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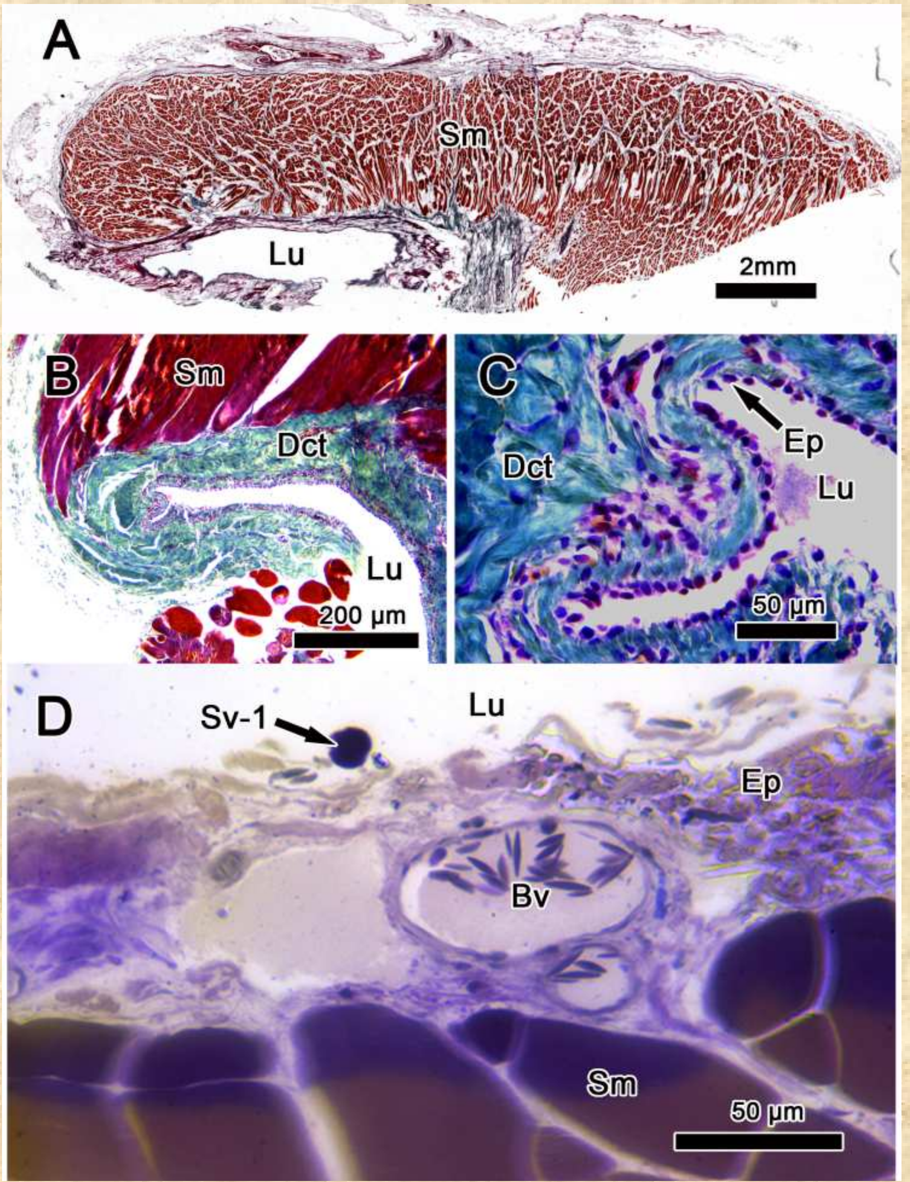

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COVER: Light micrographs of Rathke's Gland in *Macrochelys temminckii* (Chelonia: Chelydridae). *From:* Morphology of Rathke's Glands in the Alligator Snapping Turtle, *Macrochelys temminckii* (Chelonia: Chelydridae) by S.E. Trauth pp 45-51 (Manuscript 3407)

ARKANSAS ACADEMY OF SCIENCE

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Fostering Peer Evaluation Skills in Nursing Students

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Running title: Fostering Peer Evaluation Skills

Abstract

Nursing peer review is a professional practice in which nurses offer constructive, non-personal, practice-related feedback to fellow nurses. The use of nursing peer review has been recommended by the American Nurses Association (ANA 1988, 2014) and the American Nurses Credentialing Center (ANCC 2008). Despite being recommended by professional organizations, the use of peer review in nursing has not been widely implemented. Evaluation of nursing colleagues has the potential to enhance professional nursing, both in practice and in nursing education.

Fostering the skills needed to provide peers with evaluative feedback might best be accomplished if the process is started during nursing school. Teaching ways in which to give and receive feedback without causing feelings of conflict could normalize the process for future generations of nurses. The purpose of this quantitative, quasi-experimental, descriptive study was to examine the impact of teaching nursing peer review skills to student nurses. Based on results, lessons on providing constructive peer feedback will be incorporated into future semesters in a Bachelor of Science in Nursing (BSN) program at a state university in Arkansas.

Introduction

Nursing peer review is the process of providing evaluative, practice-related feedback to peers in a non-personal, constructive manner (Haag-Heitman and George 2011; Morby and Skalla 2010; Topping 2009). To maintain professional autonomy while also providing safe and effective patient care, nursing must take responsibility and be proactive in ensuring members of the profession are practicing at the highest possible level (ANA 2014; Foster 2015). Haag-Heitman and George (2011) explain that nursing peer review should occur in several forms and should not be solely retrospective, but also concurrent.

Nursing peer review is a skill that requires instruction and practice (LeClair-Smith *et al.* 2016). Learning to offer feedback to peers, as well as becoming accustomed to receiving peer input, could prepare new graduates to participate in this innovative aspect of professional practice environments by helping students develop skills needed for teamwork, collaboration, and leadership (Wong *et al.* 2016; Yoo and Chae 2011).

Background

Peer review for nurses is not a new recommendation; however, it is a practice innovation that has not been widely adopted nor implemented by most healthcare organizations (Morby and Skalla 2010). According to Roberts and Cronin (2017), nursing has yet to see widespread implementation of peer evaluation and feedback. Other health care professionals, such as pharmacists and physicians, use peer review both retrospectively and concurrently to evaluate the practice and patient outcomes of their colleagues.

In nursing education, the use of peer feedback and coaching is an emerging topic, particularly for use in undergraduate simulations and patient care scenarios (Badowski and Oosterhouse 2017; Boehm and Bonnel 2010). As part of the academic world, nurse educators have practiced peer review and evaluation routinely, both in publishing research and within their departments in institutions of higher learning (Cobb *et al.* 2001; Gazza *et al.* 2017; Harding 2010). To be accredited, nursing programs must also submit to peer review (ACEN 2013). When considering the possibility of student nurses learning to effectively participate in peer evaluation, it is worth noting that nurse educators could be uniquely positioned and qualified to help students develop a working knowledge of how best to accomplish this evaluative practice in a professional manner.

One of the goals of the peer review project was to provide a formalized lesson to students on how to offer and receive constructive peer feedback. According to Topping (2009), peer assessment benefits groups and individual learners, and has benefits for both the evaluators and the students being evaluated. Adding a lesson on peer review in the junior year of a nursing program presented students an opportunity to learn about the practice after having been briefly introduced to the concept on group projects in the previous semester. Having an initial awareness of the practice may help set the stage for students to gain a deeper understanding of the process.

Statement of the Problem

Nursing is a profession requiring knowledge and skills that must be continuously updated to reflect current research findings and evidence-based practice. Receiving peer feedback is an important way for nurses to assess the need for additional training and new knowledge. Additionally, participation in nursing peer review processes can facilitate quality improvement by providing nurses with an increased awareness of one's own practice through observation of others. Conversely, not addressing the need for increased implementation of peer review could present safety problems and contribute to poor patient outcomes. Since 1988, the ANA has advised nurses to engage in formalized peer review processes on a regular basis. George and Haag-Heitmann (2015) have advocated for the inclusion of peer review in nursing and have developed a conceptual model designed to foster the implementation of peer review programs.

Methods

Following IRB approval, the study took place during the spring semester of junior year in a pre-licensure BSN program. Students were given peer review questionnaires to complete prior to receiving a lesson on peer review. The students then took part in a simulated patient care experience in the high-fidelity simulation lab. Following this simulation activity, study participants engaged in written peer evaluation after having received classroom instruction on how to give and receive professional peer feedback.

The variable under review was the students' attitudes toward participating in nursing peer review processes before and after the intervention. Student demographic data, including age, gender, ethnicity, former occupation (if any), and previous education, were gathered with the surveys. Pre- and post-intervention responses were compared so that statistically significant changes in participants' perceptions of peer review could be detected. Participants' responses were matched pre- and post-intervention using a self-assigned identification code based on the last three letters of their mothers' maiden names, in addition to the first three letters of the mothers' birth months. Participants included this information themselves on both the pre- and post-tests.

Scope

The peer review activity was offered only to students who were in the second semester of their junior year in the pre-licensure BSN program at a state university. Students who were repeating the second semester of junior year were not eligible for inclusion because they would have already completed the simulation scenario being utilized for the peer evaluation exercise. These students would have previously received faculty feedback on their performances and, as such, there was concern that repeating students would not have been true peers with the same level of experience as first-time students. Students who had transferred into the program were not eligible for inclusion since it was not possible to know if they had received previous instruction and practice in giving peer feedback. Additionally, students who held previous licensure as health care professionals were not included since they would not have met the ANA criteria (1988) as true peers of pre-licensure students. The university used as the setting is in a rural area in the north central part of Arkansas. It is the fifth largest university in the state.

Theoretical Framework

The theoretical framework used for this project was the Feedback Intervention Theory (FIT), first proposed by Kluger and DeNisi (1996). FIT was designed to explain factors that precipitate both the positive and negative effects of feedback interventions. Additionally, the theory attempts to define how varying types of feedback, and the situations in which they are used, can result in positive or negative changes in performance.

Kluger and DeNisi (1996) wrote that there is a hierarchy of three types of feedback interventions. The three types relate to task learning, to motivation, and to self. As attention shifts from the lowest level (task learning) to the highest level (self-related), feedback becomes less effective.

Project Design

The nursing peer review study was performed using a quasi-experimental, quantitative, descriptive design. The independent variable was the peer review instruction and exercise. The variable under review was the impact of the peer feedback lesson and exercise on students' perceptions of the professional peer review process. A pre- and post-survey design was used to compare students' perceptions of professional peer feedback before and after the peer review lesson and exercise. The study sample was chosen using convenience sampling.

Students were asked to complete the Instructional Feedback Orientation Scale ([IFOS] (King *et al.* 2009) regarding perceptions of the process of peer evaluation prior to receiving the peer review lesson and again 21 days after the peer review exercise. Following the peer review lesson and the pre-planned simulation, each student completed a peer feedback form and returned the form to the facilitator. Every group member had an opportunity to evaluate the performance of another group member and to have his or her own performance evaluated.

Data Analysis

Data gathered was analyzed using IBM SPSS, version 23 (2015). Responses were based on a 5 point Likert scale. Creswell (2012) describes Likert scales as being interval data and describes the response style as being well-tested over time. Values on the scale were presented as follows: 1—strongly disagree, 2—disagree, 3—neutral, 4—agree, and 5—strongly agree. Results were grouped by area of the instrument (retention, utility, sensitivity, and confidentiality). A codebook defining the study variables was created using a spreadsheet in IBM SPSS (version 23).

Analysis of the Likert scale data gathered in the peer review study was accomplished using the *Wilcoxon signed rank test* for matched pairs. The significance level for the study was set at $p < 0.05$. Results were presented using a z-statistic.

Data from the pre-test was paired with data from the post-test so it was possible to note differences in responses from the same participants before and after the intervention. Comparing the pre- and post-test data helped the researcher determine if statistically significant differences existed in perceptions of the participants before and after receiving peer review instruction.

The accessible population for the study consisted of 38 students (n = 38). Informed consent was completed by 36 students. All 36 of the participants who consented to be enrolled in the study completed the pre-intervention survey, though some of the survey data received was ultimately discarded in accordance with exclusion criteria set for the peer review study. Pre- and post-intervention surveys were matched using a self-assigned code that participants were asked to enter on both surveys. After inclusion and exclusion criteria were applied, 31 participants (n = 31) were enrolled into the study. The majority of the participants were in the age range of 20-29 years (28), with 2 participants in the 30-39 year age group, and 1 participant in the 40-49 year group. The group included 25 females and 6 males. None of the participants had been previously licensed as health care professionals, though four participants had worked as nursing assistants.

Results

The IFOS instrument (King *et al.* 2009) is a 27 item Likert scale survey that is broken down into 4 major categories: utility, sensitivity, confidentiality, and retention. There were no statistically significant changes noted in any of the pre- and post-survey totals for the 4 major categories (Table 1). However, there were statistically significant changes noted on 3 individual questions within the *Utility* category (Table 2).

Discussion

In the current study, the areas of change noted from pre- to post-intervention were all related to the utility of peer feedback, with students reporting a significant change in how useful they perceive peer feedback to be. The researcher will attempt to build on the results when planning future research and plans to incorporate more in-depth lessons on peer review in upcoming semesters.

Table 1. Pre- and post-survey totals for the 4 major categories of the IFOS instrument (King *et al.* 2009).

Section	***z*-score** n = 31	***p*-value** n = 31	**Pre-survey Median** n = 31	**Post-survey Median** n = 31
Utility	-1.678	0.093	43	45
Sensitivity	-1.297	0.195	20	22
Confidentiality	-3.60	0.179	18	19
Retention	-0.868	0.385	6	6

Table 2. Pre- and post-survey totals on questions within the *Utility* section of the IFOS instrument (King *et al.* 2009).

Question	***z*-score** $n = 31$	***p*-value** $n = 31$	**Pre-survey Median** $n = 31$	**Post-survey Median** $n = 31$
I will usually reflect on a peer's feedback.	-2.14	0.032	4	5
Feedback from my peers motivates me to improve my performance.	-2.32	0.02	4	5
I feel relieved when I receive positive feedback.	-2.71	0.007	5	5

Because of the small sample size (n = 31), results of the study are not generalizable to the target population of all second semester, junior year BSN students.

Recommendations for future research include repeating the peer review study using a larger sample, perhaps using several schools representing other nursing degrees (vocational programs, associate, master's, and doctoral levels), and using other locations as study sites. Additional lectures and role-playing sessions, as opposed to a single session, are advised, as is a longer period of time for future research.

It is possible that performing the peer review study using a group of senior nursing students might have produced more significant results. As seniors, the students would be closer to entering practice as professionals and the information might be received and processed by participants differently than it was processed by junior year students. Students who are closer to graduation might be more receptive due to the feeling that they will indeed complete the program, whereas junior level students are perhaps not as confident that they will enter the profession and use the information. It is also possible that teaching the practice earlier, and continuing lessons on the topic throughout school, would be more effective and results would show a significant difference pre- and post-teaching. Ultimately, the most effective approach to studying the potential effects of teaching peer review to nursing students may be to teach it beginning with the first levels of nursing school and continuing the lessons and exercises throughout the students' time in school.

Regardless of when or where it is taught, peer review is a professional practice that nurses must become not only accustomed to, but proficient at performing. There has been little published research on the teaching of nursing peer review in students. Learning to give and receive peer feedback may contribute to increased professionalism within nursing and is therefore worth examining further.

Conclusions and Contributions to the Profession of Nursing

Professional nursing organizations, such as ANA (1998) and ANCC (2008), recommend including peer review on a routine and ongoing basis in healthcare organizations. The use of professional peer review may contribute to improvements in patient safety and better healthcare outcomes (Foster 2015). Bonnel and Hober (2016) describe the process as being useful as a reflective tool in education and note that peer review is not widely utilized in undergraduate nursing education. The aim of the study on nursing peer review was to foster positive perceptions among undergraduate BSN students toward peer review processes.

New methods of teaching how to participate in professional nursing peer review need to be explored through research in a variety of nursing programs. Determining the most effective method of helping nurses become accustomed to nursing peer evaluation is a worthwhile endeavor for the future of the profession. Future research on teaching peer review to nursing students is planned using a larger sample and a variety of settings. Additional research will contribute to the current body of knowledge on the professional practice of nursing peer review.

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An Updated Checklist of the Spiders (Arachnida: Araneae) of Arkansas

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Running Title: Spiders of Arkansas

Abstract

A total of 510 species of spiders representing 43 families and 215 genera are herein reported from Arkansas. The most diverse families of state spiders were Salticidae (64 species), Lycosidae (59 species), Araneidae (55 species), and Gnaphosidae (54 species). Twelve families had only a single representative species in the state. Additional taxa will surely be added to the checklist with future taxonomic studies incorporating molecular analyses and additional collecting. This updated checklist is intended to be of use to arachnologists, researchers, naturalists, professional biologists, and managers in determining the biodiversity for Arkansas. We have worked to insure that the taxonomy of the spiders documented in Arkansas is current and authoritative.

Introduction

Spiders (order Araneae) are an extremely diverse group of arachnids. There are currently over 49,000 spider species known to exist worldwide (World Spider Catalog 2021). In North America alone there are approximately 3,800 known species of spiders (Bradley 2013) with many undescribed or undiscovered species remaining.

North American spiders are divided taxonomically into 2 primary groups: Mygalomorphae and Araneomorphae. Most Arkansas spiders are araneomorphs, i.e. they possess fangs that slant toward each other (Fisher and Dowling 2017). Mygalomorphs possess fangs that point straight down and include tarantulas, trap-door spiders, and their relatives.

The major objective of this study is to provide an updated checklist of the spiders currently known from Arkansas so that state biologists, students, naturalists, resource managers, and interested parties have a comprehensive list available to them. An accurate record of state species is important for determining species ranges and for informing policies regarding land management and conservation (Milne *et al*. 2019). We derived this list from previous publications, most by Peggy Dorris, as well as other publications, collections by H. W. Robison, and from Arkansas records recorded in the Symbiota Collections of Arthropods (SCAN) database (https://scan-bugs.org/portal/). It is important to note that the Arkansas species included in the SCAN database are "ground-truthed" and based upon verifiable specimens. However, the species in the appendix were based largely upon published records and we were unable to locate the majority of specimens in existing collections. Arkansas records included in the SCAN database are marked in the appendix with an asterisk.

Historical Review of Spider Research in Arkansas

Previous species lists of the spiders of Arkansas were published primarily by Peggy Dorris and co-authors (Beck and Dorris 1982, 1983; Dorris 1968, 1969, 1972, 1980, 1985, 1989, 1991; Dorris and Burnside 1977; Dorris and Burris 1992; Dorris and Saugey 1983; Dorris and Thompson 1986; Dorris *et al*. 1995; Hill *et al*. 1995; Parker and Dorris 1995). Dorris' studies used taxonomic keys by Comstock (1982), Emerton (1902), Gertsch (1979), Heiss and Allen (1986), and Kaston (1948, 1982) to aid in identification of Arkansas spiders. Heiss also contributed significantly to our knowledge of faunal diversity of Arkansas spiders (Heiss 1977, 1984; Heiss and Meisch 1985; Heiss and Allen 1986; Heiss *et al*. 1988).

Two gnaphosid spiders new to Arkansas were described by Exline (1962). Later, McDaniel *et al*. 1979) and Peck and Peck (1982) documented spider species from Arkansas caves while Dorris and Saugey (1983) reported spiders inhabiting state abandoned mines and tunnels. Dorris *et al*. (1995) collected 102 forest litter samples from 14 localities in the Ouachita and Ozark Mountains, which revealed 17 families and

56 species among 51 genera of spiders including 19 species previously unrecorded for the state.

Most early research in the 1960s on spiders in Arkansas consisted of simple faunal lists of spiders found on cotton compiled by W. H. Whitcomb and his associates (Whitcomb 1967; Whitcomb and Bell 1964; Whitcomb *et al*. 1963a, 1963b), and as predators of the fall webworm (Whitcomb and Tadic 1963; Warren *et al*. 1967). Peck *et al*. (1971) studied spiders associated with shortleaf (*Pinus echinata*) and loblolly pine (*Pinus taeda*). Peck and Whitcomb (1978) studied the phenology and populations of cursorial spiders in a forest and a pasture. Such studies of the economic impact of spiders on various plants continued to be published as Dorris (1970) studied impact of insecticides on spider populations in a cotton field, and Heiss (1984) and Heiss and Meisch (1985) worked on spiders in rice fields. By use of pitfall traps, Dorris and Thompson (1986) documented the spiders occurring in pine and hardwood forests. Hill *et al*. (1995) reported spiders collected in pit traps in Drew County from different silvicultural systems and included a species list and observations on behavior and habitats.

Additional records of Arkansas spider species have been published in various taxonomic revisions and faunal lists (Archer 1951; Baldridge and Moran 2001; Bishop 1924; Bond and Platnick 2007; Chamberlin and Ivie 1942; Cutler 1987; Dondale and Miller 2020; Dondale and Redner 1978a, 1978b; Dupérré 2013; Edwards 2004; Gertsch 1973, 1992; Hamilton *et al*. 2018; Howell and Jenkins 2004; Maddison 1996; Marusik and Koponen 1992; Muma and Gertsch 1964; Platnick and Shadab 1976, 1983; Vogel 2004; Wallace and Exline 1978; Whitman-Zai *et al*. 2015).

William Baerg (1885–1980), a professor at the University of Arkansas and the father of American tarantula research, originally studied the brown tarantula, *Aphonopelma hentzi* (Gerard), in Arkansas (Baerg 1929, 1938, 1958). Baerg (1936) and Baerg (1959) studied the black widow and 5 other venomous spiders in the United States. Additional studies on the life history or aspects of the natural history of particular species of Arkansas spiders also have been published over the years (Eason 1964, 1969; Eason and Whitcomb 1965; Whitcomb *et al*. 1966; Vetter and Rust 2010; Hogland *et al*. 2017). More recently, studies of state spiders by Tumlison and Robison (2010) documented *Trichonephilia clavipes* (Linnaeus) in southern Arkansas while Tumlison *et al*. (2016) reported on behavior and foods of *T. clavipes*. In an informative recent monograph, Hardy (2018) described ecological observations of the Trapdoor Spider, *Myrmekiaphila comstocki*, in the Ouachita Mountains, AR.

Methods

This updated checklist of spiders is based primarily on personal collections from 1966 to 2019, published and unpublished records available to the authors, and a thorough literature and museum search. In order to develop an updated checklist of the spiders of Arkansas we embarked on a 5 part strategy including: (1) use of personal collections of Arkansas spiders by P. R. Dorris, H. W. Robison, R. Tumlison, C. E. Carlton, J. Kremers, and former SAU student collections, (2) collections of Arkansas spider specimens currently deposited in SCAN, (3) a thorough literature search of Arkansas spider records, including studies and autistic investigations specifically targeting spiders in Arkansas, as well as records gleaned from broader research, (4) use of previous collection records from museums at the Smithsonian Institution, Denver Museum of Nature &Science, Henderson State University, Southern Arkansas University, University of Arkansas, and University of Mississippi, and (5) an on-going, state-wide collection effort of litter sampling, pitfall traps, and hand collecting of spiders in the 6 physiographic regions of Arkansas (Pell 1983), namely the Ozark Mountains, Arkansas Valley, Ouachita Mountains, West Gulf Coastal Plain, Mississippi Alluvial Plain, and Crowley's Ridge. Additional unpublished records and unidentified material also were obtained from the institutions listed above. In addition, a former volunteer with the DMNS arachnology collection, Nancy Bray, has been collecting spiders from her home and environs in Arkansas, contributing significant records of Arkansas spiders to the SCAN Symbiota database.

Because of the loss of some previous spider collection records at various institutions, it was impossible to verify every spider record from Arkansas reported in previous studies. However, we were able to use previous personal collection records of Arkansas spiders from pitfall trap collections, sweeping of vegetation, hand picking, and litter substrate sampling aiding in developing this checklist of Arkansas spiders. Spiders were obtained from approximately 500 collections made between the mid-1960s and 2000 by individuals noted previously in part 1 of our strategies. Between 1960 and 2019, ca. 250 hand collections of spiders and ca. 200 pitfall trap collections were made by HWR from the Ozark and Ouachita Mountains, Arkansas River Valley, as well as collections from the Gulf Coastal and Mississippi Alluvial plains. In addition, spider collections also were made by hand collecting and sweeping vegetation with an insect net in various areas of the state. Identifications of spiders were made initially by PRD, HWR, and RT.

Taxonomic studies have changed names, added new names, redefined distributions of some taxa, and some reported species may have been misidentified in the past. Thus, some of the names of spiders found in earlier works regarding spiders in Arkansas are now excluded, and new names and taxa are included. The newly updated state checklist of the spiders including current taxonomy and new records of species occurrence in Arkansas is provided as Appendix 1. Use of available common names for species in the Appendix follows Breene *et al*. (2003). SCAN Symbiota specimens were identified using keys in Ubick *et al.* (2005).

Results and Discussion

Presently, Arkansas has 5 mygalomorph families with 8 species in total. Six species of Arkansas mygalomorphs are trap-door spiders (Euctenizidae, Antrodiaetidae, and Halonoproctidae) while the Theraphosidae contains the largest spider in Arkansas, the Texas brown tarantula (*Aphonopelma hentzi*).

Our study found a total of 510 spider species within 215 genera and 43 families currently known to inhabit Arkansas (Appendix 1). The 4 most speciose families were the Salticidae (64 species, 31 genera), Lycosidae (59 species, 16 genera), Araneidae (55 species, 25 genera) and Gnaphosidae (54 species, 17 genera). Twelve families (Atypidae, Euctenizidae, Theraphosidae, Dysderidae, Filistatidae, Leptonetidae, Liocranidae, Miturgidae, Nesticidae, Scytotidae, Segestriidae, and Theridiosomatidae) had only 1 species represented in each family.

Spiders of medical importance to humans living in Arkansas include the brown recluse spider, *Loxosceles* species in the family Sicariidae and the black widow spiders, *Lactrodectus* species in the family Theridiidae. Although 2 species of *Loxosceles* and 4 species of *Latrodectus* have been reported from Arkansas, only *Latrodectus mactans* and *Loxosceles reclusa* have been "ground-truthed" (verified by keying of specimens in collections, and indicated by an asterisk in Appendix 1). The other reported species in these 2 genera need to be verified by specimens deposited in museum collections. Much early research was done on the brown recluse in Arkansas as studies by Dillaha *et al.* (1963), Hite *et al.* (1966) and more recently by Vetter and Rust (2010) attest.

Of the 510 species of spiders documented from Arkansas, none is endemic to the state (Robison and Allen 1995), although there are a number of regional endemics known from the Interior Highlands region.

Currently, there are several new spider species being described including a widespread mountainous species (Michael L. Draney and Nina Sandlin, pers. comm.), thus our eventual number of spider species will definitely increase as more collecting is done and molecular research elsewhere is completed. Collecting also continues across the state by the authors and others in all physiographic regions and will reveal more about spider distributions in Arkansas.

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Appendix 1. An updated checklist of the spiders of Arkansas. *indicates species verified from museum collections and included in the SCAN Symbiota database (https://scan-ugs.org/portal/sitemap.php). Common names are included for those taxa listed in Breene *et al*. (2003). Some species added to this listbased on non-Arkansas literature are followed by specific citations.

INFRAORDER MYGALOMORPHAE

Antrodiaetidae – [foldingdoor spiders]
- *Antrodiaetus stygius* Coyle (Coyle 1971)
- *Antrodiaetus unicolor* (Hentz)

Atypidae – [purseweb spiders]
- *Sphodros fitchi* Gertsch and Platnick (Gertsch and Platnick 1980)

Euctenizidae
- **Myrmekiaphila comstocki* Bishop and Crosby

Halonoproctidae
- *Ummidia audouini* (Lucas)
- *Ummidia beatula* (Gertsch and Mulaik) (Godwin and Bond 2021)
- *Ummidia macarthuri* Godwin and Bond (Godwin and Bond 2021)

Theraphosidae – [tarantulas]
- *Aphonopelma hentzi* (Girard) – [Texas brown tarantula]

INFRAORDER ARANEOMORPHAE

Agelenidae – [funnel weavers]
- *Agelenopsis emertoni* Chamberlin and Ivie
- **Agelenopsis kastoni* Chamberlin and Ivie
- **Agelenopsis naevia* (Walckenaer)
- **Agelenopsis oklahoma* (Gertsch)
- *Agelenopsis pennsylvanica* (C. L. Koch)
- **Agelenopsis utahana* (Chamberlin and Ivie)
- **Coras kisatchie* Muma
- **Coras lamellosus* (Keyserling)
- *Coras medicinalis* (Hentz)
- *Coras montanus* Emerton
- *Tegenaria domestica* (Clerck) – [barn funnel weaver]

Amaurobiidae – [hackledmesh weavers]
- *Amaurobius ferox* (Walckenaer)
- *Callobius bennetti* (Blackwall)
- *Cybaeopsis tibialis* (Emerton)

Anyphaenidae – [ghost spiders]
- *Anyphaena celer* (Hentz)
- *Anyphaena fraterna* (Banks) (Platnick 1974)
- *Anyphaena maculata* (Banks)
- *Arachosia cubana* (Banks) (Platnick 1974)
- **Hibana cambridgei* (Bryant)
- **Hibana gracilis* (Hentz) – [garden ghost spider]
- *Hibana velox* (Becker)
- *Lupettiana mordax* (O. Pickard-Cambridge)
- *Wulfila saltabundus* (Hentz)

Araneidae – [orbweavers]
- *Acacesia hamata* (Hentz)
- *Acanthepeira stellata* (Walckenaer) – [starbellied orbweaver]
- *Acanthepeira venusta* (Banks)
- *Araneus bicentenarius* (McCook)
- **Araneus bonsallae* (McCook)
- *Araneus carrolli* Levi (Levi 1973)
- *Araneus cavaticus* (Keyserling) – [barn orbweaver]
- **Araneus cingulatus* (Walckenaer)
- *Araneus gadus* Levi (Levi 1973)
- *Araneus gemmoides* Chamberlain and Ivy
- *Araneus guttulatus* (Walckenaer)
- *Araneus juniperi* (Emerton)
- *Araneus marmoreus* Clerck – [marbled orbweaver]
- *Araneus miniatus* (Walckenaer)
- *Araneus nordmanni* (Thorell)
- **Araneus partitus* (Walckenaer)
- *Araneus pegnia* (Walckenaer)
- *Araneus pratensis* (Emerton)
- *Araneus thaddeus* (Hentz) – [lattice orbweaver]
- *Araniella displicata* (Hentz) – [sixspotted orbweaver]
- **Argiope aurantia* Lucas – [yellow garden spider]
- *Argiope trifasciata* (Forsskål) – [banded garden spider]
- **Colphepeira catawba* (Banks)
- *Cyclosa conica* (Pallis) [trashline orbweavers]
- *Cyclosa turbinata* (Walckenaer)
- *Eustala anastera* (Walckenaer) – [humpbacked orbweaver]
- *Eustala emertoni* (Banks)
- *Eustala cepina* (Walckenaer)
- *Gasteracantha cancriformis* (Linnaeus) – [spinybacked orbweaver]
- **Gea heptagon* (Hentz)
- **Hyposinga funebris* (Keyserling)
- *Hyposinga pygmaea* (Sundevall)
- *Hyposinga rubens* (Hentz) (Dondale *et al*. 2003)
- *Kaira alba* (Hentz) (Levi 1993)
- **Kaira hiteae* Levi
- *Larina directa* (Hentz)
- **Larinioides cornutus* (Clerck) – [furrow orbweaver]
- *Mangora gibberosa* (Hentz) – [lined orbweaver]
- *Mangora maculata* (Keyserling) – [greenlegged orbweaver]

- *Mangora placida* (Hentz) – [tuftlegged orbweaver]
- **Mastophora bisaccata* (Emerton) – [bolas spiders]
- *Mecynogea lemniscata* (Walckenaer) – [basilica orbweaver]
- *Metazygia calix* (Walckenaer)
- *Metepeira labyrinthea* (Hentz) – [labyrinth orbweaver]
- **Micrathena gracilis* (Walckenaer) – [spined micrathena]
- *Micrathena mitrata* (Hentz) – [white micrathena]
- *Micrathena sagittata* (Walckenaer) – [arrowshaped micrathena]
- *Neoscona arabesca* (Walckenaer) – [arabesque orbweaver]
- **Neoscona crucifera* (Lucas)
- *Neoscona domiciliorum* (Hentz)
- *Neoscona pratensis* (Hentz)
- *Ocrepeira ectypa* (Walckenaer)
- *Trichonephila clavipes* (Linnaeus) Foliate spider
- *Singa keyserlingi* McCook – [striped orbweavers]
- **Verrucosa arenata* (Walckenaer)

Cheiracanthiidae
- *Cheiracanthium inclusum* (Hentz) – [agrarian sac spider]
- *Strotarchus piscatorius* (Hentz)

Clubionidae – [sac spiders]
- *Clubiona abboti* L. Koch
- *Clubiona catawba* Gertsch
- *Clubiona johnsoni* Gertsch
- *Clubiona moesta* Banks
- *Clubiona obesa* Hentz
- *Clubiona pygmaea* Banks
- *Clubiona riparia* L. Koch
- *Clubiona saltitans* Emerton
- **Elaver excepta* (C. L. Koch)

Corinnidae – [antmimic spiders]
- *Castianeira amoena* (C. L. Koch)
- *Castianeira cingulata* (C. L. Koch) – [twobanded antmimic]
- **Castianeira crocata* (Hentz)
- *Castianeira crucigera* (Hentz) (Reiskind 1969)
- **Castianeira descripta* (Hentz) – [redspotted antmimic]
- *Castianeira gertschi* Kaston – [Gertsch antmimic]
- *Castianeira longipalpa* (Hentz)
- *Castianeira trilineata* (Hentz)
- *Castianeira variata* Gertsch

Ctenidae – [wandering spiders]
- *Anahita punctulata* (Hentz) (Peck 1981)
- **Ctenus exlineae* Peck
- *Ctenus hibernalis* Hentz

Cybaeidae
- *Calymmaria persica* (Hentz)
- *Cryphoeca montana* Emerton

Dictynidae – [meshweavers]
- *Dictyna bellans* Chamberlin (Chamberlin and Gertsch 1958)
- *Dictyna sylvania* Chamberlin and Ivie
- *Dictyna terrestris* Emerton (Chamberlin and Gertsch 1958)
- *Dictyna volucripes* Keyserling
- *Emblyna annulipes* (Blackwall)
- *Emblyna cruciata* (Emerton)
- *Emblyna hentzi* (Kaston)
- *Emblyna roscida* (Hentz)
- *Emblyna sublata* (Hentz)
- **Lathys immaculata* (Chamberlin and Ivie)
- *Lathys pallida* (Marx)
- *Phantyna bicornis* (Emerton)
- **Phantyna pixi* (Chamberlin and Gertsch)
- *Phantyna segregata* (Gertsch and Mulaik) – [apex mesh weaver]

Dysderidae
- *Dysdera crocata* C.L. Koch

Filistatidae – [crevice weavers]
- *Kukulcania hibernalis* (Hentz) – [southern house spider]

Gnaphosidae – [stealthy ground spiders]
- *Callilepis imbecilla* (Keyserling)
- *Callilepis pluto* Banks
- **Cesonia bilineata* (Hentz)
- *Drassodes auriculoides* Barrows
- *Drassodes gosiutus* Chamberlin
- *Drassodes neglectus* (Keyserling)
- *Drassyllus aprilinus* (Banks)
- **Drassyllus covensis* Exline
- *Drassyllus creolus* Chamberlin and Gertsch
- *Drassyllus depressus* (Emerton)
- *Drassyllus dixinus* Chamberlin
- *Drassyllus dromeus* Chamberlin
- *Drassyllus ellipes* Chamberlin and Gerstch
- *Drassyllus fallens* Chamberlin
- *Drassyllus frigidus* (Banks)
- *Drassyllus gynosaphes* Chamberlin
- *Drassyllus lepidus* (Banks)
- *Drassyllus niger* (Banks)
- *Drassyllus notonus* Chamberlin
- *Drassyllus novus* (Banks)
- *Drassyllus rufulus* (Banks)
- *Drassyllus socius* Chamberlin
- *Drassyllus texamans* (Chamberlin)
- **Gnaphosa fontinalis* Keyserling
- *Gnaphosa muscorum* (L. Koch)

Gnaphosa sericata (L. Koch)
Haplodrassus bicornis (Emerton)
Haplodrassus signifer (C. L. Koch)
**Herpyllus ecclesiasticus* Hentz – [parson spider]
**Litopyllus temporarius* Chamberlin
Micaria elizabethae Gertsch (Platnick and Shadab 1988)
Micaria laticeps Emerton (Platnick and Shadab 1988)
Micaria longipes Emerton
Micaria longispina Emerton (Platnick and Shadab 1988)
Micaria punctata Banks (Platnick and Shadab 1988)
Micaria seminola Gertsch (Platnick and Shadab 1988)
Micaria vinnula Gertsch and Davis
Nodocion floridanus (Banks)
Sergiolus capulatus (Walckenaer)
Sergiolus minutus (Banks)
Sergiolus montanus (Emerton)
**Sergiolus ocellatus* (Walckenaer)
Sergiolus tennesseensis Chamberlin
Sosticus insularis (Banks)
**Synaphosus paludis* (Chamberlin and Gertsch)
**Talanites echinus* (Chamberlin)
Talanites exlineae (Platnick and Shadab)
Trachyzelotes lyonneti (Audouin)
**Urozelotes rusticus* (L. Koch)
**Zelotes aiken* Platnick and Shadab
**Zelotes duplex* Chamberlin
Zelotes hentzi Barrows
**Zelotes laccus* (Barrows)
Zelotes pullus (Bryant)

