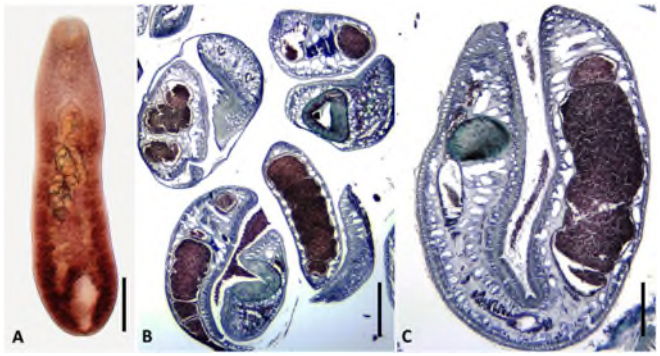

Fig. 2. Trematodes from *Fundulus chrysotus*. (A) *Homalometron* sp. Scale bar = 200 µm. (B) Several metacercariae of *Clinostomum marginatum* teased from liver. Scale bar = 1 mm. (C) Higher magnification of single *C. marginatum* metacercaria. Scale bar = 500 µm.

In conclusion, various populations of the Golden Topminnow have now been surveyed for parasites from all the major river drainages of the state (McAllister *et al*. 2020, this study). As has been shown when data from the present study is compared to that reported by McAllister *et al*. (2020), *F. chrysotus* from various Arkansas watersheds share some of the same helminth parasites. It is interesting to note, however, that none in the present study harbored myxozoans or monogeneans since McAllister *et al.* (2020) reported 8% and 18% of the Golden Topminnows they surveyed harbored *Myxobolus* sp. and *Salsuginus* sp., respectively. In addition, several watersheds within those drainages that support this species (Robison and Buchanan 2020) have not been surveyed to date (i.e., western Arkansas River Valley and along the tier of counties of extreme southern Arkansas), and additional collection efforts within these sites could reveal more information on the parasitological fauna of *F. chrysotus*, including that on the enigmatic *Homalometron* sp.

Acknowledgments

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Ecto- and Endoparasites of the Texas Deermouse, *Peromyscus attwateri* and Eastern Woodrat, *Neotoma floridana* (Rodentia: Cricetidae) from Polk County, Arkansas

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Running Title: Parasites of Texas Deermice and Eastern Woodrats

Abstract

In Arkansas, the Texas deermouse (*Peromyscus attwateri*) occurs in the western part of the state where it is restricted to the uplands of the Interior Highlands. The eastern woodrat (*Neotoma floridana*) is found statewide but is less common in the Gulf Coastal Plain. Very little is known about the parasites of either rodent in Arkansas, especially helminths from *P. attwateri* at any locality within its range. Found in/on *P. attwateri* were a coccidian (*Eimeria langbarteli*), a tapeworm (*Catenotaenia peromysci*), a nematode (*Syphacia peromysci*), 2 ticks (*Dermacenter variabilis* and *Ixodes scapularis*), and 2 mites (*Androlaelaps fahrenholzi* and *Leptotrombidium peromysci*). Eastern woodrats harbored 3 nematodes (*Eucoelus* sp., *Longistriata neotoma*, and *Trichurus neotomae*), a larval bot fly (*Cuterebra americana*), and a flea (*Orchopeas pennsylvanicus*). We document 6 new host and 5 new distributional records for these parasites.

Introduction

Arkansas supports at least 27 species of rodents (Sealander and Heidt 1990). One of these, the Texas deermouse, *Peromyscus attwateri* J. A. Allen, 1895, is a semiarboreal, small cricetid rodent that occurs in the western part of the state within the Ouachita and Ozark uplands. It also inhabits rocky portions of northern and central Texas, northward into Oklahoma and extends into southern Kansas, and Missouri (Reid 2006). This mouse prefers rocky areas including crevices along cliffs and limestone outcropping with woody vegetation.

Another rodent, the eastern woodrat, *Neotoma floridana* (Ord, 1818), is found statewide in Arkansas and also ranges from the swamplands along the lower Mississippi River, through forested uplands to southern North Carolina, west to the arid plains of eastern Colorado and Nebraska, and south to eastern Texas and Florida.

Little is known about the parasites of *P. attwateri*. Duszynski and McAllister (1995) and McAllister and Kessler (2002) reported the coccidian, *Eimeria langebarteli* from *P. attwateri* from Texas and Arkansas, respectively. Tumlison *et al.* (2015) and McAllister *et al.* (2017) reported fleas from *P. attwateri* from Arkansas. No helminth parasites, to date, are known from *P. attwateri* from any part of its range.

Moderate information is available on the parasites of *N. floridana* in Oklahoma (Murphy 1952; Boren *et al.* 1993) and Arkansas (McAllister *et al.* 2017), including a recent description by McAllister and Hnida (2020) of a new coccidian from *N. floridana* from Arkansas (incorporating 6 specimens from the current study). Fleas have only been reported from *N. floridana* nests (McAllister *et al.* 2017) and several ticks have been reported from eastern woodrats from the state (McAllister *et al.* 2016). Nothing else, however, has been published on parasites of *N. floridana* in Arkansas. Here, we document some additional parasites from *P. attwateri* and *N. floridana* from the state.

Materials and Methods

Between March and October 2019, and again during March 2020, 6 *P. attwateri* and 6 *N. floridana* were collected with Sherman live traps (H. B. Sherman Traps, Tallahassee, FL) from a limestone escarpment located ca. 4.8 km N of Mena off St. Hwy. 88 at Blue Haze Vista, Polk County (34°37'40.17"N, -94°14' 44.4228"W). In addition, a single *N. floridana* was collected on 13 March 2020 from off St. Hwy. 8 at Big Fork, Polk County (34°29'07.89"N, -93°58'03.99"W).

Rodents were killed by cervical dislocation following accepted guidelines (Sikes *et al*. 2011) and their pelage was brushed for ectoparasites. Those found were placed in a vial of 70% (v/v) ethanol; fleas and ticks were cleared in 10% potassium hydroxide, dehydrated through an ethanol series, further cleared in xylene, and slide-mounted in Canada balsam. A mid-ventral incision was made to expose the viscera and the gastrointestinal (GI) tract from the throat to anus was removed, rinsed in 0.9% saline, and organs (including heart, liver, lungs, spleen, and kidneys) were placed in individual Petri dishes. Several sections of the GI tract were cut, split lengthwise, and examined under a stereomicroscope for endoparasites. Feces from the rectum was collected from *P. attwateri* and a single *N. floridana* (from Big Fork site) and placed in 2.5% potassium dichromate. Fecal flotations were accomplished with Sheather's sugar solution (sp. gr. 1.30). Tapeworms were fixed in near boiling tap water without coverslip pressure, transferred to DNA grade ethanol, stained with acetocarmine, and mounted in Canada balsam. Nematodes were examined as temporary mounts in glycerol.

Hosts were deposited in the Henderson State University (HSU) collection, Arkadelphia, Arkansas. Voucher specimens of ectoparasites were deposited in the General Ectoparasite Collection in the Department of Biology at Georgia Southern University, Statesboro, Georgia. Endoparasites were deposited as photovouchers in the Harold W. Manter Laboratory (HWML) of Parasitology, University of Nebraska, Lincoln, Nebraska, or samples were retained for molecular analyses. Prevalence, mean intensity ± 1SD, and range of infection are provided in accordance with terminology given in Bush *et al*. (1997).

Results and Discussion

An eimerian coccidian was found to be passing in *P. attwateri* feces, a species of cyclophyllidean tapeworm and a nematode were found in the gut, and 2 mites and 2 ticks, each, occurred on the pelage. Woodrats harbored 3 species of nematodes, 1 species of flea, and a third instar bot fly larva; a single *N. floridana* was not passing coccidia. Data are presented below in annotated format.

APICOMPLEXA: EIMERIIDAE

***Eimeria langebarteli* Ivens, Kruidenier, & Levine, 1959. –** Oocysts of *E. langebarteli* (HWML 216346; Fig. 1) were passing in the feces of 4 of 6 (67%) *P. attwateri*. Oocysts were ellipsoidal, 19.8 × 13.4 (18– 22 × 13–14) µm with a length/width ratio (L/W) of 1.5 (1.4–1.7). A micropyle and oocyst residuum was absent but 1–2 polar granules were present. Sporocysts were ovoidal-ellipsoidal, (L × W) 9.2 × 5.4 (8–10 × 5–6) µm with a L/W ratio of 1.7 (1.5–2.0). A knob-like Stieda body was present but subStieda and paraStieda bodies were absent. The sporocyst residuum was composed of various granules forming either a small, compact sphere or as dispersed mass located between and across the sporozoites. An ellipsoidal posterior refractile body occurred in the sporozoites. This is the first report of measurements and accompanying photomicrographs of *E. langebarteli* from an Arkansas host.

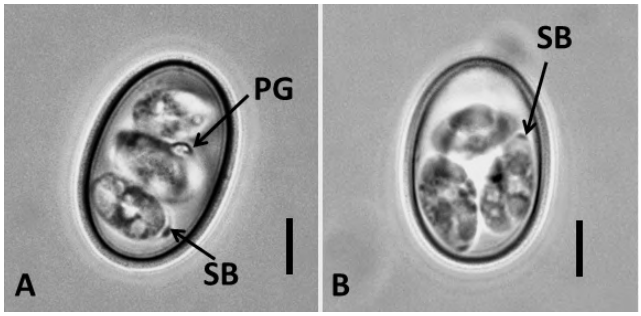

Figure 1. Coccidian from *Peromyscus attwateri*. (A) Sporulated oocyst of *Eimeria langebarteli* showing polar granule (PG) and Stieda body (SB). (B) Another sporulated oocyst of *E. langebarteli* showing SB. Scale bars = 5 µm.

McAllister and Kessler (2002) reported *E. langebarteli* from 1 of 4 (25%) *P. attwateri* from the same collection site herein; however, no mensural data or photomicrographs were provided. In the present study, it is most interesting that this rodent population has hosts passing the same coccidian species that has persisted nearly 2 decades. Duszynski and McAllister (1995) also reported *E. langebarteli* from *P. attwateri* and white-ankled mice (*Peromyscus pectoralis*) from Texas. This coccidian has now been reported from at least 6 species of cricetid rodents, including *Peromyscus* and *Reithrodontomys* from the southwestern United States and México (McAllister and Kessler 2002). Zhao and Duszynski (2001) using plastid ORF470 and nuclear *18S rDNA* sequences found that *E. langebarteli* belonged in a lineage B that included eimerians in *Mus*, *Onychomys*, *Rattus*, and *Reithrodontomys*, and which lacked an oocyst residuum.

CESTODA: CYCLOPHYLLIDEA: CATENOTAENIIDAE

***Catenotaenia peromysci* Smith, 1954. –** Two specimens were found in the small intestine of 2 of 6 (33%) *P. attwateri*. Nineteen species of *Catenotaenia*

have been described from 22 species of rodents from both Palearctic and Nearctic realms (Haukisalmi *et al.* 2010). The type host and type locality is the deer mouse (*Peromyscus maniculatus*) collected from New Mexico (Smith 1954). The geographic range of *C. peromysci* also includes Colorado, Utah, Wyoming, and Alberta and British Columbia, Canada (Smith 1954; Lubinsky 1957; Grundmann *et al.* 1976; Hwang *et al.* 2007). Other hosts include red backed voles (*Myodes gapperi*) from British Columbia, Canada (Erickson 1938). We document a new host and the southeasternmost geographic record for this tapeworm. Vouchers are being retained for molecular analyses.

NEMATODA: RHABDITIDA: TRICHOSTRONGYLIDAE

***Longistriata neotoma* Murphy, 1952. –** Several *L. neotoma* (HWML 216347; Fig. 2) were found in the small intestine of 2/7 (29%) *N. floridana* collected on 17 August and 20 October 2019. This nematode has been previously reported from *N. floridana* from Oklahoma (Murphy 1952; Boren *et al.* 1993). We document this nematode in a host from Arkansas for the first time.

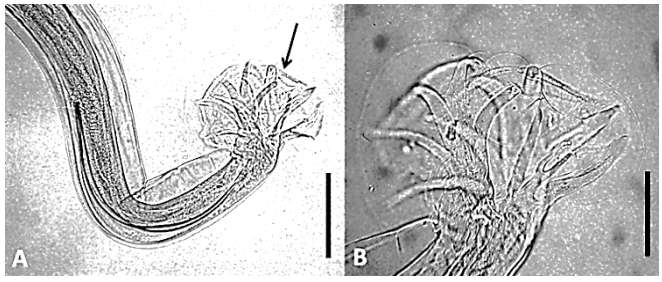

Figure 2. *Longistriata neotoma* from *Neotoma floridana*. (A) View showing posterior end of worm and copulatory bursa (arrow). Scale bar = 100 µm. (B) Close-up showing copulatory bursa and dorsal rays. Scale bar = 50 µm.

ENOPLIDA: TRICHOCEPHALIDA: TRICHURIDAE

***Eucoelus* sp.** – Species of the genus *Eucoleus* Dujardin, 1845 are primarily parasites of the esophagus and stomach of birds and mammals. The specimen (gravid female) recovered here in 1 of 7 (14%) *N. floridana* collected on 20 October 2019 was found in the small intestine and is probably undescribed. Bechtel *et al.* (2015) reported *Eucoleus* sp. from dusky-footed woodrat (*Neotoma fuscipes*) and big-eared woodrat (*Neotoma macrotis*) in California. However, their identification was made from capillariid eggs in the feces and it is impossible to reliably determine such eggs to genus, although our own record may lend weight to the possibility. The only record, to date, of *Eucoleus* specimens in North American rodents appears to be that of *E. gastricus* from the marsh rice rat (*Oryzomys palustris*) in Florida by Kinsella (1988), but that species only occurs in tunnels within the stomach lining. We here document this nematode from *N. floridana* and Arkansas for the first time. Vouchers are being retained for molecular analyses.

***Trichurus neotomae* Chandler, 1945. –** Two female *T. neotomae* (HWML 216348; Fig. 3) were taken from the cecum of 1 of 7 (14%) *N. floridana* collected on 17 August 2019. Both Boren *et al.* (1993) and McAllister *et al*. (2017) reported *T. muris* from *N. floridana* from Oklahoma but we believe this to be a misidentification and actually represent *T. neotomae*. This nematode was originally described from *N. fuscipes* from California (Chandler 1945). It has also been reported from southern plains woodrat (*Neotoma micropus*) from Texas (Charles *et al.* 2012). We report *T. neotomae* in Arkansas for the first time.

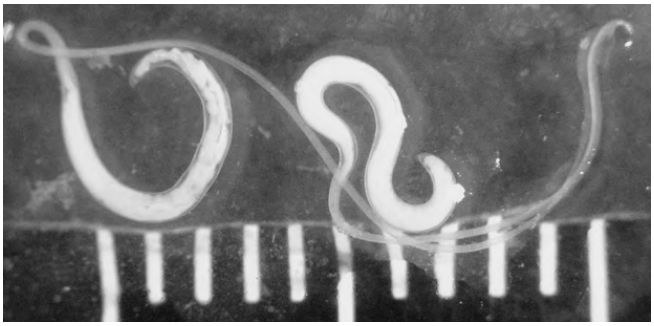

Figure 3. Stereoscopic view of 2 *Trichurus neotomae* from *Neotoma floridana*. Each scale interval = 1 mm.