Hahniidae
**Cicurina arcuata* Chamberlin and Ivie
Cicurina arkansa Gertsch
Cicurina brevis (Emerton)
Cicurina bryantae Exline
Cicurina davisi Exline
Cicurina ludoviciana Simon
Hahnia cinerea Emerton
Neoantistea agilis (Keyserling)
Neoantistea riparia (Keyserling)

Leptonetidae – [cave spiders]
**Ozarkia arkansa* (Gertsch)

Linyphiidae – [sheetweb and dwarf weavers]
Agyneta angulata (Emerton) (Dupérré 2013)
Agyneta barrowsi (Chamberlin and Ivie) (Dupérré 2013)
Agyneta fabra (Keyserling)
Agyneta girardi Dupérré
Agyneta illanoensis (Gertsch and Davis) (Dupérré 2013)
Agyneta micaria (Emerton)
Agyneta parva (Banks)
Agyneta semipallida (Chamberlin and Ivie) (Dupérré 2013)
Agyneta serrata (Emerton) (Dupérré 2013)
Bathyphantes pallidus (Banks)
Centromerus latidens (Emerton)
Ceraticelus creolus Chamberlin
Ceraticelus emertoni (O. Pickard-Cambridge)
Ceraticelus similis (Banks)
Eridantes erigonoides (Emerton) (Prentice and Redak 2013)
Erigone autumnalis Emerton
Erigone dentigera O. Pickard-Cambridge
Erigone praecursa Chamberlin and Ivie
Florinda coccinea (Hentz)
Frontinella communis (Hentz)
Frontinella pyramitela (Walckenaer) – [bowl and doily weaver]
Gonatium rubens (Blackwall)
Grammonota inornata Emerton
Grammonota maculata Banks
Grammonota ornata (O. P. Cambridge)
Grammonota texana (Banks)
Helophora insignis (Blackwall)
Islandiana flaveola (Banks)
Megalepthyphantes nebulosus (Sundevall)
Mermessus fradeorum (Berland)
Mermessus maculatus (Banks)
Mermessus tridentatus (Emerton)
Mermessus trilobatus (Emerton)
Microneta viaria (Blackwall)
Neriene radiata (Walckenaer) – [filmy dome spider]
Neriene variabilis (Banks)
Pelecopsis moesta (Banks)
Porrhomma cavernicola (Keyserling)
Tennesseellum formica (Emerton)
Tenuiphantes sabulosus (Keyserling)
Tenuiphantes zebra (Emerton)
Walckenaeria communis (Emerton) (Millidge 1983)
Walckenaeria spiralis (Emerton)

Liocranidae
Agroeca pratensis Emerton

Lycosidae – [wolf spiders]
**Allocosa funerea* (Hentz)
**Allocosa noctuabunda* (Montgomery)
Allocosa sublata (Montgomery)
Alopecosa aculeata (Clerck)

Alopecosa kochi (Keyserling)
Arctosa emertoni Gertsch
**Arctosa littoralis* (Hentz)
Arctosa rubicunda (Keyserling)
Arctosa virgo (Chamberlin)
**Gladicosa gulosa* (Walckenaer)
**Gladicosa pulchra* (Keyserling)
**Hogna antelucana* (Montgomery)
Hogna baltimoriana (Keyserling)
**Hogna carolinensis* (Walckenaer)
**Hogna frondicola* (Emerton)
Hogna lenta (Hentz)
Pardosa atlantica Emerton
**Pardosa lapidicina* Emerton
Pardosa littoralis Banks
**Pardosa milvina* (Hentz)
Pardosa moesta Banks
**Pardosa pauxilla* Montgomery
Pardosa saxatilis (Hentz)
**Pardosa xerampelina* (Keyerling)
Pirata alachuus Gertsch and Wallace
**Pirata apalacheus* Gertsch
Pirata hiteorum Wallace and Exline
Pirata montanus (Emerton)
Pirata piraticus (Clerck)
Pirata sedentarius Montgomery
Pirata seminolus Gertch and Wallace
Pirata spiniger (Simon) (Wallace and Exline 1978)
Pirata suwaneus Gertsch
Pirata sylvanus Chamberlin and Ivie
Piratula insularis (Emerton)
Piratula minuta (Emerton)
**Rabidosa punctulata* (Hentz)
**Rabidosa rabida* (Walckenaer)
**Schizocosa avida* (Walckenaer)
Schizocosa bilineata (Emerton)
Schizocosa crassipes (Walckenaer)
Schizocosa duplex Chamberlin (Dondale and Redner 1978a)
Schizocosa floridana Bryant
**Schizocosa mccooki* (Montgomery)
**Schizocosa ocreata* (Hentz)
Schizocosa retrorsa (Banks)
Schizocosa rovneri Uetz and Dondale
**Schizocosa saltatrix* (Hentz)
**Schizocosa stridulans* Stratton
Sosippus mimus Chamberlin
Tigrosa annexa (Chamberlin and Ivie)
Tigrosa aspersa (Hentz)
**Tigrosa georgicola* (Walckenaer)
Tigrosa helluo (Walckenaer)
Trabeops aurantiacus (Emerton)
Trebacosa marxi (Stone) (Wallace and Exline 1978)
Trochosa pratensis (Emerton)
**Trochosa sepulchralis* (Montgomery)
**Varacosa avara* (Keyserling)

Mimetidae – [pirate spiders]
Ero leonina (Hentz)
Mimetus epeiroides Emerton
Mimetus notius Chamberlin
Mimetus puritanus Chamberlin
Mimetus syllepsicus Hentz

Miturgidae – [prowling spiders]
Zora pumila (Hentz)

Nesticidae –[cave cobweb spiders]
**Eidmannella pallida* (Emerton)

Oecobiidae – [flatmesh weavers]
Oecobius annulipes Lucas
Oecobius cellariorum (Dugès)

Oxyopidae – [lynx spiders]
Hamataliwa helia (Chamberlin)
Oxyopes acleistus Chamberlin
**Oxyopes aglossus* Chamberlin
**Oxyopes apollo* Brady
**Oxyopes salticus* Hentz – [striped lynx spider]
Oxyopes scalaris Hentz – [western lynx spider]
Peucetia viridans (Hentz) – [green lynx spider]

Philodromidae – [running crab spiders]
Ebo latithorax Keyserling
Ebo punctatus Sauer and Platnick
Philodromus aureolus (Clerck)
Philodromus cespitum (Walckenaer)
Philodromus imbecillus Keyserling
Philodromus infuscatus Keyserling
Philodromus keyserlingi Marx
**Philodromus laticeps* Keyserling
Philodromus marxi Keyserling
**Philodromus mineri* Gertsch
Philodromus minutus Banks
Philodromus placidus Banks
Philodromus rufus Walckenaer
Philodromus vulgaris (Hentz)
Thanatus formicinus (Clerck)
**Thanatus rubicellus* Mello-Leitão
Thanatus vulgaris Simon
Tibellus duttoni (Hentz)
Tibellus oblongus (Walckenaer) Long bodied crab spider

Pholcidae – [cellar or daddylongleg spiders]
Pholcus phalangioides (Fuesslin) – [longbodied cellar spider]
Psilochorus pullulus (Hentz) (Slowik 2009)
Spermophora senoculata (Dugès) –

[shortbodied cellar spider]

Phrurolithidae

**Phrurotimpus alarius* (Hentz)
**Phrurotimpus annulatus* Chamberlin and Ivie
**Phrurotimpus borealis* (Emerton)
Phrurotimpus certus Gertsch
Phrurotimpus illudens (Gertsch)
Phrurotimpus umbratilis (Bishop and Crosby)
Scotinella brittoni (Gertsch)
**Scotinella fratrella* (Gertsch)
Scotinella pallida Banks
**Scotinella redempta* Gertsch

Pisauridae– [nursery web spiders]

Dolomedes albineus Hentz (Carico 1973)
Dolomedes scriptus Hentz
**Dolomedes tenebrosus* Hentz
Dolomedes triton (Walckenaer) – [sixspotted fishing spider]
Dolomedes vittatus Walckenaer
Pisaurina brevipes (Emerton) (Carico 1972)
Pisaurina dubia (Hentz) (Carico 1972)
Pisaurina mira (Walckenaer) – [nursery web spider]
Pisaurina undulata (Keyserling)
Tinus peregrinus (Bishop)

Salticidae – [jumping spiders]

Anasaitis canosa (Walckenaer)
Attidops youngi (Peckham and Peckham)
Attinella concolor (Banks)
Attulus floricola (C. L. Koch)
Chinattus parvulus (Banks)
Colonus puerperus (Hentz)
Colonus sylvanus (Hentz)
**Eris militaris* (Hentz) – [bronze jumper]
Eris rufa (C.L. Koch)
Ghelna barrowsi (Kaston) (Kaston 1973)
Ghelna canadensis (Banks)
Ghelna sexmaculata (Banks)
Habronattus borealis (Banks)
Habronattus coecatus (Hentz)
Habronattus decorus (Blackwall)
Habronattus viridipes (Hentz)
Hentzia mitrata (Hentz)
Hentzia palmarum (Hentz) (Richman 1989)
Lyssomanes viridis (Walckenaer) – [magnolia green jumper]
**Maevia inclemens* (Walckenaer) – [dimorphic jumper]
**Maevia intermedia* Barnes
**Marpissa formosa* (Banks)
Marpissa lineata (C. L. Koch)
Marpissa pikei (Peckham and Peckham) – [Pike slender jumper]
Menemerus bivittatus (Dufour) – [gray wall jumper]
**Metacyrba taeniola* (Hentz)
Naphrys pulex (Hentz)
Neon nelli Peckham and Peckham
Paraphidippus aurantius (Lucas)
Peckhamia picata (Hentz) – [antmimic jumper]
Pelegrina chalceola Maddison
Pelegrina exigua (Banks)
Pelegrina galathea (Walckenaer) – [peppered jumper]
Pelegrina peckhamorum (Kaston)
Pelegrina proterva (Walckenaer)
Phidippus apacheanus Chamberlin and Gertsch
**Phidippus audax* (Hentz) – [bold jumper]
Phidippus cardinalis (Hentz) – [cardinal jumper]
Phidippus clarus Keyserling
Phidippus insignarius C. L. Koch
**Phidippus mystaceus* (Hentz)
**Phidippus otiosus* (Hentz)
Phidippus princeps (Peckham and Peckham)
Phidippus purpuratus Keyserling
**Phidippus putmani* (Peckham and Peckham)
Phidippus texanus Banks
Phidippus whitmani Peckham and Peckham
Phlegra fasciata (Hahn)
**Phlegra hentzi* (Marx)
**Platycryptus undatus* (De Geer)
Plexippus paykulli (Audouin) – [pantropical jumper]
Salticus scenicus (Clerck) – [zebra jumper]
Sarinda hentzi (Banks)
Sassacus cyaneus (Hentz)
**Sassacus papenhoei* (Peckham and Peckham)
**Sassacus vitis* (Cockerell)
Synemosyna formica Hentz
Talavera minuta (Banks)
Tutelina elegans (Hentz)
Tutelina harti (Emerton)
Tutelina similis (Banks)
Zygoballus nervosus (Peckham and Peckham)
Zygoballus rufipes Peckham and Peckham – [hammerjawed jumper]
Zygoballus sexpunctatus (Hentz)

Scytodidae– [spitting spiders]

Scytodes thoracica (Latreille)

Segestriidae– [tunnel spiders]

**Ariadna bicolor* (Hentz)

Sicariidae – [sixeyed sicariid spiders]

**Loxosceles reclusa* Gertsch and Mulaik – [brown recluse]

Loxosceles rufescens (DuFour) – [Mediterranean recluse]

Tetragnathidae – [longjawed orbweavers]

**Glenognatha foxi* (McCook)

Leucage venusta (Walckenaer) – [orchard orbweaver]

Meta ovalis (Gertsch)

Pachygnatha autumnalis Keyserling (Dondale *et al*. 2003)

Pachygnatha tristriata (C. L. Koch) – [thickjawed orbweavers]

Tetragnatha caudata Emerton (Dondale *et al*. 2003)

**Tetragnatha elongata* Walckenaer

Tetragnatha guatemalensis O. Pickard-Cambridge

**Tetragnatha laboriosa* Hentz – [silver longjawed orbweaver]

Tetragnatha pallescens F.O. Pickard-Cambridge

Tetragnatha straminea Emerton

Tetragnatha versicolor Walckenaer

Tetragnatha viridis (Walckenaer)

Theridiidae – [cobweb weavers]

**Anelosimus studiosus* (Hentz)

Asagena fulva (Keyserling)

Cryptachaea porteri (Banks)

Cryptachaea rupicola (Emerton)

Dipoena nigra (Emerton)

Dipoena buccalis Keyserling

Enoplognatha marmorata (Hentz) – [marbled cobweb spider]

Euryopis funebris (Hentz)

Faiditus cancellatus (Hentz) (Exline and Levi 1962)

Hentziectypus globosus (Hentz)

Latrodectus geometricus (C. L. Koch) – [brown widow]

Latrodectus hesperus Chamberlin and Ivie – [western black widow]

**Latrodectus mactans* (Fabricius) – [southern black widow]

Latrodectus variolus (Walckenaer) – [northern black widow]

Neospintharus trigonum (Hentz)

**Parasteatoda tepidariorum* (C. L. Koch) – [house spider]

Pholcomma hirsutum (Emerton)

Phoroncidia americana (Emerton)

Phylloneta pictipes (Keyserling)

Platnickina alabamensis (Gertsch and Archer)

Platnickina antoni (Keyserling)

Platnickina tincta (Walckenaer)

Rhomphaea fictilium (Hentz)

**Robertus banksi* (Kaston)

Robertus riparius (Keyserling)

Spintharus flavidus Hentz

**Steatoda triangulosa* (Walckenaer)

Theridion albidum Banks

**Theridion australe* Banks

Theridion differens Emerton

Theridion flavonotatum Becker

Theridion frondeum Hentz

Theridion murarium Emerton

Theridion neshamini Levi

Theridion punctosparsum Emerton (Levi 1957)

Theridion rabuni Chamberlin and Ivie

Theridula emertoni Levi

Theridula opulenta (Walckenaer)

Tidarren sisyphoides (Walckenaer)

Thymoites marxi (Crosby) (Levi 1957)

Wamba crispulus (Simon)

Yunohamella lyrica (Walckenaer)

Theridiosomatidae – [ray orbweavers]

**Theridiosoma gemmosum* (L. Koch) – [ray spider]

Thomisidae – [crab spiders]

Bassaniana floridana (Banks) – [bark crab spiders]

Bassaniana versicolor (Keyserling)

Mecaphesa asperata (Hentz)

Mecaphesa celer (Hentz)

Misumena vatia (Clerck) – [goldenrod crab spider]

Misumenoides formosipes (Walckenaer) – [whitebanded crab spider]

**Misumessus oblongus* (Keyserling)

**Modysticus modestus* (Scheffer)

Ozyptila monroensis Keyserling – [leaflitter crab spiders]

Ozyptila americana Banks

Ozyptila creola Gertsch

Ozyptila distans Dondale and Redner

Synema parvulum (Hentz)

Tmarus angulatus (Walckenaer)

Tmarus rubromaculatus Keyserling

**Xysticus auctificus* Keyserling

Xysticus banksi Bryant

Xysticus bicuspis Keyserling

Xysticus elegans Keyserling – [elegant crab spider]

Xysticus ferox (Hentz)

**Xysticus fraternus* Banks

**Xysticus funestus* Keyserling

Xysticus gulosus Keyserling

Xysticus luctans (C.L. Koch)

Xysticus punctatus Keyserling
**Xysticus texanus* Banks
Xysticus triguttatus Keyserling – [threebanded crab spider]

Titanoecidae
Titanoeca americana Emerton
Titanoeca brunnea Emerton (Leech 1972)

Trachelidae
**Meriola decepta* Banks
Trachelas similis F.O. Pickard-Cambridge
Trachelas tranquillus (Hentz)

Uloboridae – [hackled orbweavers]
Hyptiotes cavatus (Hentz) – [triangle weaver]
Uloborus glomosus (Walckenaer) – [featherlegged orbweaver]

New Distributional Records for Ectoparasites (Acari: Laelapidae, Myocoptidae) of the Woodland Vole, *Microtus pinetorum* (Rodentia: Cricetidae) from Polk County, Arkansas

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Running Title: Ectoparasites of Woodland Vole

Abstract

The woodland vole, *Microtus pinetorum* is a common Arkansas rodent found statewide. To our knowledge, it has been surveyed only once in the state for ectoparasites. Here, a single specimen was examined and found to be infested with three species of mites, including *Androlaelaps fahrenholzi*, *Laelaps alaskensis*, and *Myocoptes japonensis*. This is the first time *L. alaskensis* and *M. japonensis* have been reported from Arkansas.

Introduction

At least 27 species of rodents occur in Arkansas and one of the common species in the state, the woodland vole, *Microtus pinetorum* (Le Conte, 1830), is a small cricetid rodent that ranges statewide (Sealander and Heidt 1990). Here it occurs in a variety of habitats ranging from overgrown, grassy fields and fencerows in orchards to moist woodlands (Sealander and Heidt 1990). The overall range of *M. pinetorum* is throughout the eastern and midwestern United States and extreme southern Ontario, Canada, from Maine southwestward to central Texas (Smolen 1981; Reid 2006).

Timm (1985), in a species account, provided a summation of the parasites of *M. pinetorum*. More recently, Connior *et al.* (2017) reported three species of mites from a single *M. pinetorum* collected from the Ozark Highlands of Benton County. To our knowledge, this is the only report of ectoparasites from this host in the state. Here, we document 3 mites from a *M. pinetorum* from the Ouachita Highlands.

Materials and Methods

Collections of *M. pinetorum* were attempted between 2018 and 2020 using Museum Special snap traps as well as Sherman live traps (H.B. Sherman traps, Tallahassee, FL) baited with rolled oats at the Ouachita Mountains Biological Station (OMBS), Polk County (34° 27' 43.4484" N,-93° 59' 54.3264" W). On 8 June 2020, a single neonate *M. pinetorum* was found alive on the ground. It was euthanized by cervical dislocation following American Society of Mammalogists guidelines (Sikes *et al.* 2016). The pelage was brushed over a white enamel pan for ectoparasites and specimens found were examined with a stereomicroscope. Mites were placed in vials of 70% ethanol and later cleared in lactophenol, slide-mounted in Hoyer's medium (Walters and Krantz 2009), and identified via light microscopy using Fain and Hyland (1970) and Whitaker (1982). A voucher specimen of the host is deposited in the mammal collection at Henderson State University (HSU), Arkadelphia, Arkansas. Ectoparasites are deposited in the Entomology Collection in the Department of Biology at Georgia Southern University, Statesboro, Georgia (accession no. L3848).

Results and Discussion

Three species of mites were found on *M. pinetorum* as follows:

ACARI: LAELAPIDAE

***Androlaelaps fahrenholzi* (Berlese, 1911).** – Six female specimens of this mesostigmatan mite were found recovered. This is a widespread and common Nearctic ectoparasite that has been previously reported from various rodents in Arkansas, including *M. pinetorum*, hispid cotton rat (*Sigmodon hispidus*), golden mouse (*Ochrotomys nuttalli*), and eastern woodrat, *Neotoma floridana* (Tumlison *et al.* 2015; Connior *et al.* 2017). This is the second time this mite has been found on this host in Arkansas (Connior *et al.* 2017), albeit now from a new locale in the Ouachita

Highlands.

***Laelaps alaskensis* Grant, 1947.** – A single female and 6 nymphs were taken. This species has been reported previously from *M. pinetorum* from Indiana (Mumford and Whitaker 1982; Whitaker and Mumford 2009), Mississippi (Clark and Durden 2002), and Pennsylvania (Whitaker and Lukoschus 1982). This mite has also been reported from several rodents from Indiana, Maryland, Minnesota, Mississippi, New Hampshire, New York, North Carolina, Oregon, Pennsylvania, Tennessee, and Wisconsin, and New Brunswick and the Northwest Territories, Canada (Whitaker *et al.* 2007). We document a new distributional record for *L. alaskensis* in Arkansas.

MYOCOPTIDAE

***Myocoptes japonensis* Radford, 1955.** – Three female specimens were found. Interestingly, three species of *Myocoptes* have been recorded from *M. pinetorum*, including *Myocoptes pitymys* Fain and Bochkov, 2004 from Illinois, which is probably host specific (Fain and Bochtov 2004). However, *M. japonicas* has previously been reported to be a parasite generalist that occurs on a number of host species, including *M. pinetorum* from Indiana (Mumford and Whitaker 1982) and Illinois (Pascal and Whitaker 1989), and seven other species of voles (Whitaker *et al.* 2007; Storm and Ritzi 2008). It has been previously reported from other North American hosts in Illinois, Indiana, Iowa, New York, North Carolina, Rhode Island, Oregon, Pennsylvania, and New Brunswick, Canada (Whitaker *et al.* 2007; Storm and Ritzi 2008). This is the first time *M. japonensis* has been reported from Arkansas and we document the southernmost geographic distribution record for this mite.

Overall, the distribution and host-specificity of ectoparasites of small mammals is poorly known in Arkansas. Given the paucity of information on ectoparasite diversity in the state, continuation of surveys will undoubtedly lead to additional new host and geographic records.

Acknowledgments

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Spatial and Temporal Variation in *Aedes albopictus* Prevalence Across Arkansas

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Running Title: Spatial and Temporal Variation in *Ae. albopictus*

Abstract

Aedes albopictus is a well-known vector species of mosquito that is responsible for the transmission of many arboviruses such as Zika, chikungunya, and dengue. The objective of this study was to quantify spatial and temporal variation of *Ae. albopictus* prevalence in Arkansas. We used egg abundance as a proxy for mosquito prevalence. Across 2 years, we worked with the Arkansas Department of Health to collect mosquito eggs using oviposition traps. Eggs were desiccated, counted, and later rehydrated in rearing chambers and raised through adulthood for species determination (>99% *Ae. albopictus*). We determined mean egg abundance by month, year, and latitude, and mapped egg counts using graduated colors to visually display county-specific patterns. Egg abundance was typically low in spring, peaked in late summer, and steadily declined through fall. We observed north-south differences in egg abundance, though the latitude of peak abundance varied across years and throughout the seasons. This research reveals temporal variation and spatial hotspots in *Ae. albopictus* prevalence across the state of Arkansas and highlights existing gaps that should be targeted by future sampling.

Introduction

Mosquitoes are key vectors for pathogens that cause mortality and morbidity for humans across the planet (Anoopkumar *et al.* 2017). Those in the genus *Aedes* are the primary vectors of many arboviruses including dengue, yellow fever, chikungunya, and Zika viruses (Reinhold *et al.* 2018). This genus is endemic to Africa and Asia but in recent decades has spread across much of the planet, including the United States (Kraemer *et al.* 2015). Recent models based upon environmental suitability (Kraemer *et al.* 2015) and surveillance records (Monaghan *et al.* 2019) predict distributions across most of the southeastern USA. The expanding range of these mosquitoes carries a corresponding spread of the arboviruses they carry. Indeed, researchers using niche models predict that much of the far southeast USA is highly suitable for Zika virus transmission (Messina *et al.* 2016). Interestingly, it is possible for *Aedes* populations to exhibit different disease competence depending on the geographic origin of both the mosquito and the virus (Azar *et al.* 2017). The expanding range of *Aedes* mosquitoes has created a public health crisis and a growing need for building a predictive framework of their distribution and abundance.

One of the key vectors in this genus is *Ae. albopictus*. Several characteristics make this species ideally suited for zoonotic virus transmission. First, they show both exophagic (outdoor) and endophagic (indoor) feeding preferences (Delatte *et al.* 2010). Second, they exhibit significant anthropophilic preference for feeding on humans over other vertebrate hosts (Delatte *et al.* 2010). Third, females survive better following multiple blood-feeding (Rui-De *et al.* 2008), so often feed on humans and other hosts within a short time frame (Delatte *et al.* 2010). Finally, *Ae. albopictus* is a competent vector for at least 22 arboviruses (Gratz 2004).

Ae. albopictus was first established in the USA in the 1980s and spread rapidly through the 1990s (Kraemer *et al.* 2019). Although its spread has since slowed to ~60 km per year it is expected to expand to northern states over the next 30 years (Kraemer *et al.* 2019). Grant County, Arkansas, was among the first counties to report positive cases of this species (Moore, 1999). Despite this early detection many Arkansas counties still lack documented presence records for this species (Monaghan *et al.* 2019). Researchers have posed the hypothesis that apparent absences from Arkansas counties are due to limited vector surveillance, not due to an absence of the species (Moore 1999; Monaghan *et al.* 2019).

This study aims to fill knowledge gaps surrounding *Ae. albopictus* in Arkansas. Our first objective was to broaden sampling efforts to include more counties and improve upon existing species distribution maps. Beyond this presence data we also aimed to investigate patterns of temporal (month, year) and geographic (county, latitude) variation in mosquito prevalence. This study should help improve predictive models of *Ae. albopictus* distribution and abundance and help public health efforts target under-sampled or at-risk counties.

Methods

Field collection and sample processing

Eggs were collected from June-October in 2016 and April-October in 2017. Sampling was conducted across most, but not all, Arkansas counties. Trapping locations were near Arkansas Department of Health (ADH) Local Health Unit offices, and most trapping was carried out by ADH staff. The timing and frequency of sampling was opportunistic and varied across counties. This study includes data from 541 traps that were deployed across a total of 4,048 nights (Supplementary Table 1).

Oviposition traps were used to collect eggs from gravid female mosquitoes. These traps target container-breeding mosquitoes such as those from the genus *Aedes* (United States Air Force, 2006). Traps consisted of 16oz plastic cups (black or red) filled halfway with water. A week prior to trap placement a small amount of hay or grass clippings was added to each cup and allowed to infuse. At the time of trap placement, a small rock was added to for weight and a piece of textured brown cardstock added as a laying substrate. Traps were placed near buildings at no more than 1.3 m above the ground. Locations were chosen to be protected from rain and wind.

Traps were left in place for an average of seven days, though trap duration varied from 2 to 21 days. Longer trap placement would allow more time for mosquitoes to find the water and lay eggs, so we corrected for trap duration by dividing the number of eggs by the number of trap-days. Traps missing duration data were excluded from data reporting and analyses. Results remained qualitatively similar regardless of whether we corrected for trap duration.

Oviposition papers were dried completely at room temperature before being placed in Ziploc bags and mailed to Arkansas Tech University for processing. Upon receipt we visually identified and counted all mosquito eggs using magnifying glasses and dissecting microscopes. Although we did germinate eggs and rear mosquitoes through adult stages for species identification, low germination rates (~7%) prevent accurate reporting of data on adult mosquito abundance. Instead in this paper we report egg abundance data only. Importantly, >99.7% of the 1333 successfully reared adult mosquitoes were identified as *Ae. albopictus* (Barron, *unpublished).* While rearing conditions could have favored *Ae. albopictus* over other species, this is unlikely to explain this species' prevalence since oviposition traps specifically target this genus (United States Air Force, 2006), their eggs are morphologically distinct from other mosquito genera (Bova *et al.* 2016), and species in this genus can be reared under similar conditions (Dickerson 2007). We are thus confident interpreting egg counts as an estimate of *Ae. albopictus* abundance.

Statistical analyses

All trap locations within a county were combined and assigned a single latitude for that county based on coordinates from Google Maps (Google, n.d.). For each year we also categorized the 10 northernmost counties as "North", the 10 with middle latitudes as "Middle", and the 10 southernmost counties as "South".

Count data was square root transformed (y + 0.5) to improve normality (Sokal & Rohlf 1969; St-Pierre *et al.* 2018), though results remained qualitatively similar to analyses of raw data. We present figures with raw values for easier interpretation.

We used linear regression to compare the number of eggs to trap duration and to latitude. Comparisons of mean egg abundance across months, latitude categories, and counties were made using either an ANOVA or ANCOVA (for simultaneous consideration of month and latitude). All analyses were conducted using the statistical program NCSS (NCSS LLC, 2016).

Results

Mosquito egg counts varied across months in 2017 ($F_{6,273} = 8.98$, $p < 0.001$), with a late summer peak followed by a decline through the fall (Figure 1). Data from 2016 showed the same pattern though it was not statistically significant ($F_{4,260} = 2.13$, $p = 0.08$).

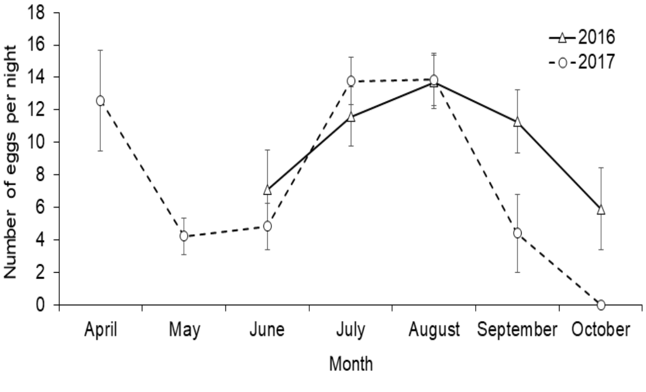

Figure 1. Mean (±SE) mosquito egg abundance by month in 2016 and 2017.

Egg abundance also varied by latitude in each year, though the direction of this pattern differed across year (Figure 2). In 2016, higher latitudes had lower egg counts ($R^2 = 0.04$, $b = -0.38$, $F_{1,263} = 11.47$, $p < 0.001$), whereas in 2017 higher latitudes had higher egg counts ($R^2 = 0.02$, $b = 0.23$, $F_{1,278} = 4.76$, $p = 0.03$). Analyses of categorical latitude regions showed similar results (Figure 3); northern counties showed the lowest number of eggs in 2016 ($F_{2,178} = 3.42$, $p = 0.03$) but the highest egg counts in 2017 ($F_{2,261} = 3.55$, $p = 0.03$).

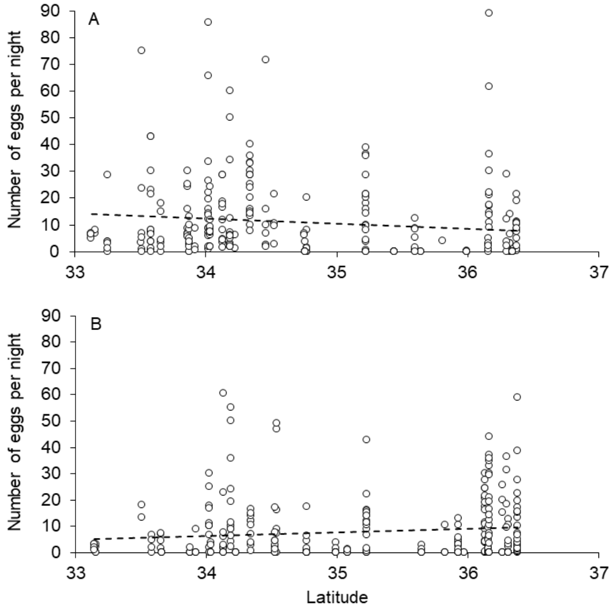

Figure 2. Mosquito egg abundance in relation to latitude in 2016 (A) and 2017 (B).

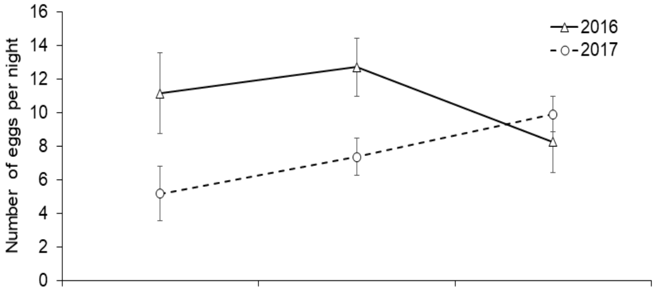

Figure 3. Mean (±SE) mosquito egg abundance versus latitudinal region. Latitudinal categories were developed by combining the 10 northernmost, 10 southernmost, and 10 middle latitude counties for each year.

When latitude and month were considered simultaneously both were significant in 2016 (month: $F_{4,259} = 2.67$, $p = 0.03$; latitude: $F_{1,259} = 13.48$, $p < 0.001$); mosquito abundance in middle latitudes was relatively consistent across the year, whereas northern and southern latitudes showed a mid-season peak (Figure 4A). In 2017 month remained significant ($F_{6,272} = 8.27$, $p < 0.001$) but latitude did not ($F_{1,272} = 1.17$, $p = 0.28$), although it should be noted that substantial latitudinal variation existed in April samples. Simultaneous consideration of month and latitude region showed relatively similar monthly patterns across latitudes (Figure 4).

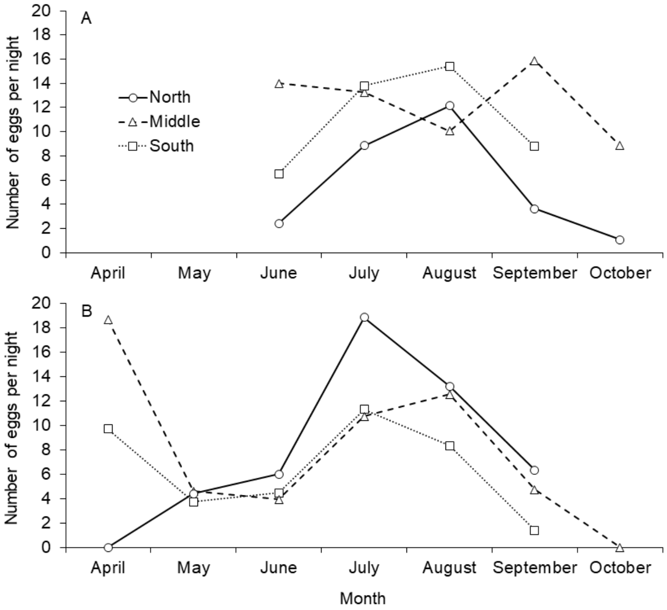

Figure 4. Mean monthly mosquito egg abundance by latitudinal region in 2016 (A) and 2017 (B).

Substantial variation existed across counties in both 2016 ($F_{32, 232} = 5.73$, < 0.001; Figure 5A; Supplementary Table 1) and 2017 ($F_{32, 247} = 5.42$, $p < 0.001$; Figure 5B; Supplementary Table 1). Furthermore, the geographical variation in mosquito abundance changed across the course of each year, as was visualized through progressive mapping of mosquito egg counts by month. In 2016 (Supplementary Video 1), mosquito abundance increasing in the south around June – our first month with data – and began to increase in the north by July. Northern counts remained high through August, after which abundance retreated toward southern counties. In 2017, similar patterns were observed (Supplementary Video 2). In May there is low abundance mostly concentrated in the south. Beginning in June, egg abundance began to increase in the north. Northern counties showed high counts through July and August, after which abundance decreased across the entire state.

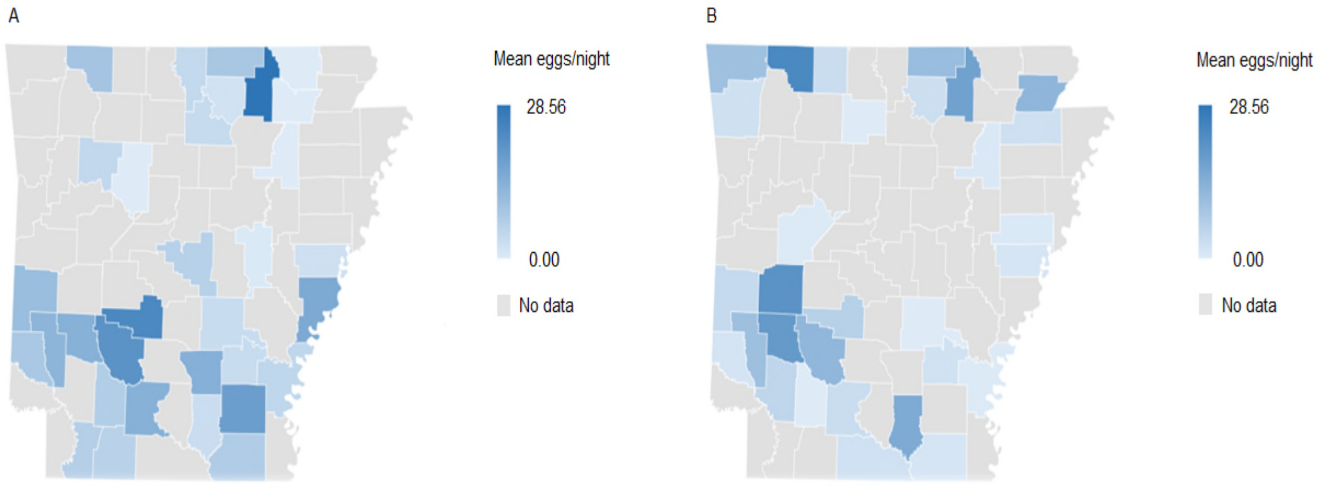

Figure 5. Mean mosquito egg abundance mapped by county for 2016 (A) and 2017 (B).

Discussion

This study generates entomological insight as the most extensive sampling effort to date for *Ae. albopictus* in Arkansas. We confirm the widespread distribution of this species throughout the state. The species was confirmed to be present in forty-one counties. Only three sampled counties (Pope, Randolph, Searcy) lacked positive counts. Considering these counties are discontinuous and bordered by positive counties we suspect these are false negatives that would be corrected with additional sampling. Thirty-one counties were not sampled, though again given the widespread occurrence of this species we expect it to be present in all Arkansas counties. Although the primary viruses this species carries are currently absent from Arkansas, the widespread distribution of this vector in Arkansas suggest future potential for local virus transmission.

Another consistent pattern we found is that *Ae. albopictus* counts were low in spring, rose to a peak in late summer, then declined through the fall. The annual emergence appears to begin in the south and spread northward with warming spring temperatures. Fall declines in abundance seem to be less dependent upon latitude, though more late-season sampling is necessary to define the end-of-season decline for this species. We would expect a corresponding peak in risk of virus transmission by *Aedes* mosquitoes in late summer. Mosquito abatement efforts may decrease or shorten this peak, during which time education campaigns should encourage strategies to decrease citizen exposure.

The Arkansas Department of Health was particularly interested in the abundance of *Ae. albopictus* in relationship to the possible spread of Zika virus through Arkansas. Although *Ae. albopictus* is in high abundance throughout the state none were known to transmit the disease (MANA Medical Associates, 2017). As of 2017, all known cases of Zika virus in Arkansas resulted from out-of-state travel. This lack of local transmission likely arises because the rarity of the virus in this region limits infected hosts and vectors and because *Ae. albopictus* is an inferior vector for this virus compared to the locally uncommon *Ae. aegypti* (Liu *et al.* 2017). However, potential for future local outbreaks of Zika virus remain a concern for several reasons. First, *Ae. albopictus* is a competent Zika virus vector (McKenzie *et al.* 2019) and can be the primary vector for Zika virus when they are widely distributed and in high abundance (Liu *et al.* 2017). Second, the abundance and northern distribution of *Ae. albopictus*, *Ae. aegypti*, and Zika virus (Kraemer *et al.* 2019) are all expected to increase in upcoming years due to climate change. For these reasons public health officials, epidemiologists, and entomologists should remain diligent surveilling for the Zika virus and its vectors in Arkansas.

Our study focused on *Ae. albopictus*, although *Ae. aegypti* is the better-known vector for arboviruses (Anoopkoomar *et al.* 2017). Currently Arkansas appears more environmentally suited *to Ae. albopictus* and it is significantly more prevalent than *Ae. aegypti* (Monaghan *et al.* 2019). *Ae. albopictus* has a quicker life cycle, thus it has a higher number of offspring and possibility of spreading any disease it is carrying at a quicker rate than *Ae. aegypti* (Anoopkumar *et al.* 2017). Previous studies have indicated *Ae. albopictus's* vector capacity is reliant on temperature as well as area of origin (Onyango *et al.* 2020; Azar *et al.* 2017). Our data could aid in determining vector capacity of the species in Arkansas and creating predictive models of the possible future impacts of *Ae. aegypti*.

Mosquito counts varied substantially in this study. The observed variation could arise from a combination of factors. From a methodological standpoint, we had considerable variation in sampling effort. Some counties sampled regularly across both seasons, whereas sampling in other counties was sporadic or absent. For example, in 2016, many northwestern counties did not submit data, and in 2017, data was lacking from central and southeastern counties. It is possible that this sampling bias could have influenced geographic and temporal patterns reported herein. Future effort should aim to implement more systematic statewide sampling of all counties.

Environmental factors such as weather could also drive the variation we observed. The year of 2016 was the second warmest year in U.S. history, closely followed by 2017. Although the difference in temperatures between the 2 years was small, 2017 had more precipitation, flooding, and hurricanes (NOAA, 2018). Previous research has indicated that precipitation rates do affect the abundance of *Ae. albopictus*, with moderate levels of precipitation leading to peak egg abundance (Kache *et al.* 2020). Warmer temperatures changing precipitation patterns could alter favorability for *Ae. albopictus* breeding. Efforts to disentangle the relative influence of temperature, precipitation, and other environmental factors would inform models of this species response to climate change and improve our ability to predict outbreaks of this species across space and time.

In conclusion, the data obtained from this study is a stepping-stone towards a better understanding of the distribution of *Ae. albopictus* in Arkansas. It conveys a pattern of lower *Aedes* abundance in the spring and fall months with peak counts in July and August. The data also indicates annual variation in geographical distribution, possibly as a result of temperature or precipitation differences. These observations could be of great significance if the species' population in Arkansas expands or becomes known to carry human viruses. More complete and systematic sampling of the species is needed before we can accurately predict local and statewide risk from this arbovirus vector.

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Investigation of Fluorescence in Selected Mammals of Arkansas

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Running Title: Fluorescence in Arkansas Mammals

Abstract

The adaptive value of fluorescence among the vertebrates has been studied most in fishes and birds, and only a few observations have been published regarding fluorescence in the pelage of mammals. Recently, reports of fluorescence in some marsupials, the platypus, and in flying squirrels have become available. We report the occurrence of fluorescent properties in some mammals from Arkansas. Most carnivores, bats, and rodents did not exhibit the property when viewed under UV light. However, opossums, rabbits, a weasel, muskrats, and moles showed substantial UV response, and a few other mammals showed minor fluorescence. Colors fluoresced included pink, green, and light cyan. Most species exhibited only 1 color, but the opossum responded with 2 colors. Potential explanations for positive responses to UV light include species signaling, mate assessment, predator avoidance, or prey location. Alternatively, the response may be an artifact without adaptive significance.

Introduction

When ultraviolet (UV) light is reflected from an object, the color is the same as the light projected (purplish), and if the color remains the same as it appeared in white light, the UV light was absorbed. Fluorescence is the property in which an object absorbs radiation of a shorter wavelength (higher energy) and emits a longer wavelength of lower energy, resulting in what is perceived as a different color. The result of UV fluorescence can be a glowing effect not visible to human eyes in white light. Human perception of UV light is limited, but many vertebrates see into the UV range (Bennet and Cuthill 1994). However, when fluorescence can make visible a color within the range of white light, an animal does not have to see into the UV spectrum, just the fluorescence itself (Marshall and Johnsen 2017). Though most mammals (with the exception of some primates) cannot discern colors representing the full spectrum in white light, many can detect UV light (Douglas and Jeffery 2014; McDonald *et al*. 2020), which opens some avenues of interpretation of adaptive use of fluorescence by mammals.

Still, little is known about occurrence of fluorescent properties in pelage of mammals, and most reported observations record the phenomenon in marsupials. Meisner (1983) in a published abstract with little detail, mentioned that the North American opossum (*Didelphis virginiana*) showed complex patterns of fluorescence, and Pine *et al*. (1985), noted that 24 of 32 species of New World didelphid marsupials fluoresced in UV light. Australian marsupials including Krefft's glider (*Petaurus notatus*), striped possum (*Dactylopsila trivirgata*), and long-nosed bandicoot (*Perameles nasuta*) exhibited different fluorescent colors when exposed to UV light (Reinhold 2021).

Among placental mammals, all species of North American flying squirrels (genus *Glaucomys*) fluoresce a pinkish hue under UV light (Kohler *et al*. 2019, Tumlison *et al*. 2019). Australian native rats including the fawn-footed mosaic-tailed rat (*Melomys cervinipes*) and the bush rat (*Rattus fuscipes*) also fluoresce, as well as the introduced black rat (*Rattus rattus*) (Reinhold 2021). Vivid red fluorescence also has been detected in the springhare (Pedetidae), an Old World placental mammal (Olson *et al*. 2021).

Even the monotreme (egg-laying) mammals recently have been shown to fluoresce a green to cyan color under UV light (Anich *et al*. 2021; Reinhold 2020). Explanations of the cause or purpose of the phenomenon in nature range from mere artifact to adaptations for navigation and orientation, species recognition, mate assessment, camouflage, and predator avoidance (Cronin and Bok 2016).

Methods and Materials

The purpose of this study was to determine whether pelage of any Arkansas mammal species fluoresces under UV light, and to offer insights and hypotheses based on our observations. We did not study whether whiskers or skin might have fluoresced. We shined light from a UVBeast™ flashlight (385-395 nm) onto preserved specimens of mammals from Arkansas, housed in the Henderson State University Collection of Vertebrates, to determine if any form of fluorescence was emitted.

Specimens in this collection had been prepared between 1990-2021. Specimens were dry skins that had been preserved by skinning, fleshing, stuffing with cotton, and drying prior to storage in specimen cabinets. The fur had not been sprayed or treated with any chemical preservatives or insecticides. One alcoholic specimen had been preserved in 10% formalin, washed, and stored in 45% isopropanol. In a few cases, living specimens also were tested.

The light was held about 15-30 cm from the specimens (depending on the size of the area we desired to illuminate) and images were taken with a Samsung Galaxy S7 phone camera. We immediately compared images taken and our visual perceptions of colors, and determined that the colors were perceptibly the same. Specimens were examined dorsally and ventrally, and for species with thicker fur, we parted the fur in order to reveal any fluorescence in underfur.

Results

We detected no fluorescence under UV light in any specimens of Chiroptera (species examined: *Perimyotis subflavus* (tricolored bat, n=7), *Lasiurus borealis* (eastern red bat, n=12), *L. seminolus* (Seminole bat, n=3), *Aeorestes* (=*Lasiurus*) *cinereus* (hoary bat, n=3), *Lasionycteris noctivagans* (silverhaired bat, n=2), *Eptesicus fuscus* (big brown bat, n=8), *Corynorhinus rafinesqii* (Rafinesque's big-eared bat, n=3), and *Tadarida brasiliensis* (Brazilian free-tailed bat, n=5)). Most Carnivora also revealed no fluorescence (species examined: *Procyon lotor* (raccoon, n=12), *Neovison vison* (American mink, n=6), *Spilogale putorius* (spotted skunk, n=2), *Mephitis mephitis* (striped skunk, n=3), *Urocyon cinereoargenteus* (gray fox, n=7), *Vulpes vulpes* (red fox, n=2), *Canis latrans* (coyote, n=2), and *Lynx rufus* (bobcat, n=5).

Flying squirrels fluoresce a pinkish coloration, especially on the white ventral pelage (Kohler *et al*. 2019; Tumlison *et al*. 2019). In our examination of another small squirrel, *Tamias striatus* (eastern chipmunk, n=12), a few specimens showed a mild pinkish appearance under UV light on ventral fur, but we believe the effect is too little to warrant further comment. Similarly, *Castor canadensis* (North American beaver, n=5) presented with mildly greenish guard hairs. Otherwise, most of the rodents we examined did not display fluorescent properties. These included: *Geomys breviceps* (Baird's pocketgopher, n=20), *Microtus pinetorum* (woodland vole, n=5), *Neotoma floridana* (eastern woodrat, n=19), *Ochrotomys nuttalli* (golden mouse, n=5), *Oryzomys texensis* (Texas marsh rice rat, n=3), *Reithrodontomys fulvescens* (fulvous harvest mouse, n=9), *Sigmodon hispidus* (hispid cotton rat, n=16), *Mus musculus* (house mouse, n=20), *Marmota monax* (woodchuck, n=10), *Sciurus carolinensis* (eastern gray squirrel, n=31), and *Sciurus niger* (eastern fox squirrel, n=23). Interestingly, fluorescence is known in bones and teeth of fox squirrels (Dooley and Moncrief 2012), and was witnessed in skeletal material within our collection, but the effect was not detected in pelage.

Fluorescent forms We found fluorescence in pelage of several species of mammals, some of which have been only vaguely mentioned in previous literature, and some here for the first time.

Didelphis virginiana (Virginia opossum, n=14). Three live adults and 3 dispersing juveniles (observed 24 April 2021) found by chance and illuminated with the UV light at night, glowed pink (Figure 1) with moderate to intense fluorescence in all 6 specimens. The dorsal underfur showed bright pink in 2 of the 8 museum specimens of opossums we examined, and a mild response was seen in another, under UV illumination. Seven of the 8 fluoresced noticeably pink on the ventral hairs (Figure 2).

Opossums sometimes have a whitish to yellowish throat patch, and in some individuals this patch continues as a streak down the thorax. Those patches fluoresced a light cyan color, making the area much brighter in UV. The patch was evident in 7 of 8

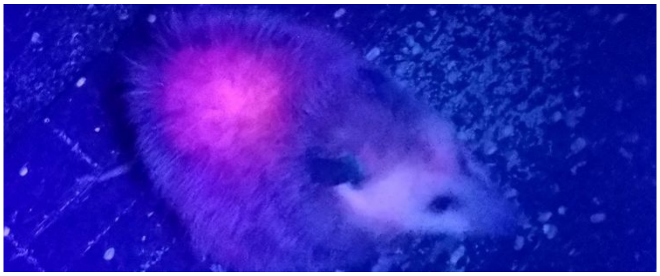

Figure 1. Image of a live opossum about 2 m distant from the UV light. On this individual, the underfur fluoresced a bright pink color, making the animal glow in the illumination. White hairs on the face fluoresced only mildly. All purplish coloration on the opossum and substrate resulted from reflectance of UV light.

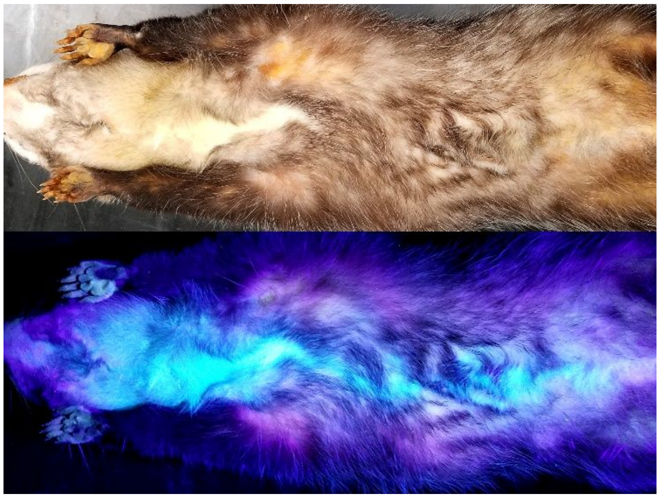

Figure 2. Comparison of the ventral pelage of an opossum (*Didelphis virginiana*) under white light (top) and UV (bottom). The whitish underfur produced a pinkish fluorescence under UV, but a midventral white streak produced a cyan color. In the black and white print version of this image, little difference is seen, but colors are vivid in the online version of this paper.

individuals we examined, and the response was most intense in specimens with darker yellow patches (as seen in white light). Yellow hairs in lab rats (Norway rats) similarly have been reported to fluoresce brilliantly (Rebell *et al*. 1956).

Other white hairs on the opossums, such as on the head, did not show this degree of response. Caution must be used in examination of greenish to cyan coloration, especially in the genital area, because urine remaining on hairs can also provide this response. However, the patterns we describe on the opossums were far anterior and limited to only the otherwise white hairs.

Scalopus aquaticus (Eastern mole, n=19). Besides 17 dry skins, we also examined an untreated frozen specimen and a specimen that had been fixed in 10% formalin and preserved in 45% isopropanol. Regardless of state of preparation, all specimens produced a vivid dull-greenish response, which was evident on dorsal and ventral perspectives (Figure 3). This indicated that preservation in fluids does not necessarily denature the effect, and that the effect likely did not result from museum preparation of the skins.

The upper shaft of hairs of moles has an expanded spatulate shield region, which was the only portion of the hair shaft that fluoresced. Examination under a dissecting microscope further revealed that the tips of the hairs were the primary locations of the greenish effect.

Figure 3. Dorsal and ventral perspective of pelage of the Eastern mole (*Scalopus aquaticus*) comparing appearance in white and UV light. All of the pelage fluoresced a greenish to cyan color when UV light was projected from the right angle. The widened spatulate tips of the hairs were the only parts of the hair shaft that fluoresced. In the color images, fluorescing green hairs can be seen, but the purplish hair at the periphery of the specimen is reflecting UV. Changing the angle of the UV source also changed which hairs reflected and which fluoresced.

Blarina carolinensis (southern short-tailed shrew, n=10). All of these shrews emitted a greenish fluorescence similar to that of the moles, but to a much lesser degree. The effect was most pronounced when the light was held vertical or posterior to the shaft. The mole hairs also reacted in that manner.

Rattus norvegicus (Norway rat, n=2).We detected green fluorescence scattered over the postcranial pelage of both specimens (Figure 4). Similar fluorescence was reported for a congener, the black rat (*Rattus rattus*; Reinhold 2021), and this fluorescence in white lab strains of the Norway rat is caused by Kynurenine (Rebell *et al*. 1956, 1957; Rebell 1966).

Ondatra zibethica (muskrat, n=3). Superficial examination of muskrat skins did not detect any fluorescence. Parting of the thick underfur, however, revealed a yellow-green response (Figure 5).The effect was limited to the posterior dorsolateral portions of the pelage, and the fluorescent part to the basal half of hair shafts of underfur hair, allowing the brown distal half of the hairs to obscure the grayish basal half of the lower shaft.

Figure 4. Norway rats (*Rattus norvegicus*) showing greenish-cyan fluorescence on the postcranial pelage. The head area reflected UV light so appears purplish.

Figure 5. Comparison of muskrat (*Ondatra zibethica*) fur under white and UV light. Fluorescent effect occurred only on the posterior dorsolateral positions on the 3 specimens examined. The darker gray portions of the hairs fluoresced, whereas the lighter gray upper portions reflected UV light (showing purple).

Mustela frenata (Long-tailed weasel, n=2). One of the 2 weasels examined produced a greenish response to UV light. The head area did not fluoresce, whereas the post-cranial brownish pelage (under white light) emitted a greenish hue under UV. In white light, the head and body were not evidently different in coloration. The fluorescence was especially distinctive when compared with skins of their near relatives, mink, which are only a slightly different shade of brown but did not fluoresce (Figure 6).

Latham (1953) found no fluorescence in the long-tailed weasel and ermine (*M. erminea*), both of which remained a dull brown under UV light, but least weasels (*Mustela rixosa* – now *M. nivalis*) fluoresced ‘a vivid lavender color’. We believe it more likely that the brown color indicated absorption of UV light, and the fluorescence was actually reflectance of the purplish (lavender) UV light. However, Toussaint *et al.* (2021) argued for the occurrence of lavender fluorescence in the ermine, though spectroscopic analysis did not reveal porphyrins (also, their specimen was in white winter pelage vs. the brown summer pelage described by Latham (1953)).

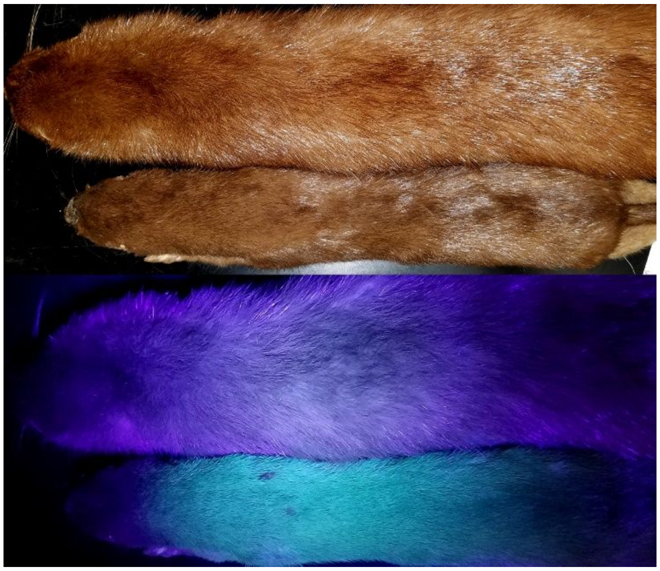

Figure 6. Comparison of mink (*Neovison vison*) and weasel (*Mustela frenata*) pelage under white light (top pair) and UV light (bottom pair). The mink skin is on top of both pairs. Note both are variants of brown in white light. Under UV, the mink largely reflects the light, creating a purplish color, whereas the weasel fluoresces a green hue.

Sylvilagus floridanus (Eastern cottontail, n=8). The dorsal pelage of the hind feet of cottontails is white under normal light, but emits a brighter light cyan coloration under influence of UV (Figure 7). Further, the brown hairs on the bottom of the foot fluoresced greenish. Other white hairs on the rump and venter also become brighter in UV. Eastern cottontails use alert postures and jumping sequences during interactions with females and rival males, and these involve lifting the hind section of the body above the plane of the shoulders (Marsden and Holler 1964), which exposes the surface of the hind foot and the tail region. Submissive postures hold the body close to the ground, largely hiding the foot and hindquarters. Fluorescence in this area might serve to amplify the display. Swamp rabbits (*Sylvilagus aquaticus,* n=7) have brownish hairs on top of the hind foot, which did not noticeably fluoresce although other white hairs of the area did show a brighter pale cyan to white.

Discussion and Conclusions

The value of fluorescence in the subphylum Vertebrata has been tested in a few species, but its

Figure 7. Comparison of dorsal surfaces of the hind foot of a cottontail rabbit (*Sylvilagus floridanus*) under white light (top) and UV light (bottom). The images show both hind feet of 1 specimen. Pale cyan fluorescence in UV light brightens the white hairs and increases the contrast with the brown hairs of the foot, which appear purplish due to reflectance of UV wavelengths.

purpose only hypothesized for many others. A primary question is whether natural fluorescence functions as a signal, or is merely a by-product of pigment structure or life history (Arnold *et al*. 2002). For example, fluorescence in marine turtles could be an artifact of diet including organisms that fluoresce, or presence of fluorescent algae on the carapace, but because males showed more intense effects, there could be an ecological role (Gruber and Sparks 2015).

Some tests have identified various adaptive purposes of UV fluorescence or UV vision. Blue tits are sexually dichromatic birds in UV light, which can be used in mate choice (Hunt *et al*. 1998). Parrots also use fluorescence for sexual signaling and mate choice (Arnold *et al*. 2002; Pearn *et al*. 2001). Many reef fishes use red fluorescence to enhance visual communication (Michiels *et al*. 2008), but they also can use it as camouflage if the background also fluoresces (Sparks *et al*. 2014). Fluorescence in the platypus may reduce visibility to UV-sensitive predators (Anich *et al*. 2021), but foraging animals may incorporate UV cues (reflectance and absorbance) from the environment, or of food items, into their foraging strategy (Honkavaara *et al*. 2002).

Fluorescent frogs may enhance their visibility to other frogs at twilight (Taboada *et al*. 2017). Some butterflies adaptively dupe bird predators by use of UV light to focus attacks on the eyespots on the back of their wings, thus avoiding fatal head grabs (Oloffson *et al*. 2010). Given all the possible interpretations to explain vertebrate ability to see UV light, or to fluorescence and thus make the UV visible, detailed studies are needed to examine any adaptive hypothesis for each mammalian species determined to fluoresce. If adaptive, fluorescence should adjust invisible UV light into the visible spectrum to some advantage. Based on our observations, we offer some recommendations and considerations for future study.

Kohler *et al*. (2019) suggested a possible link between fluorescence and nocturnality. All of the mammals we found to fluoresce are crepuscular to chiefly nocturnal. However, many nocturnal species did not fluoresce, including all bats and most rodents.

Moles are subterranean and have tiny eyes, thus ability to detect UV would seem to be of little value. Glösmann *et al*. (2008) reported that European moles could see UV light and offered an adaptive explanation as the ability to detect leakage of light where tunnel systems might need repair. Thus, an adaptive purpose of UV vision is possible, but this possibility would not explain a purpose for green fluorescent pelage that we observed in all specimens of moles.

Fluorescence appeared only in the basal portions, and in small areas, of muskrat underfur. Underfur hairs in muskrats have a long brown upper shaft, which does not fluoresce and also covers the reactive basal parts of the shaft. How moles or muskrats might adaptively use their obscured fluorescence is particularly unclear. Any adaptive value might be related to factors other than intraspecific interactions. However, we examined dry specimens of muskrats. As a wetland species, their fur often is wet, which causes hairs to matt and may allow the hidden fluorescent area to become exposed, and thus an adaptive possibility exists.

Underfur hairs of opossums typically have a short black tip and a long white to grayish remainder. The pink glow produced on some opossums was visible on that entire whitish portion of the shaft. Pine *et al*. (1985) noted the same fluorescent portions for other didelphid marsupials. All 6 of our live adult and juvenile opossums seen during springtime fluoresced dorsally, whereas only 2 of the 8 museum specimens did so (though most fluoresced ventrally).

Shrews showed a minor UV reaction of greenish color similar to that of the moles. Arguably, the taxonomic relationship between these mammals may indicate a shared response due to phylogeny. However, mink and weasel also are phylogenetically related but none of our sample of mink reacted to UV light, whereas 1 of 2 weasels produced a strong response. The head fur of that weasel absorbed UV but the body fluoresced a distinctive green. Additional specimens should provide better insight, but we hypothesize that the presence and distribution of UV coloration may differ among ages or stages of molt. Some species of owls can be aged via examination of which feathers fluoresce, because younger feathers following molt have more porphrins so fluoresce brighter, and older feathers less (Weidensaul *et al*. 2011). Further, Bollinger (1944) noted that fur of the brushtail possum (*Trichosurus vulpecula*) was more fluorescent when in the new growth phase. Thus, in species where UV response is inconsistent across specimens, we argue that age effects or stages of molt might be examined to explain such observations.

Our observations of fluorescence deep within the fur of muskrat might be related to patchy or wavy molt patterns (Ling 1970) with newer hairs fluorescing whereas older hairs do not, and have no other adaptive value. Seasonal molt in some species, regardless of age, also may relate to variation. The adaptive shift between white winter fur and brown summer fur of several arctic mammals is well known. Hypothetically, similar seasonal adjustments in hair pigmentation to utilize UV might be expected.

Small sample sizes in many studies prevented examination of data stratified by age and sex, which might reveal patterns, but good sample sizes used by Kohler *et al*. (2019) in flying squirrels and Olson *et al*. (2021) in springhares found no variation by age, sex, location, or time of collection.

Mammals we observed fluoresced green, cyan, and pink. Moles appeared green both dorsally and ventrally, but opossums showed pink dorsal to ventral underfur, and cyan in the white patch of the throat and mid-venter. Different fluorescing colors on the same individual could mean different signals, multiple effects to achieve 1 signal, only 1 may be adaptive, or both may simply be artifacts from pigmentation in the pelage. Our samples were too small to compare sexes and ages, but not all opossums showed the same intensity, or even presence, of fluorescence.

Time and method of preservation may affect pelage of prepared skins of mammals. Labile pigments in some hairs may change after death (Pine *et al*. 1985), and if those fluoresced while the animal was alive, studies of museum specimens may not reveal the property. Chemicals used in preparation of wet specimens or tanning solutions may alter pigment structure and remove or reduce fluorescent properties. Specimens preserved in alcohol after fixation in formalin may be less likely to retain the effects, though our 1 alcoholic mole specimen retained its fluorescent effects. We present data from museum specimens that did retain fluorescent properties, but we note that some museum specimens may have variably lost or retained the property. For example, all of the live opossums we examined fluoresced dorsally but not all museum preparations did so. Pine *et al*. (1985) and Olson *et al*. (2021) also suspected that fluorescence might be brighter in live animals, and might degrade in museum specimens over time.

Finally, it should be noted that the best test of fluorescence is by use of fluorescent spectroscopy to determine wavelengths of perceived responses. We have provided new observations that warrant further study with more technical projects.

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Distal Excurrent Ducts and Penile Morphology of the Urogenital System in the Mississippi Mud Turtle, *Kinosternon subrubrum hippocrepis* (Chelonia: Kinosternidae)

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Running Title: Distal Excurrent Ducts and Penile Morphology in the Mud Turtle

Abstract

We examined the distal excurrent ductal morphology and penile anatomy of the urogenital system in the Mississippi Mud Turtle, *Kinosternon subrubrum hippocrepis,* from a small sample of individuals collected in Arkansas in order to provide additional information regarding turtle urogenital anatomy. Specifically, we focused on the basic anatomy and histology of distal excurrent ducts (ductus deferens and ureter), associated structures (urogenital papillae), and penile histology in this kinosternid turtle. In addition, we provide an overview of the gross urogenital anatomy in this turtle, given that little detailed information exists on this topic in the chelonian literature.

Introduction

Recent descriptions and illustrations of the male squamate urogenital system have added much clarity to the basic functional morphology as interpreted through histological and ultrastructural studies of this anatomical region (e.g., Trauth and Sever 2011; Rheubert *et al.* 2015; Pewhom and Srakaew 2018; Trauth 2018, 2020). On the other hand, even the most general gross morphological illustrations of the male turtle urogenital system (MTUGS), which includes the testes, kidneys, penis, and associated excurrent ducts, have been largely neglected in most turtle species. For instance, the gross anatomy of the MTUGS is usually depicted in the form of schematic diagrams in comparative vertebrate anatomy textbooks (e.g., Kardong 2015) and in laboratory dissection guides (e.g., Ashley 1962). As a whole, macroscopic illustrations or actual anatomical displays through the use of photomicrographs encompassing the entire MTUGS appear infrequently in books on turtle reproductive biology (e.g., Kuchling 1999) and in the general literature (Fox 1977). Traditionally, the primary research regarding the MTUGS has focused on elucidating the male sexual cycle through examination of testicular histology (for review of sexual cycle literature, see Miller and Dinkelacker 2008) and by reporting on either kidney microanatomy (Solomon 1985) or macroanatomy (Thigpen *et al.* 2020). Other MTUGS studies have centered specifically on penile morphology (Gerecke 1932; Abe 1956; Seshadri 1956; Majupuria 1959; Zug 1966; Kelly 2002, 2004) or on extra-testicular excurrent ducts, specifically the proximal efferent ductules (Waqas *et al.* 2015). Very few microscopic studies have examined the histology of the distal excurrent ducts (i.e., the ductuli deferentia and the ureters) of the urogenital region, a distinct portion of the ducts which lies between the anteriormost structures (testes, epididymides and kidneys) and the posteriormost structure, the penis, that when unerect, lies along the ventral surface of the cloaca (Nicholson and Risley 1940; Zug 1966; Blüm 1986). Herein, we refer to this specific anatomical segment of excurrent ducts as the distal excurrent ductal region (DEDR).

In the present study, we histologically examined the DEDR and penile anatomy of the Mississippi Mud Turtle—MMT (*Kinosternon subrubrum hippocrepis*). This species is a small, semi-aquatic kinosternid turtle commonly found within mostly lowland habitats throughout much of Arkansas (Trauth *et al.* 2004; Powell *et al.* 2016). In addition, we provide gross morphological features of the entire MTUGS for the first time in this turtle family.

Materials and Methods

We collected 3 adult male specimens of the MMT from Arkansas. Each turtle was humanely sacrificed with an intra-pleuroperitoneal injection of dilute sodium pentobarbital in accordance with IACUC protocol guidelines at Harding University. We measured the standard carapace length (SCL) in each turtle and permanently assigned each a museum number (Arkansas State University Museum of Zoology [ASUMZ]: ASUMZ 33828 [SCL = 89 mm] and 33830 [SCL = 79 mm], captured 9 May 2018 from White County; ASUMZ 34058 [SCL = 76 mm], captured 9 June 2019 from Lonoke County). Specimens are deposited in the herpetological collection in the Arkansas Center for Biodiversity Collections at Arkansas State University.

One of us (SET) removed the entire urogenital system from each turtle, photographed the gross anatomy, and then placed tissues into 10% neutral buffered formalin (NBF) in preparation for histological examination. Following fixation, the tissues were temporarily placed into vials of 70% ethanol and were readied for light microscopy following the paraffin embedding techniques outlined in Presnell and Schreibman (1997). In brief, the histological steps included dehydrating tissue in increasing ethanol solutions (70 to 100%), clearing in 100% xylene, infiltrating in paraffin overnight in a paraffin oven (56°C), embedding in paraffin using plastic molds (tissues positioned to yield either transverse or longitudinal sections), sectioning with a rotary microtome into 10 µm serial strips (affixed onto glass microscope slides coated with Haupt's adhesive prior to floating strips in 2% NBF 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 the microscope slides with Permount© (Fisher Scientific Products).

For histosection photomicroscopy, SET used a Leica MC 120 HD camera atop a Leica DM 2000 LED compound light microscope. For macrophotography, SET used a Canon T4i digital single lens reflex camera fitted with a 50 mm macro lens. Most descriptions of urogenital structures follow the terminology found in Zug (1966), Blüm (1986), Kuchling (1999), and Trauth (2018, 2020). Microscope slides are currently housed in the Trauth Histo-herpetology Laboratory in Morrilton, Arkansas.

Results

Gross Urogenital System Morphology

The gross anatomy of the urogenital system (ventral aspect) of a reproductively active male MMT is shown in Figure 1, and we provide here a brief description of the structural morphology. The paired, ellipsoidal-shaped testes are positioned ventromedial to the paired kidneys and appear a light tawny brown in color. The kidneys are deep brown, exhibit superficial convolutions, and extend cranially and caudally away from the testes. Each highly looped and tightly bound epididymis lies ventrolateral to each testis and projects a silvery-white sheen due to the presence of sperm and seminal fluids within the ductus epididymis. Short paired excurrent ducts (ductus deferens and the ureter) lead caudally away from the testes and kidneys. These ducts appear superficially together (Fig. 1; see also excurrent duct configurations in Figs. 4 – 6) as they lie ventral to the supportive dorsal musculature and connective tissues within the urogenital complex. (Both excurrent ducts descend deeply to connect with the urogenital sinus near the anterior extension [coprodeum] of the cloaca; see Figs. 2 and 3 for gross anatomical positioning of ducts.) The urinary bladder attaches ventrally to the urogenital sinus. A greatly expanded and darkly pigmented penis terminates as the

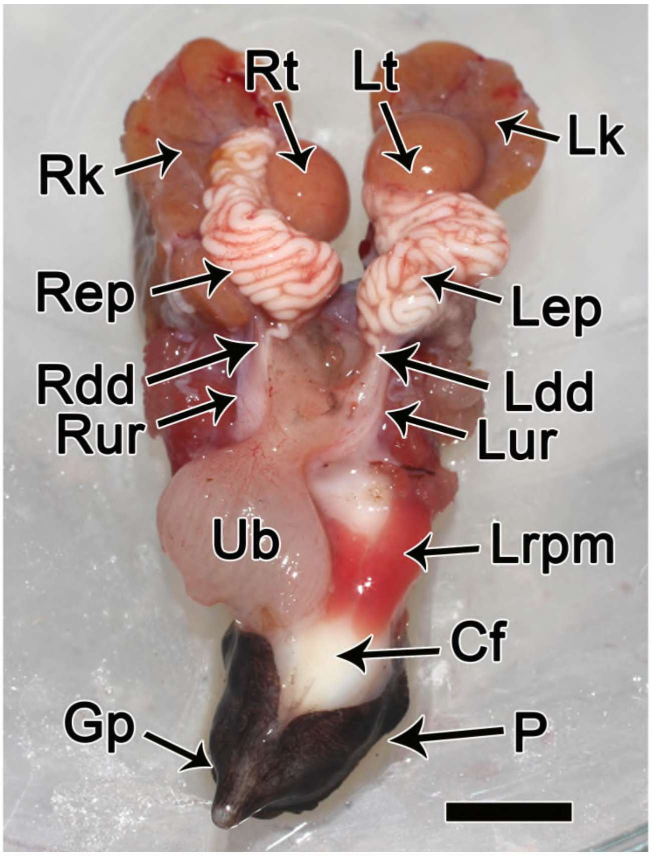

Figure 1. Ventral aspect of the urogenital system of a recently sacrificed, reproductively active MMT (ASUMZ 33828). Cf, corpus fibrosum; Gp, glans penis; Ldd, left ductus deferens; Lep, left epididymis; Lk, left kidney; Lrpm, left retractor penis muscle; Lt, left testis; Lur, left ureter; P, penis; Rdd, right ductus deferens; Rep, right epididymis; Rk, right kidney; Rt, right testis; Rur, right ureter; Ub, urinary bladder. Scale bar = 5 mm.

narrow-projecting glans penis (Fig. 1). Retractor penis muscles attach to the ventrolateral surfaces of the corpus fibrosum of the penis (see Figs. 8 and 9 for penile structural anatomy).

Light Microscopy

DEDR.—The positioning of the DEDR in relation to the other anatomical structures of the MTUGS is revealed by examining 2 longitudinal histosections of the tissue complex (Figs. 2 and 3). In Figure 2, a dorsal slice shows the coprodeum of the cloaca nestled between the more anterior testes and kidneys and the dorsal musculature of the carapace; the epididymis as well as the DEDR are not visible in this image. However, in Figure 3, which depicts a deeper and more ventral histosection in comparison to Figure 2 and represents an anatomical section at the level of the epididymis, one pair of excurrent ducts is now evident as small, oblong, transverse slits on the left side of the tissue complex (Fig. 3). The interior lining of the coprodeum exhibits a highly-folded epithelium; this anatomical feature is visible inside the relatively thick muscularis externa layer that surrounds the coprodeum and is shown in Figures 4 – 7.

The entire DEDR is best illustrated through a composite series of cranial-to-caudal, transverse histosections (Figs. 4 – 6) beginning at a point of attachment between each ureter and its respective kidney (Fig. 4A). At this juncture, each ductus deferens is transitioning from a coiled ductus epididymidis into a straight duct lying medial to each ureter. In a more caudal section (Fig. 4B), each ureter has become detached from its respective kidney and is now incorporated into a rapidly expanding smooth muscle/connective tissue mass along with its ipsilateral ductus deferens. Eventually, the ductus deferens and ureter move together more medially to lie in close proximity to the anteriormost region of the coprodeum (Figs. 4C; 7A) prior to its transition to become the urodeum (Fig. 6B) of the cloaca. The coprodeum remains characterized by a relatively thick muscularis externa (Figs. 4C; 6B; 7).