OXYURIDA: SYPHACIIDAE

***Syphacia* (*Seuratoxyuris*) *peromysci* Harkema, 1936. –** Two immature female specimens were found in the small intestine of 1 (17%) *P. attwateri* collected on 15 March 2020. This nematode has been previously reported from *Peromyscus* spp. (*P. gossypinus*, *P. leucopus*, *P. maniculatus*, and *P. polionotus*), western harvest mouse (*Reithrodontomy megalotis*), and spotted ground squirrel (*Xerospermophilus spilosoma*) from Arizona, Minnesota, New Mexico, North Carolina, Utah, Wisconsin, and Quebéc, Canada and México (Kruidenier *et al.* 1961; Pulido-Flores *et al.* 2005; Falcón-Ordaz *et al.* 2016). The life cycle is direct with hosts being infected via perianal contact with ova. This is the first time *S. peromysci* has been reported from *P. attwateri* and Arkansas is also a new

geographic locality. Vouchers are being retained for molecular analyses.

ACARI: TROMBICULIDAE

***Leptotrombidium peromysci* Vercammen-Grandjean & Langston, 1976. –** One larval *L. peromysci* (L3843) was collected from a single (17%) *P. attwateri* collected on 15 March 2020. This chigger has been reported from several species of small and medium-sized mammals in the eastern U.S. and South Dakota (Walters *et al.* 2011), including white-footed mouse (*Peromyscus leucopus*) from Arkansas (Connior *et al.* 2017). This represents the second record of this species from Arkansas as well as a new host record for this chigger mite.

LAELAPIDAE

***Androlaelaps fahrenholzi* (Berlese, 1911).** A single nymphal *A. fahrenholzi* (L3843) was found on 1 of 6 (17%) *P. attwateri* collected on 15 March 2020. This is a widespread and common Nearctic ectoparasite that has been previously reported from various rodents in other states (Whitaker *et al.* 2007), including woodland vole (*Microtus pinetorum*), hispid pocket mouse (*Sigmodon hispidus*), golden mouse (*Ochrotomys nuttalli*), and *N. floridana* from Arkansas (Tumlison *et al.* 2015; Connior *et al.* 2017). We document a new host record for *A. fahrenholzi*.

IXODIDA: IXODIDAE

***Dermacentor variabilis* (Say, 1821). –** Two larval American dog ticks (L3843) were collected from a single (17%) *P. attwateri* collected on 20 March 2020. This is a commonly collected tick from a variety of mammalian hosts, including several rodents (Cricetidae, Sciuridae) from Arkansas (McAllister *et al.* 2016). There are several records of *D. variabilis* from domestic dogs and cats from Polk County (McAllister *et al.* 2016). This is, however, the first time *D. variabilis* has been reported from *P. attwateri.*

***Ixodes scapularis* Say, 1821. –** A single larval *I. scapularis* (L3843) was taken from a *P. attwateri* collected on 20 March 2020; this same host also co-harbored the *D. variabilis* above. The blacklegged tick is a common species in Arkansas on a wide variety of hosts, including medium to large-sized mammals as adults but immatures are often found infesting the same hosts as well as small mammals, birds, and reptiles, especially lizards (see McAllister *et al.* 2016). We document *I. scapularis* from *P. attwateri* for the first time.

INSECTA: SIPHONAPTERA: CERATOPHYLLIDAE

***Orchopeas pennsylvanicus* (Jordan, 1928). –** Three of 7 (43%) eastern woodrats, collected on 15 March and 20 October 2019, harbored 1 male (L3828) and 2 female (L3837) *O. pennsylvanicus* (Fig. 4) from the Blue Haze Vista site respectively, and another *N. floridana* collected on 13 March 2020 was infested with 18 (9 male and 9 female, L3843) *O. pennsylvanicus* from the Big Fork site. This flea was previously reported by Schiefer and Lancaster (1970) from woodrat "nests" from northwestern Arkansas. The eastern woodrat hosts a variety of generalist flea parasites (Durden *et al.* 1997) and *O. pennsylvanicus* occurs in eastern North America as an ectoparasite of woodrats (Lewis 2000); it has no known medical-veterinary importance. We document the first report of this flea (with genuine voucher specimens) on eastern woodrats from Arkansas.

Figure 4. Female *Orchopeas pennsylvanicus* (L3837) from *Neotoma floridana*. Scale bar = 500 µm.

DIPTERA: OESTRIDAE

***Cuterebra americana* (Fabricius, 1775). –** A single third-instar larval woodrat bot fly, *C. americana* (L3836; Figs. 5A–B) was found on the subcutis of the throat region of 1 of 7 (14%) *N. floridana* collected on 19 October 2019. The host of preference for *C. americana* is the eastern woodrat (Beamer *et al.* 1943; Sabrosky 1986). Eggs of cuterebrids are typically laid on sticks, rocks or other surfaces at or near the entrance to woodrat nests (Beamer *et al.* 1943). Flies of the genus *Cuterebra* are common in most temperate and tropical regions in the Western Hemisphere (Sabrosky 1986; Colwell *et al.* 2005). Larvae of these flies infest lagomorphs and rodents (Colwell *et al.* 2005).

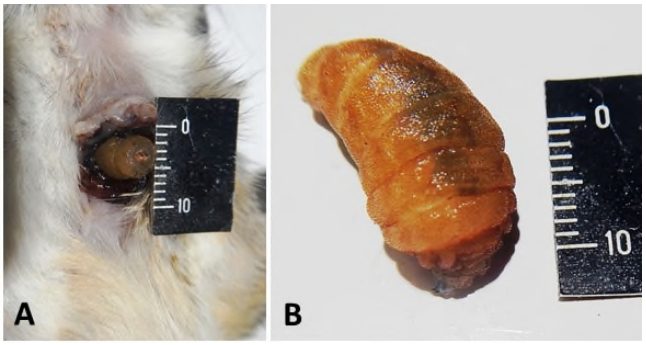

Figure 5. *Cuterebra americana* from *Neotoma floridana*. (A) View showing bot *in situ*. (B) Extracted specimen. Scale intervals = 1 mm.

Although botflies are relatively large compared to their hosts, they rarely cause mortality from myiasis. This appears to be the first report of a *N. floridana* from Arkansas to be infested with larval *C. americana*. Sabrosky (1986) documented adult *C. americana* from Baxter and Washington counties in northern Arkansas.

In summary, we have provided novel ecological information on 2 rodents from Arkansas by documenting 6 new host and 5 new distributional records for their parasites. As Arkansas supports at least 27 species of rodents (Sealander and Heidt 1990), additional surveys are warranted to identify and report their parasites in the state.

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The Arkansas Game and Fish Commission issued a Scientific Collecting Permit to CTM. We thank Drs. S.L. Gardner and G. Racz (HWML) for expert curatorial assistance, and J.M. Kinsella (Missoula, MT) for assistance with helminth identifications.

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Observations of Undescribed Diel Activity in the Wolf Spider *Rabidosa rabida* Show Cathemeral Behavior

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Running Title: First Description of *R. rabida* Diel Activity

We describe the diel activity patterns of animals as being either diurnal, nocturnal, or crepuscular. These terms do not appropriately describe animals that are active equally during both day and night. Fortunately, there is another term to cover this situation. This term is cathemeral. There are a number of terms in the literature used to describe widespread diel activity including circumdiel (Stiles *et al.* 2017), metaturnal (Tattersall 1987), "around-the-clock activity" (Bloch *et al.* 2013) and cathemeral. Terms are used inconsistently in the literature. We chose cathemeral due to its previous, though limited, use with spiders.

Tattersall first used the term cathemeral in 1987. He defined cathemeral as an organism's activity being distributed approximately evenly through the 24 hours of the daily cycle or when significant amounts of activity, particularly feeding or traveling, occur within both the light and dark portions of the cycle (Tattersall 1987). The term cathemeral is not common in ecological or behavioral literature. Most applications are limited to primate behavior, where the term was first defined. We expect that cathemeral behavior is more common in arthropods than in the primates, due to arthropod diversity of physiology and behavior.

We find the only applications of the term cathemeral to arthropods in a paper describing jumping spider and Philodromid spider circadian rhythms (Mezofi *et al*. 2019). Spiders are an ecologically diverse group of arthropods both physically and behaviorally (Foelix 2011). Most spiders have never had their diel activity described. This gap in our ecological knowledge may lead us to miss important characteristics of arthropod communities that are now under threat from a changing environment.

Rabidosa rabida is a large wolf spider commonly found in the Eastern half of North America (Brady & McKinley 1994). We know very little about the ecology of this spider. While working with *R. rabida*, we discovered that there were no clear descriptions of its diel behavior in the field. Looking at the original descriptive literature for this spider, we find that there are almost no descriptions of behavior, as is common in biological literature from the 1800s (Walckenaer 1837; Simon 1898). We decided to make the first known, clearly reported observations of the diel activity of *Rabidosa rabida* to decide if this spider is nocturnal, diurnal, or cathemeral.

We captured *R. rabida* at the Harding University Gilliam Biological Research Station in White County, Arkansas, in July 2019. We captured 26 adult, female spiders nocturnally. To each, we attached a Biomark Inc. mini HPT8 RFID tag with a drop of cyanoacrylate on the opisthosoma. We released marked spiders at the location where we had previously captured them. The following days we tracked each spider using a Biomark HPR Lite RFID microreader. Capturing individuals during diurnal and nocturnal periods allowed us to observe and record behaviors of individual spiders during both diel periods. We made observations of behavior during the morning, midday, and mid-afternoon times. Crepuscular observations were limited due to the difficulty of finding spiders by either the visual or spotlighting methods under twilight lighting conditions. In previous fieldwork, we made personal observations of behaviors similar to the reported activity for this species made here. We also made observations of unmarked spiders during diurnal tracking to confirm the observations made of marked spiders. Males and juveniles lacked the body size necessary to attach tags but were included in the untagged observations, which included over 200 spiders of all sexes and sizes.

Twenty-six spiders were marked nocturnally. We recaptured 17 of these spiders diurnally. These recaptures provided us with nocturnal and diurnal observations of multiple individual spiders in both diel periods. These observations, along with additional notes on unmarked spiders, give us confidence that our observations here are ordinary in this population.

We have previously observed spiders moving both vertically and horizontally, hunting, eating, molting, mating, and demonstrating cannibalism of mates

nocturnally (Figure 1). Diurnally we were able to observe spiders moving vertically and horizontally, hunting, and eating (Figure 1). Vertical movements were limited diurnally compared to nocturnal behavior but horizontal movements were more common or possibly more visible diurnally. We were able to observe 1 case of a spider molting in the vegetation during daylight (Figure 1). We have in previous studies, observed wasp predators carrying paralyzed spiders away during the daylight. While we did not observe any predator avoidance behaviors here, we suggest that these behaviors should be either diurnal or cathemeral, if these behaviors are present in this spider. Nocturnal predation on this species has never been explored and the scientific literature contains no descriptions of predator avoidance behaviors as of yet.

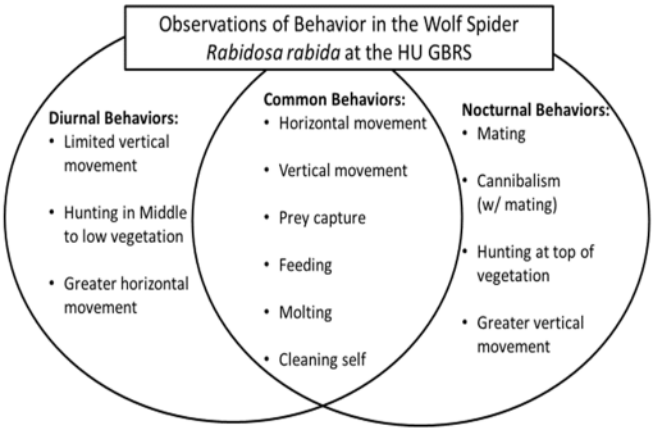

Figure 1: Venn diagram of observed behavior in *Rabidosa rabida* compared between daylight hours (diurnal behaviors), after dark (nocturnal behaviors), and behaviors observed at both times (Common behaviors).

According to the definition given by Tattersall (1987), we can classify an animal as cathemeral if it performs important functions during both day and night. We observed *R. rabida* hunting, eating, moving, and molting during both diurnal and nocturnal diel periods causing us to conclude that we should classify its behavior as cathemeral.

The description made here is the first published description of the field diel activity in *Rabidosa rabida*. Previous work, focusing on pesticide influences on behavior, indirectly suggest cathemerality in this species, in a laboratory setting, but did not directly provide a clear description of diel patterns or make field observations (Tietjen & Cady 2007). We have still not documented the full range of times and conditions that this spider is active during the day nor have we made enough daylight observations to be confident of all of the behaviors occurring during daylight.

The changing climate is making changes to arthropod communities that we cannot at this time describe due to a lack of basic knowledge about the animals in these communities. We need to describe multiple traits of these communities before they are at best changed, or worse, lost forever. These observations of an influential predator are a start to this work, but we need much more work for this species and many others in Arkansas and around the world.

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Regional Variation in Ventral Body Color and Pattern in the Western Ratsnake, *Pantherophis obsoletus* (Reptilia: Serpentes: Colubridae), in Arkansas

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Running Title: Ventral Color and Pattern in the Western Ratsnake

Pantherophis obsoletus, the Western Ratsnake (a.k.a., black ratsnake or chicken snake), is a large colubrid species widely distributed throughout the central and southcentral United States west of the Mississippi River (Powell *et al.* 2016). This species has received considerable attention with its early taxonomic history found in Neill (1949) and Dowling (1952) and its current phylogeographic status examined by Burbrink *et al.* (2000), Burbrink (2001), and Gibbs *et al.* (2006). Dorsal body color and pattern played an important role in resolving the early taxonomic issues within this ratsnake species complex, whereas mtDNA was utilized in the more recent phylogeographic analyses of ratsnake groups. As a whole, however, phenotypic plasticity in body color and pattern is a hallmark feature of all North American ratsnake complexes. For example, few species show as much regional variation as do ratsnakes in the southeastern United States (Gibbons and Dorcas 2005).

Burbrink *et al.* (2000) noted that color pattern classes were found not to be concordant within the evolutionary history of some ratsnake species, and this is the case in the Western Ratsnake. The black dorsal pattern may feature considerable blotching, which is the typical dorsal color for adult Western Ratsnakes in Arkansas, but the background color may also include a variable array of red, brown, gray, and white between the mostly black dorsal scales (Burbrink 2001; Trauth *et al.* 2004). The ventral surface scales may contain markings in the form of 2 or 3 connected rows of scale blotches, scales may be completely black at midbody, or scales may possess a range of colors from white, yellow, tan, or darkly mottled in some areas. In addition, venters may also be totally unpatterned.