As the urogenital sinus appears ventral to the coprodeum (Fig. 5A), each pair of excurrent ducts are now firmly positioned ventromedial to the coprodeum. The ducts also lie embedded in a smooth muscle/connective tissue mass that incorporates them but also includes the coprodeum and the urogenital sinus. Also, as observed within the coprodeum, the urogenital sinus characteristically possesses some folding of the epithelial lining. With the appearance of the urogenital papillae along the lateral surfaces of the urogenital sinus (Fig. 5B), the excurrent ducts begin to lose their outer muscular walls, flatten, and extend toward the tip of each papilla. Each ureter now lies dorsal to each ductus deferens. The ductus deferens and ureter then empty their contents independently into the urogenital sinus through 2 distinct urogenital orifices (Figs. 5C; 6A); the ductus deferens opens cranially, and the ureter opens caudally.

The urogenital sinus begins to narrow into a butterfly-shaped cavity in a region just posterior to the orifices of the urogenital papillae (Fig. 6B). Small, papilla-like structures can be seen projecting from its dorsal wall and, to a lesser extent, from its ventral epithelial lining. Also, a thinning of the muscularis externa of the coprodeum has occurred. A slightly more posterior section reveals the merging of the urogenital sinus with the urodeum (Fig. 6C). At this point the muscular wall of the coprodeum has lost most of its structural integrity and has expanded ventrally to cojoin with the thin bands of muscle and loose connective tissue surrounding the urogenital sinus; the

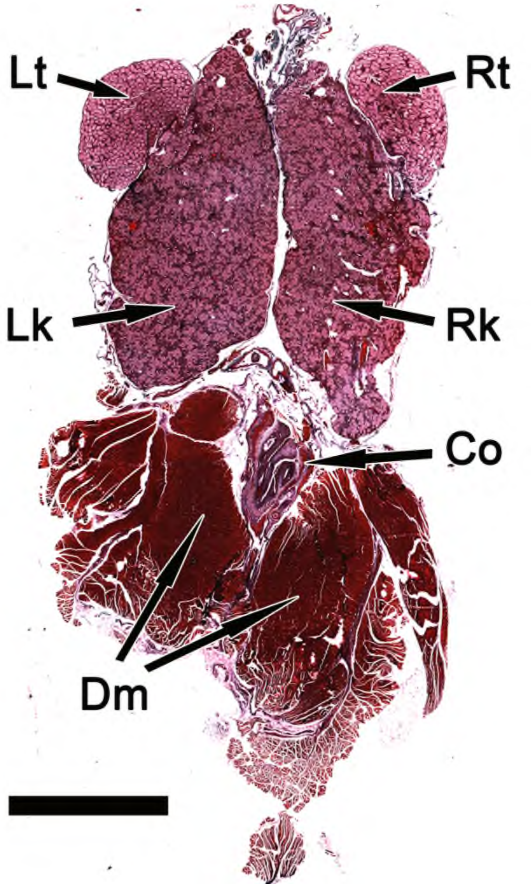

Figure 2. Photomicrograph of a longitudinal dorsal section through the urogenital structures of a reproductively active male MMT (ASUMZ 34058). See text for explanation of structures. Co, coprodeum; Dm, dorsal musculature; other abbreviations same as in Fig. 1. Scale bar= 5 mm. Pollak.

combined cavity is now referred to as the urodeum of the cloaca.

The epithelial lining of the ductus deferens reveals morphological variation along its pathway from the epididymis to the urogenital papilla. Initially, the duct exhibits a thin epithelial layer of low columnar cells but as it approaches the urogenital papilla, the lining changes to an irregular pseudostratified columnar epithelium (Fig. 7C). Its smooth muscular wall (Figs. 4C; 6B), however, remains intact, being dominated by a relatively thick outer circular band of smooth muscle compared to a much thinner inner longitudinal band. Both layers remain unchanged until entering the base of the urogenital papilla (Fig. 7B, C). By comparison, the epithelial lining of the ureter contains a transitional epithelium throughout its entire length (Fig. 7A, B).

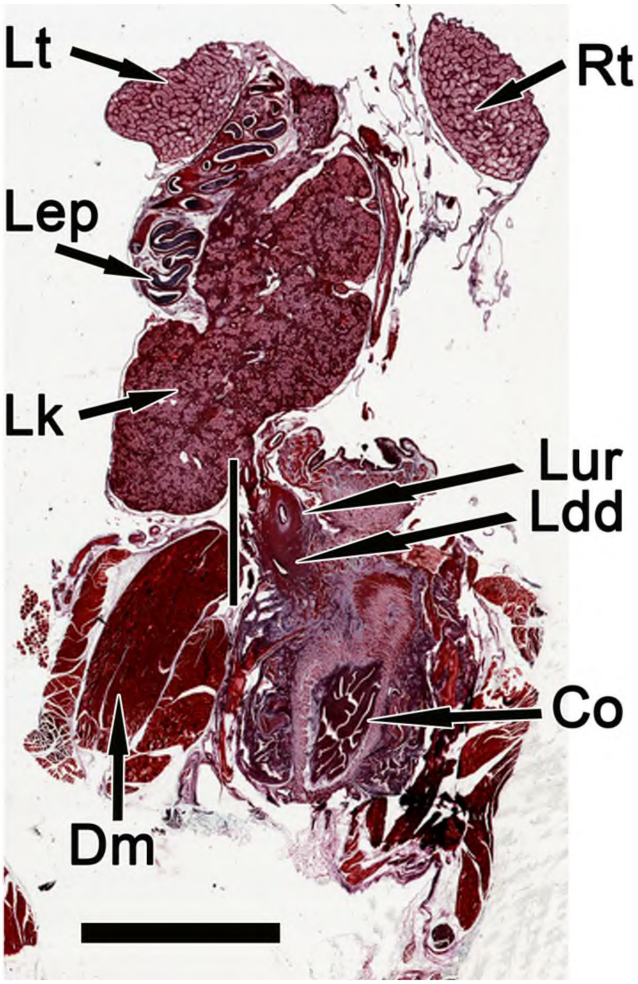

Figure 3. Photomicrograph of a longitudinal dorsal section of ASUMZ 34058 (Fig. 2) showing the emergence of the DEDR (area delineated by the vertical bar) anterior to the coprodeum. Notice the extensive internal foldings of the coprodeal lining. The left ureter (Lur) and the left ductus deferens (Ldd) are wrapped in a smooth muscle/connective tissue sheath. Abbreviations same as in Figure 1. Scale bar = 5 mm, Pollak.

Its muscular wall contains a much-reduced muscularis band compared to that of the ductus deferens.

The structural wall of the coprodeum (Fig. 7) remains pronounced as this cavity accompanies the 2 excurrent ducts posteriorly toward the urogenital sinus. The columnar epithelial lining of the coprodeum has numerous goblet cells along its entirety, and a well-defined muscularis externa comprised of longitudinal and circular bands of smooth muscle is evident. These bands mostly disappear during its transition to become the urodeum (Fig. 6C).

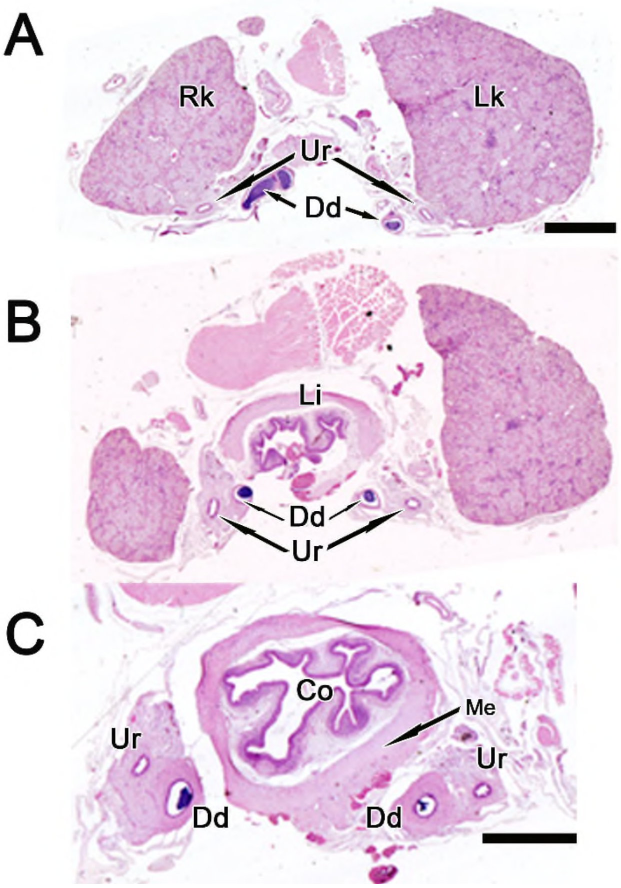

Figure 4. Photomicrographs of transverse sections showing the positioning of the distal excurrent ducts of ASUMZ 33830 in relation to the coprodeum (Co). See text for further explanation of ducts and structures. Li, large intestine; Me, muscularis externa. Abbreviations same as in previous figures. Scale bars= 1 mm; H&E.

Penile anatomy.—The penis of the MMT consists of a proximal shaft and a distal glans penis. The shaft contains of 2 types of erectile tissue: the ventral corpus fibrosum and the dorsal surface layer, the corpus spongiosum (Fig. 8). Anteriorly, these 2 tissue components appear as a circumferential layer surrounding a cavernous interior comprised mostly of blood vessels and loose connective tissue (Fig. 8A); however, the two are clearly separate entities (see diagonal line in Fig. 8D), posteriorly, prior to the glans.

The corpus fibrosum consists of a thick, dense plate of connective tissue that provides the primary support for the penis. Proximally, the corpus fibrosum rises as a broad, slightly curved structure (Fig. 8A), but more distally a superficial partitioning of it into a bi-lobed mass occurs as the anterior medial sinus of the seminal groove penetrates deeply into the plate (Fig. 8B – F). However, at the proximal region of the glans (Fig. 8G, H), these 2 masses unite to form a much

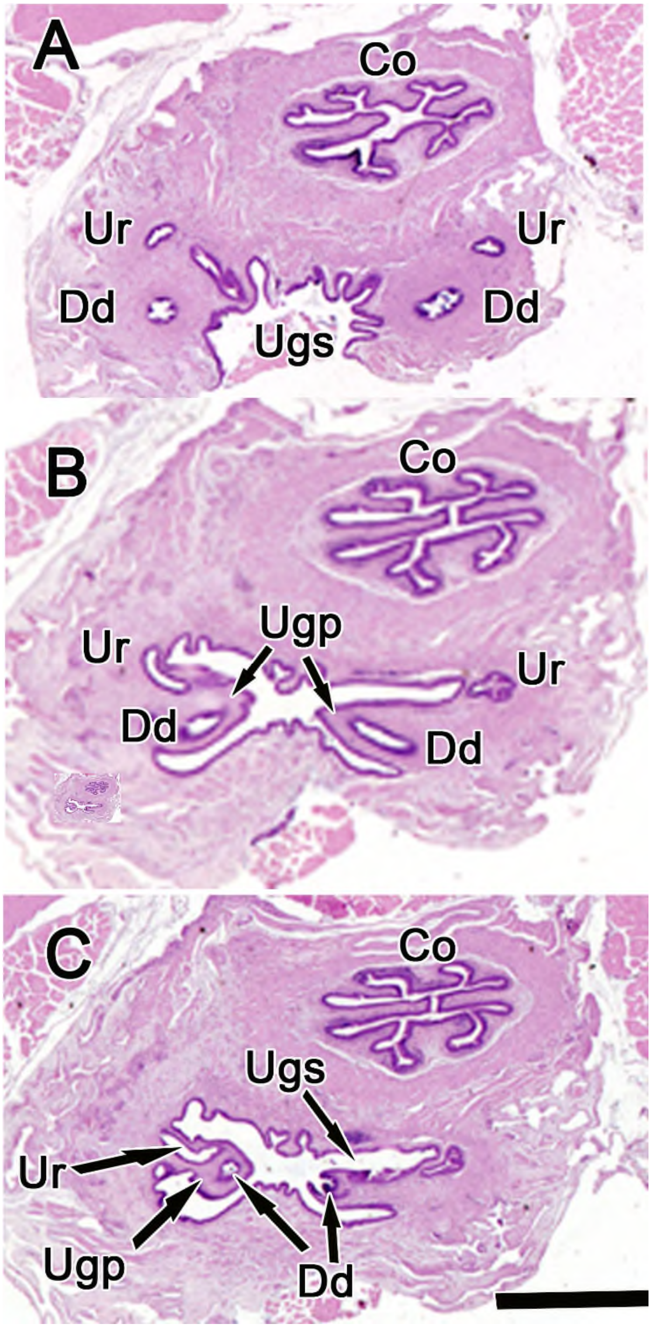

Figure 5. Continuation of Figure 4. Photomicrographs showing the positioning of the coprodeum in relation to the urogenital sinus and excurrent ducts. See text for further explanation of ducts. Ugp, urogenital papilla. Abbreviations as in previous figures. Scale bar = 1 mm for A – C, H&E.

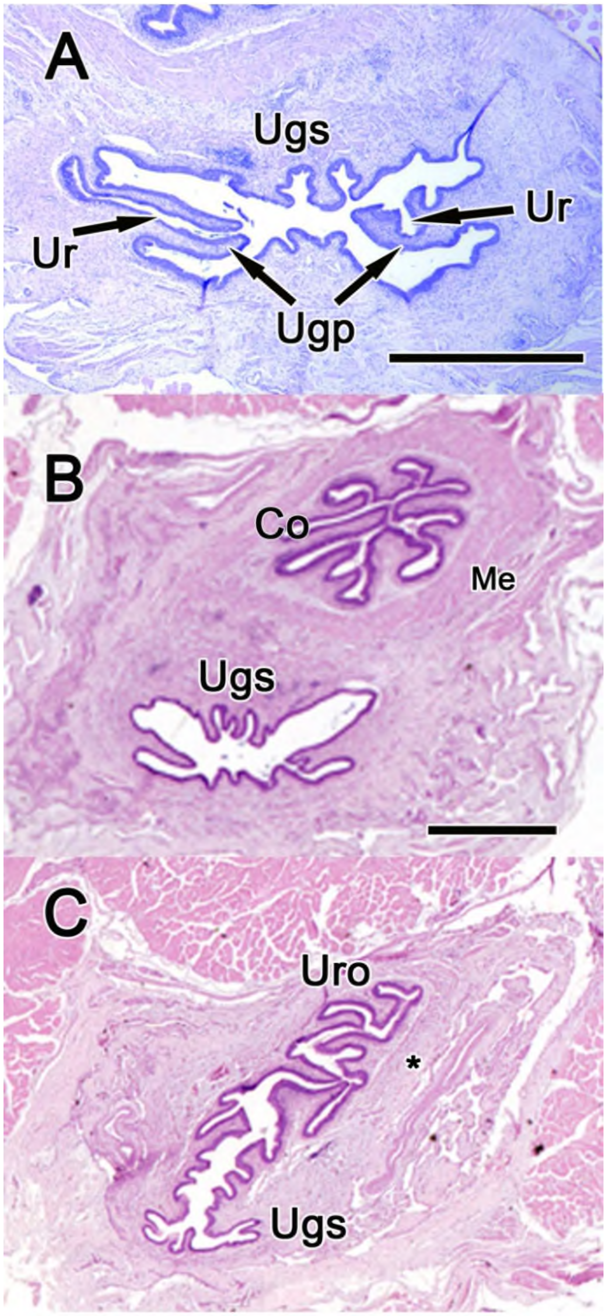

Figure 6. Continuation of Figure 5. Photomicrograph in A shows urogenital sinus just posterior to orifices of the ductus deferens (Fig. 5C). B. Remnants of the urogenital papillae remain in urogenital sinus caudally from urinary orifices. C. Merging of the urogenital sinus with the urodeum (Uro) of the cloaca. Asterisk in C demarks a thinning of the muscularis externa (Me, as seen in B). See test for further explanation of structures. Abbreviations as in previous figures. Scale bar = 1 mm for A; scale bar in B = 1 mm for B and C; H&E.

reduced, oval-shaped, rod-like structure (Fig. 8H). Paired retractor penis muscles shown in Figure 1 attach to the ventrolateral surfaces of the corpus fibrosum (Fig. 8B – F). The corpus fibrosum extends distally beyond the glans and ends in a point (Fig. 1).

The corpus spongiosum arises as a highly vascularized connective tissue layer that is anteriorly bisected dorsally by the seminal groove (Fig. 8). The seminal groove superficially separates the corpus spongiosum into 2 seminal ridges (Fig. 8A – D); however, a medial connection between the 2 ridges is present (see arrow in Fig. 8B). The corpus spongiosum also gives rise to the entire glans penis as it rests atop the corpus fibrosum. The seminal groove extends from the urethral opening (not shown here) along the proximal wall of the urogenital sinus and posteriorly into the glans.

There are paired surface depressions (sinuses) associated with the seminal groove. They lie atop each seminal ridge (Fig. 8E, F). In addition, a single sinus, the anterior medial sinus of the seminal groove (Fig. 8B – D), extends anteriorly and medially beneath the corpus spongiosum.

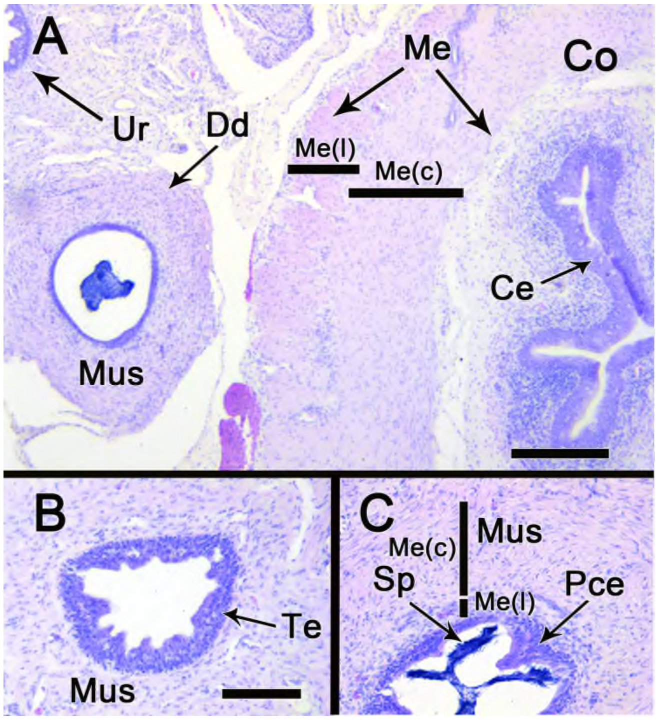

Figure 7. Photomicrographs of epithelial linings and wall structure of the excurrent ducts and coprodeum. Me(c) and Me(l) represent circular and longitudinal smooth muscle bundles of the muscularis externa. Ce, columnar epithelium; Mus, muscularis; Pce, pseudostratified columnar epithelium; Sp, spermatozoa. See text for further explanation of structures. Abbreviations as in previous figures. Scale bar in A = 250 µm; in B – C, 100 µm. H&E.

The glans penis is a highly folded structure consisting of 2 types of pleats. The largest fold, the plica externa, forms the lateral and distal borders of the glans (Figs. 8H; 9). Anterior sinuses of the glans penetrate anteriorly within the enlarged proximal bases of each plica externa (Figs. 8H; 9A) and then increase in size within the paired proximal portions of plica externa. These anterior sinuses terminate as a smaller fold, the plica media, arises (Fig. 9A, B) along the medial surface of the plica externa. The plica media forms a pair of papilla-like folds on each side of the seminal groove and also has a distal fold, the distomedial triangular fold. The seminal groove enters the glans singly (Fig. 9A, B) and then trifurcates with 2 branches leading into each lateral fold of the plica externa (see triple arrow set in Fig. 9C) and a medial third branch that leads distally onto the distomedial triangular fold near the termination of the glans (Fig. 9D). The posterior sinuses of the seminal groove occupy the distal cavities of the plica externa.

Discussion

As pointed out earlier, morphological descriptions of the MTUGS are not available for most turtle species and even fewer microscopic studies on turtles have examined the histology of the distal excurrent ducts. Thus, comparing our results with those of other turtle species is not possible. Instead, we will compare our results with those from several microscopic studies on squamates.

The intricate terminal anatomy of the DEDR in the MMT differs markedly to the comparable anatomical region for these ducts found in male squamate reptiles. For example, colubrine snakes possess a single common chamber, the ampulla urogenital papilla (AUP), in which the ductus deferens and ureter dump their reproductive materials and nitrogenous wastes, respectively, directly into before these materials exit from the AUP into the cloaca through urogenital papillae (Trauth and Sever 2011). On the other hand, crotaline snakes lack an AUP, but possess an expanded distal segment of the ureter, the ampulla ureter (AUR), which receives reproductive components from the ductus deferens. All urogenital products are then dumped into the urodeum of the cloaca from the AUR through urogenital papillae (Trauth and Sever 2011). No similar terminal modifications of this excurrent duct system were observed in the MMT.

Considerable variability exists in the structural morphology related to the release pathways of urogenital products in male lizards (Fox 1977). An

AUP is present in only a few lizard families (e.g., in Gerrhosauridae, Gymnophthalmidae, Teiidae, and Varanidae). In addition, some lizard species, such as skinks (family Scincidae), possess excurrent ducts that unite prior to the orifice of the urogenital papilla (Rheubert *et al.* 2015; Trauth 2018, 2020). This type of adjoining of the excurrent ducts cranial to the urogenital papilla was not observed in the MMT. Instead, the seminal materials from the ductus deferens exit the urogenital papilla anteriorly through a separate terminal orifice. The ureter then follows, dumping its contents through a urinary orifice found posterior to the genital orifice of the urogenital papilla (Figs.5C; 6A). The position of these ducts differs from their anatomical counterparts described by Nicholson and Risley (1940) in Blanding’s Turtle, *Emys blandingii*, and by Ashley (1962) in the Red-eared Slider, *Trachemys scripta elegans*, in which the urinary orifice lies in an anterior position. However, as is the case with these 2 turtle species, total separation of excurrent duct orifices is present on the urogenital papilla of the MMT. Because of this papillary configuration, some authors have referred to the reproductive papilla as the genital papilla as mentioned by Nicholson and Risley (1940) and Ashley (1962). That papillary designation would not apply to the MMT.

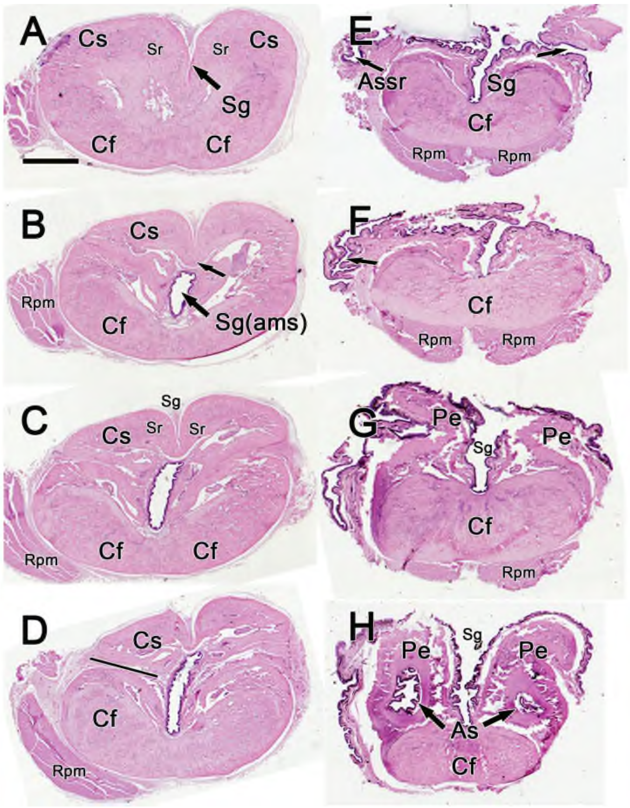

Figure 8. Photomicrographs of transverse sections of penile morphology in ASUMZ 33828. See text for explanation of structures. As, anterior sinus of the glans; Assr, anterior sinus of the seminal groove atop the seminal ridge; Cf, corpus fibrosum; Cs, corpus spongiosum; Pe, plica externa; Rpm, retractor penis muscle; Sg, seminal groove; Sg (ams), anterior medial sinus of the seminal groove; Sr, seminal ridge. Scale bar in A = 1 mm for A – H. H&E.

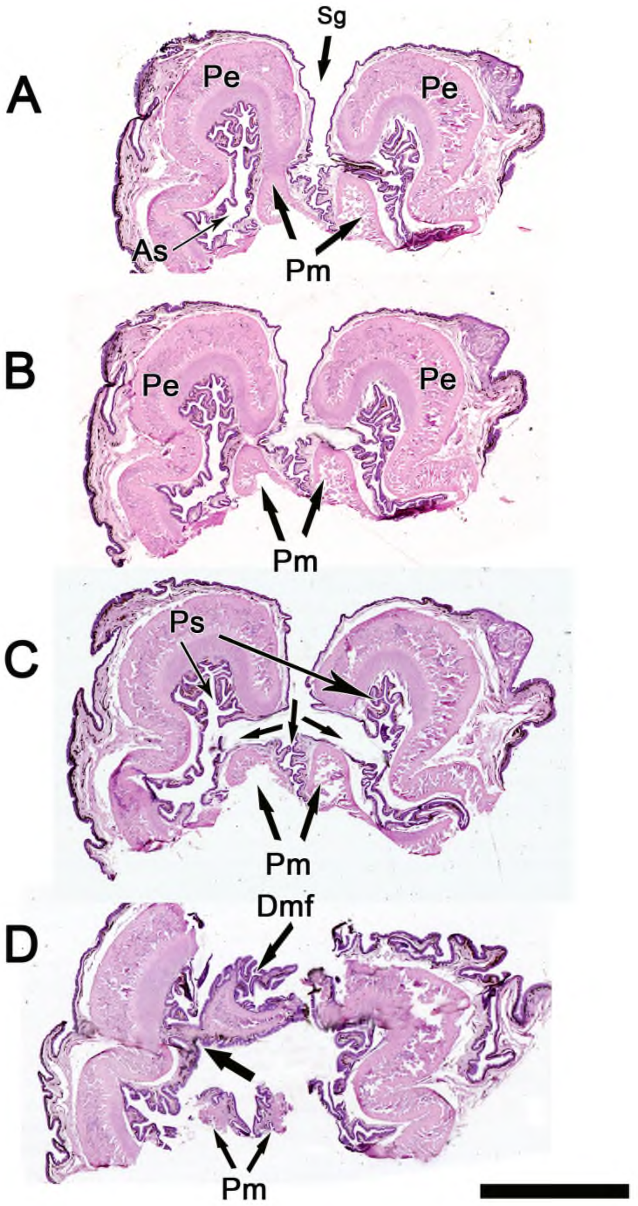

Figure 9. Continuation of Figure 8. Photomicrographs of penile morphology in the distal region of glans penis. Dmf, distomedial triangular fold of the plica media (Pm). Arrow in D points to attachment of the Dmf to the plica externa. See text for explanation of structures. Additional abbreviations as in Figure 8. Scale bar = 2 mm. H&E.

In his classic paper on penile morphology in cryptodiran turtles, Zug (1966) provided an in depth descriptive analysis of penile structure in 4 genera within the family Kinosternidae, including the genus

Kinosternon. Our histological observations on the penis in the MMT are in agreement with his findings, except that we noted the presence of a new sinus of the seminal groove, the anterior medial sinus.

In conclusion, we have demonstrated that a histological mode of discovery is an effective method for revealing anatomical relationships among distal urogenital ducts in the MMT. Moreover, we have shown that examining urogenital morphology can be a beneficial avenue of research for comparing differing urogenital anatomies among reptilian species. We recommend that future researchers on turtle urogenital morphology consider incorporating histological analyses into their research protocol.

Acknowledgments

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Morphology of Rathke's Glands in the Alligator Snapping Turtle, *Macrochelys temminckii* (Chelonia: Chelydridae)

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Running Title: Rathke's Glands in the Alligator Snapping Turtle

Abstract

I examined the morphology of Rathke's glands (RG) in the Alligator Snapping Turtle, *Macrochelys temminckii*, using light microscopy and scanning electron microscopy. This species possesses 4 pairs of RG (i.e., an axillary and 3 inframarginals) that are embedded beneath marginal bones and are named primarily according to the anatomical location of their orifices. These holocrine-type, exocrine, integumentary glands are anatomically and ultrastructurally similar to one another. Each gland contains a single and highly vascularized secretory lobule, which is bounded by a thick tunic of asymmetrically arranged striated muscle bundles. Two types of secretory vacuoles were identified within the holocrine cells of the glandular epithelium. The results of this study generally support my previous findings on RG in *Chelydra serpentina*, the Snapping Turtle (ST); however, some lack of cellular and structural conformity was evident compared to glands in this close relative as well as to RG in other turtle species. For example, epithelial cell layer depth and configuration and glandular lumen composition were inconsistent with prior observations. Moreover, the dearth of secretory cells and their products within the lumen of glands suggests that storage or temporary retention of glandular materials differs markedly from the conditions found in RG of other cryptodiran turtle.

Introduction

There are limited detailed descriptions of scent or musk glands, now formally known as Rathke's glands (RG) in turtles, which occur in living members of 13 of the 14 chelonian families (Vallen 1944; Waagen 1972; Ehrenfeld and Ehrenfeld 1973; Solomon 1984; Plummer and Trauth 2009; Trauth and Plummer 2013). Rathke's glands are large, exocrine, integumentary glands, which can number from one to 5 pairs in turtles. Most RG are located in the ventrolateral aspect of the trunk and release a foul-smelling secretion through external epidermal pores. The glands are named based upon either the general location of their pores (i.e., axillary and inguinal) or a pore's proximity to marginal scutes (e.g., 1st, 2nd, or 3rd inframarginal). One or more lobules are sheathed within a thick striated muscle covering; the secretory epithelium is characterized by ovoid-to-spherical holocrine cells (Ehrenfeld and Ehrenfeld 1973; Solomon 1984; Plummer and Trauth 2009; Trauth 2012; Trauth and Plummer 2013; Trauth 2017). Secretions released by these cells are primarily glycoproteins and, to a lesser extent, lipids, as well as various acids (Seifert *et al.* 1994; Weldon *et al.* 2008). The function of RG secretions as well as their comparative anatomy among most chelonians remains largely unknown despite our increasing knowledge about their morphology and glandular chemistry (Weldon *et al*. 2008). Few detailed histological investigations have focused on RG (Zangerl 1941; Ehrenfeld and Ehrenfeld 1973; Solomon 1984; Plummer and Trauth 2009; Trauth 2012; Trauth and Plummer 2013; Trauth 2017).

My primary objective in the present study was to report on the histology and ultrastructure of RG in the *M. temminckii*. The results of this study provide additional information relevant to enhancing knowledge about comparative anatomies of these glands among turtles.

Materials and Methods

I removed the RG from 4 *M. temminckii* collected from northeastern Arkansas and sacrificed with an intra-pleuroperitoneal injection of sodium pentobarbital in accordance with IACUC protocol guidelines at Arkansas State University. The glands were dissected from beneath the marginals (lateral edges of carapace) using a Dremel Multi-Max™ oscillating tool. The RG were then usually bisected into equal halves before fixation in vials of either 10% neutral buffered formalin, NBF (see below for procedures for paraffin sectioning—LM-Paraffin), and scanning electron microscopy (SEM) for 48 h or in a 2% glutaraldehyde (GTA) solution buffered with 0.1 M sodium cacodylate at a pH of 7.2 (see below for procedures for plastic sectioning—LM-Plastic) for 2 h. For postfixation of GTA-fixed glands, I used 1% w/v osmium tetroxide, buffered as above, for 2 h.

Turtles were sexed, measured (standard carapace length [SCL in mm]), and macro-photographed. Each turtle was assigned an Arkansas State University Museum of Zoology (ASUMZ) number and documented as follows (ASUMZ no., sex, SCL, date of collection): ASUMZ 31793, female, 282 mm, 30 May 2011; ASUMZ 21291, male, 251 mm, 3 June 2011; ASUMZ 33188, female, 327 mm, 2 May 2014, and ASUMZ 33268, 186 mm, 23 August 2014. Turtles were deposited in the herpetological collection in the Arkansas Center for Biodiversity Collections at Arkansas State University.

The RG were prepared for LM-Paraffin, LM-Plastic, and SEM in the former Electron Microscopy Facility at Arkansas State University. Following NBF fixation, the tissues were placed into vials of 70% ethanol and were readied for LM-Paraffin in accordance with the paraffin embedding techniques outlined in Presnell and Schreibman (1997). In brief, the procedures included dehydrating tissue in increasing ethanol solutions (70 to 100%), clearing in 100% xylene, infiltrating in paraffin overnight in a paraffin oven (56°C), embedding in paraffin using plastic molds (glands positioned to yield sagittal sections), sectioning with a rotary microtome into 10 µm serial strips (affixed onto glass microscope slides coated with Haupt's adhesive prior to floating strips in 2% NBF 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 tissue and muscle. Cover slips were then adhered to the microscope slides with Permount© (Fisher Scientific Products).

For LM-Plastic for plastic-embedded glands, I cut gland halves into a minimum of 4 pieces, dehydrated gland portions in a graded series of increasing ethanol solutions (50-100%), placed gland tissues in a 50/50% acetone/plastic mixture for overnight infiltration, and then embedded tissues in Mollenhauer's Epon-Araldite #2 (Dawes 1988). For thick sectioning (approximately 1 µm in thickness) and staining, I used glass knives on an LKB Ultrotome (Type 8800) and used Ladd® multiple stain (LMS), respectively.

For SEM, I dehydrated gland halves in a graded series of increasing ethanol solutions (50-100%), followed by several fluid exchanges in 100% ethanol. An Autosamdri-815 critical point drier (Tousimis Research Corporation, Rockville, MD) was used (31°C, 1072 psi, ventilation rate ~100 psi/min) to remove excess ethanol. Gland samples were then mounted on 25.4 mm aluminum pin stub specimen mounts and coated with gold using a Cressington 108 sputter coater (Cressington Scientific Instruments Ltd, Watford, UK). Tissues were then examined using a Vega TS 5136XM digital scanning electron microscope (Tescan USA Inc., Cranberry Township, PA) at 19.5 kV.

For 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 autofocus macro lens. Most descriptions of RG anatomy follow the terminology found in Plummer and Trauth (2009), Trauth (2012), Trauth and Plummer (2013), and Trauth (2017). Microscope slides are currently catalogued and housed in the Trauth Histo-herpetology Laboratory located in Morrilton, Arkansas.

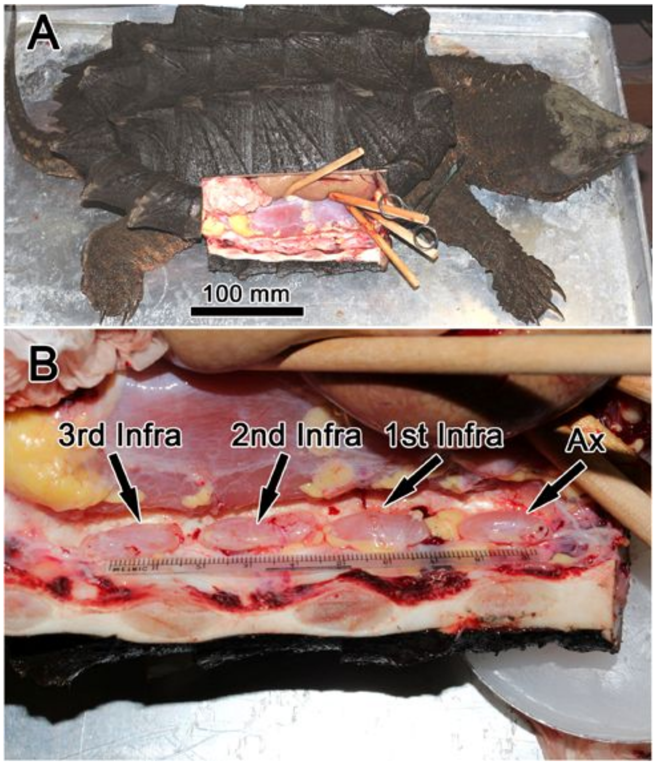

Figure 1. Exposed RG in *M. temminckii* (ASUMZ 33188). A. Dissection of glands using dorsal and lateral incisions into carapace and marginals. B. Arrows identify axillary gland (Ax) and 3 inframarginal glands (1st Infra, 2nd Infra, 3rd Infra) along bridge. Metric ruler is shown below glands in B.

Results

Gross Morphology

The RG of *M. temminckii* are located beneath the posterolateral edge of costal scute 1 (immediately posterior to tip of third rib) and extend to the middle of costal scute 3 between tips of ribs 4 and 6 (Fig. 1). The orifices of these epidermal glands are mostly inconspicuous and were not identified in the present study; however, the three inframarginal orifices are embedded in the posterolateral surfaces of three bridge scutes, whereas the orifice of the axillary gland appears along the interface between abdominal skin and the 4th marginal scute (Waagen 1972). Internally, the glands are aligned in a linear series within slight depressions along the tips of interior marginal bones and are surrounded by a scattering of fatty deposits and loose connective tissue. Gland dimensions are variable, but usually fall between 12 – 20 mm in length, 4 – 6 mm in width, and 3 – 4 mm in depth.