Most photographic records of live adult specimens of Western Ratsnakes, which are used for species recognition, display only the dorsal color pattern (e.g., 5 photographs in Werler and Dixon 2000; 5 photographs in Trauth *et al.* 2004). In fact, photographic documentation of the ventral body color and pattern in the Western Ratsnake is normally lacking in most reference books and is often unavailable for most specimens prior to deposition into museum collections.

Matthews (2015) emphasized the need for veteran professional ichthyologists and herpetologists to publish any of their high-quality undocumented biological data on poorly-studied aspects of species (i.e., he urged the publication of unpublished information that may be hidden in personal field notes or in species voucher records.). He also stressed the continued value of such biological information, especially that which pertained to life history traits or other basic attributes of an organism's biology. In following this theme, I report, herein, on variation in ventral body color and pattern in a small sample of adult Western Ratsnakes from Arkansas, which I found in my personal repository of snake photographs.

In the spring of 1989 and then again in 1991, my students (Natural History of Vertebrates class) and I collected Western Ratsnakes from several localities mostly from the southwestern region of the state. Snakes were returned to the lab at Arkansas State University for processing and deposition into the university herpetological collection; snakes were assigned museum (ASUMZ) numbers. Venters of all snakes (except ASUMZ 34059) were photographed using a Minolta 35-mm single lens reflex camera and Kodachrome 64 color slide film. Images were taken in sunlight immediately following sedation of snakes using a pleuroperitoneal injection of a dilute sodium pentobarbital solution. Specimens were later fixed with 10% formalin for 48 hr and placed into 70% ethanol for preservation. One additional snake, ASUMZ 34059 collected in 2019, was included as an outlier for this snake sample. In the following, I provide detailed information pertaining to all snakes (i.e., ASUMZ no., snout-vent length and tail length [each to the nearest mm], no. of ventrals, sex, date of collection, collection locality [decimal degrees, WGS84 (all cases), and county] along with a brief description of the ventral body color and pattern (Figs.

1-4). Color slides remain in my possession.

ASUMZ 12900. 936, 196, 232, male, 14 April 1989, 34.411747°N, 91.073231°W, Monroe Co. This ratsnake has a uniform cream background color from the chin to around ventral 69; thereafter, this background color progressively fades, being obscured by a burnt orange and black color. Medially, bold black markings occur repetitively in sets of 3 or 4. These markings are interconnected in a zigzag pattern beginning at ventral 73, and they reappear in this pattern periodically toward the vent. The burnt orange color begins abruptly at about ventral 70 and increases in intensity to become suffused with black caudally. These combined colors tend to obscure the black repetitive interconnected markings. The subcaudals are almost entirely black.

ASUMZ 12901. 944. 197, 227, male, 15 April 1989, 33.558231°N, 93.168367°W, Nevada Co. This ratsnake possesses a uniform cream background from the chin to around ventral 70. A pink color of mostly light intensity begins at ventral 70 and extends to ventral 155; thereafter, the venter is mostly pale. The subcaudals appear mostly pale and possess lateral dark oblong markings.

ASUMZ 12902. 1446, 424, 236, female, 15 April 1989, 33.314128°N, 92.96976°W, Union Co. This large ratsnake possesses a cream background overlaid with diffuse pink from the chin posteriorly to around ventral 198. A scattering of mostly isolated pink lateral blotches are interspersed from ventral 110 to 198; these are lost from around ventral 198 caudally toward the vent. All ventrals exhibit lateral, dark crescent-shaped markings as mentioned by Burbrink (2001). These dark markings extend dorsally to meet dark scales of the dorsum. The subcaudals lack the pink color of the venter, although they retain the lateral, dark crescents on a mostly pale background.

ASUMZ 12903. 1063, 361, 231, female, 15 April 1989, 34.026058°N, 93.946281°W, Howard Co. This ratsnake possesses a cream color on the chin and neck which is replaced by a mostly brown color intermixed with some yellow pigment starting on ventral 12. The

Figure 1. Ventral body color and pattern in the Western Ratsnake (top to bottom): ASUMZ 12900, 12901, 12903, and 12902.

Figure 2. Ventral body color and pattern in the Western Ratsnake (ASUMZ 17397).

brown color intensifies at around ventral 88 and lessens near ventral 170. Hidden in the background are the characteristic medial interconnected zigzag bold markings. From ventral 170 to the vent, the brown pigment becomes mottled into a peppery, dull black background. There are black markings which fringe the lateral edges of each ventral. The subcaudals consist of a mixture of mostly black along the margins and brown within the central areas.

ASUMZ 17397. 770, 155, 239, male, 16 March 1991, 33.354658°N, 93.509881°W, Lafayette Co. This ratsnake possesses a cream background covered with a light yellow pigment beginning at ventral 22; the yellow color spreads caudally onto the broad dark central regions of the scales. Medial double sets of faint yellow blotches of 2 or 3 scale rows begin at around ventral 28 and continue through ventral 142 after which they merge into mostly dark blotches. A series of lateral dark blotches on 2 or 3 ventrals begin at ventral 6 and re-occur every 3 to 4 scale rows until merging with the dark dorsal scales. Most mid body and posterior ventrals possess a pattern of alternating light

Figure 3. Ventral body color and pattern in the Western Ratsnake (above, ASUMZ 17615; below, 17614).

Figure 4. Ventral body color and pattern in the Western Ratsnake (ASUMZ 34059).

lateral edges (as yellow spots) interspersed between 2 to 3 ventrals. The lateral edges of the subcaudals are mostly dark, whereas the medial surfaces are covered with a light yellow fringe, creating the appearance of a mid-ventral line.

ASUMZ 17614. 1239, 253, 245, male 27 April 1991, 34.167103°N, 94.014781°W, Howard Co. This ratsnake possesses a cream background which is intermixed and eventually overtaken by dominate black marking. Medial black markings start at ventral 41as weakly interconnected rows of 3 or 4 scales that eventually alternate in position in the characterist zig-zag pattern as seen in ASUMZ 12900, 17615, and 34059. This marking pattern continues to around ventral 187; thereafter, the black markings mostly merge with one another. Lateral dark blotches on 2 or 3 ventrals, as seen in ASUMZ 17397, begin at ventral 12 and re-occur every 3 to 4 scale rows. They merge with the dark dorsal scales. The subcaudals are almost entirely black.

ASUMZ 17615. 1279, 282, 238, male 26 April 1991, 33.572022°N, 94.072997°W, Ouachita Co. This ratsnake possesses a cream background which is mostly overtaken by medial black blotches toward the vent. Starting at ventral 12, bold medial black blotches of interconnected rows of 3 or 4 scales alternate in position in the characterist zigzag pattern as seen in ASUMZ 12900 and 17614. This color pattern dominates the venter. Lateral dark blotches on 2 or 3 ventrals, as seen in ASUMZ 17397, begin at ventral 8 and re-occur every 3 to 5 scale rows. They merge with the dark dorsal scales. The subcaudals are black..

ASUMZ 34059. 1086, 225, 237, male 29 June 2019, 35.191844°N, 92.715053°W, Conway Co. This ratsnake possesses a cream blackground that is overtaken by medial black blotches caudally. These medial blotches begin weakly on 2 scale rows fromventral 36 to ventral 64; thereafter, the pattern of 2 or 3 interconnected and zigzag markings extend caudally on the venter. Less conspicuous were the lateral dark blotches. They begin on ventral 7 and re-occur every 3 to 4 scale rows caudally. The subcaudals contained a mixture of dark and pale scales.

Despite the lack of concordance with respect to body color and pattern throughout the range of Western Ratsnakes, the yellowish brown ventral color, as seen in ASUMZ 12903, is worth mentioning here. This ventral color bears a striking resemblance to some Western Ratsnakes from central Texas, which were formerly known as Texas Ratsnakes (Werler and Dixon 2000; Boundy and Carr 2017). For instance, in central and west-central Texas, Western Ratsnakes have a ventral body color that is a mixture of tan to yellowish brown as revealed in a specimen from near Waco, Texas (Fig. 5). One might argue that there appears to be some genetic influence from Texas Ratsnakes resulting in the yellowish brown venter observed in ASUMZ 12903. For reasons explained below, I support this possibility; i.e., that ASUMZ 12903 expresses this ventral body coloration by being a member of a subpopulation within a remnant gene pool that is localized in the Blackland Prairie region (MacRoberts *et al*. 2011) of southwestern Arkansas (Fig. 6). Burbrink *et al.* (2000) illustrated (i.e., their Fig. 5) the presumed divergence and dispersal pattern

of the western mtDNA clade, one of three clades of *Pantherophis obsoletus* complex, following the post-Wisconsin glacial period (Auffenberg and Milstead 1965). The gene flow dispersal pattern of the western clade appears to have originated from refugia in southern Texas and dispersed northward and eastward, possibly following the Prairie Peninsula Corridor for herpetofaunal movements (Auffenberg and Milstead 1965). For example, there are populations of other reptiles, such as eastern collared lizards and western diamondback rattlesnakes, that moved eastward during a period of warming and aridity (Xerothermic Phase, occurring between 6,000 and 3,000 yr B.P.) contributing to desert and prairie extensions into Arkansas (Dowling 1956; Smith 1957).

Figure 5. Ventral body color and pattern of a recently-deceased Western Ratsnake from McGregor, McLennon County, Texas. Photographed on 28 June 2019.

This particular ratsnake was collected in the community of Center Point not far from Blackland Prairie regions in Howard County . By comparison, Western Ratsnakes just outside the Blackland Prairie region (e.g., ASUMZ 17397, 17614, and 17615; also, the outlier, ASUMZ 34057) possess the more typical Western Ratsnake venters. A genetic analysis of ratsnakes from the Blackland Prairie would be necessary to determine if such individuals show an affinity to Texas populations.

Another Western Ratsnake (ASUMZ 12901) deserves mentioning because of its unusual pink color pattern. This specimen was collected south of the Blackland Prairie but within a sandhill region of Nevada County. No other ratsnake that I can recall (Trauth *et al.*2004) has possessed this coloration. In addition, Boundy and Carr (2017) do not include pink in their color description of the species.

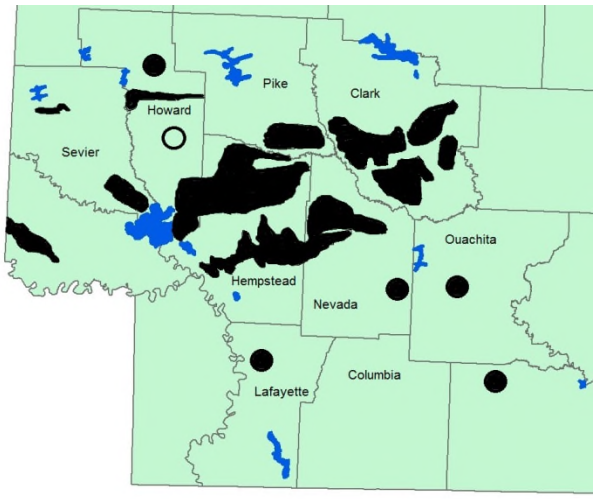

Figure 6. Map of the Blackland Prairie of southwestern Arkansas (adapted from MacRoberts *et al.*, 2011). Open circle represents collection site for ASUMZ 12903; closed circles represent collection sites of other Western Ratsnakes examined in the present study.

I extend a grateful thanks to my students for field assistance and to C. Wherry for her thoughtful comments regarding body color and pattern in these ratsnakes. I also thank H. Trauth for providing the photograph of the Western Ratsnake from Texas.

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Red-shouldered Hawk (*Buteo lineatus*) predation on North American Racer (*Coluber constrictor*) in the Arkansas Ozarks

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Running Title: Hawk preys on racer

The Red-shouldered Hawk (*Buteo lineatus*) has a catholic diet that includes small mammals, snakes, lizards, turtles, anurans, salamanders, birds, insects, crayfish, and other terrestrial and freshwater invertebrates (Bent 1937; Dykstra *et al*. 2003; Fisher 1893; Fitch 1963; Howell and Chapman 1998; McAtee 1935; Platt and Rainwater 2019; Portnoy and Dodge 1979; Roble 2013; Strobel and Boal 2010). Roble (2013) compiled Red-shouldered Hawk predation records for 27 species of snakes, including reports of the North American Racer (*Coluber constrictor*) from Georgia (Howell and Chapman 1998) and Massachusetts, Maine, and Florida (Fitch 1963). These reports were based on prey items recovered from stomachs and observations of prey brought to nests. Regional variation in ophiophagy in Red-shouldered Hawk populations is poorly known. Here I report two observations of Red-shouldered Hawk preying on North American Racers in Arkansas.

On 19 April 2018, I photographed an adult Red-shouldered Hawk after it captured a mature racer in Izard County, Arkansas (36° 9.43' N; 92° 9.32' W). The hawk dropped from an elevated perch along a fence line and caught the racer in the adjacent pasture. After manipulating it on the ground for several minutes, the hawk carried the writhing racer about 230 m (measured with Google Earth Pro) to a barbwire fence along the road (Fig. 1). The racer's head was severely damaged and bloodied but its slowly twisting body made it difficult for the hawk to balance on the wire. The hawk paused on the wire for ~15 seconds before carrying the racer over the distant tree line (175 m).

The snake's large size and slate-gray dorsum shading gradually to an unmottled pale bluish-white venter identified it as a North American Racer, most likely *C. constrictor priapus* based on geography (Trauth *et al*. 2004). The unusually pale venter suggests it may represent an intergrade with *C. constrictor flaviventris*, which occurs in the northern tier of counties in Arkansas east to Fulton County (Trauth *et al*., 2004).

A second instance of possible predation was recorded on 27 April 2017 (13:20 h), when Jerald Britten observed an adult Red-shouldered Hawk struggling with a large racer (~ 1.2 m in length) in Marion County, Arkansas (36° 15.48' N; 92° 34.75' W). By the time Britten retrieved his camera (13:31 h), both hawk and snake appeared exhausted and were nearly motionless (Fig. 2, top panel). The photograph in the

Figure 1. Red-shouldered Hawk (*Buteo lineatus*) grasping a North American Racer (*Coluber constrictor*) in Izard County, Arkansas (Photograph: Gary R. Graves).

Figure 2. Red-shouldered Hawk (*Buteo lineatus*) grappling with a North American Racer (*Coluber constrictor*) in Marion County, Arkansas (photographs: Jerald A. Britten).

bottom panel (Fig. 2) was taken at 13:35 h. The hawk was on its back and gripped the racer with one foot. Britten left the scene at 13:47 h to avoid stressing the hawk. When he returned at 14:20 h, the hawk had righted itself (Fig. 2, middle panel). Bloody abrasions and cuts were visible on the racer's head and body. Britten again left the scene. When he returned at 15:05 h, both hawk and snake were gone. The predation attempt was probably unsuccessful because a large racer with fresh cuts on its head and body was observed in the area on 29 April.

These observations represent the first predation reports from Arkansas for Red-shouldered Hawk on North American Racer. Interactions between these species are probably common given their statewide occurrence and similar habitat preferences.

Acknowledgements

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First Record and Notes on the Ecology of the Boreal Chorus Frog (*Pseudacris maculata*) in Arkansas

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Running Title: First Record and Notes on the Boreal Chorus Frog in Arkansas

Pseudacris maculata, boreal chorus frog, is a small hylid frog found throughout the midwestern United States. In northwestern Arkansas, all *Pseudacris* were previously referred to as *P. triseriata* (Trauth *et al*. 2004). However, the majority of populations of *P. triseriata* in Arkansas were redescribed as *P. fouquettei* (Lemmon *et al*. 2008) based on genetic data from Lemmon *et al*. (2007). Based on these genetic data, Lemmon *et al*. (2007) suggested *P. maculata* occurs in extreme northwestern Arkansas; however, no specimens of *P. maculata* from Arkansas were included in the study. Thus, our study was conducted to confirm occurrence in the state and to examine the ecology of this species in Arkansas, specifically regarding habitat, diet, reproduction, and parasites.

Populations of chorus frogs were sampled from select areas of the northwestern portion of the state (Benton and Madison counties) to determine if *Pseudacris maculata* occurs in Arkansas as suggested by Lemmon *et al*. (2007). Between March 2008 and March 2016, opportunistic data were collected during the spring breeding season by listening for breeding choruses of male *Pseudacris* frogs in roadside ditches, fishless ponds, and ephemeral wetlands (Fig. 1). When a *Pseudacris* population was located, a sample of individuals was collected and identified using mitochondrial DNA analysis. Methods for the mitochondrial DNA analysis followed Moriarty and Cannatella (2004). DNA was extracted from tissue using the Qiagen DNeasy kit. Two primers (16sc/16sd) were used to amplify the region of the 16S rRNA mitochondrial genes via polymerase chain reaction. When *P. maculata* were positively identified, a subsample was necropsied for parasite infections, diet, and reproductive notes. Specimens were placed in individual bags on ice and within 48 hrs frogs were overdosed with a 10% v/v ethanol solution (HACC 2004). A mid-ventral incision from mouth to cloaca was made to expose the gastrointestinal tract. Specimens were examined for select protists, including the gall bladder for myxozoans and the rectum for opalinids and ciliates (McAllister 1987; 1991). For helminths, the entire gastrointestinal tract was examined. Trematodes were stained with acetocarmine and mounted in Canada balsam for identification. Reproductive status of females was noted by the presence of ovarian eggs. When females were gravid, clutch size was determined by counting yolked ovarian follicles. Additionally, food items were identified to the lowest taxon possible.

Voucher specimens of parasites that were new host records were deposited in the Harold W. Manter Parasitology Lab (HWML), Lincoln Nebraska. Voucher specimens of *Pseudacris maculata* were deposited in the Sternberg Museum of Natural History (MHP), Fort Hayes, Kansas, Henderson State University Herpetological Collection (HSU), Arkadelphia, Arkansas, and Arkansas State University Herpetological Collection (ASUMZ), State University, Arkansas.

The only confirmed site for *Pseudacris maculata* was in Benton Co. near Pea Ridge (N 36°27’26; W 94°03’36). On 2 March 2008, a male *Pseudacris* frog was the first specimen from Arkansas to be genetically identified as *P. maculata* (MHP 14025). All other populations that we sampled were genetically confirmed to be *Pseudacris fouquettei.* Ten *P. maculata* were collected in 2015 and 2016, respectively. We collected limited data on food habits of *P. maculata* as only a few frogs that were necropsied contained food in their stomachs. However, 3 of 20 frogs had a single food item each: terrestrial isopod, gastropod (Hydrobidae), and Hirudinae (only contained half of the mid body). Most breeding activity that we observed occurred during February and March at this site with calling choruses and both males and females present. Three female *P. maculata* collected were gravid and had the following clutch sizes: SVL 28 mm- 480 eggs; SVL 29 mm- 185 eggs; SVL 30 mm-371 eggs. Three species of

endoparasites were found in *Pseudacris maculata*: *Opalina* sp., *Myxidium melleni,* and *Langeronia microcirra* (HWML 98399). *Opalina* and *Myxidium* were collected in 2015 with 2 of 10 frogs infected with *Opalina* and 4 of 10 frogs infected with *Myxidium. Langeronia* were collected in 2016 with 6 of 10 frogs infected with an average of 2.5 trematodes per host (range 1—5).

Figure 1. Typical breeding habitat of *Pseudacris maculata*.

This study is the first to report a genetically confirmed population of *Pseudacris maculata* in Arkansas. The breeding season we observed in Arkansas is similar as previously reported elsewhere (Dodd 2013). Our egg count range of 185—371 falls within the reported range of 137—793 (Pettus and Angleton 1967). Our limited data over food habits do not elucidate much regarding diet. However, chorus frogs eat mainly small invertebrates (Dodd 2013) as we found in our study.

Opalina sp. and *Myxidium* have been reported from every hylid host that inhabits Arkansas (Muzzall and Sonntag 2012; McAllister *et al*. 2013), including the newly documented *Hyla squirella* from Arkansas (Connior *et al*. 2014). Both of these parasites are ubiquitous in amphibians. The trematode *L. microcirra* is a new host record and distributional record for the state. Although we were only able to confirm one population of *P. maculata,* we suspect further systematic distributional surveys will produce additional breeding populations within the extreme northwestern portion of Arkansas. In fact, during March 2020, some small populations of chorus frogs were heard in the vicinity of the known locale but were not collected or analyzed for species identification.

Acknowledgments

We thank E. M. Lemmon and lab staff (FSU) for the DNA analysis, C. T. McAllister and C.R. Bursey provided parasite identifications, and S. Chordas III provided food item identification. We thank P. Pilitt (USNPC), R. Tumlison (HSU), and S. E. Trauth (ASUMZ) for curatorial assistance. The Arkansas Game and Fish Commission provided Scientific Collecting Permits to MBC and KR.

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New County Records of Three *Baptisia* Species in Arkansas, with an Updated Distribution Map

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Running title: New Arkansas *Baptisia* records

The genus *Baptisia* (Fabaceae), known by the common names "wild indigo" and "false indigo," is found throughout the eastern United States (USDA, NRCS 2020). Five species of the genus are native to Arkansas: *B. alba* var. *macrophylla*, *B. australis*, *B. bracteacta*, *B. nuttalliana*, and *B. sphaerocarpa*, with occasional hybridization events between *B. sphaerocarpa* and *B. bracteata* (Kartesz 2015). These species are normally found in habitats with high rates of natural disturbance, and historically, these habitats have been prairies, barrens, and open-understory forests (Kartesz 2015; USDA, NRCS 2020). Today, *Baptisia* species are also found in areas of high human disturbance, including old-fields and roadsides.

Baptisia species serve essential roles in their ecosystems. They have deep root systems and rapidly recover from disturbance. *Baptisia* species are often among the first spring forbs to emerge and serve as nectar sources and host plants for butterflies. For example, the Texas frosted elfin (*Callophrys irus hadros*) is a presumably rare butterfly that is dependent on *Baptisia* as a host for their larvae (Albanese *et al*. 2007; Peterson *et al.* 2010). The Texas frosted elfin—a subspecies of the frosted elfin (*Callophrys irus*) complex—was listed as a species of greatest conservation need in the 2015 Arkansas Wildlife Action Plan (Fowler 2015). Additionally, two more Arkansas butterfly species rely on *Baptisia* spp. as larval hosts—the wild indigo duskywing (*Erynnis baptisiae*) and the hoary edge (*Achalarus lyciades*)—and the plants serve as important nectar sources for many other species (Opler and Wright 1999; Covell 2005; Powell and Opler 2009; Gobeil and Gobeil 2016). With the conversion of Arkansas prairie and barren habitats in the last century, as well as extensive fire suppression, the range of Arkansas *Baptisia* species has probably been reduced (Stephens *et al*. 2008).

In March and April of 2018, we located six new Arkansas county records of *Baptisia* species while surveying for the Texas frosted elfin. Plants were found predominantly during driving surveys of roads or in walking surveys of open habitats with regular disturbance. Specimens were identified as *Baptisia* spp. by the distinct trifoliate leaves, asparagus-like immature raceme, and the pale green coloration. Species were identified by flower color—*B. alba*, white; *B. nuttalliana*, *B. bracteata*, and *B. sphaerocarpa*, yellow; *B. australis*, blue (Larisey 1940). Inflorescence type was used to distinguish species with the same flower color. *Baptisia sphaerocarpa* and *B. bracteata* inflorescences have numerous flowers borne in long (<15cm) racemes, whereas flowers of *B. nuttalliana* are solitary, occasionally in short (>5cm) racemes. *Baptisia sphaerocarpa* displays vertical racemes while *B. bracteata* displays horizontal racemes (Larisey 1940). Current distribution records were utilized from the 2013 *Atlas of Vascular Plants of Arkansas* (Gentry *et al.* 2013) as well as records from the Biota of North America Program (Kartesz 2015). New county records were located for three species: *B. alba*, *B. nuttalliana*, and *B. sphaerocarpa* (Fig. 1).

For *B. alba*, these new records in conjunction with the previously known distribution suggest that the species has existed unobserved in Polk and Perry Counties, and our finding has filled in the research gaps. The same can be said for our discovery of *B. nuttalliana* in Hot Spring County. The discovery of *B. sphaerocarpa* in Pike, Howard, and Clark Counties, however, indicates a notable southward range extension for the species in Arkansas.

All of our records were found along roadsides, railroads, and utility rights-of-way, although some plants within stands penetrated the edges of nearby forest areas. These observations suggest that landscapes managed for anthropogenic purposes are functioning as simulated prairie for these plants and

Fig. 1. Updated distribution of three *Baptisia* species across the counties of Arkansas. Previously known occurrences are shown in dark gray (Kartesz 2015), and new county records are shown with black dots in striped counties.

possibly allowing for their expansion across the state. Additionally, our specimens were found near areas suspected to have large pre-settlement areas of prairie, suggesting that these plants have succeeded because of pre-adaptation to the high levels of disturbance of areas managed for human use. Though remaining prairie areas are reduced and connectivity is low in Arkansas, roads are probably serving as dispersal corridors for *Baptisia* species.

One voucher specimen was collected for each population. These specimens are deposited in the Hendrix College Herbarium (HXC), listed by specimen accession numbers, and are available to interested researchers.

New records of distribution

Baptisia alba (L.) Vent. var. *macrophylla* (Larisey) Isley

Perry County: 4 km west of Casa on Hwy 10. Stand of 40 individuals growing alongside railroad track located on 29 April 2018. N 35.0257° W 93.0879°, elevation 125 m. Accession Number: HXC006052.

Polk County: 1.5 km east of Rich Mountain on Hwy 59. Stand of 8 individuals growing alongside Hwy 59 located on 22 May 2018. N 34.6853° W 94.3235°, elevation 460 m. Accession Number: HXC006054.

Baptisia sphaerocarpa Nutt.

Pike County: 4.9 km east of Newhope on Hwy 70. Stand of 5 individuals growing alongside Hwy 70 located on 21 April 2018. N 34.2426° W 93.8305°, elevation 170 m. Accession Number: HXC006055.

Howard County: 5.0 km west of Newhope on Hwy 70. Stand of approximately 1,000 individuals growing alongside Hwy 70 found on 15 April 2018. N 34.2127° W 93.9329°, elevation 180 m. Accession Number: HXC006050.

Clark County: 3.4 km north of Gurdon on Hwy 67. Stand of approximately 50 individuals growing with *B. nuttalliana* alongside Hwy 67. N 33.9480° W 93.1449°, elevation 61 m. Accession Number: HXC006053.

Baptisia nuttalliana Small

Hot Spring County: 19.8 km east of Amity on Amity Rd. Stand of approximately 100 individuals found alongside Amity Rd on 9 May 2018. N 34.3634° W 93.3316°, elevation 140 m. Accession Number: HXC006051. Note, the site was disturbed by heavy machinery in 2019, but plants appear to be re-sprouting.

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ARKANSAS ACADEMY OF SCIENCE

Secretary's Report

ARKANSAS ACADEMY OF SCIENCE
SUMMER 2020 EXECUTIVE COMMITTEE MEETING MINUTES
June 2 – 2:00p.m.
Carried out via Zoom Video®

This meeting was in place of the business meeting that would have been held at the 2020 meeting that was canceled due to the COVID-19 pandemic. President Stephen Addison organized and presided. The meeting was called to order at 2:00pm.

Those participating included Stephen Addison, Jack Jackson, Collis Geren, John G. Chamberlin, Monsour Mortiazavi, Sharon Hamilton, Todd Tinsley, Andy Sustich, Ivan Still, Panneer Selvam, Abdel Bachri, Ebo Tei, Joseph Onyilagha

1. **Matters arising from the cancellation of the 2020 meeting**

a) Current officers. It has been suggested that the current officers remain in position until with the normal progression resuming with the 2021 meeting. These officers were approved by vote of the general membership at the 2019 meeting. This suggestion was made by Collis Geren. Panneer Selvam moved the motion and Abdel Bachri provided the second. Approval was unanimous.

b) 2021 Meeting. It has been suggested that since the 2020 meeting was canceled that the 2021 meeting be held at UAFS. This would then mean that the 2022 meeting would be at OBU, and the 2023 meeting would be at UAPB. Abdel provided the motion and Panneer provided the second. Approval was unanimous. Jack provided information that much was already in place. After some discussion April 9 and 10 of 2021 was selected as the date of the meeting so as not to conflict with Easter.

2. **Secretary's Report: Colis Geren**

Collis presented a review of the minutes of the Fall 2019 executive committee meeting. Collis also provided a current membership list for the participants in the Zoom Video® meeting

3. **Treasurer's Report: Andy Sustich**

Since Collis included the treasurer's report in the minutes, it was not necessary to repeat as changes have been minor. The end of 2020 AAS financial statement is presented at the end of these minutes.

The Treasurer's report was approved unanimously.

4. **Historian's Report: Abdel Bachri**

No report due to COVID 19 cancelling the meeting

5. **Journal of the Arkansas Academy of Science Editor-In-Chief Report: Ivan Still**

a) Journal (JAAS #73) Report:

Twenty eight manuscripts were submitted for consideration of publication in volume 73 (2019) of the JAAS. These manuscripts included 16 Articles, 1 review and 11 General notes, all being submitted by the electronic manuscript submission process on the Journal website.

By the beginning of May, manuscripts were checked for style, grammar, format, etc., to ensure compliance with the "Instructions to Authors". Abstracts were sent to potential reviewers by mid to late May. Dr. Still handled Physical Science papers, Invertebrate biology and some Vertebrate biology papers, while Doug Barron handled

Ecology/Environmental papers, and our new Associate Editor for Vertebrate Biology, Christina Blanco handled 6 papers for her first year! The majority of manuscripts were sent out electronically for review by the beginning of June.