Light Microscopy

The basic internal structure of RG in *M. temminckii* consists of a single lobule, which possesses a secretory epithelium that rests upon a thin basement membrane (Figs. 2; 3A, B). A thick layer of dense connective tissue lies between the lobule and its striated muscle covering (Fig. 2B, C). In general, the secretory epithelium is comprised of a single cell layer, which produces roughly oblong-to-spheroid shaped holocrine cells (Fig. 3). These epithelial cells proliferate outward into the glandular lumen (Fig. 3). At some point following their release from the secretory epithelium, secretory cells lose their structural integrity and degenerate, dumping their cellular contents into the lumen (Fig. 3C). The mostly flocculent cellular debris becomes the material that is eventually passed into a duct leading to the exterior.

Secretory cells are also characterized by the presence of two different types of secretory vacuoles: Type 1 and Type 2 (Fig. 3). Type 1 secretory vacuoles are generally larger than those of Type 2 (Fig. 3B) and appear as singular, mostly spherical masses. Their matrix is not removed during tissue preparation. When stained with LMS, Type 1 secretory vacuoles stain purple in color, a positive indication of the presence of carbohydrate substances.

Type 2 secretory vacuoles, in contrast, are much smaller and appear mostly devoid of material (Fig. 3B,C). Type 2 secretory vacuoles also usually contain lipoidal material that is normally referred to as lipid droplets. Soluble lipids within these lipoid droplets are mostly removed from these vacuoles during histological preparation. Although not observed during the present study, osmiophilic, membrane-bound, lipoidal granules are often found within Type 2 secretory vacuoles. Holocrine cell apoptosis releases cellular materials; the persistent presence of the cell's nucleus surrounded by varying levels of glandular constituents characterizes the cellular debris (Fig. 3D).

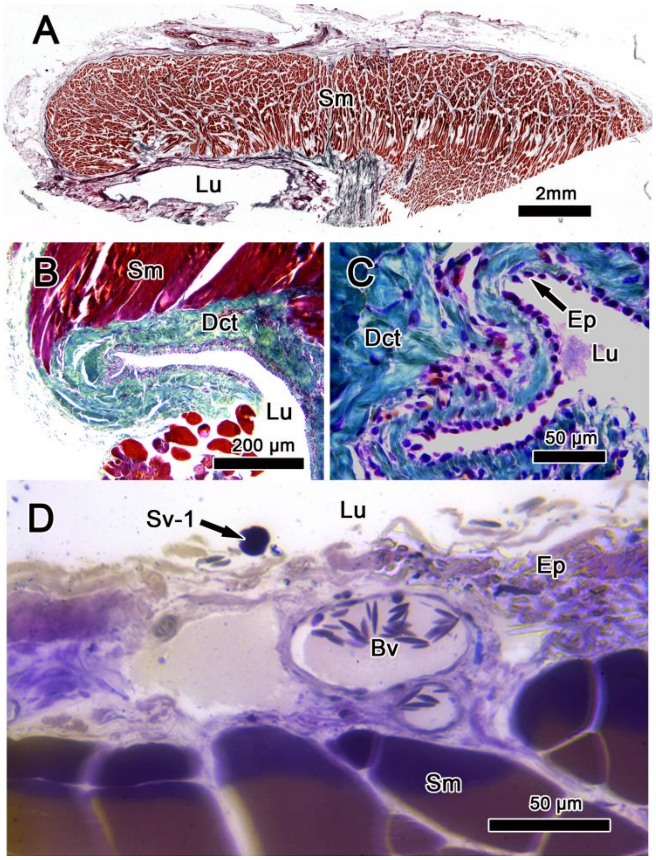

Figure 2. Light micrographs of RG in *M. temminckii*. A. Sagittal section of 2nd inframarginal gland (ASUMZ 21291) showing thick tunic of striated muscle (Sm) and glandular lumen (Lu). Pollak. B. Sagittal section of portion of 3rd inframarginal gland (ASUMZ 33188) showing thick layer of dense connective tissue (Dct) region surrounding lumen. Pollak. C. Magnification of B showing uniformly singular layer of holocrine cells of the secretory epithelium (Ep). Pollak. D. Secretory epithelium of an axillary gland (ASUMZ 31793) subtended by a blood vessel (Bv). A Type 1 secretory vacuole (Sv-1) is identified. LMS.

Scanning Electron Microscopy

When viewed sagittally, secretory lobules of the RG in *M. temminckii* appear to be asymmetrically positioned with respect to their muscular tunic (Fig. 4).

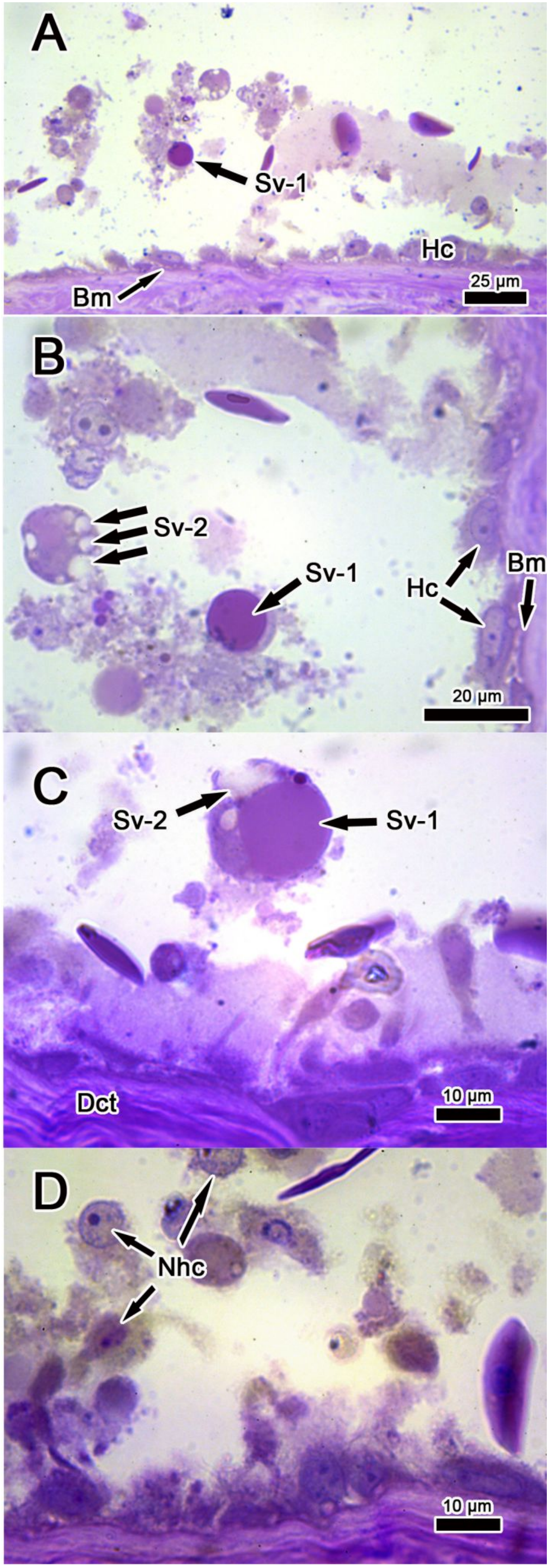

Figure 3. (at left). Light micrographs of the 2nd inframarginal gland in *M. temminckii* (ASUMZ 33268). A. Section showing secretory epithelium with scattered holocrine cells (Hc) along the basement membrane (Bm). A holocrine cell containing a dark-staining, Type 1 secretory vacuole is identified. B. Magnification of A revealing several Type 2 secretory vacuoles (Sv-2) along the periphery of a cell and lying adjacent to a Sv-1. C. Image of a holocrine cell exhibiting Sv-1 and Sv-2. Note that the Sv-2 has ruptured, releasing material into the lumen. D. Image of several holocrine cell nuclei (Nhc) from apoptotic cells. See text for further explanations. LMS for A–D.

The unattached (free) surface of each gland is dominated by a thick mass of striated muscle (exposed surface shown in Fig. 1), whereas the attached surface is comprised primarily of a broad layer of dense connective tissue (Fig. 4B). This dense layer is continuous with the innermost layer of dense connective tissue that encapsulates the lobule (Fig. 2B,C).

A single surface layer of holocrine cells characterized the interior lining of the secretory epithelia in all RG in *M. temminckii* (Fig. 5). Basal cells were not observed using SEM. Numerous intercellar bridges appear to link together all surface cells (Fig. 5A). An abundance of minute, spherical, secretory blebs were observed. Type 1 secretory vacuoles were infrequent (Fig. 5C).

Discussion

Several common morphological and histological features occur in RG found in non-marine turtles studied thus far. For example, the glands of *Sternotherus odoratus* (Ehrenfeld and Ehrenfeld 1973), *Apalone mutica* and *A. spinifera* (Plummer and Trauth 2009), *Kinosternon subrubrum* (Webb 2010), *Chelydra serpentina* (Trauth 2012), *Terrapene carolina* and *T. ornate* (Trauth and Plummer 2013), and *Sternotherus carinatus* (Trauth 2017) exhibit the following shared features: 1) a single lobule or, in exceptional cases, multiple lobules (2 lobules occur in softshell turtles [Plummer and Trauth 2009]) are present; 2) a thin-to-relatively thick layer of dense connective tissue immediately encases the secretory epithelium of a lobule; 3) lobules are wrapped in some manner by a tunic of striated muscle; 4) lobules receive a rich supply of blood from capillaries that lie in close proximity to the basement membrane of the secretory epithelium; 5) holocrine cells of the secretory epithelium generate 2 types of secretory vacuoles, and 6) glandular lobules exhibit lumina packed with either freshly released holocrine cells, previously released apoptotic holocrine cells, and/or cellular debris.

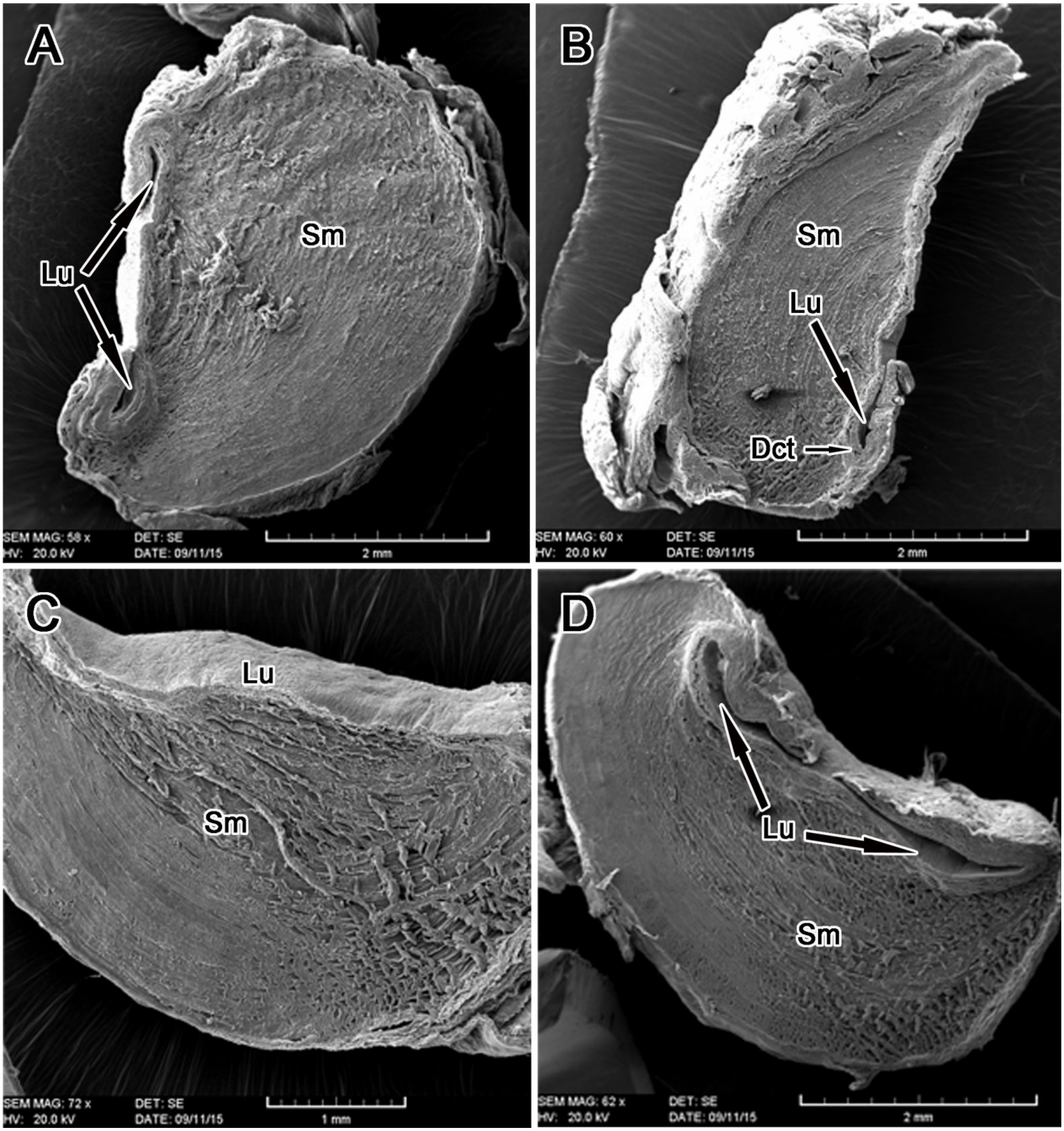

Figure 4. Scanning electron micrographs of sagittal sections (A – D) through the interior of RG in *M. temminckii* (ASUMZ 33188) showing the asymmetrical positioning of the secretory epithelium. A. 1st inframarginal gland. B. 2nd inframarginal gland. C. Axillary gland. D. 3rd inframarginal gland. Abbreviations same as in previous figures.

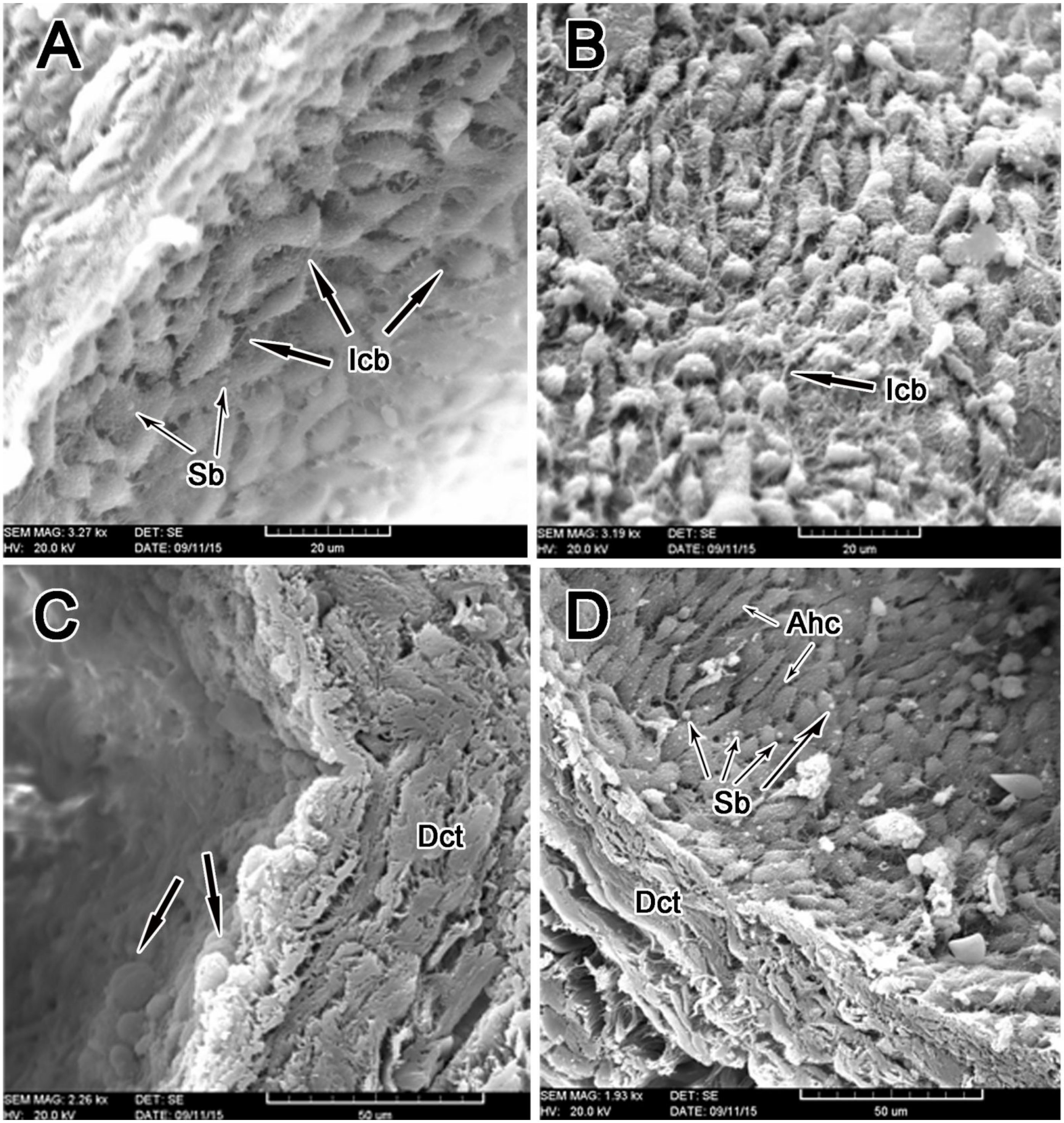

Figure 5. Scanning electron micrographs of the secretory epithelia of RG of *M. temminckii* (same as shown in Figure 4A – D). A. Abundance of intercellular bridges (Icb) radiating between holocrine cells; secretory blebs (Sb) reside on plasma membranes. B. Image reveals intercellular bridges as shown in A. The epithelial lining appears to be a single cell layer. C. Circular secretions, presumably Type 1 secretory vacuoles (arrows), are present. Dct, dense connective tissue. D. Attenuated epithelial cells (Ahc) dominate the surface layer; secretory blebs are numerous.

All *M. temminckii* examined in the present study possess RG that differ in some respect compared to the features listed above, except for some similarities found with *Chelydra serpentina*, the Snapping Turtle

(ST), a closely related species. For example, the lobules of all RG in *M. temminckii* were asymmetrically arranged, being greatly displaced from the core of its muscular tunic. This anatomy was similar to ST; on the other hand, most turtles listed above exhibit centrally located lobules. Another feature, inconsistent with all other turtles (including ST), was the apparent lack of secretory material being stored within lobules in *M temminckii*. This finding was most peculiar and remains equivocal, given that gland preparation for all turtle species employed in 4 previous studies was similar.

The basic morphology of the secretory epithelium was also puzzling when comparing the RG of *M. temminckii* with other turtles. The notable shape and configuration of the single cell layer of holocrine cells, clearly evident in both light and scanning electron micrographs, was remarkably different from the consistency found in turtles species mentioned previously. Also, the microstructure of this single cell layer revealed intercellular bridges, for the first time, by examination using SEM. Transmission electron microscopy will be necessary in order to resolve the nature of the cellular layers, intercellular connections and methods of liberating secretory material from RG in *M. temminckii*.

In conclusion, solving the intricacies related to comparative and functional morphologies of RG will require additional histological and ultrastructural investigations. These methods of inquiry offer a unique opportunity for microscopists to collaborate with behavioral and chemical ecologists.

Acknowledgments

Collection of turtles was authorized by a scientific collecting permit from the Arkansas Game and Fish Commission (Permit nos. 120820101 in 2011 and 020520134 in 2014). I thank P. Weldon for his helpful comments and information regarding Rathke's glands.

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Eimeria lancasterensis (Apicomplexa: Eimeriidae), Three Nematodes (Heligmosomoidea: Boehmiellidae, Heligmonellidae), and a Flea (Siphonaptera: Ceratophyllidae) from the Eastern Fox Squirrel, *Sciurus niger* (Rodentia: Sciuridae) in Arkansas

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Running Title: Parasites of Eastern Fox Squirrel

Abstract

In Arkansas, the eastern fox squirrel (*Sciurus niger*) is a common inhabitant of the state. Although information is available on ectoparasites of this host in Arkansas, little is known about the endoparasites of this squirrel. A single specimen from Montgomery County was examined and found to harbor the following: a coccidian (*Eimeria lancasterensis*), three nematodes, *Boehmiella wilsoni*, *Citellinema bifurcatum*, and *Sciurodendrium hassalli*, and a flea, *Orchopeas howardi*. We document these nematodes from an Arkansas *S. niger* for the first time, and add mensural and molecular information on *E. lancasterensis* from this host.

Introduction

The eastern fox squirrel, *Sciurus niger* (L., 1758) is the largest tree squirrel in the Western Hemisphere that occurs naturally in temperate forests over most of eastern North America (Hall 1981; Koprowski 1994). In Arkansas, *S. niger* is found statewide (Sealander and Heidt 1990). It inhabits a diversity of deciduous and mixed-forest habitats, but is more common in forest patches (Nixon and Hansen 1987). Fox squirrels feed heavily on tree seeds during much of the year (Koprowski 1994).

Although *S. niger* has been the subject of several studies of its coccidian parasites (Knipling and Becker 1935; Levine and Ivens 1965; Joseph 1972, 1973a, b, 1975; McAllister and Upton 1989; Spurgin and Hnida 2002; Motruik-Smith *et al.* 2009; Ozmen *et al.* 2009), there are no surveys reporting coccidia in any specimen from Arkansas.

Eastern fox squirrels have also been reported to be host of a suite of helminth parasites (Rausch and Tiner 1948; Flyger and Gates 1992). In Arkansas, Davidson (1976) examined some *S. niger* from the Ozarks in Stone County for parasites. There are no other reports of any helminth parasite from this host in the state. Here we report new records for parasites from a *S. niger* from the Ouachitas of Arkansas as well as include additional figures, mensural, and sequence data for a coccidian.

Materials and Methods

On 16 October 2020, an adult squirrel was hit and killed by an automobile on St. Hwy. 8, 3.2 km west of Black Springs, Montgomery County (34° 27' 16.29" N, -93° 46' 20.2872" W). It was opportunistically collected and immediately taken to the lab and processed for parasites. The pelage was brushed over a white enamel tray for ectoparasites. Any found were placed in a vial of 70% (v/v) ethanol and later cleared in 10% (w/v) 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% (w/v) saline, and organs (including heart, liver, lungs, spleen, and kidneys) were placed in individual Petri dishes. Several 10 cm sections of the GI tract were cut, split lengthwise, and examined under a stereomicroscope for endoparasites. Feces from the rectum was collected and placed in 2.5% (w/v) potassium dichromate. A fecal flotation was accomplished with Sheather's sugar solution (sp. gr. 1.30). Nematodes were examined as temporary mounts in glycerol.

For analysis of the DNA sequence of the *Eimeria* species, feces in 2.5% (w/v) potassium dichromate was sent to the Fish and Wildlife Disease Laboratory at SUNY-ESF. DNA was extracted using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research Corp, Irvine, CA) with modifications described in Whipps *et al.* (2020). PCR was performed in 50 µL reaction volumes in Quick-Load® Taq 2X Master Mix (New England Biolabs, Ipswich, MA), 0.25 µM of each primer and 3 µL of template DNA. Overlapping fragments targeting the SSU ribosomal DNA were amplified with primers Eimeria1F (5'-GAT TCA TAG TAA CCG AAC GG) with 18R (Whipps *et al.,* 2003), and Eimeria2F (5'-GGG CAT TCG TAT TTA ACT GTC) with 18R. Amplifications were performed on a C1000™ Thermal Cycler (BioRad Laboratories, Hercules, CA) with initial denaturation at 95°C for 3 min, followed by 35 cycles of 94°C for 30 sec, 56°C for 45 sec, 68°C for 90 sec, and a final extension at 72°C for 7 min. Product amplification was evaluated by observation on a 1% (w/v) agarose gel and the remainder of the sample purified using the E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek, Norcross, GA). DNA was quantified using a DNA spectrophotometer (NanoDrop Technologies Wilmington, Delaware). Sequencing used amplification primers with the ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1, using the ABI3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were assembled manually in BioEdit (Hall 1999) and identity analyzed by GenBank BLAST search.

A host photovoucher was deposited in the Eastern Oklahoma State College Collection, Idabel, OK. Voucher specimens of ectoparasites were deposited in the General Ectoparasite Collection in the Department of Biology at Georgia Southern University, Statesboro, GA. Endoparasites were deposited in the Harold W. Manter Laboratory (HWML) of Parasitology, University of Nebraska, Lincoln, NE, or samples were retained for molecular analyses.

APICOMPLEXA: EIMERIORINA: EIMERIIDAE

***Eimeria lancasterensis* Joseph, 1969 –** Oocysts (Fig. 1A–C, HWML 216668) of this coccidian were being passed in feces. Oocysts (*n* = 20) were ellipsoidal, 23.5 × 14.3 (18–29 × 11–19) µm, with a length/width ratio (L/W) of 1.6 (1.3–1.8). Bilayered wall was 1.4 (1.1–1.7) with a smooth, occasionally lightly pitted or sculptured, colorless to light yellow outer layer, ~2/3 total thickness; inner layer light yellow. Micropyle and oocyst residuum was absent but 1–3 sometimes bilobed polar granule(s) were present. Sporocysts (*n* = 20) were ellipsoidal, (L × W) 11.5 × 6.7 (10–13 × 6–8) µm with an L/W ratio of 1.7 (1.4–2.2). Nipple-like Stieda body was present but subStieda and paraStieda bodies were absent. Sporocyst residuum was composed of various-sized granules forming a compact sphere, or a dense irregular mass located between and across the sporozoites, or a combination of both within the same sporocyst. Sporozoites (not measured) were elongate, anterior end tapered, posterior end rounded with a large, ellipsoidal posterior refractile body. The 1,472 nucleotide SSU DNA sequence for this specimen was submitted to GenBank (accession MZ831509). Our sequence was identical to a sequence from *E. lancasterensis* from eastern gray squirrels*, Sciurus carolinensis* Gmelin in Italy (GenBank accession KT360976) over 1,224 nucleotides.

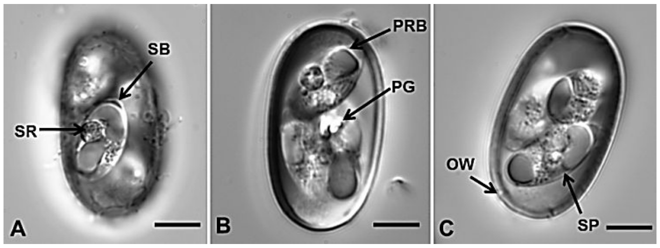

Figure 1. Sporulated oocysts of *Eimeria lancasterensis* from *Sciurus niger* from Montgomery County, Arkansas. A. Stieda body (SB) and sporocyst residuum (SR). B. Polar granule (PG) and posterior refractile body (PRB). C. Oocyst wall (OW) and sporocyst (SP). Scale bars = 10 µm.

This is one of the most prevalent coccidians infecting members of the rodent family Sciuridae. It has been reported previously from *S. niger* in Texas (McAllister and Upton 1989a), Nebraska (Spurgin and Hnida 2002), and Virginia and Wyoming (Motriuk-Smith *et al.* 2009). In addition, this coccidian has been reported from *S. carolinensis* from Italy (Hofmannová *et al.* 2016), from a red squirrel, *Sciurus vulgaris* (L.) in Turkey (Ozmen *et al.* 2009) and from *S. carolinensis* in Massachusetts (Joseph 1969, 1972), Florida (Forrester *et al.* 1977), Texas (McAllister and Kessler 2002), and Arkansas (McAllister and Kessler 2002). Although the latter authors reported *E. lancasterensis* in the state from *S. carolinensis*, no mensural data or photomicrographs were provided. Therefore, this is the first report of measurements, accompanying photomicrographs, and molecular data on *E. lancasterensis* from an Arkansas host.

NEMATODA: HELIGMOSOMOIDEA: BOEHMIELLIDAE

***Boehmiella wilsoni* Lucker, 1943.** – Two specimens (HWML 112234) were found in the stomach. *Boehmiella* spp. are principally characterized by having chitinized sheaths on the lateral and externo-dorsal rays of the bursa. They have short, complex unbranched spicules and females are didelphic. This nematode was described infecting *S. carolinensis* in Florida, Georgia, Minnesota, and West Virginia (Lucker 1943; Rausch and Tiner 1948). Coyner *et al.* 1996) reported it from *S. niger* from Florida, and Whitaker and Mumford (2009) from Indiana. Davidson (1976) reported *B. wilsoni* from *S. carolinensis* from Stone County, Arkansas. More recently, *B. wilsoni* was found in Deppe's squirrel, *Sciurus deppei* Peters in México (Falcon-Ordáz and García-Prieto 2004); in brown agouti, *Dasyprocta variegata* Tschudi in Bolivia (Mollericona *et al.* 2016); and in Ferreira's spiny tree-rat, *Mesomys hispidis* (Desmarest) in Brazil (Andrade-Silva *et al.* 2020). We document *B. wilsoni* in a *S. niger* from Arkansas for the first time.

TRICHOSTRONGYLOIDEA: HELIGMONELLIDAE

***Sciurodendrium hassalli* (Price, 1928).** – Approximately 30 specimens (HWML 112233) were found in the small intestine. Price (1928) originally described this nematode from *S. carolinensis* from Maryland. *Sciurodendrium* spp. are loosely coiled parasites and are characterized by having most of the cuticular ridges discontinuous and scalloped. Species are determined by the pattern of the bursal rays and females are monodelphic. The distribution of *S. hassalli* in sciurids is widespread. Chandler (1942) reported 100% prevalence in fox squirrels from eastern Texas, while Eckerlin (1993) found 50% prevalence in *S. niger* from Maryland and Virginia. It has also been reported from *S. niger* from Florida (Coyner *et al*. 1996), Ohio (Katz 1938) and Tennessee (Reiber and Byrd 1942). Davidson (1976) reported *S. hassalli* from *S. carolinensis* from Stone County. We document *S. hassalli* from an Arkansas eastern fox squirrel for the first time.

***Citellinema bifurcatum* Hall, 1916.** – Two specimens (HWML 112232) were recovered from the small intestine. The type host is the Wyoming ground squirrel, *Urocitellus elegans* (Kennicott) (see Hall 1916). *Citellinema* spp. are tightly coiled parasites characterized by an asymmetrical bursa with a greatly reduced dorsal ray. The spicules are short (380–400 µm) and deeply bifurcated and females are didelphic. It is a common among sciurids where it occurs in squirrels over a range from Colorado, Wyoming, and Saskatchewan, Canada to Maine (Reiber and Byrd 1942). This nematode has also been reported from *S. niger* from Florida (Coyner *et al.* 1996), Indiana (Whitaker and Mumford 2009), Tennessee (Reiber and Byrd 1942), and Ohio (Katz 1938). Davidson (1976) reported *C. bifurcatum* from *S. carolinensis* from Stone County. This nematode is reported from an Arkansas eastern fox squirrel for the first time.

ARTHROPODA: INSECTA: SIPHONAPTERA: CERATOPHYLLIDAE

***Orchopeas howardi* (Baker, 1895).** – a single female (L3851) was recovered. This flea is a common ectoparasite of sciurids, including *S. niger* (Whitaker *et al.* 1976; Lewis 2000). Schiefer and Lancaster (1970) and McAllister *et al.* (2013) reported *O. howardi* previously from *S. niger* from the Arkansas Ozarks. Other hosts from the state include *S. carolinensis*, southern flying squirrel, *Glaucomys volans* (L.), and raccoon, *Procyon lotor* (L.) (McAllister *et al.* 2017). This flea has been reported to transmit North American strains of the causative agent of sporadic epidemic typhus (*Rickettsia prowazekii*), which is maintained enzootically in flying squirrel populations (McDade 1987). Human cases of this disease have been serologically confirmed and recorded in Arkansas (McDade 1987). We report *O. howardi* from a host from the Ouachita uplands of the state for the first time.

In conclusion, we document, for the first time, three nematodes from a *S. niger* from Arkansas. Two of these, *S. hassalli* and *C. bifurcatum*, which have direct life cycles, are proposed to be core species of *S. niger* (Kinsella 1991) and we concur. Although only a single *S. niger* was examined herein it yielded these new records as well as extra mensural and molecular data on the coccidian, *E. lancasterensis*. Additional eastern fox squirrels in Arkansas should be examined for parasites from the southern and eastern parts of its range in the state.

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Energy Content of Seeds of Palmer's Pigweed (*Amaranthus palmeri*) in the Diet of Scaled Quail (*Callipepla squamata*) in Southeastern New Mexico

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Running Title: Energy Content of Seeds of Palmer's Pigweed in Southeastern New Mexico

Abstract

Palmer's pigweed (*Amaranthus palmeri*) is a common grassland plant that occurs across much of North America. It is often considered a weed but is an important source of food for many game birds. We analyzed the energy content of seeds of Palmer's pigweed 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 Palmer's pigweed from New Mexico averaged 16.6 J/kg (4.0 kcal/g), and was among the lowest values obtained when compared to those of many seeds previously reported from the diet of scaled quail and other granivorous birds.

Introduction

Understanding the energy content of food items is important for conservation and management of wildlife and their food plants, as it helps wildlife managers make decisions on planting or removing potential food items that may be invasive or weedy. 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*). A previous study (Hunt *et al.* 2020) measured the energy content of seeds of common sunflowers (*Helianthus annuus*) consumed by free-living scaled quail, and a study (Saunders and Parrish 1987) conducted in Kansas measured the assimilated energy of some potential food items by captive scaled quail, including two species in the genus *Amaranthus* (commonly referred to as amaranths or pigweeds). Studies have measured energy content of some known and potential food items of other birds living in the same areas, such as mourning doves (*Zenaida macroura* — Schmid 1965; Shuman *et al.* 1988; Hunt *et al.* 2019); some of the items measured are known or potential food for scaled quail.

A study of feeding habits of scaled quail in southeastern New Mexico determined that seeds of Palmer's pigweed (*Amaranthus palmeri*) made up a substantial portion (5.3%) of the total mass of crop contents, and were present in 35.0% of the crops (Hunt and Best 2001*b*); another study (Davis *et al.* 1975) reported smaller amounts of seeds of Palmer's pigweed. Seeds of Palmer's pigweed are also reported to be a 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 pigweeds from Kansas has been measured (Saunders and Parrish 1987), no such measurements have been conducted on seeds of *A. palmeri* from sand-scrub habitat of New Mexico. We used an oxygen bomb calorimeter to determine the energy content of seeds of Palmer's pigweeds from Eddy and Lea counties in New Mexico.

Methods and Materials

This study is an offshoot of 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*). The study area is at the Waste Isolation Pilot Plant in eastern Eddy County and 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). A number of 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*). The study site is heavily grazed by cattle.

In late summer and autumn in 1981 and 1982, 178 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 and no residue from containment within the crop was detected. 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). We used seeds thus collected rather than raw seeds from the habitat to ensure that the samples included food actually consumed by scaled quail.

Samples of seeds of *Amaranthus palmeri* 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 came from 11 individual scaled quail with crops that contained enough seeds for analysis; 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 (Jessup 1960). Results are reported in J/kg; kcal/g are given in parentheses for comparisons with previous studies.

Results

The 11 samples analyzed contained an average of 16.6 J/kg (4.0 kcal/g — range, 15.5-22.6 J/kg [3.7-5.4 kcal/g]; standard deviation, 2.0 J/kg [0.5 kcal/g] — Table 1). This figure is comparable to that previously obtained for an unspecified species of *Amaranthus* (19.2 J/kg [4.6 kcal/g], Saunders and Parrish 1987) and less than many other tested food items in the diet of scaled quail and other seed-eating game birds.

Table 1. Gross caloric value (energy content) of seeds of Palmer's pigweeds (*Amaranthus palmeri*) in the crops of scaled quail (*Callipepla squamata*) collected from Eddy and Lea counties, New Mexico, summer and autumn, 1981-1982.

Sample No.	Energy in J/kg (kcal/g)
SQ038-81a	15.9 (3.8)
SQ038-81b	15.9 (3.8)
SQ038-81c	15.5 (3.7)
SQ038-81d	16.3 (3.9)
SQ039-81a	16.3 (3.9)
SQ039-81b	22.6 (5.4)
SQ040-81	16.3 (3.9)
SQ004-82a	15.5 (3.7)
SQ004-82b	15.5 (3.7)
SQ021-82	16.3 (3.9)
SQ023-82	16.7 (4.0)

Discussion

Our study demonstrates that seeds of Palmer's pigweed have an energy content less than most food items from previous studies. For example, Robel and Harper (1965) 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 energy content equal to or greater than that which we measured for Palmer's pigweed; the greatest energy content in that study was for hulled domestic sunflower-seed chips (27.6 J/kg [6.6 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 that 8 of the 9 items tested contained more energy than the Palmer's pigweed measured in this study; only corn (*Zea mays*, 17.1 J/kg [4.1 kcal/g]) and wheat (*Triticum aestivum*, 16.7 J/kg [4.0 kcal/g]) were comparable. 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 all had greater energy content than the Palmer's pigweed tested in our study. Davison (1958) categorized some types of amaranth as "inferior" food plants for northern bobwhites, meaning they were poorly digestible or lacking in availability. Our study seems to indicate that amaranth may also be inferior because of lower energy content.