Authors were informed if their paper was accepted with the need for minor or major revision or whether their paper was rejected in July. Authors were asked to return their revisions to their handling editor via Scholarworks by August 31, with the page charges submitted directly to Andy Sustich. Three manuscripts required major corrections. One General Note was withdrawn by the author/ did not meet the deadline for corrections. Once reviews were returned to handling editors, control of manuscript processing was returned to me.

The Journal was published electronically January 21, 2020. The total number of manuscripts that were published this year is 27 (down from the 36 in Volume 72), consisting of 16 Articles, 1 review and 10 General Notes. We also published a Tribute to Mostafa and an In Memoriam for our departed friend and colleague, Dr. Doug James. Dr. Still thanked the Associate Editors and reviewers for their help in the preparation of volume 73. Volume 73 is 198 pages long (including cover pages).

Due to the cancellation of the meeting, Dr. Still mailed journals to individual members and universities over the subsequent months as COVID closures took effect. The final batch were sent out May 16. Total cost to date was $288.41. The receipts were sent to Andy for reimbursement after this Excom meeting.

Download statistics for the on-line journal were: 5463 full-text downloads in the month of April 2020, 51,781 in the past year, and 116,355 total downloads in the three years being on Scholarworks from a total of 2,338 manuscripts in the journal. A member inquired about the Journal being added to the journal list on ResearchGate, however ResearchGate is currently not adding journals while they work on improving their link features.

Issues that arose post publication of V73:

Two issues arose after the hard copy journal had been published. Processed manuscripts that are ready for publishing are uploaded at the end of November, with authors automatically being notified by the system.

When the Journal was published on-line at the end of January, one author emailed to say that an earlier version had been published, not their revised one. This was due to the authors not following instructions and uploading a wrongly formatted copy of their manuscript in the wrong file format, and then, failing to note the notifications as the manuscript moved to publication. The online version has been corrected with a note regarding the discrepancy with the hard copy.

In a separate issue, the scientific integrity of data in a published manuscript was questioned by a coauthor of the manuscript (in April). The Editorial Board investigated the issue and the corresponding author finally agreed (5/21/2020) to the retraction of the manuscript (performed 5/26/2020).

b) Volume 74: Current Progress

Although the annual meeting was cancelled due to COVID-19, we have accepted submissions for volume 74 (2020). Sixteen manuscripts were submitted prior to the original date of the annual meeting, and are currently under review. If these manuscripts are accepted, this will constitute 75 pages of a much reduced volume!

The reduction in the size of the volume led to other points of discussion:

i. Other elements to be added in: Minutes from this meeting and membership?

ii. Hard copy or e-copy only?

After discussion, these points were resolved as evidenced in this publication.

c) Pursuant to recruitment of Associate Editors, Managing Editor and future Editor-in-Chief

Dr. Still sent to Dr. Selvam updated info for the Journal that was placed in the newsletter. The need for an Associate Editor for Invertebrate Biology was highlighted. Dr. Amber Harrington is willing to join the Editorial Board as Associate editor for Physical science (effectively taking over Mostafa's role for Physical Science manuscripts). In addition, the job descriptions for the Managing Editor and Editor-in-Chief were included in the Newsletter (unfortunately it transpired that Panneer had not sent the Newsletter to Dr. Alroobi for upload to the Academy website). The duties are appended to this report, and are found after the list of Editorial Board members in this volume. Dr. Still had attempted to recruit at his College meeting at ATU in Fall, however although one person responded, that faculty member subsequently backed out.

Points of discussion:

Executive committee members and Academy representatives need to canvas their university departments for volunteers to the posts.

d) Review of comparable Journals from sister State Academies with regard to page costs.

At the request of the President, Dr. Still initiated this review, but due to pressures of the COVID outbreak, Dr. Still was only able to survey 19 state academies and comparable journals that some of our members publish in/reference in their manuscripts). Naturally, the size of the membership will also play into the finances too. This complete survey was shared with the Executive committee and is available upon request.

Our cost of $50 per page with open access exposure at no additional cost, with only one author needing to be a member (frequently that member is a life member), an annual membership of $30, and given the size of the academy, remain reasonably competitive to our neighboring state academies (Tennessee, Oklahoma, Texas), especially given the number of published articles in the Journal. Academies that have lower page charges frequently do not publish many articles and/or are sporadic in publication. There is an increasing trend to move to on-line only journals (thereby decreasing costs), and either an opt-in for a hard copy, or increased membership charge for including a hard copy journal.

Conclusions and Options

Cost per page of printing the hardcopy Journal is dependent on the size of the published journal; however, we have maintained a cost of about $15 per copy from 2008 onwards, when Dr. Still took over the Managing Editor and essentially a Production Editor role. Cost per page has decreased recently to about $12. It should be noted that the low cost of producing the Journal is in part because there are no Editorial costs charged on top of that, because the University of Arkansas does not currently charge for maintenance of the Journal website, and Dr. Still does not charge for digital processing of the manuscripts for both electronic and hard copy publishing. A few years ago, a life member approached Dr. Still offering editorial help, but he basically retracted the offer when Dr. Still indicated that these are volunteer activities. The small survey of national (non-state) societies that faculty publish in/reference generally indicate that those societies have higher publication costs and/or membership costs presumably to cover the dedicated personnel and resources to run their journal and society as a whole. However, it does seem reasonable to propose reducing the current page charge for the JAAS, similar to that of Oklahoma ($35 per page) and maintain a hard copy for now. The reduced page charges would cut finances into the Academy by approximately $2,300 (based on vol. 73), unless that loss of revenue promotes the submission of more articles. Another point to consider is that, depending on Dr. Still's replacement, the Academy might need to consider increased costs if a third party is brought in to edit submitted manuscripts and the Academy meeting report and program. Moving to an on-line only publication may streamline issues (post publication issues are a lot easier to resolve), further reduce costs (financial, and we should consider environmental too) and even make recruiting Managing Editor and Editor-in-Chief positions easier.

Points of discussion:

i. Do we reduce page costs, and if so to what level?

ii. Do we move to an on-line only publication/opt in hardcopy option, but there is the issue of the statement in membership "AAS members receive one copy of the JAAS with their membership and institutional members receive two copies."

iii. Timeline for any changes?

After discussion (during which Dr. Still's internet connection decided to go down!), it was decided that the status quo would be maintained for now, and these discussions would be tabled.

The Journal Editor-in-Chief's Report was approved unanimously. Ivan subsequently provided requested descriptions of the Managing Editor and Editor-in-Chief position directly to the Executive committee again, so that they could directly circulate the duties to their respective institution:

Duties of the Editor-in-Chief (Constitutional)

The Editor-in-Chief is an elected member of the Executive Committee of the Arkansas Academy of Science and is responsible for oversight of the publishing the Journal of the Arkansas Academy of Science. The Editor-in-Chief works closely with the Managing Editor and members of the

JOURNAL Editorial Board (the Academy "Publication Committee") in all aspects of the publication process. The peer-reviewed JOURNAL has evolved over the past decade, and is now globally available through the JOURNAL website (https://scholarworks.uark.edu/jaas/). The JOURNAL is thus published on-line and also as a hard copy JOURNAL that is distributed to Academy members, and member Institutions.

Specific duties:

1. Receives manuscripts submitted for publication and cooperates with Managing Editor and Associate Editors in the review, revision and acceptance process
2. Liaises with the Treasurer of the Academy of the Arkansas Academy of Science with regard to the financial management of the JOURNAL, and prepares reports for the Executive Committee and the Annual General Business meeting regarding the status of the JOURNAL.
3. Prepares the next issue of the JOURNAL by assembling the final copies of manuscripts accepted for publication
4. Works with the printer in the technical preparation of the Journal.
5. Arranges for the distribution of copies of the Journal at the next annual meeting, and mailing of copies to Academy members and Institutional Members.

Duties of the Managing Editor

1. **Publication of the Journal of the Arkansas Academy of Science**

The Managing Editor acts as the primary contact person during manuscript submission in March/April each year. All manuscripts are submitted a minimum of 2 days prior to the annual meeting electronically via: https://scholarworks.uark.edu/jaas/, the JOURNAL website. The Managing Editor ensures that manuscripts and their authors are in compliance with the policies and instructions to authors as laid out on the JOURNAL website and cooperates with the Editor-in-Chief to perform initial Editorial review. The Managing Editor assigns manuscripts to appropriate Associate Editors who subsequently submit manuscripts to referees for critical review for scientific content, originality and clarity of presentation. This process is handled via the JOURNAL server. Associate Editors are assigned based on their areas of expertise. The Editor-in-Chief, Managing Editor and Associate editors, (the Publication Committee) cooperate in the acceptance, rejection or revision of all manuscripts.

Author-revised manuscripts will be the manuscripts that will be entered into the final on-line and hard copy JOURNAL. However, manuscripts frequently require finishing touches to formatting to maintain the quality of the JOURNAL. Thus, the Managing Editor subsequently ensures that accepted revised manuscripts meets publication standards for the JOURNAL on-line and in the hard copy. The Managing Editor also collects the Secretary's and Treasurer's reports, the annual meeting report and assembles the meeting reports with the final copies of manuscripts into the completed JOURNAL for publication. The on-line system allows a relatively simple way of assembling the on-line JOURNAL. The Institutional Repository Coordinator at University of Arkansas (currently Cedar Middleton) can aid with this final assembly.

2. **Liaison with associated organizations**.

The Journal of the Arkansas Academy of Science is an Open Access Journal. The University of Arkansas Libraries have partnered with the Academy to archive and make volumes of the JOURNAL and Proceedings freely available worldwide online at https://scholarworks.uark.edu/jaas/. This repository is indexed in the Directory of Open Access Repositories. The Managing Editor is the contact person for the Directory of Open Access Journals and the International directory, SHERPA, and handles any issues with maintaining status within these directories.

The exact disposition of these duties may be discussed between the Editor-in-Chief and the Managing Editor.

7. <u>Webmaster: Rami Alroobi</u>

Although Rami could not attend, he supplied the following electronically.

Website: The AAS page is functional. Page Visits: 10516.

Recent changes: In the past few weeks some changes have been applied to the Journal part upon the request of Dr. Ivan Still.

Issues: Our account with the hosting company,

i.e. register.com, has some problem when accessing the billing & payment information and the account holder information. I am working with them on that. I will update you about it in the future if needed.

Twitter: The blue bird account of the academy is functional although it is not seeing a lot of activity (Following: 71, Followers: 20). To keep it alive I sometimes post scientific articles/news on there. If you wish you can send me content that you think can be posted there and can add more life to the AAS twitter.

https://urldefense.proofpoint.com/v2/url?u=https-3A__twitter.com_AcademyArkansas-3Flang-3Den&d=DwIF-g&c=7ypwAowFJ8v-mw8AB-SdSueVQgSDL4HiiSaLK01W8HA&r=oh8DYTzxEO6ZbntCiHS-8g&m=2S5jroqEPtZszICIYz2RHS6pZiPMBnxgrzyz8fWPhS4&s=v2xanzJZHDnshdA0a2ij2l9TrK5IdVfX1LatP6qGx4c&e=

8. Newsletter: Panneer Selvam

Panneer did not have anything specific to report: the completed Spring 2020 newsletter was circulated by email at the appropriate time, including comments received from other committee members. In discussion of the JAAS editor positions, it was revealed that the newsletter was not sent to Rami to post on the Academy website. Panneer will rewrite the newsletter and get it out by November 2020 for review to the Board.

9. Committee Reports:

Nominations Committee

Nominating Committee: has not met – Mostafa was previously the chair. Steve will assume the chair. As noted earlier, current officers will remain in position until with the normal progression resuming with the 2021 meeting. These officers were approved by vote of the general membership at the 2019 meeting

Undergraduate Research Awards: Stephen Addison

Four applications were received:

a. Chloe Cline (Henderson State), Mentor: Martin Campbell, Synthesize and measure the anti-malarial activity of an organic compound. Requests $1,000.

b. Brandon Fagen (Harding), Mentor: David Donley, Determine the impact of bacterial metabolites on microglial activation and response to beta-amyloid. Requests $1,000

c. Willow Newman (UCA), Mentor Ginny Adams, Determine which minnow species living in the headwater streams of the King's river are most likely to survive increased intermittent conditions based on their respective thermal tolerances. Requests $896.64.

d. Grace Davenport (UCA), Mentor: Ginny Adams, Persistence and stability of Fish assemblages in Ozark streams in the White river drainage of Arkansas, specifically Janes, piney, Big, and Sylamore creeks. Requests $906.

Steve moved we approve all four with Todd as the second. Approval was unanimous.

Outreach Committee Report- Edmund Wilson Chair

Committee members: Stephen Cooper, Biology, Harding University; Gija Geme, Chemistry, Southern Arkansas University; Antoinette Odendaal, Biology, Chemistry, Engineering, Southern Arkansas University.

We have committed to having a booth at Thunder of the Rock StemFest, October 24-25, 2020 at the Little Rock Air Force Base. Of course, this is uncertain now because of COVID-19, last year there were over 13,000 students. Ed asked for help finding the best things to present on behalf of the Arkansas Academy of Science. We thought perhaps showcasing some Arkansas animals and some neat chemical and biological experiments. We need to be able to minimize the cost and maximize the Wow! Factor. We also need volunteers.

The second area of emphasis is the development of materials for our website to direct learning activities for K-12. We do not have to develop the materials ourselves but rather gather information placed on our website, with comments, for great hands-on learning experiences. Of course, developing such materials is also welcome. Come help us find volunteers for our committee from each AAS college campus!

10. Business Old and New:

i. Fellows: Status - Collis Geren

As the 2019 minutes show, Panneer and Collis were to contact Jim Coleman as to willingness to be nominated while Steve was going to contact Carolina Cruz Niera. Since Coleman has taken a

job elsewhere, his nomination is moot. Steve still needs to contact Niera.

Andy has provided Collis with a complete nomination package for Stan Trauth while Ivan and Collis have a package for Mostafa. Todd will need to provide Colis with a nomination letter for Joyce Harden, a seconding letter, a CV for Joyce, and a concise list of her contributions to the Academy.

ii. Membership dues: Andy and Collis need the information on dues paid for the cancelled April meeting. Specifically Andy needs the dollar amounts while Collis needs the names with the type of membership purchased.

11. Meeting was adjourned

Subsequent to the meeting Collis made a motion by E-mail for the Executive Committee to approve rolling 2020 dues over to 2021 which was seconded by Steve. All responding members voted to approve.

Minutes prepared by Secretary Collis Geren, August 7 2020.

Treasurer's Report
ARKANSAS ACADEMY OF SCIENCE
2020 FINANCIAL STATEMENT
December 9, 2020

Balance – December 9, 2020	**$164,264.61**
Balance – December 7, 2019	**$158,834.34**
Net Gain	**$5,430.27**

DISTRIBUTION OF FUNDS

Checking Account Dec. 9, 2020 Arvest Bank	$15,544.77
PayPal Account: Available funds on Dec. 9, 2020	$457.17
Certificate of Deposit Dec. 9, 2020 Includes Phoebe and George Harp Endowment Arvest Bank	$53,450.82
Certificate of Deposit Dec. 9, 2020 Arvest Bank	$53,450.82
Certificate of Deposit Dec. 9, 2020 Arvest Bank	$41,361.03
TOTAL	**$164,264.61**

INCOME

1. INTEREST (Interest Earned Year to Date December 9, 2020)	
a. Checking Account, Arvest Bank	$0
b. CD1 (Arvest Bank)	$664.75
c. CD2 (Arvest Bank)	$664.75
d. CD3 (Arvest Bank)	$514.39
All interest was added to the CDs	**$1,843.89**
2. JOURNAL	
a. Page Charges	$3,700.00
b. Subscriptions, University of Arkansas	$0
Total	**$3,700.00**
3. MEMBERSHIP	
a. Individual/Associate (reimbursements for double payments)	$465.00
b. Individual collected at the meeting	$1,710.00
c. Institutional	$1,100.00
Total	**$3,275.00**
4. MEETING INCOME	**$0**
5. MISCELLANEOUS INCOME	
a. Unspent/returned UG awards	$39.78
b. Gifts: PayPal Charitable Giving Fund	$5.70
Total	**$45.48**
TOTAL INCOME	**$8,864.37**

EXPENSES

1. STUDENT AWARDS	$0
2. AWARDS (Organizations)	
a. Arkansas State Science Fair	$0
b. Arkansas Junior Academy of Science	$0
c. Arkansas Junior Science and Humanities Sym.	$0
Total	**$0**
3. UNDERGRADUATE RESEARCH AWARDS	
a. None this year	$0
Total	**$0**

4. JOURNAL

a. Volume 73 Printing Cost	$2,559.87
b. Journal Mailing Cost	$288.41
Total	**$2,848.28**

5. MISCELLANOUS EXPENSES

a. Reimburse Collis for Plaques	$175.60
b. Reimburse Rami for Website registration	$163.49
c. Reimburse Andy for Quicken subscription	$49.99
d. Dues: National Assoc. of Academies of Science	$175.00
e. PayPal fees	$21.74
Total	**$585.82**

6. MEETING EXPENSES **$0**

TOTAL EXPENSES **$3,434.10**

ARKANSAS ACADEMY OF SCIENCE
COST OF JOURNAL

VOLUME	COPIES	PAGES	PRINTER CHARGE	TOT. VOL. COST	COST/ COPY	COST/ PAGE
41 (1987)	450	116	$7,122.79	$7,811.25	$17.36	$67.34
43 (1989)	450*	119	$8,057.24	$8,557.24	$19.02	$71.91
44 (1990)	450*	136	$9,298.64	$9,798.64	$21.77	$72.05
45 (1991)	450*	136	$9,397.07	$9,929.32	$22.06	$73.01
46 (1992)	450*	116	$9,478.56	$10,000.56	$22.22	$86.21
47 (1993)	400	160	$12,161.26	$12,861.26	$32.15	$80.38
48 (1994)	450	270	$17,562.46	$18,262.46	$40.58	$67.63
49 (1995)	390	199	$14,725.40	$15,425.40	$39.55	$77.51
50 (1996)	345	158	$11,950.00	$12,640.75	$36.64	$80.00
51 (1997)	350	214	$14,308.01	$15,008.01	$42.88	$70.13
52 (1998)	350	144	$12,490.59	$13,190.59	$37.69	$91.60
53 (1999)	350	160	$13,686.39	$14,386.39	$41.10	$89.91
54 (2000)	350	160	$14,149.07	$14,849.07	$42.43	$92.81
55 (2001)	360	195	$16,677.22	$17,498.22	$48.61	$89.73
56 (2002)	350	257	$18,201.93	$19,001.93	$54.29	$73.94
57 (2003)	230	229	$14,415.12	$15,715.12	$68.33	$68.62
58 (2004)	210	144	$7,875.76	$9,175.76	$43.99	$63.72
59 (2005)	215	226	$16,239.04	$17,835.84	$82.96	$78.92
60 (2006)	220	204	$11,348.06	$12,934.30	$58.79	$63.40
61 (2007)	195	150	$8,196.84	$9,914.69	$50.84	$66.10
62 (2008)	220	166	$2,865.00	$2,967.49	$13.49	$17.88
63 (2009)	213	206	$3,144.08	$3,144.08	$14.76	$15.26
64 (2010)	232	158	$2,713.54	$2,764.30	$11.91	$17.50
65 (2011)	200	194	$2915.12	$2,963.03	$14.82	$15.27
66 (2012)	200	216	$3,087.91	$3,180.29	$15.90	$14.72
67 (2013)	200	238	$3,311.42	$3,396.32	$16.98	$14.27
68 (2014)	180	192	$2,812.75	$2,944.08	$16.36	$15.33
69 (2015)	180	170	$2,622.87	$2,622.87	$14.57	$15.43
70 (2016)	180	307	$3,179.53	$3,320.76	$18.45	$10.82
71 (2017)	180	262	$2,839.45	$2,839.45	$15.77	$10.83
72 (2018)	180	229	$2,681.40	$2,779.35	$15.44	$12.14
73 (2019)	180	166	$2,559.87	$2848.28	$15.56	$17.16

The Total Volume Cost equals the printer's charge plus the other miscellaneous charges (e.g. Mailing Costs).

- On Volume 43 the Academy received 523 copies, but the printer did not charge us for the extra 73 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 44 the Academy received 535 copies, but the printer did not charge us for the extra 85 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 45 the Academy received 594 copies, but the printer did not charge us for the extra 144 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 46 the cost was greater than usual due to the high cost of a second reprinting of 54 copies by a different printer.

2020 ARKANSAS ACADEMY OF SCIENCE MEMBERSHIP

LIFE MEMBERS

FIRST	LAST NAME	INSTITUTION
Steven	Addison	University of Central Arkansas
Edmond J.	Bacon	University of Arkansas-Monticello (ret.)
Vernon	Bates	Ouachita Mountains
Floyd	Beckford	University of Virginia's College at Wise
Don	Bragg	USDA Forest Service
Calvin	Cotton	Geographics Silk Screening Co.
Betty	Crump	Ouachita National Forest
James	Daly	UAMS (retired)
Leo	Davis	Southern Arkansas University (ret.)
Mark	Draganjac	Arkansas State University
Jim	Edson	University of Arkansas-Monticello
Kim	Fifer	UAMS
Collis	Geren	University of Arkansas-Fayetteville
John	Giese	Ark. Dept. of Env. Qual. (ret.)
Walter	Godwin	University of Arkansas-Monticello (ret.)
Anthony	Grafton	Lyon College
Joe M.	Guenter	University of Arkansas-Monticello
Joyce	Hardin	Hendrix College
George	Harp	Arkansas State University (ret.)
Phoebe	Harp	Arkansas State University (ret.)
Gary	Heidt	University of Arkansas-Little Rock
Mostafa	Hemmati	Arkansas Tech University
Shahidul	Islam	University of Arkansas-Pine Bluff
Cynthia	Jacobs	Arkansas Tech University
Art	Johnson	Hendrix College
Cindy	Kane	UAMS
Scott	Kirkconnell	Arkansas Tech University (retired)
Roger	Koeppe	University of Arkansas
Christopher	Liner	University of Arkansas
Roland	McDaniel	FTN Associates
Grover P.	Miller	UAMS
Mansour	Mortazavi	University of Arkansas-Pine Bluff
James	Peck	University of Arkansas-Little Rock
Kannan	Ragupathy	University of Arkansas-Fort Smith
Michael	Rapp	University of Central Arkansas
Dennis	Richardson	Quinnipiac College
Jeff	Robertson	Arkansas Tech University
Henry	Robison	Southern Arkansas University (retired)
Benjamin	Rowley	University of Central Arkansas
David	Saugey	U.S. Forest Service (retired)
Panneer	Selvam	University of Arkansas-Fayetteville
Ivan	Still	Arkansas Tech University (retired)
Suresh	Thallapuranam	University of Arkansas-Fayetteville
Stanley	Trauth	Arkansas State University (retired)

LIFE MEMBERS

FIRST	LAST NAME	INSTITUTION
Gary	Tucker	FTN Associates
Renn	Tumlison	Henderson State University
Scott	White	Southern Arkansas University
James	Wickliff	University of Arkansas
Robert	Wiley	University of Arkansas-Monticello
Steve	Zimmer	Arkansas Tech University (retired)

REGULAR MEMBERS

FIRST	LAST NAME	INSTITUTION
Ginny	Adams	University of Central Arkansas
Meredith	Akins	University of Arkansas-Ft. Smith
Chiraz	Amrine	Arkansas Tech University
Souvik	Banerjee	University of Arkansas-Ft. Smith
Doug	Barron	Arkansas Tech University
Sandhya	Baviskar	University of Arkansas-Ft. Smith
Keith	Blount	University of Arkansas-Monticello
David	Bowles	US. National Park Service
Tom	Buchanan	University of Arkansas-Ft. Smith
Joshua	Burns	University of Arkansas-Ft. Smith
Martin	Campbell	Henderson University
John	Chamberlin	Chamberlin Research, Little Rock, AR
Puskar	Chapagain	Southern Arkansas University-Magnolia
Stephen	Chordas III	Ohio State University
Rajib	Choudhury	Arkansas Tech University
Shannon	Clardy	Henderson State University
Kim	Cloud	University of Arkansas-Ft. Smith
Matthew	Connior	Northwest Arkansas Community College
Steven	Cooper	Harding University
David	Donley	Harding University
Phillip	Dukes	Southern Arkansas University-Magnolia
Eric	Fuselier	Crafton-Tull
Kristie	Garner	University of Arkansas-Ft. Smith
Abby	Geis	Arkansas College of Osteopathic Medicine
Gary	Graves	Smithsonian Institute
Gaumani	Gyanwali	Rich Mountain Community College
Mohammad	Halim	University of Arkansas-Ft. Smith
Sharon	Hamilton	Ouachita Baptist University
Lionell	Hewavitharana	Southern Arkansas University-Magnolia
Stacey	Hickson	Southern Arkansas University-Magnolia
Anthony	Holt	University of Arkansas C. C. at Morrilton
Jack	Jackson	University of Arkansas-Fort Smith
Kailash	Jajam	University of Arkansas-Little Rock

Arkansas Academy of Science Business Meeting Report

REGULAR MEMBERS

FIRST	LAST NAME	INSTITUTION
David	Jamieson	Crowder College
Qinglong	Jiang	University of Arkansas-Pine Bluff
Thurmond	Jordan	Audobon Society
Dave	Mayo	University of Arkansas-Fort Smith
Chris	McAllister	Eastern Oklahoma State College-Idabel
Gerhard	Mensch	Mensch Wold Lab
Matthew	Moran	Hendrix University
Rebecca	Mroczek	University of Arkansas-Fort Smith
Kristina	Nath	Rich Mountain Community College
Henry	North	Harding University
Antoinette	Odendaal	Southern Arkansas University-Magnolia
Joseph	Onyilagha	University of Arkansas-Pine Bluff
Rajvardhan	Patil	Arkansas Tech University
Mike	Plummer	Harding University
Christin	Pruett	O Ouachita Baptist University
Brett	Servis	Henderson State University
Jeffrey	Shaver	University of Arkansas-Ft. Smith
Mikel	Shinn	AR Dept Environmental Quality
Hamed	Shojaeo	Arkansas Tech University
Twanda	Simmons	Arkansas State University-Beebe
Amy	Skypala	University of Arkansas-Ft. Smith
Ryan	Stork	Harding University
Andy	Sustich	Arkansas State University-Jonesboro
Ebo	Tei	University of Arkansas-Pine Bluff
Todd	Tinsley	Hendrix College
Susanne	Wache	Southern Arkansas Community College
Daoyuan	Wang	University of Arkansas-Pine Bluff
Grady	Weston	Harding University
Matthew	White	Arkansas College of Osteopathic Medicine
Jessica	Young	Arkansas Tech University
Matthew	Young	Arkansas Tech University
Zahra	Zamanipour	Henderson State University

STUDENT MEMBERS

FIRST	LAST NAME	INSTITUTION
Parker	Fane	Harding University
Samantha	Gibson	University of Arkansas-Pine Bluff
Carlin	Hill	Arkansas College of Osteopathic Medicine
Grace	Hoss	Arkansas College of Osteopathic Medicine
Olivia	Loudermilk	Harding University
Kate	Main	Arkansas College of Osteopathic Medicine
Brooke	Nelson	Arkansas College of Osteopathic Medicine
Brandon	Parker	Mensch Wold Lab
Zachary	Pierce	Arkansas College of Osteopathic Medicine
Audrey	Thomas	University of Arkansas-Ft. Smith
Kyla	Wilson	John Brown University

SPONSORING/SUSTAINING MEMBERS

FIRST	LAST NAME	INSTITUTION
Abdel	Bachri	Southern Arkansas University-Magnolia
Shannon	Clardy	Henderson State University
Eugene	Jones	Connect4Business
Edmond	Wilson	Harding University

Journal Acknowledgements and Editorial Board

The Arkansas Academy of Science gratefully acknowledges the Editorial board for volume 74 of the Journal during 2020.

Editorial Board for 2020

The editorial staff also extends our heartfelt appreciation for the expertise, assistance and valuable time provided by our colleagues who act as reviewers for the Journal. Our expert reviewers are recruited from within Arkansas, North America, Europe, South America, Australia and Asia. Only through the diligent efforts of all those involved that gave freely of their time, can we continue to produce a high quality scientific publication.

Recruitment to Editorial Positions: Duties of the Editor-in-Chief and Managing Editor

We are looking for members who would like to become the Editor-in-Chief and Managing Editor when Dr. Still retires after publication of Volume 75 at the end of 2021. If you are interested in either of these positions, please contact Dr. Still (jarksci@gmail.com) and Dr. Addison (saddison@uca.com) by email and provide your contact information. The duties for these positions are provided below.

Duties of the Editor-in- Chief

The Editor-in-Chief is an elected member of the Executive Committee of the Arkansas Academy of Science and is responsible for oversight of the publishing the Journal of the Arkansas Academy of Science. The Editor-in-Chief works closely with the Managing Editor and members of the *JOURNAL* Editorial Board (the Academy "Publication Committee") in all aspects of the publication process. The peer-reviewed *JOURNAL* has evolved over the past decade, and is now globally available through the *JOURNAL* website (https://scholarworks.uark.edu/jaas/). The *JOURNAL* is thus published on-line and also as a hard copy *JOURNAL* that is distributed to Academy members, and member Institutions.

Specific duties:

1. Receives manuscripts submitted for publication and cooperates with Managing Editor and Associate Editors in the review, revision and acceptance process
2. Liaises with the Treasurer of the Academy of the Arkansas Academy of Science with regard to the financial management of the *JOURNAL,* and prepares reports for the Executive Committee and the Annual General Business meeting regarding the status of the *JOURNAL*.
3. Prepares the next issue of the *JOURNAL* by assembling the final copies of manuscripts accepted for publication
4. Works with the printer in the technical preparation of the Journal.
5. Arranges for the distribution of copies of the Journal at the next annual meeting, and mailing of copies to Academy members and Institutional Members.