Palmer's pigweed is considered to be an important agricultural pest because it has spread widely beyond its original range in the desert Southwest to much of North America, grows as a weed in many crops including corn and cotton (*Gossypium hirsutum*), and is resistant to many commonly used herbicides (Ward *et al*. 2013). Palmer's pigweeds are often associated with disturbance (Sauer 1955), often being found in dumps, along roadsides and railway rights-of-way, in farms, and in fields. 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 Palmer's pigweeds grow abundantly. Although Palmer's pigweed usually flowers and fruits in summer and autumn, the plant has been observed to flower in all months of the year in the southwestern United States and Mexico (Sauer 1955). Individual plants are highly prolific; some have been recorded producing up to 600,000 seeds in a single year (Ward *et al*. 2013). Availability of seeds of Palmer's pigweeds helps explain their prevalence in the diet of scaled quail (Hunt and Best 2001*b*) and other granivorous birds of the area, despite the relatively poor energy content demonstrated herein.

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Distribution and History of the Roseate Spoonbill (*Platalea ajaja*) in Arkansas

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Running Title: Roseate Spoonbill in Arkansas

Abstract

The Roseatte Spoonbill (*Platalea ajaja*) is a rare bird in Arkansas and was not reported from the state until 1959, when it was seen in southwestern Arkansas. By use of online sources for citizen science, we elucidate the history of occurrence and present analysis of seasonal distribution of this bird in Arkansas. Individuals arrive in Arkansas as early as April, observations peak in August, and the birds may remain to late October when colder weather promotes southward migration. Most observations are of a few birds, but a maximum of 128 has been counted at one location and time. Most of the birds seen had not developed breeding plumage so were believed to be younger birds migrating northward in spring and summer to forage. The birds have been observed in 28 counties, but most observations have been in Chicot and Desha Cos. of southeastern Arkansas, bordering the Mississippi River. Several other wading birds such as storks, herons, egrets, and ibis have been reported as associates in flight or foraging. Nesting was discovered for the first time in 2020, in Ashley Co. of southeastern Arkansas.

Introduction

Populations of the Roseate Spoonbill (*Platalea ajaja*) apparently declined due to habitat loss and the millinery trade for plumes. These are wading birds that use a spatula-shaped bill to forage in shallow water. Mature birds have a pink body with red on the wings and part of the tail, and otherwise an almost orange tail, whereas juveniles are light pinkish (Dumas 2020).

The Roseate Spoonbill breeds along the coasts and increasingly into the interior of Texas, Louisiana, and south Florida, but there are no records of breeding in Alabama or Mississippi (Dumas 2020). The bird has only a short documented history in Arkansas, as it was not reported by Howell (1911), Wheeler (1924), or Baerg (1931, 1951). Further, the species was not reported in earlier literature from neighboring Oklahoma (Nice 1931), though it was observed there in 1940, and several more records have been documented in Oklahoma in more recent years (Shackford 1991). Still, based on reports in Allen (1942), in which some Roseate Spoonbills were known to use the Mississippi Valley to wander as far north as Wisconsin, it seems plausible that the birds might have passed undetected through Arkansas.

This bird was first reported in Arkansas in 1959, from Miller County in southwestern Arkansas (James and Neal 1986). James (1974) did not include it in a discussion of threatened native birds of Arkansas because it had never been considered a breeding bird in the state. The few reports made by 1985 were from southwestern Arkansas (Hempstead, Howard, Little River, and Miller Cos.), with exceptions from Jefferson County (southeastern Arkansas) in 1973 and Pulaski County (central Arkansas) in 1985 (James and Neal 1986). Observations in Arkansas and other southeastern states, farther inland than the distribution of known breeding grounds, were thought to represent immatures ranging northward during late summer (James and Neal 1986; Dumas 2020). Oberholser (1974) previously had interpreted distributional records of the Roseate Spoonbill in Texas to indicate dispersal along watercourses northward and inland after the breeding season, in late summer and fall.

Methods and Materials

To determine distribution and dates of occurrence, 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 locations and dates of sightings, but also comments describing habitat, behavior, numbers of birds seen, and their avian associates.

Data were gathered into a spreadsheet and sorted various ways to reveal the history and timing of the presence of this rare bird in Arkansas. Duplicate accounts of the same observations made by different observers were deleted prior to analysis, to create a dataset with unique observations.

Increasing numbers of observations can be attributed to both an increasing number of birds over time and to an increasing number of observers making reports. We followed the method of Whitfield *et al.* (2018) to evaluate whether numbers were increasing over time by plotting the maximum group size reported at one sighting each year over the years of observation.

We examined the seasonal distribution of Roseate Spoonbills in North America to interpret whether reports of the birds in Arkansas seemed to show N-S migrations, or whether the birds might also migrate E-W. This was accomplished in eBird (eBird 2021) by use of the science tab, then application of the tab for abundance animation. We also used eBird to elucidate the distribution of breeding Roseate Spoonbills in North America for comparison with observations of breeding in Arkansas.

Results and Discussion

Distribution – The first report of a Roseate Spoonbill in Arkansas was from a swampy lake near the Red River in southwestern Arkansas (Miller Co.), observed in the fall (20 September) of 1959 (James and Neal 1986). The earliest reported date of observation was in the spring, on 21 April 2010 in northeastern Arkansas (Poinsett Co.). We report here our new observation of early arrival, on the first weekend of April in 2021, in Ashley Co.

Reported locations of Roseate Spoonbills in Arkansas, including a total of 28 counties, are included in Fig. 1. As these records are composited from citizen science, it must be understood that the data do not represent a systematic survey, and that less accessible habitats also may support summer residents and perhaps nesting pairs (Tumlison *et al.* 2020). Stars on the map represent records of observations in adjacent states, which help illustrate that these birds disperse along rivers and forage in adjacent lowlands. Observations along the Mississippi River in Mississippi, Tennessee, and Missouri indicate that Roseate Spoonbills are likely to be found more commonly in NE Arkansas than has been reported, and in the 4 counties without current records in eastern Arkansas. Patterns of distribution relate to appropriate wetland habitat, which tends to be found along river systems in lowland regions of the state (West Gulf Coastal Plain and Mississippi Alluvial Plain). In Arkansas, these rivers include the Red, Ouachita, Arkansas, White, and Mississippi.

Although sightings of Roseate Spoonbills have occurred in 28 counties (Fig. 1), about 71% of the 287 unique statewide observations occurred in only 4 counties. Most (52.7%) were in the 2 southeastern counties bordering the Mississippi River (29.7% in Chicot Co. and 22.9% in Desha Co.). Bald Knob National Wildlife Refuge (BKNWR) accounted for almost all of the observations in White Co. (10.8% of the total statewide observations), and 7.5% of the total observations were on farms in Lafayette Co. in southwestern Arkansas.

Sightings of only 1-2 birds occur from the time of first documented arrival, on 21 April, through 9 October. Those observations account for 44.4% of the reported sightings. Groups of 3 birds appear by 11 May, 4 by 19 July, and 5+ by 22 July. Groups of 20+ birds appear earliest on 13 August but are most common in September and October.

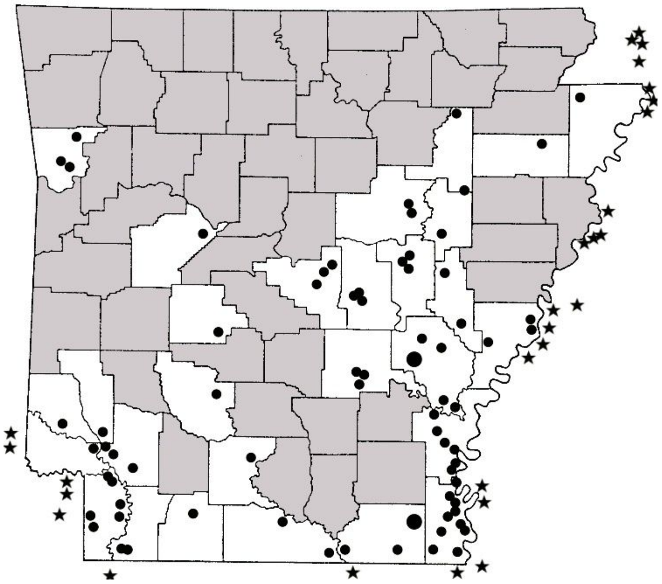

Figure 1. Distribution of the Roseate Spoonbill (*Platalea ajaja*) in Arkansas (1959-2020) based on literature and records compiled from citizen science websites. Dots indicate locations of observation (but multiple observations during the same or different years are not represented by additional dots). Stars represent locations reported in eBird for adjacent states. The enlarged dot in Ashley Co. (southeastern Arkansas) represents the verified breeding location in Arkansas, and the large dot in Arkansas Co. is the location of the second likely breeding record.

Ten or more birds have been documented at one time in Ashley, Chicot, Desha, Lafayette, Lonoke, Miller, Monroe, Prairie, Pulaski, and White Counties. The largest numbers of birds seen simultaneously have been repeated observations in 2005 and 2010 at Camp Nine in Desha County (with a maximum of 128 birds counted on 25 September 2005). Observers have reported a group of 65 birds at the Mississippi Levee in Chicot County, and as many as 30 individuals were seen at BKNWR in White County on 11 September 2017.

Examination of historical distribution shows the longest term of continued occurrence in the lowlands of southeastern Arkansas along the Mississippi River, and in southwestern Arkansas near the Red River system (Fig. 2). From 1959-1969, the species was recorded only from Hempstead, Jefferson and Miller Cos., and during the decade of 1970-1979, the bird was reported from only Miller and Jefferson Cos. It would be almost 2 more decades before Roseate Spoonbills were recorded again from those counties.

From 1980-1989, observations were reported from a total of 7 counties, all of them new (Chicot, Clark, Desha, Garland, Howard, Little River, and Pulaski). In the decade from 1990-1999, again 7 counties were reported but 4 of these were new (Ashley, Lonoke, Mississippi, and Union).

Interest in birds and reporting of records increased after 2000 (Tumlison *et al.* 2020), but apparently an actual influx of Roseate Spoonbills occurred as well. From 2000-2009, occurrence was reported in 12 counties including the addition of 5 more counties (Phillips, Prairie, Monroe, White, and Yell). From 2010-2020, observations increased dramatically with records from 20 counties, including 9 new counties (Arkansas, Columbia, Crawford, Jackson, Lafayette, Phillips, Poinsett, Sevier, and Woodruff).

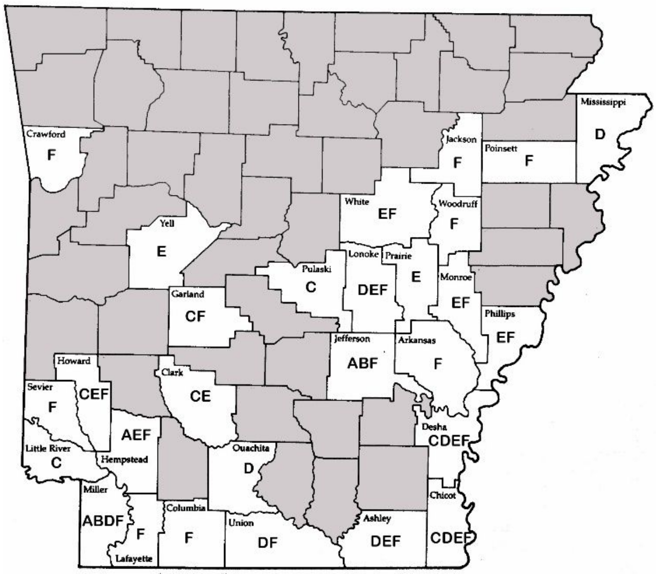

Figure 2. Historical distribution of the Roseate Spoonbill in Arkansas. Unshaded counties have records, and lettering represents time frames for the records: A = 1959-1969, B = 1970-1979, C = 1980-1989, D = 1990-1999, E = 2000-2009, and F = 2010-2020.

Roseate Spoonbills have been present but are very uncommon in Arkansas from April through June. Sparse observations during May (Chicot, Hempstead, Monroe, and Woodruff Cos.), and June (Chicot, Desha, Lafayette, and White Cos.) account for only 9 of 287 unique observations (Fig. 3). Of the remaining available records, 27 (9.7%) occurred in July, 122 (43.7%) in August, 98 (35.1%) in September, and 30 (10.8%) in October. The latest date of observation in Arkansas was a group of 5 birds seen on 12 November 2005 in Desha Co.

The great increase in August likely includes birds that nested along the coasts of Louisiana and Texas and migrated northward to feeding grounds. Chronological data from eBird showed that populations from southern Louisiana and southeastern Texas appeared to migrate N along the Mississippi and Red Rivers, and populations in Florida moved N into the Florida panhandle or into Georgia and South Carolina. Few records occurred in Mississippi and Alabama, and there was little evidence of E-W migration. As fall approaches, the birds begin a seasonal migration southward and are mostly absent from Arkansas by late October.

Age-identified birds reported from April through July were immature individuals, therefore hatched in the previous breeding season, and a few unmated adults also were noted. The latter include accounts of

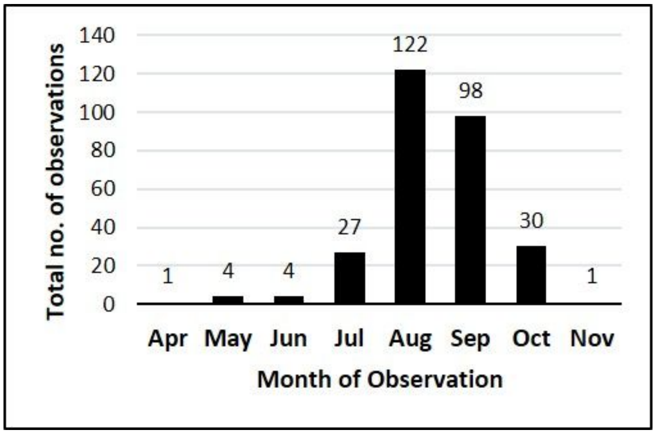

Figure 3. Monthly frequency of reported observations of Roseate Spoonbill (*Platalea ajaja*) in Arkansas, 1959-2020.

single birds in Poinsett Co. (21 April), Hempstead Co. (3 July), and Pulaski Co. (9 July). Spoonbills identified as adults otherwise do not appear until late July and have been seen mostly in August and September. Adults migrating northward after the breeding season usually are single birds, and have been documented scattered over several counties, including Ashley, Chicot, Desha, Hempstead, Jefferson, Lafayette, Lonoke, Miller, Poinsett, and Pulaski. Eight adults were seen together in Chicot Co. on 10 August, along with 57 young birds.

The pattern of monthly occurrence also must be viewed cautiously, as reports are based on citizen science and the communicated presence of a rare species often results in multiple reports of the same birds over several days or weeks if the birds remain at the same site. This attribute inflates the numbers of observations of those birds, although these same data reflect continued presence over time. Further, the same individuals may move short distances and thus be documented at various locations. Strings of sightings only short distances from each other (e.g., the string of sightings in Chicot and Desha Cos. in southeastern Arkansas, see Fig. 1) may sometimes represent the same individual spoonbills that moved among a variety of locations.

The general pattern of historic occurrence indicates that Roseate Spoonbills seen in Arkansas from April-July represent unmated individuals and juveniles, then fledged birds and post-nesting adults enter the state from July-September. These likely are mostly dispersers from April-August following the breeding season farther south, an interpretation consistent with other observers (Oberholser 1974; Dumas 2020). After northward dispersal and foraging through late summer, individuals apparently return southward with onset of colder weather.

The increasing maximum group size of flocks seen since about the year 2000 (Fig. 4) indicates that more Roseate Spoonbills are becoming summer migrants into Arkansas. From 1966 to about 2000, only 1-2 birds were seen together at a time. During the last 20 years, the size of the largest flocks has increased appreciably (although there is much yearly variation, with a maximum group of 128 in 2005). This kind of plot helps reduce the bias of more observers and greater frequency of reporting through citizen science portals (Whitfield *et al*. 2018) and complements the observation that the birds also are being observed in more counties in recent years (see Fig. 2).

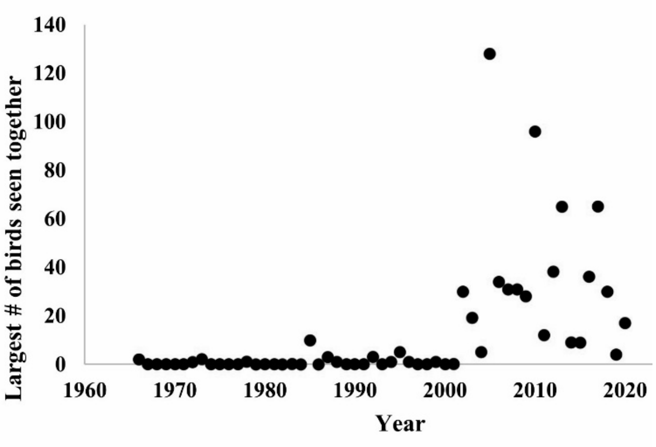

Figure 4. Maximum group size of single flocks of Roseate Spoonbills reported in Arkansas, plotted by year. Increases since 2000 indicate expanding range into Arkansas.

Associates – Avian species found associated at feeding sites help define the nature of a foraging habitat used by a bird of interest. In Arkansas, reported feeding associates of the Roseate Spoonbill include egrets (Chicot, Crawford, Desha, Jefferson, Miller, Sevier, and White Cos.), herons (Chicot, Desha, and White Cos.), White Ibis (Chicot Co.), and Wood Storks (Chicot and Lafayette Cos.). Several times, only the term “egret” or “heron” was mentioned without species definition. When the species was listed, egrets included Cattle, Great, and Snowy, and herons specified only Great Blue. In May, June, and July 2020, KR observed those 3 egret species, Great and Little Blue Herons, and White and White-faced Ibis along with Roseate Spoonbills at 2 nesting sites in Arkansas and Ashley Cos.

These species are wading birds that commonly tend to associate and feed in shallow waters. Unlike most associated species, Roseate Spoonbills feed in a characteristic pattern of swinging the spatulate bill from side to side to intercept small fish, crustaceans, and aquatic insects (Dumas 2020), which is a pattern also sometimes seen in White Ibis (Kushlan 1977).

Reproduction – Roseate Spoonbills were not known to breed successfully in Arkansas until discovery of nests on 24 May 2020 near Montrose (Ashley Co., Fig. 1). The species had been reported in Ashley Co. 28 years earlier - a single individual seen on 15 July 1992 in Overflow National Wildlife Refuge (ONWR), located several km southwest of the Montrose breeding location. Ten years later, Roseate Spoonbills were again reported at ONWR between 28 July and 31 August, usually with only 1-3 individuals sighted, until 21 birds were observed on 13 August and

30 on 20 August 2002. Spoonbills were reported at ONWR also between 10 August and 9 September in 2011, 2014, and 2018.

All birds for which information was available were categorized to be in immature or first year plumage, except for 1 adult observed on 30 July 2002. In neighboring Chicot Co., numerous observations of the birds over 33 years (since 1987) reported adults only in August and September (after the breeding season).

Thus, for the known history of presence in Ashley Co., there had been evidence only of immature birds found in the late season, but no breeding pairs in Ashley Co., or any other part of Arkansas, during the expected nesting season of April-August known in Louisiana and Texas (Oberholser1974; Dumas 2020).

On 24 May and 18 June of 2020, a photographer (Jami Linder) captured images of wetland birds near Montrose in Ashley Co. of southeastern Arkansas (Zellers 2020). The site was a wetland within a farming area near Montrose. The head of the Arkansas Game and Fish Commission's nongame migratory bird program (KR) subsequently identified the species in the images. Finding Roseate Spoonbills among the birds, she requested the photographer to try to get images of nestlings, and filed a report of rare birds on ARBIRD-L. On 24 May 2020, 2 birds were seen on nests, and on 18 June, 8 adults were seen. Other Roseate Spoonbills were carrying nesting material at the time. Discovery of the new species nesting in Arkansas was popularized in a state newspaper (Arkansas Democrat-Gazette, 21 June 2020, p 1A).

Zellers (2020) reported that Linder and Rowe had spotted about 32 Spoonbills, including 20 adults, 8 young that were still bound to nests, and 4 young that were learning to fly. Those observations were made on 1 July (KR, *pers. obs.*).

Some Roseate Spoonbills at the Ashley Co. site were incubating eggs while others had young almost ready to fledge. Roseate Spoonbill eggs hatch after 22 days of incubation, and the birds fledge after about 6 weeks of development (White *et al.* 1982). It is unknown why the range in the timing of nest building was spread over several weeks at the Arkansas site. A couple of hypotheses include the age of the adults (perhaps first-time nesters breed later than older adults), or the timing of nesting might have been related to limited availability of quality nest sites.

The birds left the Ashley Co. nest site during the third week of August 2020, and in 2021 returned the first weekend of April (KR, *pers. obs.*). Current studies are to locate foraging areas and emergent wetlands near the breeding site.

Presence of Roseate Spoonbills in breeding plumage and during the breeding season at another location in southeastern Arkansas indicates another likely nesting site (Zellers 2020). Adult Roseate Spoonbills were observed roosting in trees on the Hampton Reservoir near Lodge Corner, Arkansas Co., on June 3 (KR, *pers. obs.*, see Fig. 1). The landowner (Rick Hampton) previously had seen these birds on the reservoir only in late summer. The birds were roosting at the edge of the reservoir, but sites with likely nests were completely inaccessible by boat or foot and use of a spotting scope did not allow conclusive examination of the presumed nesting site. At least 2 nests were suspected in young cypress and buttonbush.

A breeding abundance map for Roseate Spoonbills generated in eBird showed a northward extension along the Mississippi valley, about two-thirds up the state of Louisiana. Thus, new records of nesting in Arkansas would be expected along the Mississippi, but the new breeding records we report are considerably extralimital to the historically known breeding range.

Management and Conservation – The property that supported the nesting birds in Ashley Co. is managed through the WRP as waterfowl habitat, located within a farming region used primarily for rice and soybean row crops. Other wetland birds associated with the Roseate Spoonbills at the nest site included Anhinga, Cattle and Snowy Egrets, Little Blue and Great Blue Herons, Yellow-crowned and Black-crowned Night Herons, Least Bitterns, White-faced Ibis, and Common Gallinules (Zellers 2020). Some of these birds also were breeding at the site.

Roseate Spoonbills forage in emergent wetlands with water depths up to 20 cm (Powell 1987), but depths of about 12 cm or less are preferred (Lewis 1983), likely because depths below 13 cm are linked to higher nest production rates (Lorenz 2014). Nests in inland forested swamps have been reported in small trees and shrubs such as buttonbush (*Cephalanthus occidentalis*), and under the canopy of hardwood trees such as water oak (*Quercus nigra*) and elms (*Ulmus* sp.) (Dumas 2020). Linder's photos of the Ashley Co. nests in Zellers (2020), and posted by the Arkansas Democrat Gazette newspaper https://www.arkansasonline.com/galleries/29987/album/ showed some of the stick nests in lower branches of bald cypress (*Taxodium distichum*) trees just above the level of buttonbush, and the observers also mentioned nests constructed on the buttonbush. Buttonbush may reach a height of about 5m (16 ft.) (Ogle *et al.* 2020).

Management favoring early successional hemimarsh wetlands (such as the Wetland Reserve Program, WRP) in southeastern, and perhaps southwestern, Arkansas could aid in improving foraging habitat for Roseate Spoonbills. More mature WRP wetlands with established bottomland hardwood tree species could create additional breeding habitat for Roseate Spoonbills (Zellers 2020). This bird is not an historic breeder in Arkansas, but its seasonal natural presence is an attractor for bird enthusiasts, and therefore is also of economic value to the state. Furthermore, several species of wading birds that associate with Roseate Spoonbills benefit from the same management strategies. Restoration of such wetlands also is important for soil hydrology.

Acknowledgments

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Is Technological Progress a Random Walk? Examining Data from Space Travel

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Running title: Forecasting Space Exploration Progress

Abstract

Improvement in a variety of technologies can often be successfully modeled using a general version of Moore's law (Moore 1965) (i.e., exponential improvements over time). Another successful approach is Wright's law, which models increases in technological capability as a function of an effort variable such as production. While these methods are useful, they do not provide prediction distributions, which would enable a better understanding of forecast quality.

Farmer and Lafond (2016) developed a forecasting method which produces forecast distributions and is applicable to many kinds of technology. A fundamental assumption of their method is that technological progress can be modeled as a random walk with drift.

We demonstrate a class of technology, space exploration, in which random walk with drift does not occur. This shows the need for alternative approaches suitable in such technological domains.

Introduction

The recognition that technology progresses in a predictable way is now widespread. Some of the earliest research in this area was conducted by the aeronautical engineer Theodore Paul Wright. Wright described a phenomenon he observed while supervising the production of aircraft, as the batch size of a model of aircraft increased, the per-unit cost to manufacture those aircraft decreased at a predictable rate. The approximate relationship was a 20% drop in cost for every doubling of production volume (Wright 1936). This phenomenon has been attributed by many researchers, to "learning by doing" where productivity is improved through the accumulation of experience. Subsequent research indicated that this pattern holds for a variety of industries although the rate of cost decline varies by industry (Hax and Majluf 1982). This relationship between effort and per-unit cost has been referred to by various names such as learning curves and experience curves (Henderson 1968). Contemporary research into technology foresight uses the term Wright's law, so in this paper we will be using this term.

A more popularly known trend is Moore's law. Originally this phenomenon was described by one of the co-founders of Intel, Gordon Moore, in 1965. Moore famously noted a regular doubling of the number of components that could be built into an integrated circuit and hypothesized that this trend would continue (Moore 1965). The trend soon slowed somewhat but then continued with a doubling time, for that domain, of approximately 18 months to 2 years. Just like Wright's law, Moore's law has been found to be generally applicable to a variety of technologies as shown below.

Before we can develop models of technological improvement, we must first define a metric for improvement. While many legitimate metrics of technological performance exist, one of the simplest to use is cost per performance. This metric has two important advantages for researchers, the data may be available, and the metric captures a general notion of the development of a technology at a given time. Let us review the general applicability of Moore's law in terms of cost with a few examples.

First, we can consider the cost to sequence a human genome. This cost is not only declining exponentially, but it is also declining much more rapidly than the rate of Moore's law as applied to computer processors. More specifically, we see that sequencing a genome today is approximately 100,000 times cheaper than sequencing a genome in 2001 (NHGRI 2020).

Solar electricity is another source of exponential improvement which is having a massive impact on our society. Like most exponential technologies, the initial slow rate of improvement led many to dismiss its importance. Today the situation is much different with many believing that the plummeting cost of solar is a primary cause of the decline of the coal industry (Plumer 2020; Gimon *et al*. 2019; Our World in data 2021).

Finally, we can consider nanotechnology. It is not immediately obvious how progress in nanotechnology should be quantified since it is not a specific technology but rather a scale at which technological effort is undertaken. However, it is often argued that number of nanotechnology publications is reasonable since an exponential increase in the number of publications suggests a commensurate increase in effort to improve the technology and thus, one might reasonably conjecture, the capability of the technology (Palmberg *et al.* 2009; Hullman 2006). Figure 1 shows just such an exponential increase in the number of nanotechnology related scientific publications over time.

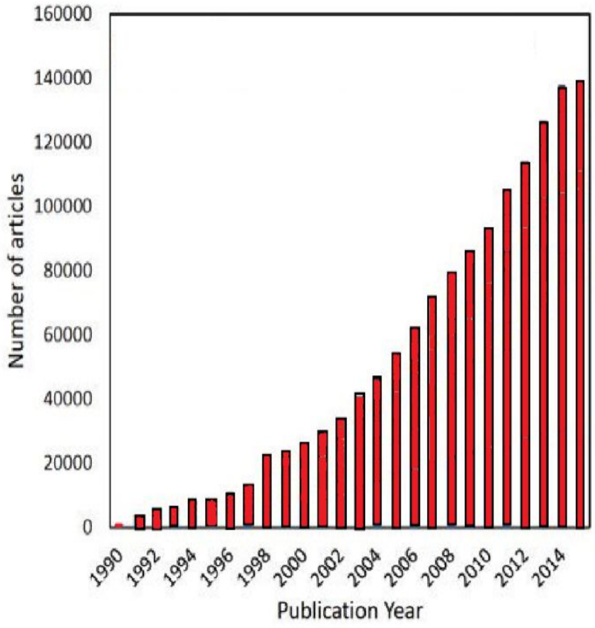

Figure 1. Nanotechnology publications by leading countries. Based on Kwon (2016).

Progress in Space Technology

While many technologies have displayed exponential improvement, one technology that is often missing from the literature on exponential improvement is space exploration. It would be surprising if such improvement did not occur in that domain since it would imply there is something innately different about space exploration technology. In response to this conundrum, a primary focus of our research has been to find exponential trends in space exploration technology that have been previously unnoticed.

As mentioned earlier, however, we must first define our metric of improvement before attempting to find these trends. With this in mind, we investigated spacecraft lifespan as a metric, which we define as the length of time a spacecraft sent to at least one extraterrestrial body operates. The data we used is maintained as a Google spreadsheet located at https://docs.google.com/spreadsheets/d/1ZtfkjbcTOoZTbETUkOY5Hlq5SY5GREvFYjgzmKZQww4/edit#gid=117287008. The data covers deep space missions to extraterrestrial bodies (except the Sun) beginning in 1959 and continuing to the present day. All of the data was collected from public sources. Our previous investigations had shown signs of exponential improvement for the domain (Berleant *et al.* 2017; Berleant *et al.* 2019). An important difference between this metric and other metrics for technological progress is that it does not directly measure empirical properties of the spacecraft such as mass, thrust, or fuel efficiency. However, it can be argued that good metrics for technological progress should capture the utility to the user since this is what results in the societal impact of the technology (Magee *et al.* 2014). Mission lifespan does have this advantage as a metric.

While this initial analysis of trends in mission lifespan was encouraging, there were some problems with using mission lifespan for modeling improvements in space exploration technology. Many models of technological progress use least-squares regression. When building such a model using mission lifespan as the dependent variable, this leads to absurd scenarios where predicted lifespan is longer than the entire history of spacecraft technology (Berleant *et al.* 2019). Therefore, the use of this metric required other modeling techniques to really be useful.

Determining Forecast Quality

The search for other techniques required to model space exploration technology dovetails with another problem brought about by using least-squares regression for modeling improvements in technology. Namely, while Moore's law (Moore 1965) and Wright's law (Wright 1936) have been quite successful in modeling the increase in a wide variety of technologies, they do not provide forecast distributions. This is important because no forecast is 100% accurate and these distributions would give us an idea of the range of outcome values we might encounter. Farmer and Lafond (2016) mention this problem in relation to technology foresight and describe why understanding forecast uncertainty is so important for policy considerations.

So where does forecast uncertainty arise? Hyndman (2014) lists four primary sources:

1.) The assumption of the continuation of past trends
2.) Model quality
3.) Parameter uncertainty
4.) Random shocks

The first assumption is a prerequisite for an extrapolation-based approach. Factor (2) can be optimized by modeling techniques such as optimizing model fit and distribution of residuals. Parameter uncertainty can theoretically be minimized using simulations although this increases the complexity of forecasting (Ibid). In this article we focus on the impact of random shocks. This can be done by using the standard literature of time series analysis.

There are important differences between the methods used by us and those used by Farmer and Lafond (2016). Their method assumes that parameter uncertainty is the largest source of forecast uncertainty. More specifically, the method assumes that forecast variances grow with the square of the time horizon in the presence of parameter uncertainty but only linearly when there is no parameter uncertainty (Sampson 1991). Parameter uncertainty was likely more of an issue since most of the time series data was short and had to be aggregated (Farmer and Lafond 2016).

Another important assumption of their method is that improvements in each technology can be modeled as a random walk with drift. More specifically:

$$y_t = y_{t-1} + \mu + \varepsilon_t \qquad (1)$$

where y_t is the performance of the technology at a given time step, μ is the "drift" or trend, and ε_t is an i.i.d. noise process. Each technology is modeled with a different mean and variance parameter for the noise component of the model. Due to the limited size of the time series, they were unable to perform unit root tests to justify this approach theoretically; however the empirical results they derived were consistent with this model.

We did not have this problem since our space mission data is large enough to perform unit root tests, and our results indicated that the data was not generated by a random walk as we demonstrate below. Therefore, space exploration is one technology that would benefit from an approach other than the random walk model. In the following section we detail how unit root tests, autocorrelation patterns, and backtesting demonstrate that this data does not have a unit root.

Methods and Results

As stated before, we used time series modeling to describe improvements in space exploration technology. This approach does not consider parameter uncertainty, suggesting prediction intervals would likely be too narrow in backtesting (Hyndman 2014). Since this did not happen for mission lifespan models it is likely that parameter uncertainty is not a significant issue for them. Each point forecast was based on an ARIMAX model, which is a combination of a linear regression and an ARIMA model fitted on the residuals. The regressor for the model was the order of launch. This approach was chosen over a standard ARIMA model since this performed better on backtesting.

All analysis was conducted using the R statistical packages *tseries, forecast, and stats*. First, we had to determine if the time series was stationary or not. This is done usually by examining the autocorrelation function (ACF) of the data. Figure 2 displays a plot of the autocorrelation of mission lifespan which decays very slowly. While this is usually considered a sign of nonstationarity, the first differences tell a different story. Figure 3 shows the plot of the first differences of the data which displays several significant lags. This is inconsistent with time series that contain stochastic trend (i.e., a random walk) but is consistent with time series that contain a deterministic trend. This is because the first differences of a time series describe the changes from one period to the next, the first differences of a random walk should therefore be uncorrelated.

The next step is to determine the autoregressive components of the model. A plot of the Partial Autocorrelation Function (PACF) is displayed in Figure 4. This plot shows significant autocorrelations for lags 1, 2, and 5. Autoregressive models display decaying autocorrelations alongside partial autocorrelations with significant lags typically equal to the appropriate autoregressive parameter. These plots indicate that an autoregressive parameter no larger than 2 would be appropriate. An Augmented Dickey-Fuller (ADF) test statistic of -3.4854 was generated at 5 lags with a p-value of 0.0473 which further indicates stationarity. The lag length of 5 was originally chosen

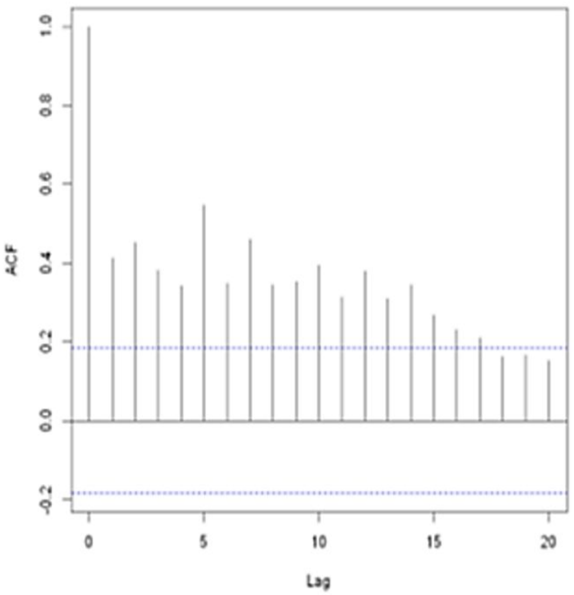

Figure 2. Autocorrelation of Mission Lifespan.

for the purpose of finding the optimal model to predict progress in mission lifespan. However, since the focus of this paper is to compare random walk and non-random walk models, this will not be expounded on. The significant autocorrelation at lag 5 of the partial autocorrelation plot may be an indication of seasonality. This hypothesis can be further supported by Figure 2 which displays a noticeable spike at approximately every 5 lags. While it can be demonstrated that seasonal models produce better forecasts for mission lifespan we will not elaborate on this topic. As stated earlier, the focus of the paper is whether or not a random walk model is appropriate for predicting progress in mission lifespan.

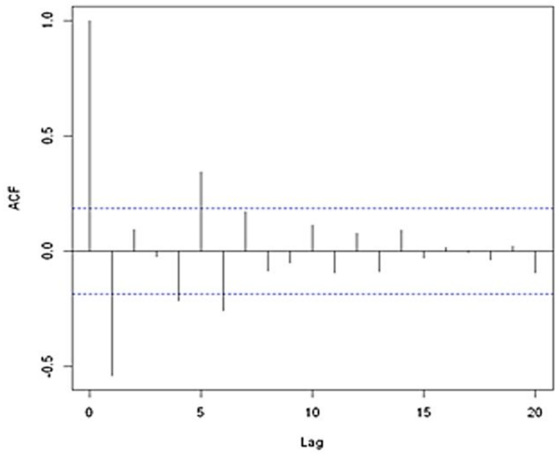

Figure 3. Autocorrelation of Mission Lifespan Differences.

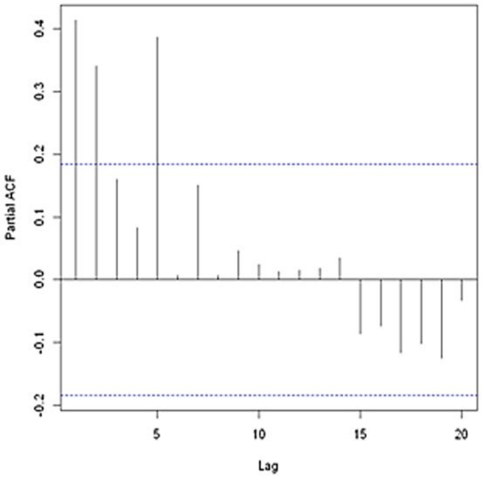

Figure 4. Partial Autocorrelation of Mission Lifespan.