Duties of the Managing Editor

Publication of the Journal of the Arkansas Academy of Science

The Managing Editor acts as the primary contact person during manuscript submission in March/April each year. All manuscripts are submitted a minimum of 2 days prior to the annual meeting electronically via: https://scholarworks.uark.edu/jaas/, the *JOURNAL* website. The Managing Editor ensures that manuscripts and their authors are in compliance with the policies and instructions to authors as laid out on the *JOURNAL* website and cooperates with the Editor-in-Chief to perform initial Editorial review. The Managing Editor assigns manuscripts to appropriate Associate Editors who subsequently submit manuscripts to referees for critical review for scientific content, originality and clarity of presentation. This process is handled via the *JOURNAL* server. Associate Editors are assigned based on their areas of expertise. The Editor-in-Chief, Managing Editor and Associate editors, (the Publication Committee) cooperate in the acceptance, rejection or revision of all manuscripts.

Author-revised manuscripts will be the manuscripts that will be entered into the final on-line and hard copy *JOURNAL*. However, manuscripts frequently require finishing touches to formatting to maintain the quality of the *JOURNAL*. Thus, the Managing Editor subsequently ensures that accepted revised manuscripts meet publication standards for the *JOURNAL* on-line and in the hard copy. The Managing Editor also collects the Secretary's and Treasurer's reports, the annual meeting report and assembles the meeting reports with the final copies of manuscripts into the completed *JOURNAL* for publication. The on-line system allows a relatively simple way of assembling the on-line *JOURNAL*. The Institutional Repository Coordinator at University of Arkansas (currently Cedar Middleton) can aid with this final assembly.

The Managing Editor is responsible for the distribution of hard copies of the *JOURNAL* at the next annual meeting, and where necessary prepares journals for mailing to the Institutional members of the Academy and to Members of the Academy who were unable to attend the annual meeting.

Liaison with associated organizations.

The Journal of the Arkansas Academy of Science is an Open Access Journal. The University of Arkansas Libraries have partnered with the Academy to archive and make volumes of the *JOURNAL* and Proceedings freely available worldwide online at https://scholarworks.uark.edu/jaas/. This repository is indexed in the Directory of Open Access Repositories. The Managing Editor is the contact person for the Directory of Open Access Journals and the International directory, SHERPA, and handles any issues with maintaining status within these directories.

Instructions to Authors

The *JOURNAL OF THE ARKANSAS ACADEMY OF SCIENCE* is published annually

A. General Policies

In order for a manuscript to be considered for publication in journal, it is the policy of the Arkansas Academy of Science that:

1) at least one of the authors of a paper submitted for publication in the JOURNAL must be a member of Arkansas Academy of Science,
2) only papers presented at the annual meeting are eligible for publication,
3) manuscript submission is due at the annual meeting.

B. General Requirements

The *JOURNAL OF THE ARKANSAS ACADEMY OF SCIENCE* is published annually. Original manuscripts should be submitted either as a **feature article** or a shorter **general note**. Original manuscripts should contain results of original research, embody sound principles of scientific investigation, and present data in a concise yet clear manner. Submitted manuscripts should not be previously published and not under consideration for publication elsewhere. The *JOURNAL* is willing to consider **review articles**. These should be authoritative descriptions of any subject within the scope of the Academy. Authors of articles and reviews must refrain from inclusion of previous text and figures from previous reviews or manuscripts that may constitute a breach in copyright of the source journal. Reviews should include enough information from more up-to-date references to show advancement of the subject, relative to previously published reviews. During submission, Corresponding authors should identify into which classification their manuscript will fall.

For scientific style and format, the CBE Manual for Authors, Editors, and Publishers Sixth Edition, published by the Style Manual Committee, Council of Biology Editors, is a convenient and widely consulted guide for scientific writers and will be the authority for most style, format, and grammar decisions. Special attention should be given to grammar, consistency in tense, unambiguous reference of pronouns, and logically placed modifiers. To avoid potential rejection during editorial review, all prospective authors are strongly encouraged to submit their manuscripts to other qualified persons for a friendly review of clarity, brevity, grammar, and typographical errors before submitting the manuscript to the *JOURNAL*. Authors should rigorously check their manuscript to avoid accidental plagiarism, and text recycling. Authors should declare any and all relevant conflicts of interest on their manuscripts.

To expedite review, authors should provide the names and current e-mail address of at least three reviewers within their field, with whom they have not had a collaboration in the past 2 years. The authors may wish to provide a list of potential reviewers to be avoided due to conflicts of interest.

C: Review Procedure

Evaluation of a paper submitted to the *JOURNAL* begins with critical reading by the Managing Editor. The manuscript is then submitted to referees for critical review for scientific content, originality and clarity of presentation. To expedite review, authors should provide, in a cover letter, the names and current e-mail address of at least three reviewers within the appropriate field, with whom they have not had a collaboration in the past two years. Potential reviewers that the authors wish to avoid due to other conflicts of interest can also be provided. Attention to the preceding paragraphs will also facilitate the review process. Reviews will be returned to the author together with a judgement regarding the acceptability of the manuscript for publication in the *JOURNAL*. The authors will be requested to revise the manuscript where necessary. Time limits for submission of the manuscript and publication charges will be finalized in the accompanying letter from the Managing Editor (see "Proposed timetable for manuscript processing"). The authors will then be asked to return the revised manuscript, together with a cover letter detailing their responses to the reviewers' comments and changes made as a result. The corresponding author will be responsible for submitting the total publication cost of the paper to the Treasurer of the Academy, when the revised manuscript is returned to the Editor assigned to your manuscript. Failure to pay the publication charges in a timely manner will prevent processing of the manuscript. If the time limits are not met, the paper will be considered withdrawn by the author. Please

note that this revised manuscript will be the manuscript that will enter into the bound journal. Thus, authors should carefully read for errors and omissions so ensure accurate publication. A page charge will be billed to the author of printed errata; however, no charge is made for errata that are only "printed" in the on-line journal (contact the Editor-in-Chief for more details). All final decisions concerning acceptance or rejection of a manuscript are made by the Managing Editor and/or the Editor-in-Chief.

Please note that all manuscript processing, review and correspondence will be carried out electronically via the *JOURNAL* web site at https://scholarworks.uark.edu/jaas/, and the authors are able to monitor progress on their manuscript as their article is moved to final publication. Thus, authors are requested to add the e-mail addresses of the editors (jarksci@gmail.com) to their accepted senders' list to ensure that they receive all correspondence.

Reprint orders should be placed with the printer, not the Managing Editor. Information will be supplied nearer publication of the *JOURNAL* issue. Authors are able to download a finished electronic copy of their manuscript from the *JOURNAL* website.

D: Policies to Maintain Quality of the Peer Review Process, Academic Honesty and Integrity

The *JOURNAL* adheres to the highest standards of academic honesty and integrity. Authors of articles and reviews must refrain from inclusion of previous text and figures from previous reviews or manuscripts that may constitute a breach in copyright of the source Journal. Authors of reviews should include enough information from more up-to-date references to show advancement of the subject, relative to previously published reviews. Authors should check their manuscript rigorously to avoid accidental plagiarism, and text recycling. Authors should declare any and all relevant conflicts of interest on their manuscripts.

The *JOURNAL* maintains a strict peer review policy with reviewers from relevant fields drawn from around the world to produce a high quality scientific publication. Evaluation of a paper submitted to the *JOURNAL* begins with critical reading by the Managing Editor. The manuscript is then submitted to referees for critical review for scientific content, originality and clarity of presentation. Editors and reviewers are expected to declare all potential conflicts of interest that may affect handling of submitted manuscripts. To expedite review, authors should provide the names and current e-mail address of at least three reviewers within their field, with whom they have not had a collaboration in the past two years. Authors may wish to provide a list of potential reviewers, or editorial staff to be avoided due to conflicts of interest.

Allegations of misconduct will be pursued according to COPE's guidelines (available at http://publicationethics.org/resources/guidelines).

Neither the JOURNAL editorial board, the University of Arkansas nor bepress.com accepts responsibility for the opinions or viewpoints expressed, or for the correctness of facts and figures.

E: Copyright, Licensing and Use Policy

The Journal of the Arkansas Academy of Science is an Open Access Journal. The University of Arkansas Libraries have partnered with the Academy to archive and make volumes of the JOURNAL and Proceedings freely available worldwide online at http://scholarworks.uark.edu/jaas/ repository (indexed in the Directory of Open Access Repositories).

All articles published in the *JOURNAL* are available for use under the following Creative Commons license: Attribution-NoDerivatives 4.0 International (CC BY-ND 4.0) (https://creativecommons.org/licenses/by-nd/4.0/). Thus, users are able read, download, copy, print, distribute, search, or link to the full texts of these articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author. Authors retain copyright over their material published in the *JOURNAL*, however appropriate citation of the original article(s) should be given. Authors may archive a copy of the final version of their articles published in the *JOURNA*L in their institution's repository.

F: Proposed Timetable for Manuscript Processing

It is the policy of the Arkansas Academy of Science that 1) at least one of the authors of a paper submitted for publication in the *JOURNAL* must be a member of Arkansas Academy of Science, 2) only papers presented at the annual meeting are eligible for publication, and 3) manuscript submission is due at the annual meeting. Thus, manuscripts should be submitted to the *JOURNAL* website:

https://scholarworks.uark.edu/jaas/, two days before the meeting. Authors who have submitted manuscripts via the system previously, should use the contact/email and password that was used previously. New authors should follow instructions on the site to establish their profile. Authors can subsequently update their profile with any changes to their contact and account information as necessary

After the meeting all correspondence regarding response to reviews etc. should be directed to the Managing Editor. Publication charges ($50 per page) are payable by check (we are unable to accept PO numbers or credit cards) when the corresponding author returns their response to the reviewers' comments. **Publication charges, made payable to the Arkansas Academy of Science, must be sent to Andrew T. Sustich, Ph.D. Treasurer, Arkansas Academy of Science, PO Box 419, State University, AR 72467-0419.** Please note that the corresponding author will be responsible for the total publication cost of the paper and will submit one check for the entire remittance by the set deadline. If page charges are not received by the deadline, publication of the manuscript will occur in the following year's *JOURNAL* volume (i.e. two years after the meeting at which the data was presented!) The check **must** contain the manuscript number (assigned at time of submission). All manuscript processing, review and correspondence will be carried out electronically. Thus, authors are requested to add the editors' e-mail addresses to their accepted senders' list to ensure that they receive all correspondence.

Timetable

Please note: All manuscripts must be properly formatted PRIOR to submission as a MS Word document.

All manuscripts must be submitted a minimum of 2 days prior to the annual meeting electronically via: https://scholarworks.uark.edu/jaas/, the *JOURNAL* website. The entire review and publication procedure will be handled via the server. Authors who have submitted manuscripts via the system previously, should use the contact/email and password that was used previously. New authors should follow instructions on the site to establish their profile. Authors can subsequently update their profile with any changes to their contact and account information as necessary. Should you have any problems, please contact the Managing Editor (jarksci@gmail.com).

End of April: Initial editorial review. Associate Editors are assigned.

End of May: Manuscripts sent to reviewers.

End of July: All reviews received. Editorial decisions made on reviewed manuscripts. Manuscripts returned to authors for response to reviewers' critiques. For accepted manuscripts, additional details and due dates for manuscript return will be given in the acceptance letter. Please email the Managing Editor if you fail to receive your review by the 31st July.

End of August: Authors return revised manuscripts as a MS Word document to the *JOURNAL* website, as per due dates in the acceptance letter, typically 28 days after editorial decision/reviewers, critiques were sent. Corresponding author submits publication charges to Andrew T. Sustich, Ph.D. Treasurer, Arkansas Academy of Science, PO Box 419, State University, AR 72467-0419. The Managing Editor will send an email reminder approximately 1 week prior to the final due date.

The prompt return of revised manuscripts as a MS Word document and payment of publication costs is critical for processing of the *JOURNAL* by the *JOURNAL* staff. If the corresponding author will be unable to attend to the manuscript within the framework of this schedule, then it is the responsibility of the corresponding author to make arrangements with a coauthor to handle the manuscript. NB. The corresponding author will be responsible for submitting the total publication cost of the paper by August 31st. FAILURE TO PAY the publication charges by the deadline will prevent processing of the manuscript, and the manuscript will be added to the manuscripts received from the following year's meeting.

PREPARATION OF THE MANUSCRIPT

A. General considerations

Format the manuscript as a published paper. If you are unfamiliar with the *JOURNAL*, please access last year's journal at http://scholarworks.uark.edu/jaas to familiarize yourself with the layout.

1. Use Microsoft Word 2007 or higher for preparation of the document and the file should be saved and uploaded as a Word Document.
2. The text should be single spaced with Top and Bottom margins set at 0.9″, Left and Right

margins 0.6″. Except for the Title section, the manuscript must be submitted in two column format and the distance between columns should be 0.5″. This can be performed in MS Word by clicking on “Layout" on the Toolbar and then “Columns” from the drop-down menu. Then select "two" (columns).

3. Indent paragraphs and subheadings 0.25″.
4. Use 11 point font in Times New Roman for text. Fonts for the rest of the manuscript must be
   a) Title: 14 point, bold, centered, followed by a single 12 point blank line.
   b) Authors’ names: 12 point, normal, centered. Single line spaced. Separate last author line from authors' address by a single 10 point blank line.
   c) Authors’ addresses: 10 point, italic, centered. Single line spaced. Separate last author line from corresponding author's email by a single 10 point blank line.
   d) Corresponding author’s email: 10 point, normal, left alignment. Please note that all authors (including email addresses) must be included in the electronic submission form, but only the corresponding author’s email is to be included in the uploaded manuscript file.
   e) Running title: 10 point, normal, left alignment.
   f) Main text: 11 point, justified left and right.
   g) Figure captions: 9 point, normal.
   h) Table captions: 11 point normal.
   i) Section headings: 11 point, bold, flush left on a separate line, then insert an 11 point line space. Section headings are not numbered.
   j) Subheadings: 11 point, bold, italic and flush left on a separate line.
5. Set words in italics that are to be printed in italics (e.g., scientific names).
6. In scientific text, **Arabic numerals** should be used in preference to words when the number designates anything that can be counted or measured: 3 hypotheses, 7 samples, 20 milligrams. However, numerals are not used to begin a sentence; spell out the number, reword the sentence, or join it to a previous sentence. Also, 2 numeric expressions should not be placed next to each other in a sentence. The pronoun “one” is always spelled out.
7. Use of footnotes is not permitted
8. A **feature article** is 2 or more pages in length. Most **feature articles** should include the following sections: Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgments, and Literature Cited.
9. A **general note** is generally shorter, usually 1 to 2 pages and rarely utilizes subheadings. A note should have the title at the top of the first page with the body of the paper following. Abstracts are not used for general notes.
10. A **review article** should contain a short abstract followed by the body of the paper. The article may be divided into sections if appropriate, and a final summary or concluding paragraph should be included.