To illustrate our thesis, a model containing a single autoregressive parameter will be used although this pattern can be observed with higher numbers of parameters. One of the most used is methodology based on Autoregressive Integrated Moving Average (ARIMA) model by Box *et al.* (2015).

The ARIMAX model is an extension of Autoregressive Integrated Moving Average (ARIMA) model. The ARIMA model has three parameters, namely: p, d and q, where p is the autoregressive term, q is the moving average term and d indicated the series is differenced to make it stationary (Smarten 2018).

When an ARIMA model includes other time series as input variables, the model is sometimes referred to as an ARIMAX model. Pankratz (1991) refers to the ARIMAX model as dynamic regression.

By using Maximum Likelihood Estimation (MLE), we obtained the following equations for the time series using one autoregressive parameter. For an ARIMA (1,0,0) model we have:

$$Y_t = -0.5367Y_{t-1} + \varepsilon_t \tag{2}$$

where Y_t is the value of the time series at time *t* and ε_t is an error term. For an ARIMAX (1,0,0) model we obtain:

$$Y_t = 0.0727X_t + n_t \tag{3}$$

where Y_t is the value of the time series at time *t*, X_t is the order of launch, and n_t is an ARIMA (Autoregressive Integrated Moving Average) model fitted to the model residuals.

The Akaike information criterion (AIC) is an estimator of out-of-sample prediction error and thereby relative quality of statistical models for a given set of data (Akaike 1977). The Bayesian Information Criteria (BIC) (Schwartz 1978) or Schwarz criterion (also SBC, SBIC) is a criterion for model selection among a finite set of models. It is based, in part, on the likelihood function. The ARIMA (1,0,0) model has AIC and BIC scores of 501.13 and 506.23 respectively, while the ARIMAX (1,0,0) model has AIC and BIC scores of 472.78 and 483.04 respectively. ACF plots of residuals for both models displayed in Figure 5 and Figure 6 demonstrate that the residuals of the ARIMAX (1,0,0) model more closely resemble white noise with the aforementioned significance 5. This is a further indication that the ARIMAX model should be preferred.

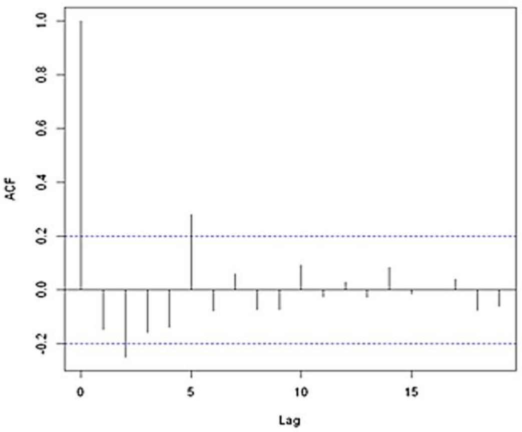

Figure 5. Autocorrelation of ARIMA (1,0,0) Residuals.

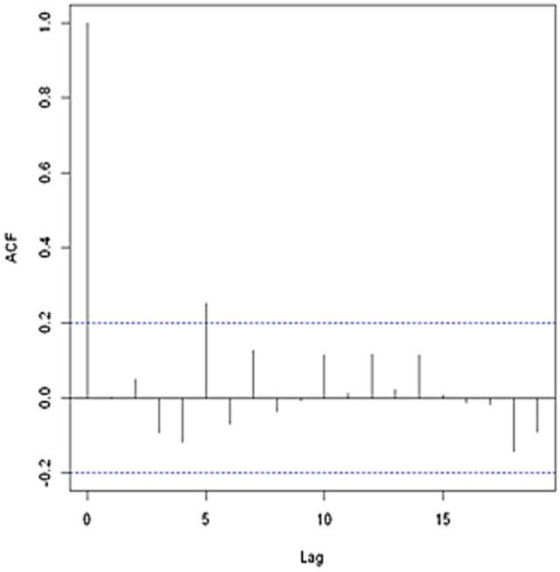

Figure 6. Autocorrelation of ARIMAX (1,0,0) Residuals.

Backtesting

Forecasting using the assumption of stationary data also produces superior forecasts as measured by backtesting. As an example, Figure 7 shows the results of backtesting with an ARIMA (1,1,0) model. The notation indicates that our model is based on the first differences of the time series and predicts the current period using the value immediately preceding it. This model was produced using 96 data points with 17 data points withheld for validation. This amounts to 85 percent and 15 percent respectively. The blue and gray regions represent the 80% and 95% prediction intervals of the model respectively. The red line consists of the 17 data points that were withheld for validation and the blue line represents the point forecast of the model.

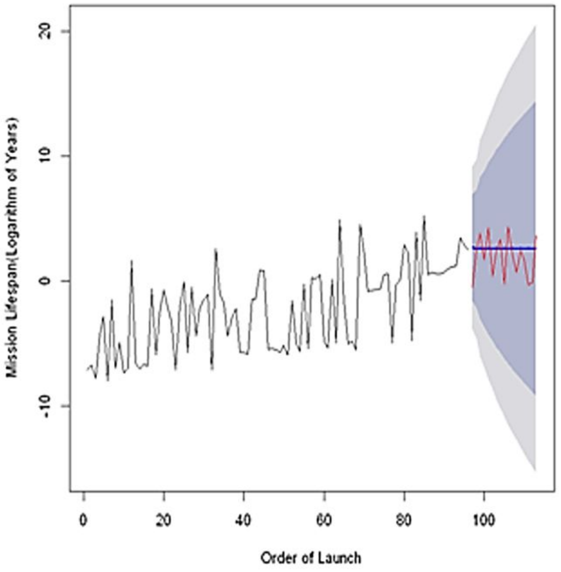

Figure 7. Forecast using an ARIMA (1,1,0) model. Log scaling prevents the left-hand region of the graph from being compressed and thus losing detail relative to the right-hand region. The wide prediction intervals indicate that the forecast assumes a random walk.

Figure 8 displays an ARIMAX (1,0,0) model trained with the same data. Notice that in both cases we produce models with accurate forecasts but the model which assumes stationarity provides a narrower range of possibilities and therefore, supports a narrower prediction. Due to this and the other aforementioned reasons, ARIMAX models produce better predictions than ARIMA models for improvements in mission lifespan.

We can be reasonably certain that the time series will remain stationary as long as our estimate of the model's autoregressive parameters, φ_p , are less than 1. Therefore, the general pattern displayed in Figure 5 should remain valid for future forecasts as long as the data generating process does not change.

If our parameter estimate is either equal to 1 or a unit root test is statistically significant then this model is no longer reasonable.

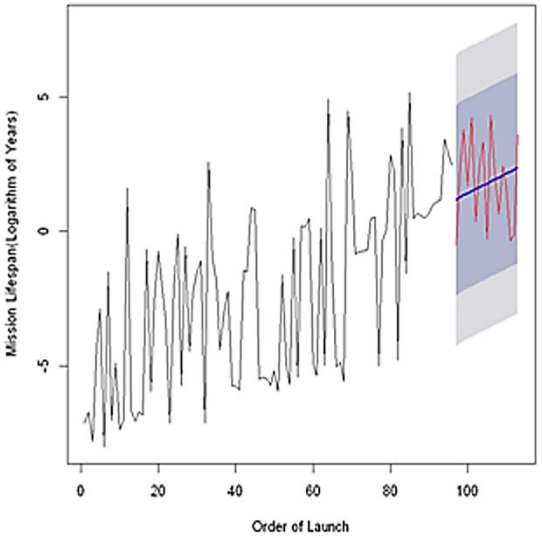

Figure 8. Forecasts for an ARIMAX (1,0,0) model with a linear trend. Log scaling prevents the left-hand region of the graph from being compressed and thus losing detail relative to the right-hand region. The assumption of no random walk and a linear trend results in a better prediction as indicated by the narrower prediction interval.

Discussion & Conclusion

It seems that a random walk model can be useful for modeling improvement in a wide variety of technologies. Nevertheless, it appears from our analysis of mission lifespan data that not all technologies follow a random walk model and thus require a different approach.

But why is this so? One possible explanation is that space exploration is a fundamentally different kind of technology. For example, one might conjecture that space exploration is not primarily a commercial activity whereas the technologies most often analyzed are primarily commercial. The literature of endogenous growth theory suggests that economic forces play a significant role in producing technological development, lending support to that possibility (Romer 1990). This becomes more apparent when we measure improvements in terms of cost. Basic economics would hold that if the price elasticity of demand were held constant then a decrease in cost should lead to an increase in demand which would lead to a decrease in cost via Wright's law, and so forth (Magee *et al.* 2014). It is easy to see how this could give rise to a random walk pattern and how it should not apply to non-commercial technologies.

Another possible reason is that much of the original data did not have a unit root and this was undetected since it was so short. This is likely a problem due to the lack of comprehensive databases on technological performance. The Santa Fe Institute's performance curve database is a noteworthy attempt to correct this problem, but ultimately researchers need more data. Most modeling of technological performance improvements is based on Maximum Likelihood Estimation which is notorious for mis-specifying parameters when data is limited (Bishop 2006).

In closing, we hope this advances the discussion on what methods might best be used to model improvements in technological performance. Technological advancement has a great effect on the future of society and thus increasing our understanding of how it develops can help deepen understanding of social change.

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Novel Reproductive Data on Pealip Redhorse, *Moxostoma pisolabrum* (Cypriniformes: Catostomidae), from Northeastern Arkansas

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Running Title: Pealip Redhorse Reproduction

Abstract

Little is known about the natural history of the Pealip Redhorse (*Moxostoma pisolabrum*), particularly on its reproductive biology in Arkansas. We examined 11 female *M. pisolabrum* collected in late February 2020 and 2021 from the Black River, Lawrence County. Egg mass (g) represented 9–14% of the total weight of these gravid females. This is the first time information on female reproduction in this species has been reported from any population of *M. pisolabrum* in the state.

Introduction

The Pealip Redhorse, *Moxostoma pisolabrum* Trautman and Martin, 1951, is a slender sucker with a short head and a distinctive pea-shaped thickening in the middle of the upper lip (Fig. 1). This species was formerly recognized as a subspecies of the Shorthead Redhorse, *Moxostoma macrolepidotum* (Lesueur, 1817). It was elevated to species status by Nelson *et al.* (2004), based on Harris *et al.* (2002). The overall range of this species is watersheds in the Ozark uplands and adjacent areas, in southeastern Kansas, Missouri, Oklahoma, and Arkansas (Miller and Robison 2004; Robison and Buchanan 2020). In Arkansas, it inhabits clear, gravel-bottomed medium to larger river systems of the state, including the Arkansas, White, and St. Francis drainages (Robison and Buchanan 2020).

Figure 1. *Moxostoma pisolabrum* from the Black River. (A) Lateral view showing specimen. (B) Ventral view showing pea-shaped thickening in middle of upper lip (arrow). Photos by CTM.

Little is known about the reproductive biology of *M. pisolabrum*, including the timing of spawning. Tuberculate males were observed in early March in Crooked Creek, Marion County, Arkansas (Robison and Buchanan 2020). However, nothing is known about reproductive data for females throughout its range. Presumably, reproduction is similar to *M. macrolepidotum* from Kansas and Illinois (Cross 1967; Burr and Morris 1977; Sule and Skelly 1985). In Illinois, *M. macrolepidotum* spawned in mid-May (Burr and Morris 1977). Pflieger (1997) noted that schools of *M. pisolabrum* (reported as *M. macrolepidotum*) were observed on gravelly riffles in the Moreau River of central Missouri during late April. Here, we document novel reproductive information for *M. pisolabrum* from northeastern Arkansas.

Materials and Methods

During 21–22 February 2020 and again between 25–27 February 2021, 11 female *M. pisolabrum* (mean ± SD total length [TL] = 455.7 ± 43.4, range 400–540 mm) were collected by a local commercial fisherman with hoop nets from the Black River at Black Rock, Lawrence County (36° 06' 4.3848" N, -91° 05' 7.9224" W). Fish were transferred to large (625 liter) aerated tanks containing habitat water and killed by immersion in a concentrated tricaine methanesulfonate solution. They were weighed on an Ohaus digital scale to the nearest 0.1 g. A mid-ventral incision was made from the lower operculum to the anus. Egg masses were

Table 1. Reproductive data on female *Moxostoma pisolabrum* from the Black River at Black Rock, Lawrence County, Arkansas. Specimens 1-3 were collected in February 2020 and specimens 4-11 were collected in February 2021.

Specimen no.	TL (mm)	Total wt g (lbs)	Egg mass g (lbs)	% of Total wt
1	400	648.6 (1.43)	86.2 (0.19)	13.3%
2	435	907.2 (2.0)	108.9 (0.24)	12.0%
3	464	1,215.6 (2.68)	158.3 (0.35)	13.0%
4	540	1,973.1 (4.35)	231.3 (0.51)	11.7%
5	490	1,274.6 (2.81)	131.5 (0.29)	10.3%
6	485	1,111.3 (2.45)	149.7 (0.33)	13.4%
7	415	707.6 (1.56)	90.7 (0.20)	12.8%
8	405	657.7 (1.45)	77.1 (0.17)	11.7%
9	454	830.1 (1.83)	108.9 (0.24)	13.1%
10	420	743.9 (1.64)	104.3 (0.23)	14.0%
11	505	1,510.5 (3.33)	136.1 (0.30)	9.0%

collected and weighed to the nearest 0.1 g on the same scale. Voucher specimens were photographed.

Results and Discussion

All 11 female *M. pisolabrum* possessed large yolked egg masses (Fig. 2) that ranged in weight from 77.1 to 231.3 g (Table 1). Egg mass accounted for 9 to 14% of body weight, with no apparent relationship to length or weight of the females, though we note our sample size was modest. At the same time, a single tuberculate male *M. pisolabrum* (535 mm TL, 1,388 g) was sexually mature and producing milt.

Although our sample size is modest, we suggest that spawning of *M. pisolabrum* in northeastern Arkansas may occur as early as late February. Additional collections of *M. pisolabrum* in other parts of the state are recommended to add to our knowledge of the natural history of this fish.

Figure 2. Egg mass complement from *Moxostoma pisolabrum*.

Acknowledgments

The Arkansas Game and Fish Commission issued a Scientific Collecting Permit to CTM and HWR. We thank Dwight Ferguson, commercial fisherman (Black Rock), for collecting the *M. pisolabrum* and providing his site for the processing of fish.

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Selected Helminth Parasites (Cestoda, Nematoda) of Bobcat, *Lynx rufus* (Carnivora: Felidae), in Northeastern Arkansas

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Running Title: Endoparasites of Bobcats

Abstract

The bobcat, *Lynx rufus* is a relatively common Arkansas carnivore that ranges statewide. Although there is a great deal of information on the natural history of this species in the state, there have been few studies where parasites have been documented in Arkansas bobcats. Here, a single specimen was examined and found to be infected with a tapeworm, *Taenia rileyi* and two nematodes, *Toxoascaris leonina* and *Toxocara cati*. We document the first record of *T. rileyi* from Arkansas and the first report of *T. leonina* from a bobcat in the state.

Introduction

The bobcat, *Lynx* (syn. *Felis*) *rufus* (Schreber, 1777), is a common moderately-sized stealthy felid that occurs from southern Canada slightly above the 50th parallel south through much of the central USA as far southward as Rio Mezcala, México, just below the 18th parallel (Larivière and Walton 1997); it is found statewide in Arkansas (Sealander and Heidt 1990). In eastern Arkansas, *L. rufus* primarily feeds on rabbits followed by moles and small rodents (Tumlison and McDaniel 1990). Although a great deal is known about various aspects of the natural history and ecology of *L. rufus* in Arkansas (Fritts and Sealander 1978; Tumlison and McDaniel 1981, 1986, 1988, 1990; Tumlison 1983; Rucker *et al.* 1989), little is known about its parasites in the state.

In addition to a suite of ectoparasites, *L. rufus* is host to protozoans, blood parasites, trematodes, cestodes, nematodes, and acanthocephalans (Miller and Harkema 1968; Pence and Eason 1980; Watson *et al.* 1981; Tiekotter 1985; Marchiondo *et al.* 1986; Smith *et al.* 1995; Reichard *et al.* 2004; see also references in Hiestand *et al.* 2014). To our knowledge, the only report of endoparasites from this host in the state was by Heidt *et al.* (1988). They reported eight species of intestinal parasites. Here, we document three helminths from a *L. rufus* from the Ozark Highlands.

Materials and Methods

A single adult male *L. rufus* was collected on 26 February 2021 by a local furbearer in Black Rock, Lawrence County (36°06’53”N, -91°04’25.73”W), using a leg-hold trap and killed by .22 caliber gunshot. The pelage was retained and not available for ectoparasite examination. A blood sample was taken from the heart, smeared on a glass slide, air dried, fixed in absolute methanol, and stained with Wright’s Giemsa stain. Fifty high power fields were examined under oil immersion (1,000×) with a light microscope. A mid-ventral incision from throat to anus was made and all organs and 10cm pieces of the intestinal tract were placed in Petri dishes containing 0.9% saline. Feces from the rectum was placed in 2.5% potassium dichromate and examined for coccidia after flotation in Sheather’s sugar solution (specific gravity = 1.30). A stereomicroscope was used to scan the material in Petri dishes and when parasites were found, they were rinsed of mucus and fixed in nearly boiling tap water. Cestodes were placed in an individual vial containing 10% neutral buffered formalin and a piece of the posterior proglottid was saved in 70% DNA grade ethanol for potential molecular characterization. Nematodes were preserved in 70% ethanol, cleared in lactophenol, and observed as temporary mounts. Helminths were deposited in the Harold W. Manter Parasite Collection (HWML), University of Nebraska, Lincoln, Nebraska.

Results and Discussion

The blood was negative for any intraerythrocytic hematozoans and the bobcat was not passing coccidia in its feces at the time of death. However, three species of helminths were found in *L. rufus* as follows:

CESTODA: CYCLOPHYLLIDEA: TAENIIDAE

***Taenia rileyi* Loewen, 1929.** – Nine tapeworm specimens (Fig. 1A, HWML 112230) matched the description of *T. rileyi* (Rausch 1981). The rostellum (Fig. 1B) has 42 hooks (21 large, 21 small) that measured 230 and 180 µm long, respectively. *Taenia rileyi* has been reported in bobcats from Georgia, Illinois, Minnesota, Nebraska, “New England”, North Carolina, Oklahoma, South Carolina, Texas, Utah, Virginia, and West Virginia (Hiestand *et al.* 2014; McAllister *et al.* 2019). Larval *T. rileyi* have been reported in cricetid rodents in Florida and Georgia and may serve as intermediate hosts of this tapeworm (Kinsella 1988, 1991). Hiestand *et al.* (2014) reported *T. rileyi* occurred in high prevalence (70%) that caused intense infections in Illinois bobcats; it is also considered to be a bobcat-host specific helminth. We here document *T. rileyi* from Arkansas for the first time.

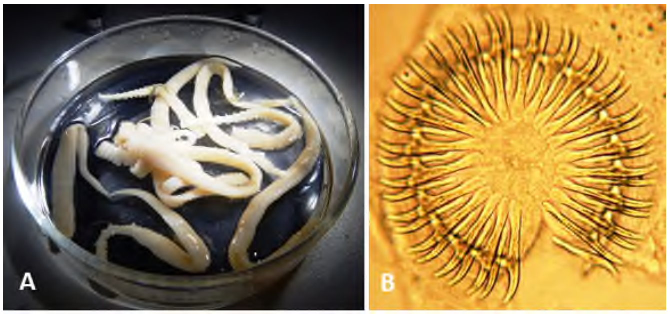

Figure 1. *Taenia rileyi* from *Lynx rufus* from Lawrence County, Arkansas. (A) Recovered tapeworms. (B) Rostellar hooks.

NEMATODA: ASCARIDIDA: ASCARIDIDAE

***Toxoascaris leonine* (von Linstow, 1902).** – A single female specimen (HWML 112231) was recovered from the intestine. This nematode is a common parasite found in domestic and wild canids and felids. Sprent (1959) provided a host list that included a variety of canids, felids, and procyonids. The life cycle includes oral ingestion of the infective ova, and then the egg-hatched larvae mature in the small intestine of the definitive hosts (Sprent 1959). It is cosmopolitan in distribution and the paratenic hosts are usually rodents (primarily rats and mice). This nematode poses potential threats to public health due to aberrant larva migrans (Beaver and Bowman 1984). Shoop *et al.* (1991) reported it in the state from mixed-breed cats (*Felis catus*) from Conway County.

***Toxocara cati* Schrank, 1788.** – Three female and a single male specimen (HWML 112251) were recovered from the intestine. Specimens fit the description of *T. cati* by possessing a ventriculus posterior to the esophagus (Sprent 1956). Heidt *et al.* (1988) previously reported *T. cati* from bobcats from Montgomery County. It was also reported from *F. catus* from Conway County (Shoop *et al.* 1991).

In the only previous report of parasites on eight Arkansas bobcats, Heidt *et al.* (1988) documented the following helminths (1 cestode, 7 nematodes): *Taenia taeniaformis*, *Ancylostoma* sp., *Spirometra mansoides*, *Physaloptera rara*, *T. cati*, *Strongyloides* spp., *Trichurus* spp., and *Capillaria* spp. Interestingly, these authors did not report *T. leonina* and *T. rileyi* that we found in the current study from *L. rufus*.

Overall, comparative data on the helminth parasites of mammals in the Order Carnivora in Arkansas is poorly known. Given this lack of information on endoparasite diversity on carnivores in the state, continuation of helminth surveys will surely lead to additional new host and geographic records.

Acknowledgments

The Arkansas Game and Fish Commission issued a Scientific Collecting Permit to CTM. We thank D. Ferguson, furbearer (Black Rock) for providing the bobcat carcass for examination and Dr. J. Mike Kinsella (Missoula, MT) for identifying and depositing the cestode and providing Fig. 1. We also thank Dr. Gabor Racz (HWML) for expert curatorial assistance and technical help with the cestode.

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History, Distribution, and Reproduction by the Swallow-tailed Kite (*Elanoides forficatus*) in Arkansas

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Running Title: Swallow-tailed Kite in Arkansas

Abstract

The Swallow-tailed Kite (*Elanoides forficatus*) is a rare bird in Arkansas, and its historical populations are believed to have declined over much of the last century due to loss of bottomland hardwood forests and associated wetlands. However, sightings have increased in the recent 2 decades. By use of online sources for citizen science, we elucidate the current distribution of this bird in Arkansas, and comment on the status of reproduction. Swallow-tailed Kites arrive in Arkansas as early as March and remain to mid-September, but numbers of reported sightings have a bimodal occurrence with peaks in May and August.

Introduction

The Swallow-tailed Kite (*Elanoides forficatus*) is a monomorphic (sexes do not differ in appearance) raptor with black and white markings that present a striking contrast. The head, neck, underside, axillaries, and anterior part of the underwing are white, whereas the back, upperwing coverts, and all rectrices and remiges are black. The bird is named for the obvious character of a very long and deeply forked tail, which is used in very graceful movement in the air and which distinguish it from other kites even in silhouette (Meyer 2020).

The original breeding range for the Swallow-tailed Kite included most of the southeastern United States and extended up the Mississippi River toward the Great Lakes. However, by 1940 the breeding range appeared to have been restricted to southeastern Atlantic and Gulf Coastal states (Meyer 2020).

Due to lack of recent sightings, Howell (1911) believed that the Swallow-tailed Kite was extremely rare or no longer occurred in Arkansas by 1910, though he documented that it had bred in Newport (Jackson Co.) in 1884 and in Little River Co. in 1890. Along the Arkansas River drainage into Oklahoma, the bird was considered to have been abundant, yet had disappeared from Oklahoma by 1910 (Nice 1931).

Smith (1915) reported a record from Winslow (Washington Co.), which accounted a Swallow-tailed Kite seen on 8 October 1913 by a farmer. Black (1935) noted that there had been no further record in the Winslow area, but commented that the bird apparently was once common there as a transient. Baerg (1931) also did not add any new records, but 20 years later reported observations from Newport in 1935 and on 10 July 1949, when pairs were seen and believed to be nesting (Baerg 1951).

Howell (1911) and Baerg (1931) regarded the former population of Swallow-tailed Kites in Arkansas to have been numerous, nesting in the cypress swamps in the lowlands of Arkansas. At the time of early exploration of the deltaic region (Mississippi Alluvial Plain) of eastern Arkansas by Europeans, "the skies were filled with" a variety of bird species, including Passenger Pigeons, Whooping Cranes, Carolina Parakeets, Ivory-billed Woodpeckers, and Swallow-tailed Kites (Grimmett 1989). However, James and Neal (1986) wrote that this bird had not been recorded in the state since the 1940s because there were no reports in the Audubon Society files (and the 1935 and 1949 reports by Baerg (1951) were from second hand accounts).

The status of Swallow-tailed Kites in Arkansas after about 1900 is unclear and James and Neal (1986) reported no records since the 1940s and no reports in Audubon Society files. Before about 1910, regular breeding by Swallow-tailed Kites in eastern Texas was observed in coastal prairies and timbered watersheds (Brown *et al.* 1997). However, the species had completely disappeared by the mid-1910s. This decline in population was the result of human interference causing destruction of feeding grounds and nesting sites (Brown *et al.* 1997).

The most recent summary of information regarding the Swallow-tailed Kite in Arkansas was compiled by Chiavacci *et al.* (2011). At that time, this bird had become a frequent visitor of the Dale Bumpers White

River National Wildlife Refuge (NWR), though sightings of the Swallow-tailed Kite were still considered to be rare. Evidence of nesting attempts was seen in the presence of constructed nests, nest-building and incubation behavior, and the documented presence of nestlings in 2005 and 2008. However, no attempts at nesting were successful. The birds had been observed in forested wetlands consisting heavily of various oak trees.

With the increasing use of citizen science web sites, data regarding current distribution, habitat, and reproduction of many species of birds are growing, with more sightings being reported in recent years. Our objectives were to compile current information to look for patterns of distribution, arrival, and departure of the birds from Arkansas on a monthly basis, as well as over the years. Further, we consolidate information to date about this bird in Arkansas.

Methods and Materials

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 associated birds, prey, and appearance. Many records are duplicated on those sites, but use of all sources allowed us to reduce chances of missing important data.

We sorted our spreadsheet of data to determine the locations and timing of presence of the birds in Arkansas, and made maps of distribution. Because multiple observers often independently report their sightings, we filtered the data by eliminating redundant reports for birds seen within an 8 km (5 mi.) radius and within 5 days of each other. We did not eliminate those reports in which presence of the bird extended over more than 5 days, or when the species was seen at the same location but in different years.

Results and Discussion

Distribution - The first recent observation (since the 1940s) of a Swallow-tailed Kite in Arkansas was in Calhoun Co. on 7 April 1986. The earliest reported date of observation in Arkansas was in southeast Arkansas (Drew Co.) on 25 March 2020. Records of the birds across appropriate habitats in Arkansas continue through the spring and summer, with the last bird sighting reported on 16 September (these kites usually migrate southward from the US by mid-September (Meyer 2020).

The birds tend to become summer residents in bottomland and marshland habitats near river systems (Arkansas, Sulphur, and White) and their tributaries. Reported locations, including rare observations, are included in Fig. 1. As these records were compiled from “citizen science,” we note that the data do not represent a systematic survey, and that kites may go undetected in less accessible habitats.

Most sightings record only 1-2 individuals, but high-quality bottomland or wetland habitats are revisited often by the birds, e.g.: Arkansas County at Dale Bumpers White River NWR and Miller County at Sulphur River Wildlife Management Area (WMA). The most birds observed at one time were in Miller Co., Sulphur River WMA, on 11 July 2020, where 8 birds (including 4 adults, 1 second year bird, and 3 fledglings) were reported. Due to likelihood of seeing these birds, these sites are visited often by enthusiastic birders wishing to see this rare species in Arkansas, resulting in more numerous reports.

Examination of historical distribution shows the longest term of continued occurrence in the east central region of Arkansas (Fig. 2). From 1986-1991, the species was recorded only in Calhoun and Van Buren Cos. From 1992-1996, the bird was reported in Pike and Scott Cos., then from 1997-2001 sightings expanded

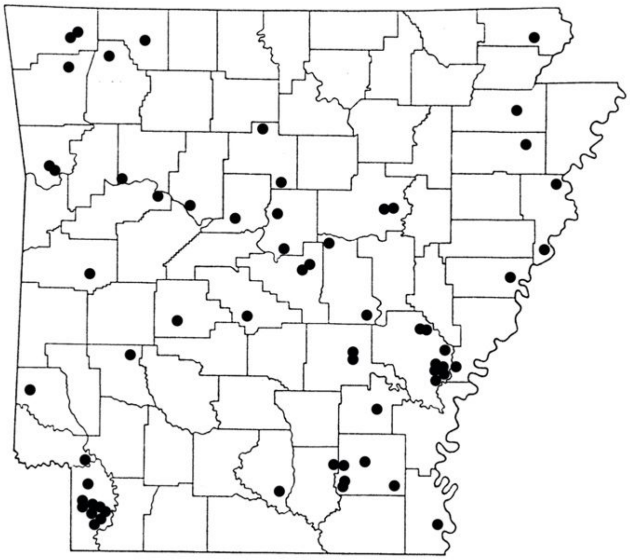

Figure 1. Distribution of Swallow-tailed Kites (*Elanoides forficatus*) in Arkansas based on literature and records compiled in arbirds.org and ebird.org. Dots indicate locations of observation.

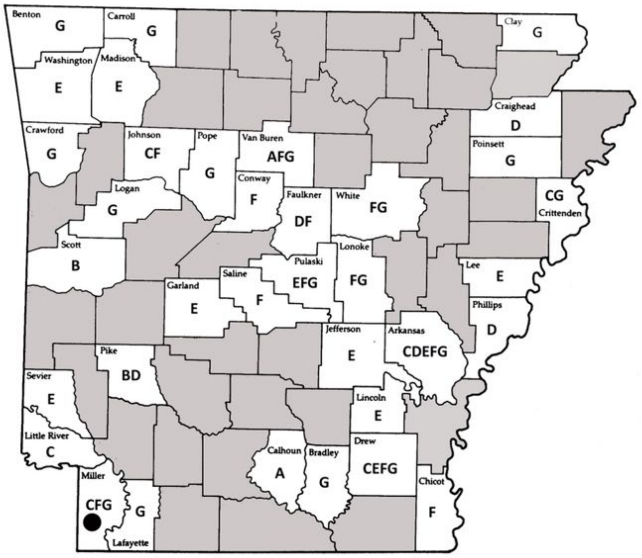

Figure 2. Historical distribution of Swallow-tailed Kites in Arkansas. Unshaded counties have records, and lettering represents time frames for the records: A = 1986-1991, B = 1992-1996, C = 1997-2001, D = 2002-2006, E = 2007-2011, F = 2012-2016, and G = 2017-2020. Dot indicates the county in which successful nesting has been observed.

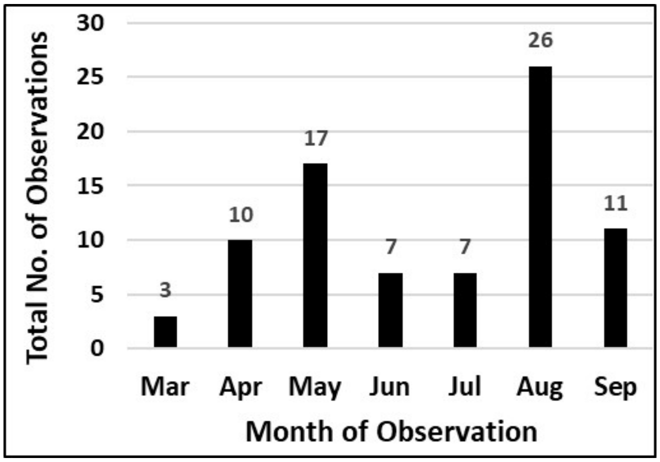

Figure 3. Monthly chronological frequency of reported observations of Swallow-tailed Kites in Arkansas, 1986-2020 (with likely redundant reports of the same birds removed). Months not listed had no reported observations.

into Arkansas, Crittenden, Drew, Johnson, Little River, and Miller Cos.

Sightings of the Swallow-tailed Kite were reported in 5 counties from 2002-2006 including the addition of Craighead, Faulkner, Phillips, and Pike Cos. Interest in reporting increased after 2010. Between 2007-2011 observations were reported from 10 cos., with new records from Garland, Lee, Lincoln, Madison, Pulaski, Sevier, and Washington Cos. Reports of the Swallow-tailed Kite continued to increase from 2012-2016 with reports from 12 counties including the addition of Chicot, Conway, Lonoke, Saline, and White Cos.

From 2017-2020, sightings were reported from 17 counties. Of those, first time reports came from Benton, Bradley, Carroll, Clay, Crawford, Lafayette, Logan, Poinsett, and Pope Cos. To date, Swallow-tailed Kites have been recorded in 35 counties.

Sightings of Swallow-tailed Kites in Arkansas begin in March and end in September. However, the distribution of sightings is bimodal (Fig. 3). Birds seen in April and May include transients but also birds that attempt nesting (Chiavacci *et al*. 2011; Zellers 2020a, b). The August peak in observations also likely is augmented by birds wandering from other areas during the post-breeding period (Meyer 2020). The number of counties in which sightings have been reported also peaks in August (Fig. 4). Interestingly, of the 35 counties with reported sightings, 20 only had sightings during the expected southern migration period of August to September. In Florida, a similar bimodal occurrence of sightings seems to be associated with increased foraging activity after eggs hatch, followed by a second peak associated with independence of young and gathering of birds in group foraging areas prior to southern migration. Further, migrations of tracked birds in GA, SC, and FL tended to be somewhat N-S, with no migrating birds wandering more westward (Ken Meyer, *pers. comm.*).

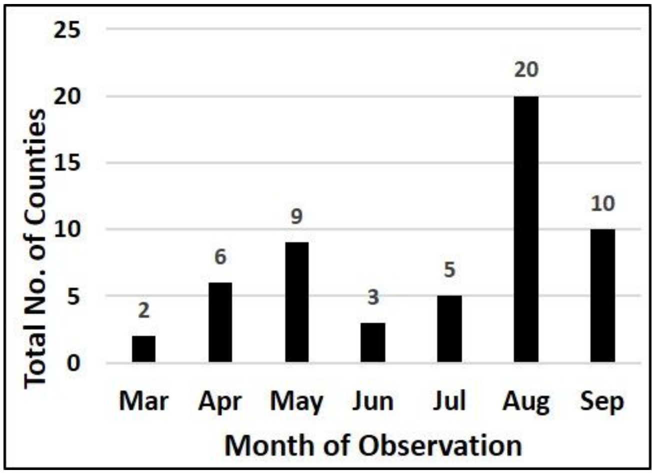

Figure 4. Total number of counties in Arkansas from which reports of Swallow-tailed Kites originated, stratified by month. Birds attempting to nest were found in the same county across multiple months, and some counties had multiple-month sightings without reports of nesting, but reports occurred only in August and September from 20 of the 35 counties with reported records. Data from 1986-2020, with likely redundant reports of the same birds removed. Months not listed had no reported observations.

Citizen reports of the Swallow-tailed Kite often coincide with sightings of its closest relative in Arkansas, the Mississippi Kite (*Ictinia mississippiensis*). Arkansas Swallow-tailed Kites have been reported foraging with flocks of 4-25 Mississippi Kites. Of 11 reports of mixed flocks, 3 were in April-May, and 8 in July-Sept, which may indicate appearance in group migrations because none were found in June and most were in August. Other flock associates included up to 28 Common Nighthawks (*Chordeiles minor*) and a group of Turkey Vultures (*Cathartes aura*) and Black Vultures (*Coragyps atratus*).

Nesting - Observations of nesting by Swallow-tailed Kites in Arkansas were documented in the late 1800s, and might have occurred rarely until the 1940s, but not since then (Baerg 1951; James and Neal 1986). In 1998, a pair was observed during the breeding season in White River NWR (Chiavacci *et al*. 2011). Their study of potential nesting in White River NWR, conducted from 2001-2009, detected 5 nests. However, none was able to produce fledglings. Interpreted failure of nests appeared to be due to abandonment by the nesting pair, young kites being killed likely by either a hawk or owl, and eggs in 1 nest probably preyed upon by a snake (Chiavacci *et al*. 2011). The young kites killed by another raptor were estimated to be about 2 weeks old, indicating that nesting success was possible.

Chiavacci *et al*. (2009, 2011) reported attempted nesting in White River NWR beginning in 2002. Although a few other details of nesting have been reported in Arkansas (constructed nests, birds performing nest building behavior), the best documentation of nesting would be the identification of nests with eggs or developing young, such as has been reported in White River NWR. Sighting of a mature kite feeding fledglings in July 2020 confirmed successful nesting of Swallow-tailed Kites in the Sulphur River WMA, making this the first documented successful breeding pair since 1890 (Zellers 2020a,b). Sulphur River WMA contains over 6,475 ha (16,000 acres) of bottomland and wetlands, was created in the 1950s from wetland mitigation lands, and provides habitat for other species of rare and threatened animals (Zellers 2020a,b).

Few details of nest composition have been reported. Zellers (2020b) commented that nests can be found in dominant trees along the edge of wetlands. Chiavacci *et al*. (2011) reported that nests in White River NWR often were found in oak trees 30 m or taller that projected an average of 7.2 m above surrounding trees, consistent with the observations for these kites elsewhere (Brown *et al*. 1997; Meyer 2020). Studies in Texas found nests to be comprised of small hardwood sticks and twigs and may incorporate Spanish moss (Brown *et al.* 1997).