Title of a Paper (14 point, bold, centered)

A.E. Firstauthor[1*], B.F. Second[1], C.G. Third[2], and D.H. Lastauthor[1] (12 point font, normal, centered)

[1]Department of Biology, Henderson State University, Arkadelphia, AR 71999
[2]Arkansas Game and Fish Commission, 915 E. Sevier Street, Benton, AR 72015 (10 point font, italic, centered)

[*]Correspondence: Email address of the corresponding author (10 point, normal, left alignment)

Running title: (no more than 65 characters and spaces) (10 point, normal, left alignment)

Figure 1. Layout of the title section for a submitted manuscript.

B. Specific considerations

1. Title section
(see Fig. 1 above for layout).

i. It is important that the title be short, but informative. If specialized acronyms or abbreviations are used, the name/term should be first indicated in full followed by the short

form/acronym.

ii. Names of all authors and their complete mailing addresses should be added under the Title. Authors names should be in the form "A.M. Scientist", e.g. I.H. Still. Indicate which author is the corresponding author by an asterisk, and then indicate that author's email address on a separate line (see A.4 for format.)

iii. Please include a Short Informative **Running title** (not to exceed 65 characters and spaces) that the Managing editor can insert in the header of each odd numbered page.

iv. Insert a single 10 point blank line after the "Running Title" and add a Continuous section break. DO NOT INSERT A PAGE BREAK.

2. Abstract

An abstract summarizing in concrete terms the methods, findings, and implications discussed in the body of the paper must accompany a **feature article** (or **a review article**). That abstract should be completely self-explanatory. A short summary abstract should also be included for any review article. When submitting a General Note via the electronic submission system, an abstract should be inserted into the appropriate part of the submission form. This facilitates the review process, and visibility of the published General Note on the web. However, an abstract is not required in the body of the actual manuscript. Please review your title and abstract carefully to make sure they convey your essential points succinctly and clearly.

3. Introduction

An appropriately sized introduction should be included that succinctly sets the background and objectives of the research.

4. Materials and Methods

Sufficient details should be included for readers to repeat the experiment. Where possible reference any standard methods, or methods that have been used in previously published papers. Where kits have been used, methods are not required: include the manufacturer's name and location in brackets e.g. "RNA was prepared using the RNeasy Plus Micro Kit (Qiagen, USA)."

5. Tables and figures (line drawings, graphs, or black and white photographs) should not repeat data contained in the text. Tables, figures, graphs, pictures, etc., have to be inserted into the manuscript with "text wrapping" set as "top and bottom" (not "in line with text"). Figures, tables, graphs and pictures can occupy one column (3.4″ wide) or a maximum of two columns wide (7.3″). In the event that a table, a figure, or a photograph requires larger space than a single column, the two column format should be ended with a "Continuous Section Break" and the Table/figure should be placed immediately afterward. The two column format should continue immediately after the Table/figure. To save space, where possible place Tables/Figures at the top or bottom of the column/page.

Tables and figures must be numbered, and should have titles and legends containing sufficient detail to make them easily understood. Allow two 9 point line spaces above and below figures/tables. Please note that Figure and Table captions should be placed in the body of the manuscript text AND NOT in a text box.

i. Tables: A short caption in 11 point normal should be included. Insert a solid 1.5 point line below the caption and at the bottom of the table. Within tables place a 0.75 point line under table headings or other divisions. Should the table continue to another page, do not place a line at the bottom of the table. On the next page, place the heading again with a 0.75 point line below, then a 1.5 point line at the bottom of the table on the continued page. Tables can be inserted as Tables from Excel, but should not be inserted as pictures from PowerPoint, Photoshop etc., or from a specialized program, as the Editorial Board cannot guarantee maintaining the quality of the print in those other formats.

ii. Figures: A short caption should be written under each figure in 9 point, normal. Figure 2 shows an example for the format of a figure inserted into the manuscript. All figures should be created with applications that are capable of preparing high- resolution PhotoShop compatible files. The figure should be appropriately sized and cropped to fit into either one or two columns. Figures should be inserted as JPEG, TIFF images or PhotoShop compatible files. Arrows, scale bars etc., must be integral to the figure: i.e. not "added over" the figure once place in the word document: "independent arrows, etc., will be lost in manuscript formatting. While the

JOURNAL is printed in black and white, we encourage the inclusion of color figures and photographs that can be viewed in the online version. Please note that the figures directly imported from PowerPoint frequently show poor color, font and resolution issues. Figures generated in PowerPoint should be converted to a high resolution TIFF or JPEG file (see your software user's manual for details). If a figure/table is taken from a powerpoint slide, the figure title/legend from that slide should be removed: the only title and legend that should be associated with the figure should be the caption as described at the start of this section, and as shown in the example Figure 2.

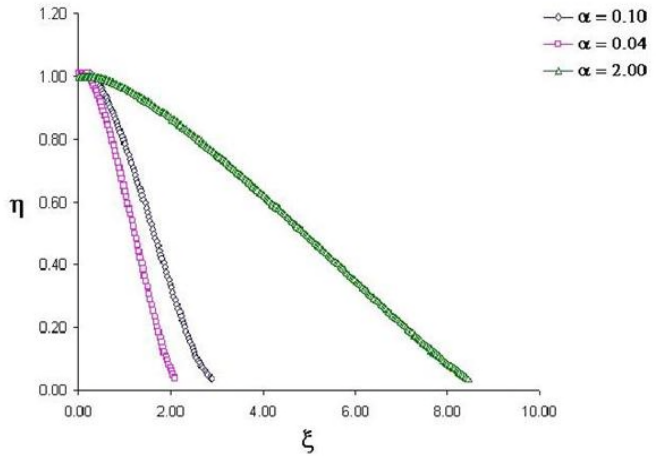

Figure 2. Electric field, η, as a function of position ξ, within the sheath region for three different wave speeds, α.

6. Chemical and mathematical usage

i. The Journal requires the use of the International System of Units (SI). The **metric system of measurements** and **mass** must be employed. **Grams** and **Kilograms** are units of **mass** not weight. Non-SI measurements may be included, secondarily, in parentheses.

ii. Numerical data should be reported with the number of significant figures that reflects the magnitude of experimental uncertainty.

iii. Chemical equations, structural formulas and mathematical equations should be placed between successive lines of text. Equation numbers must be in parentheses and placed flush with right-hand margin of the column.

7. Biological Specimens

i Common names

Due to the variability in use of English common names, the common name should be appended with the scientific name at first mention. Use full common names in the abstract. Authors should then be consistent with the use of common names of organisms in their manuscripts**.**

ii Deposition of materials and sequences in publicly available domains

Cataloguing and deposition of biological specimens into collections is expected. Publication of manuscripts will be contingent on a declaration that database accession numbers and/or voucher specimens will be made available to interested researchers. Where possible, collector and voucher number for each specimen should be stated in the Results section. The location of the collection should be stated in the Methods section. This will facilitate easy access should another researcher wish to obtain and examine the specimen in question.

8. Literature Cited

All cited literature must be included in the Literature Cited section at the end of the manuscript and formatted as given below. No reference should be placed in the manuscript as a footnote.

i Authors should use the Name – Year format as illustrated in *The CBE Manual for Authors, Editors, and Publishers* and as shown below. The *JOURNAL* will deviate from the form given in the *CBE Manual* only in regard to placement of authors' initials and abbreviation of journal titles. Initials for second and following authors will continue to be placed before the author's surname. Note that authors' names are in bold, single spacing occurs after periods. If a citation has 9 authors or more, write out the first 7 and append with *et al*. in the Literature Cited section. **Journal titles should be written in full.** Formats for a journal article and a book are shown below along with examples.

ii. Please note how the literature is "cited in text as", i.e. in the introduction, results etc. In general, cite in text by "first author *et al*." followed by publication date. **DO NOT USE NUMBERS, etc.** Also note that in the Literature Cited section, references should be single line spaced, justified with second and following lines indented 0.25". If

in doubt, see previous issue for format.

Accuracy in referencing current literature is paramount. Authors are encouraged to use a reference databasing system such as Reference Manager or Endnote to enhance accurate citation. Do not cite abstracts and oral, unpublished presentations. Unnecessary referencing of the authors own work is discouraged; where possible the most recent reference should be quoted and appended with "*and references therein*".

General form:

Author(s). Year. Article Title. Journal title volume number(issue number):inclusive pages.

Author(s) [or editor(s)]. Year. Title of Book. Publisher name (Place of publication). Number of pages.

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Specific examples:

Standard Journal Article

Davis DH. 1993. Rhythmic activity in the short-tailed vole, *Microtus*. Journal of Animal Ecology 2:232-8

Cited in text as: (Davis 1993)

Steiner U, JE Klein, and **LJ Fletters.** 1992. Complete wetting from polymer mixtures. Science 258(5080):1122-9.

Cited in text as: (Steiner *et al*. 1992)

Zheng YF and **JYS Luh.** 1989. Optimal load distribution for two industrial robots handling a single object. ASME Journal of Dynamic System, Measurement, and Control 111:232-7.

Cited in text as: (Zheng and Luh 1989)

In press articles

Author(s). Expected publication Year. Article Title. Journal title *in press*.

Cited in text as: (First author *et al*. *in press*)

Kulawiec M, A Safina, MM Desouki, IH Still, S-I Matsui, A Bakin, and **KK Singh.** 2008. Tumorigenic transformation of human breast epithelial cells induced by mitochondrial DNA depletion. Cancer Biology & Therapy *in press.*

Cited in text as: (Kulawiec *et al*. *in press*)

Books, Pamphlets, and Brochures

Box GEP, WG Hunter, and **JS Hunter.** 1978. Statistics for experiments. J Wiley (NY). 653 p.

Cited in text as: (Box *et al*. 1978)

Gilman AG, TW Rall, AS Nies, and **P Taylor, eds.** 1990. The pharmacological basis of therapeutics. 8th ed. Pergamon (NY). 1811 p.

Cited in text as: (Gilman *et al*. 1990)

Engelberger JF. 1989. Robotics in Service. MIT Press Cambridge (MA). 65 p.

Cited in text as: (Engelberger 1989)

Book Chapter or Other Part with Separate Title but Same Author(s) – General format is given first.

Author(s) or editor(s). Year. Title of book. Publisher's name (Place of publication). Kind of part and its numeration, title of part; pages of part.

Hebel R and **MW Stromberg.** 1987. Anatomy of the laboratory cat. Williams & Wilkins (Baltimore, MA). Part D, Nervous system; p 55-65.

Cited in text as: (Hebel and Stromberg 1987)

Singleton S and **BC Bennett**. 1997. Handbook of microbiology. 2nd ed. Emmaus (Rodale, PA). Chapter 5, Engineering plasmids; p 285-96.

Book Chapter or Other Part with Different Authors – General format is given first.

Author(s) of the part. Year. Title of the part. *In:* author(s) or editor(s) of the book. Title of the book. Publisher (Place of publication). Pages of the part.

Weins JA. 1996. Wildlife in patchy environments: Metapopulations, mosaics, and management. *In*: McCullough DR, editor. Metapopulations and wildlife conservation. Island Press (Washington, DC). p 506.

Johnson RC and **RL Smith.** 1985. Evaluation of techniques for assessment of mammal populations in Wisconsin. *In:* Scott Jr NJ, editor. Mammal communities. 2nd ed. Pergamon (NY). p 122-30.

Dissertations and Theses – General format is given first.

Author. Date of degree. Title [type of publication –

dissertation or thesis]. Place of institution: name of institution granting the degree. Total number of pages. Availability statement.

The availability statement includes information about where the document can be found or borrowed if the source is not the institution's own library.

Stevens WB. 2004. An ecotoxilogical analysis of stream water in Arkansas [dissertation]. State University (AR): Arkansas State University. 159 p.

Millettt PC. 2003. Computer modeling of the tornado-structure interaction: Investigation of structural loading on a cubic building [MS thesis]. Fayetteville (AR): University of Arkansas. 176 p. Available from: University of Arkansas Microfilms, Little Rock, AR; AAD74-23.

Published Conference Proceedings – General format is given first.

Author(s)/Editor(s). Date of publication. Title of publication or conference. Name of conference (if not given in the 2nd element); inclusive dates of the conference; place of the conference. Place of publication: publisher. Total number of pages.

Vivian VL, ed. 1995. Symposium on Nonhuman Primate Models for AIDS; 1994 June 10-15; San Diego, CA. Sacramento (CA): Grune & Stratton. 216 p.

Scientific and Technical Reports – General format is given first.

Author(s) (Performing organization). Date of publication. Title. Type report and dates of work. Place of publication: publisher or sponsoring organization. Report number. Contract number. Total number of pages. Availability statement if different from publisher or sponsoring organization. (Availability statement may be an internet address for government documents.)

Harris JL and **ME Gordon** (Department of Biological Sciences, University of Mississippi, Oxford MS). 1988. Status survey of *Lampsilis powelli* (Lea, 1852). Final report 1 Aug 86 – 31 Dec 87. Jackson (MS): US Fish and Wildlife Service, Office of Endangered Species. Report nr USFW-OES-88-0228. Contract nr USFW-86-0228. 44+ p.

Electronic Journal Articles and Electronic Books should be cited as standard journal articles and books except add an availability statement and date of accession following the page(s):

Available at: www.usfw.gov/ozarkstreams. Accessed 29 Nov 2004.

Online resources

Citation depends on the requirement of the particular website. Otherwise use the "electronic journal article" format.

US Geological Survey (USGS). 1979. Drainage areas of streams in Arkansas in the Ouachita River Basin. Open file report. Little Rock (AR): USGS. 87 p. <www.usgs.gov/ouachita> Accessed on 2 Dec 2005.

Cited in text as: (USGS 1979)

Multiple Citations are Cited in text as:

(Harris and Gordon 1988; Steiner *et al*. 1992; Johnson 2006).

8. Submission of Obituaries and *In Memoria*

The Executive Committee and the Journal of the Arkansas Academy of Science welcome the opportunity to pay appropriate professional honor to our departed Academy colleagues who have a significant history of service and support for the Academy and Journal. The editorial staff will consider obituaries for former executive committee members to be included in the Journal. Additional obituaries not meeting these criteria will be forwarded to be posted on the Academy website. We would request that paid up members of the Academy that wish to write an obituary provide a one to two page professional description of the scientist's life that should include details of his/her contribution to the Academy and publication record. The format should follow the two column format and 11pt Times New Roman font. A color or black-and-white photograph to fit in one column should also be provided.

BUSINESS & SUBSCRIPTION INFORMATION

Remittances and orders for subscriptions and for single copies and changes of address should be sent to Dr. Collis Geren, Former Vice Provost of Research & Sponsored Programs and Dean of the Graduate School (Retired), University of Arkansas at Fayetteville, AR 72701, (email: cgeren@uark.edu).

Members may receive 1 copy with their regular membership of $30.00, sustaining membership of $35.00, sponsoring membership of $45.00 or life

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