Chiavacci *et al*. (2011) documented nests at White River NWR in Arkansas Co. from 2002-2008, on dates ranging from 9 April–28 May. Additionally, a likely breeding pair was spotted in Miller Co. on 6 June 2015, assumed to be tending a nest (which could not be located from the ground). The most recent documentation was on 11 July 2020 at Sulphur River WMA (Miller Co.), where adults were seen feeding fledglings (Zellers 2020a,b). This sighting is thought to be the first successful breeding pair in Arkansas in over a century. While not all nests are successful, this nesting activity can be used to create a timeline of reproductive effort for this species in Arkansas. Suggested management includes conservation of sites where Swallow-tailed Kites have been known to attempt nesting (including super-emergent trees), minimizing disturbance at such sites from 1 April to 31 July, and uneven-age management of forest to create irregular canopy (Chiavacci *et al*. 2009).

Foods - Adult Swallow-tailed kites are known to consume vertebrates such as frogs, small reptiles, birds, and fishes, along with a variety of insects (Meyer 2020). Reports from Arkansas have included comments about seeing the birds feeding on dragonflies, caterpillars, a small rodent, and foraging over fields with large grasshopper populations. Meyer (2020) noted an important aspect of the diet of the Swallow-tailed Kites includes adults and larvae of stinging and biting insects.

Acknowledgments

We thank the many bird enthusiasts who systematically collect and report observations of birds, and Dan Scheiman for his efforts in updating the eBird data base for Arkansas birds, which greatly facilitated this study. Ken Meyer (Avian Research and Conservation Institute, Gainesville, FL) provided information about this species in FL, GA, and SC. We thank 2 anonymous reviewers for their many helpful suggestions.

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Remote Ornithology: Studying Nesting Behavior of Bermuda Petrels via Live Webcam

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Running Title: Webcam Study of Nesting Bermuda Petrels

The Bermuda Petrel *Pterodroma cahow* (hereafter, Cahow) is one of the rarest seabirds in the world (Fitzpatrick 2019; Brinkley and Sutherland 2020). After being presumed extinct for 300 years, the species was rediscovered in 1951 (Murphy and Mowbray 1951). Since then, an aggressive restoration program has increased the population (Madeiros *et al*. 2012; Brinkley and Sutherland 2020). A relocation scheme using artificial concrete nest burrows has succeeded in re-establishing a breeding population in Nonsuch Island, off the main island of Bermuda (Carlile *et al*. 2012).

Incubation behavior of *Pterodroma* petrels is difficult to study because of their burrowing and nocturnal behavior (Warham 1990). Although a lot of information is available about petrel breeding biology in general, little is known about the behavior of incubating parents. The advent of webcams has afforded an opportunity to fill this gap in knowledge.

In 2011, Nonsuch Expeditions, a Bermuda-based tour company, installed a live web camera inside an artificial nest burrow in Nonsuch Island, Bermuda, to livestream infrared video and audio. In 2016, they collaborated with the Bird Cams project at the Cornell Lab of Ornithology, resulting in 20 million minutes of footage from three seasons (Cornell Lab Bird Cams 2019). Jeremy Madeiros, Senior Conservation Officer (Terrestrial), in Bermuda's Department of Environment and Natural Resources, made periodic health checks of the nest and posted public updates on Twitter or YouTube (on egg and parental mass, etc.).In this note, we supplemented our own observations with those updates. We also included some observations from a second nest nearby with a webcam.

We observed the Cahow nest (Colony A, nest #831) via webcam for 167.3 hours in 2019, noting behaviors and involuntary movements of parents incubating a single egg. This is the first time an entire study has been done from remote via Webcam. Both parents were moved to the larger and more elevated Nonsuch Island by biologists in 2006 as nearly fledged nestlings from two separate nesting islands nearby. This parental pair has been together since at least 2009. They had produced a nestling successfully for 5 years in a row since 2014 (J. Madeiros via video posted 21 March 2019). The website provided 4 hours of recording at any given time, enabling us to backtrack and make up to 4 hours of observation per access of camera feed. We monitored the nest for all but 9 days of the 55-day observation period. We missed those 9 days due to time conflicts. To ensure a balanced round-the-clock coverage, we monitored our coverage of each hour of the 24-hr clock throughout the study. We coordinated our observation efforts to cover all hours of the day and night. Although some time periods of the 24-hr clock were opportunistically covered better (e.g., 1000-1100 hrs Bermuda time) than others (0200-0300 hrs Bermuda time), we accomplished our goal to observe the nest at all times of the day and night. The percentage of total time observed ranged from 2% at 0200 h to 7% at 1100 h (Bermuda time) (Fig. 1). Almost equal time was spent observing the nest during day (81.4 hours) and night (85.9 hours).

Sexes look alike in Cahows, making sex identification via the webcam feed impossible. So, we based sex identifications on online postings made by Jeremy Madeiros. He reported using external cloacal examination to sex the birds within three weeks of egg-

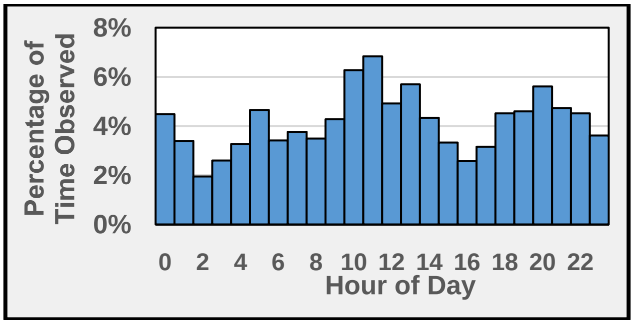


Figure 1. Time of observations.

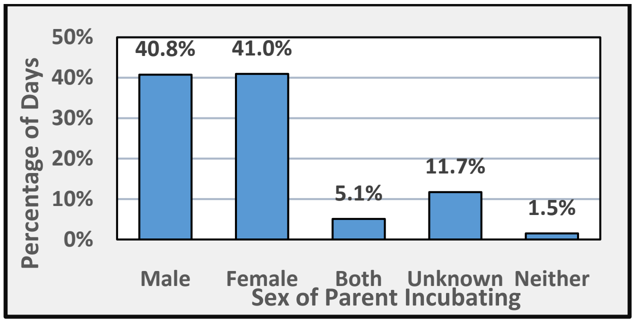


Figure 2. Sex of incubating parent vs. percentage of observation time.

laying. Outside this period, he found that nesting birds consistently showed a significant difference in mass and bill length, which are greater in adult males than adult females (as cited as a personal communication in Brinkley and Sutherland 2020).

We tracked the relative roles played by each parent in incubation (Fig. 2). Both parents contributed equally to incubation. Each was observed an equal proportion of observation time when they were solo in nest. The nest was left unattended only 1.5% of the observed time (Fig. 2).

The female (weighing 359g) laid a single egg (weighing 59g) on 10 January 2019. Our observations started on 11 January. An onsite health check on 17 January recorded the male parent's mass as 397g. Our observations were terminated on 6 March on the 55th day, 5 days after the known incubating time of 50 days from the same parents from the 2018 season. The mean incubation period reported for Cahows is 52.4 days (Warham 1990) and 53 days (Madeiros *et al.* 2012). The first Nonsuch Island chick hatched on 3 March 2019, in another nest. A health check of our nest on6 March showed that the male had lost 85g since the last check, down to 312g. On 11 March 2019, the 60th day since laying, the egg was examined and found to be not viable. The parent continued incubation. Cahows are known to incubate for "up to a month" after failed incubation (J. Madeiros via video posted 21 March 2019).

We tracked several parental incubation behaviors (with codes used and percentage of observed time in parentheses). Sedentary behaviors of resting (R, 56%) and sleeping with head tucked back (S, 31%) accounted for most of the observed behaviors. We categorized behavior as "resting" when parents' head was upright with eyes open. Wheelwright and Boersma (1979) found that incubating Fork-tailed Storm Petrels spend majority of time sleeping, "often tucking bill under scapulars". Warham (1990) indicated that petrels might sleep with head erect but eyes covered by nictitating membrane. "Comfort movements" (Warham 1990), i.e., preening (P, 5%) and nest maintenance (N, 3%) were also observed. Nest maintenance (rearranging fibers and/or digging ground) was observed 153 times with a mean time of 2.06 minutes per observation. Vigorous nest maintenance of 4–13.5 minutes was observed 22 times. Both parents were observed together in 21 different intervals. During 19 of these, allopreening (PA) was observed constituting 8% of the time while both parents were together. Moving (M) and shuffling around occupied only 1% of observed time. In addition, we noted the following other (O) less commonly observed behaviors: "yawning", wing stretching, head scratching, and adjusting egg with bill, together accounting for 0.5% of observed time. We counted 102 "yawns" (parent momentarily opening mouth wide), 42 wing stretches (parent extending one wing), 45 head scratches (all done over wing), and 41 egg adjustments (parent touching egg with bill to adjust its position) during the observation period. On 5 occasions the pair called to each other while both were in the nest, and on 1 occasion apparent copulation was observed. The attentive period, defined as proportion of time spent incubating was 98.5%; i.e. both parents were away (A) with the egg unattended for only 1.5% of observed time (Fig. 3). This high attentiveness is supported by studies on petrels in general (Warham 1990). We found no evidence of nest-ventilating behavior, wherein parent rises to its feet to expose egg (Grant *et al.* 1982).

Orientation of incubating parent on the nest was noted breaking directions down into 12 equal sectors as in the numbers on a clock, with the camera at 6:00 and 12:00 facing directly away from the camera. The camera was mounted on the side, facing so that the entrance was located at 11:00. The incubating parent

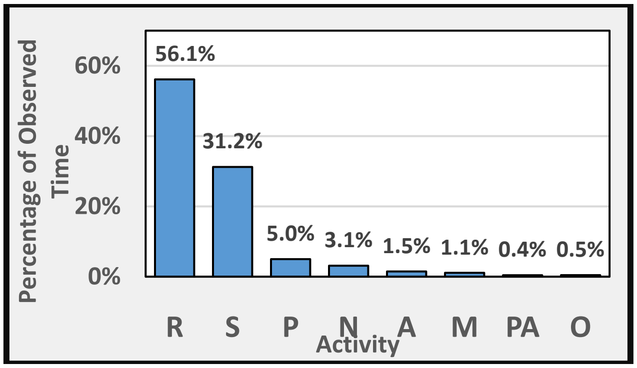


Figure 3. Behaviors of incubating parent as percentage of observation time (R=Resting, S=Sleeping with head tucked back, P=Preening, N=Nest maintenance, A=Away, M=Moving, PA=Allopreening, O=Other)

faced the entrance of the nesting burrow, orienting within 30° of the opening, 49% of the time. The exact opposite orientation was also common, with the parent facing within 30° of opposite the opening 28% of the time. Orientation perpendicular to the opening was rare (Fig. 4). These observations are supported by Warham (1990), who found that, generally, burrowing petrels face the entrance, apparently to greet a partner or deter intruders.

The presence of a rigidly stationary camera gives the opportunity to observe behaviors and involuntary movements that would be impossible to obtain otherwise. For the first time, breathing rates and headshaking rates were quantified in a seabird. We opportunistically quantified breathing rates (in breaths per minute) of the incubating parent for 189 times during the observation period, by counting the rhythmic heaving movements of the body for a minute. The breath rate of the male was significantly higher than that of the female (Table 1 and Fig. 5; 2-sample 2-tailed *t*-test, $p < 0.0001$, $t = 4.12$). A 95% confidence interval for the difference (male – female) in breath rate was [1.3, 3.7] breaths per minute.

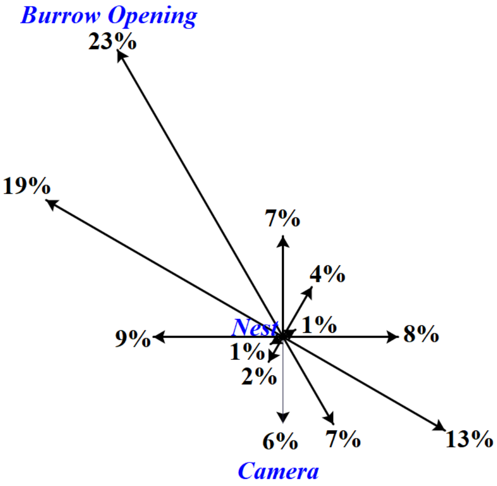


Figure 4. Orientation of incubating parent.

Tube-nosed seabirds are known for their head-shaking behavior while at nest to remove salty secretions from their nostrils. When we observed these head shakes, we recorded the rate of the shakes. Male parent shook head significantly more than the female (Table 1 and Fig. 5; 2-sample 1-tailed *t*-test, $p = 0.016$, $t = 2.44$). A 95% confidence interval for the difference (male – female) in number of shakes was [1.4, 14.0] shakes per minute. It is not clear why this difference was observed, since both parents spent equal proportion of time incubating, and presumably, equal amounts of time foraging and getting exposed to salt. Excel was used to create graphs and calculate basic statistics.

There were 5 incubation shifts (parental "changing of the guard") during our study. This agrees with typical frequency of incubation shifts in other petrels (Brown 1966; Warham *et al.* 1982; Thomas *et al.* 1983; Jouventin *et al.* 1985). Our observations, plus information provided by J. Madeiros on when the parents exchanged places, enabled us to track which days the parents were on the nest. The male was observed at the nest with the female absent for stretches of up to 13 days. Similarly, the female went up to 11 days without relief from her mate (Table 2). The female's return on 22 January, after being away for 8 days, was a surprise. She was expected to be away for 2-3 weeks, leading to a tweet from J. Madeiros

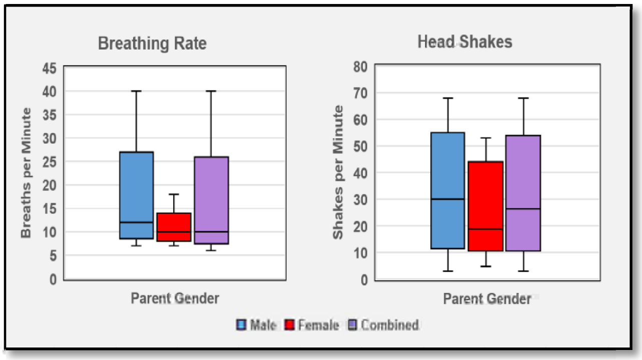


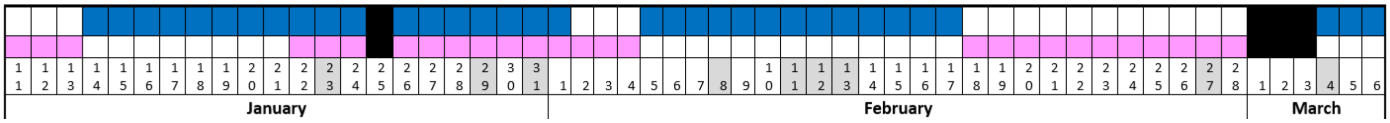
Figure 5. Involuntary movements*

Table 1. Involuntary movements

	Breathing Rates (breaths per minute)			Head Shakes (shakes per minute)		
	Male	Female	Combined*	Male	Female	Combined*
Number of Observations	62	65	189	49	37	96
Median	12	10	10	30	19	26.5
Mean	12.5	10.0	10.7	33.1	25.4	29.6
Standard Deviation	4.4	1.9	1.8	16.0	13.3	14.8

*Combined includes observations when sex of the parent was unknown.

Table 2. Sex of parent in the nest by date. Top line (blue) Male, Bottom line (pink) Female, Black (sex unknown), Shaded dates indicate dates when the authors did not make observations.



January																				
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February																											
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1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28

March					
⬛	⬛	⬛	🟦	🟦	🟦
⬛	⬛	⬛			
1	2	3	4	5	6

Figure 6. Parent burying inviable egg. From CahowCam, Cornell Lab Bird Cams project.

speculating that she probably found food closer to Bermuda than expected. However, the male stayed in the nest with the female for 11 more days (Table 2). Both parents were in the nest for only portions of 11 out of the 55 days (19%) of our observations, supporting Warham's (1990) observations from other petrels that parents seldom return to an incubation partner without relieving it. Both parents often called and preened each other while together, as in the Fork-tailed Storm Petrel (Boersma and Silva 2001). Warham (1990) wrote that incubation shifts are frequent but "rarely seen" in gadfly petrels and other burrowers, but we observed them. On 20 March 2019, one of the parents buried the egg before departing the nest and concluding the effort. It used its breast and wings to push soil and debris from the tunnel to help cover the egg (Fig. 6). It also used its feet to fling debris from the back and over its body on to the egg in front. This is the first time egg-burying behavior has been recorded in petrels.

We do not know if the behaviors we observed are characteristic of the species, considering that we only observed a single nesting pair for one season, and the nesting attempt was unsuccessful. We encourage future research using our methods to ascertain if behaviors remain consistent across successful nesting attempts. It is typical of this species to lay one egg per year. By producing a nestling successfully for 5 previous years, this pair had outperformed the norm of reproducing once every other year or 2 out of every 3 years for this species. Egg failures can be up to 40% in the entire colony (J. Madeiros, video posting dated 21 March 2019).

The incubating parents shared the nest burrow with a pholcid spider and several ants. Sometimes the ants swarmed on the apparently unperturbed parent. On 16 May 2019, the webcam filmed a land crab (*Cardisoma guanhumi*) eat the inviable egg. In the other nest with a webcam, a land crab was filmed on 29 May 2019, entering the burrow with a nestling inside. The crab scurried away after the nestling woke up and moved. This suggests that land crabs eat eggs but not nestlings. It is not clear if land crabs can be classified as egg predators, because they may only scavenge unattended or inviable eggs. The endangered Bermuda skink (*Plestiodon longirostris*) was seen inside the nest burrow post-season (17 May), when it was empty. For five years in a row since 2017, another webcam in the island recorded a Leach's Storm-Petrel (*Oceanodroma leucorhoa*) visiting and staying in an active Cahow nest burrow (Cornell Lab Bird Cams 2021). The list of intruders also includes an aggressive young Cahow prospecting for a nest site (Cornell Lab Bird Cams 2017).

This study would not have been possible without the collaboration between The Cornell Lab of Ornithology, Nonsuch Expeditions, and Jeremy Madeiros of Bermuda's Department of Environment and Natural Resources. Technology such as this opens many new avenues of data collection resulting in observations previously deemed impossible or impractical. Furthermore, making this video stream public allows for remote observations from around the world. The authors were able to perform these observations from Arkansas and New York without having to visit Bermuda. This study is especially unique since the authors have never seen a Cahow in person, yet they were able to make observations from the comforts of their offices or homes thousands of kilometers away. We encourage similar efforts to further the ability to do research of this kind and make the information widely available. It will also help surpass logistic and financial hurdles in conducting field studies and encourage the future use of webcams by teachers for collaborative citizen science investigations in their classrooms. The study was initiated as a special high school science project by one of the authors (TC).

Acknowledgments

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Bird Usage of Black *Marasmius* Fibers as Nest Material

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Running Title: Bird Usage of *Marasmius* Fibers as Nest Material

Fungal fibers are used for nest construction by 176 species of birds (Elliott *et al*. 2019). At least 98 bird species use the black rhizomorphs of Marasmioid Basidiomycetes fungi as nest material (Hansell 2000; Aubrecht *et al.* 2013; Caballero 2020). The Yellow-olive Flycatcher (*Tolmomyias sulphurescens*), a common bird of forests and forest edges in Central and South America, principally uses these black *Marasmius* fibers for nesting (Fig. 1; Anciães *et al.* 2012; Menezes *et al.* 2014). Several hypotheses have been proposed to explain this phenomenon. There are conflicting reports on whether the fibers have antibiotic properties (Melin *et al*. 1947; Meng *et al*. 2011; Ramesh and Pattar 2010; Seupaul 2021). These slender fungal filaments have been shown to have anti-carcinogenic properties (Rosa *et al.* 2009), but whether this is a factor in nest material selection by birds has not been examined. An additional selective advantage proposed of these so-called horse-hair fungi, owing to their resemblance to long black horse hairs, is that they are longer than locally available grass fibers and hence afford flexibility and convenience in the construction of long pendulous nests (Freymann 2008). This too remains to be addressed empirically. Another possible advantage of choosing horse-hair fungi may be that they are more water-resistant than grass material. This has been tested and supported by data (Freymann 2008).

Two more hypotheses have either been incompletely tested or untested thus far. The first is that these fungal filaments are physically stronger than grass fibers, and thus they are preferred for their durability (Freymann 2008; Aubrecht *et al*. 2013). To test this, Freymann (2008) conducted experimental trials comparing tensile strengths of Marasmioid filaments used by Streak-backed Orioles (*Icterus pustulatus*) in Costa Rica, with grass fibers extracted from nest linings from the same nests. He demonstrated that the fungal filaments were stronger than the grass lining material. An obvious drawback of his study was that he compared the physical performance of fungal filaments with grass linings of nests, and not to grass fibers used in the main nest structure. Such a comparison would be necessary to establish if fungal filaments afford greater durability in terms of higher load bearing than alternate grass material used in similar ways by coexisting bird species that also construct pendent fiber nests.

The second hypothesis is that using *Marasmius* fibers in nest provides some advantage in the control of temperature exchange between the interior and exterior of the nest. Until now, this hypothesis has not been tested empirically.

In this study, we tested these two hypotheses. We compared tensile strengths of *Marasmius* fibers obtained from six nests of Yellow-olive Flycatchers in Belize, Central America, with grass fibers extracted from a Yellow-tailed Oriole (*Icterus mesomelas*) nest from the same general area. Both species build pendent nests made of fibers. We also compared nest interior temperatures of the five *Marasmius* nests with that of the control grass nest and concurrent ambient temperatures.

All nests were inactive and empty at the time of collection in June 2019. We could not determine exactly when they were in use, but the intact condition of the nests indicated that they were in use that summer. The flycatcher nests were predominantly or exclusively made of black fungal fibers. The oriole nest was comprised of grass material only. We provisionally identified the flycatcher nest fibers as *Marasmius* in the field based on their black color (Fig. 1) and their thin and wiry form (Hedger 1990; Hedger *et al.* 1993; Koch *et al.* 2020) resembling horse hairs.

To confirm that the black fibers were indeed aerial rhizomorphs of *Marasmius* fungi, a sample of fibers was sequenced with NS1 [5’(GTA GTC ATA TGC TTG TCT C)3’] and NS8 [5’(TCC GCA GGT TCA CCT ACG GA)3’] primers used for higher fungi. Examination of 1659 base pairs (bp) of the full 18S rRNA sequence

Figure 1. A Yellow-olive Flycatcher nest (left) on an Ant-*Acacia* tree, near an active wasp nest (right). Photo by David Oakley.

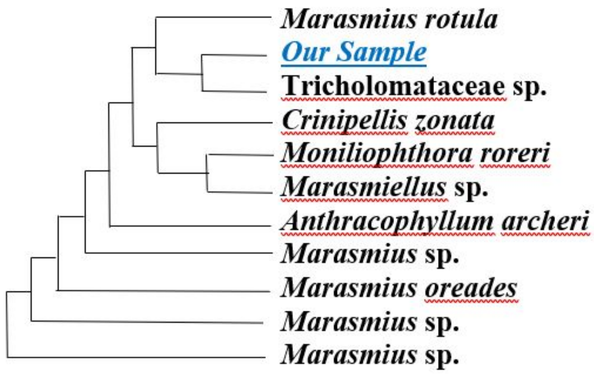

Figure 2. Maximum Likelihood Phylogeny Tree as reconstructed from our sequencing data.

of the sample showed 99% similarity to *Marasmius* sp. (Bit-score=3020, E-Value= 0.0). The sequenced region (bp 54-1712) covered approximately 93% of the total 18S rRNA gene sequence. The Maximum Likelihood Phylogeny Tree as reconstructed from this data revealed that the sample was closely allied to other marasmoid fungi (Fig. 2). Partial sequencing of small subunit rRNA gene of five samples (bases 1-949) indicated that one was *M. oreades* (GenBank Accession No. OK103912.1) and the rest *Marasmius* sp. (GenBank Accession Nos. OK103913.1, 103916.1, 103918.1, 103919.1).

To test the tensile strength hypothesis, six fibers were selected from each of the six flycatcher nests. They were compared to six grass fibers from the oriole nest. Each fiber was cut into a standardized length of 15 cm. An eXpert 7600 Series Universal Tester by ADMET was used to measure the tensile strength of the fibers. Each fiber was attached on each end to the hydraulic clamps of the universal tester and stretched to its breaking point. The force gauge transmitted the load at the time it broke to an online database (in pounds), which we converted to a force in Newtons.

Since the oriole nest grass fibers were significantly thicker, and thus presumably stronger, than the *Marasmius* fibers, we controlled for this variable by measuring the diameter of all fibers (control and experimental) with a micrometer. This enabled us to present results as force per cross-sectional area in N/mm^2 (Fig. 3). We assumed that the cross-sectional areas may be reasonably modeled by a circular cross-section with the diameter measured at an arbitrary position on the fiber.

The mean load per cross-sectional area at breaking point for the 6 grass fibers (896 N/mm^2) was higher than the mean for the 36 black fibers (456 N/mm^2). The *p*-value from a 2-sample, 2-tailed *t*-test for difference in means was 0.0074, strongly indicating that this difference was significant. On average, the grass fibers withstood an additional 440 N/mm^2 strain more than the *Marasmius* fibers. A 95% confidence interval for the difference (Grass – *Marasmius*) in mean load per cross-sectional area is [162.75, 717.37] N/mm^2.

Our results indicate that the brown grass fiber nest material used by the oriole had a significantly stronger tensile strength than the black *Marasmius* fiber nest material used by the flycatcher. Tests indicated that the grass fibers are stronger than *Marasmius* fibers, even after correcting for the greater thickness of the grass fibers. Therefore, we rejected the hypothesis that *Marasmius* fibers are chosen for their superior strength.

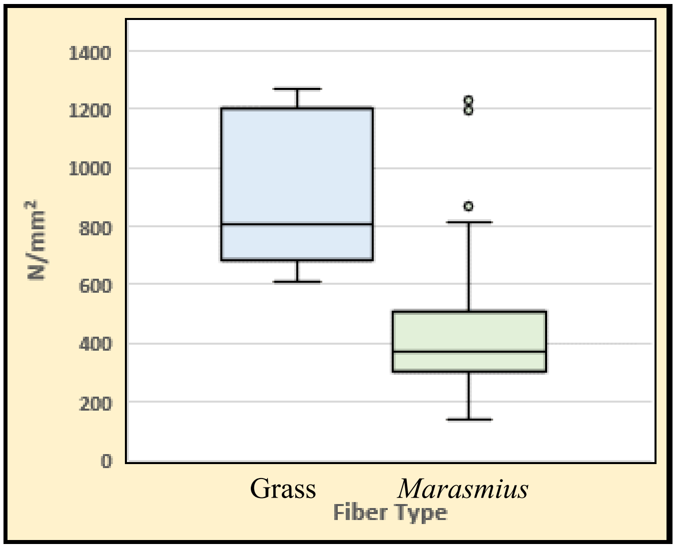

Figure 3. Comparison of tensile strengths between grass (oriole) and fungal (flycatcher) nest material. (Excel method for finding quartiles.)

Our tensile strengths data suggests that *Marasmius* fibers do not have a tensile strength advantage to at least one stronger nesting material that was readily available in the area. This seems to contradict the results of Freymann (2008), but it should be reiterated that he compared tensile strength of *Marasmius* fibers used as the main structural component of nests to the grass fibers used to line the same nests. In contrast, our study compares the *Marasmius* fibers used exclusively in a nest of one species to brown grass fibers used exclusively in the nest of a different local species.

We tested the temperature buffering hypothesis by suspending the black fiber nests and control nest from small trees at 4.5m above ground and 1.8m from one another. This study was conducted at the Crystal Paradise Resort, Cayo District, Belize. HOBO MX2201 data loggers were placed inside the empty nests and set to record the temperature every 30 minutes for 24-hour time increments. Temperatures were recorded for the ambient environment, the interior of the grassy control nest and one *Marasmius* nest. In addition, 2 of the other 4 *Marasmius* nest temperatures were recorded on a rotating basis. The mean of the temperatures in the *Marasmius* nests was computed. The absolute value of the differences in the ambient temperature from the oriole nest and from the average flycatcher nest temperatures was computed (Fig. 4). This produced ordered pairs of absolute temperature difference for each time for each type of nest. The mean of these differences was -0.021°C with the oriole sample producing the higher degree of temperature buffering. A one-sample *t*-test was performed on the difference of these pairs, producing a *p*-value of 0.47. While both types of nests provided some degree of temperature moderation, there is no statistical difference in the temperature moderation ability of the two types of nests.

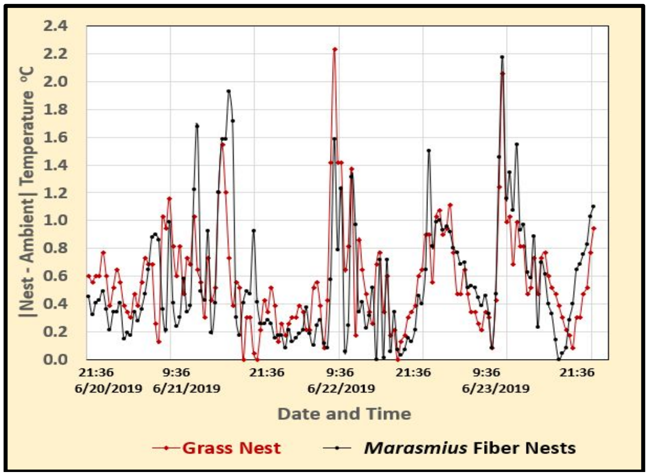

Figure 4. Absolute differences in internal and external temperatures for grass and *Marasmius* nests

Therefore, we reject both the hypothesis that *Marasmius* nests provide greater tensile strength and that they provide greater temperature moderation than that provided by other readily available nesting material. In fact, we tested one such material and found it provided the same temperature moderation and greater tensile strength.

Nest microclimate is crucial for successful incubation and brooding, and it directly impacts daily energy requirements of adults (Gill 2007). Birds adopt a range of strategies to promote thermal inertia, from nesting communally (Lowney *et al.* 2020) to placement of nests in cavities and burrows (Ar and Piontkewitz 1994). The choice of nest materials plays a role in buffering external temperatures (Mainwaring *et al.* 2014). Within species, nests in colder climates have better heat retaining features, aided in part by the nest materials chosen by parents (Kern and van Ripper 1984; Briskie 1995; Rohwer and Law 2010). Given the importance of nest materials in thermoregulation, and the widespread usage of *Marasmius* in tropical birds, it is surprising that this is the first time this nest material has been examined for possible regulation of nest microclimate.

There are two limitations in our study. First was the small sample size, particularly of the grass control material. Using a larger sample size, particularly for the control material, from multiple nests examining different nesting materials, would have strengthened this study. The second was the availability of only five data loggers, requiring us to take data over three days for the five different available black nests and the single nest from the grassy material. Ideally, we would have preferred taking the data concurrently from more nests of both types. Despite these limitations, our study should spur future inquiries examining these and other hypotheses, using and building on the technologies and methods we used.

Our study suggests that neither tensile strength nor temperature moderation is a factor in the frequent usage of black fungal fibers in Yellow-olive Flycatcher nests. Perhaps these fibers are chosen because of their water resistance (Freymann 2008) or simply due to their easy availability in forested environments, compared to grassy material that may be more common in open habitats. Also, *Marasmius* fibers may be better suited for cup nests compared to hanging nests, with a possible tradeoff between physical strength and other properties. Nestlings may be benefitted by these fiber linings in some way (Aubrecht *et al.* 2013). There is some evidence that uneven distribution of *Marasmius* may be involved in selective use differences by birds across geographical areas and habitats (Aubrecht *et al*. 2013).

The complex web of interactions between *Marasmius*, birds, ant-acacia trees, acacia-ants, and social hymenopterans (Fig. 1) make teasing out cause-and-effect challenging (Young *et al*. 1990; Flaspohler and Laska 1994; Menezes *et al*. 2014). Also, there is evidence that many species of Marasmiod fungi form aerial rhizomorphs, and birds selectively use them for different roles (lining, support) in nest (Koch *et al*. 2020). Given these complexities, we encourage more research to solve the mystery of why these black *Marasmius* fibers are preferred by the Yellow-olive Flycatcher and other tropical birds.

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Steven L. Stephenson of the University of Arkansas – Fayetteville helped with fungal identification and provided import permits. Nawaf Alshammari sequenced the fibers in the labs of Macrogen in Saudi Arabia. Haris Rana performed some of the data collection during a summer High School Research Program in Shawn Bourdo's lab at Center for Integrative Nanotechnology Sciences, University of Arkansas at Little Rock. Anant Deshwal assisted in data collection. Andy Tut collected all the nests. David Oakley provided the photograph. Becky Mroczek helped us interpret the sequencing data. The temperature study was part of the Belize Coastal Caribbean Biology undergraduate summer study abroad course at the University of Arkansas- Fort Smith. Three anonymous reviewers made suggestions that enabled us to improve the manuscript.

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ARKANSAS ACADEMY OF SCIENCE

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Historian

R. Panneer Selvam
Newsletter Editor

Stephen Addison
Undergraduate grants

Secretary's Report

ARKANSAS ACADEMY OF SCIENCE
104th Annual MEETING MINUTES

Zoom Meeting Hosted by UCA

April 10 – 11:00a.m.

Meeting called to order at 11:00 am CDT by President Stephen (Steve) Addison.

1. **President's Report**

Steve Addison welcomed attendees to the first ever Zoom general Business Meeting of the Academy. He thanked everyone for participating. Steve noted he had been president for two consecutive years which is a first for the Academy. Further, during that time he has not been able to attend an in-person annual spring meeting of the Academy. We have missed the comradery of the live academy meetings and are quite ready for them to return.

2. **Local Arrangements Committee: Jack Jackson**

Jack Jackson reported the meeting to be progressing smoothly. He specifically named Ragupathy Kannan and Clint Brooks as valuable support staff for the meeting. A complete list of those who staffed the meeting is in the Resolutions included in the Business Meeting Minutes (Appendix B).

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

The minutes of the previous year's meeting will be available to the membership in this year's journal. Any corrections should be sent to the secretary.

Current membership is 128 of which 55 are life members while 73 are annual memberships.

To date 11 institutions have paid their annual dues for 2021.

Two people have been nominated to become Fellows of the Arkansas Academy Science. More than 200 e-mails have been sent asking for members to vote for or against these nominations. Votes will be counted if they are received by April 14.

Members are reminded they can nominate potential fellows or honorary fellows up to October 1 of the year prior to their initial consideration. Those nominations should be to the secretary (information on nomination criteria are given at the end of this report). The membership will vote on the nominees at the spring meeting. Those approved will be awarded the following year. This year's vote is, of necessity electronic, but the expectation is that it will be in person in future years.

The Academy has had plaques prepared for Jack Jackson, Stephen Addison, and Ivan Still for their service to the Academy. If those persons will electronically send me an appropriate USPS address, I will mail their plaques to them.

We are arranging a luncheon late in the summer to award Peggy Smith and Liz Adams the plaques for Doug James and Kim Smith as the first two named Fellows of the Arkansas Academy of Science.

For your information, the following are the rules adopted for the fellowship programs of the Academy.

FELLOWS

The Fellows of the Arkansas Academy of Science are a group of distinguished scientists, technologists, engineers, and mathematicians selected in recognition of their outstanding contributions to science, technology, engineering, and mathematics (STEM) in Arkansas. Any Member of the Academy who has made a distinguished or substantial contribution to the areas of teaching, research, and service in any area of STEM in Arkansas may be nominated. AAS Fellows serve as ambassadors for the Society and as such are encouraged to engage in outreach and other activities that will benefit and promote both AAS and the STEM professions in Arkansas.

Arkansas Academy of Science members are invited to submit nominations for Fellows. A potential Fellow must be an active member of the Academy for a minimum of 3 years and have contributed to STEM in one or more of the following: (a) outstanding STEM research, (b) inspired teaching of STEM, or (c) significant leadership in the Academy. Nominations and seconds for Fellows should be submitted to the Secretary of the Academy no later than October 1 of the year previous to the year of actual consideration. A nomination consists of 1) a cover letter from the primary nominator, 2) a second supporting letter from a co-sponsor, 3) a CV of the nominee, and 4) a concise document outlining the nominee's specific contributions to STEM in Arkansas and AAS in particular. The Executive Committee will determine if the nominee's qualifications are complete during the fall Executive Committee Meeting. A majority vote of the Executive Committee on any nominee will result in that nomination being presented to the Academy Membership during the following Spring Business Meeting for possible approval. The Secretary will distribute a list of nominees to the Academy Membership at least 10 days prior to the Spring Business Meeting. Fellows will receive a plaque at the subsequent year's business meeting. Fellows are appointed for life. There are no restrictions on the number of Fellows elected each year.

HONORARY FELLOWS

The Honorary Fellows of the Arkansas Academy of Sciences are a group of distinguished individuals, selected in recognition of their outstanding contributions to STEM in Arkansas, who are not members of AAS. Any individual who has made a distinguished or substantial contribution in any area of STEM in Arkansas may be nominated. AAS Honorary Fellows serve as ambassadors for the Society and as such are encouraged to engage in outreach and other activities that will benefit and promote both AAS and STEM in Arkansas.

Arkansas Academy of Science members are invited to submit nominations for Honorary Fellows. Candidates must have spent a significant portion of their professional careers in Arkansas or contributed substantial research findings pertaining to Arkansas. Nominations and seconds for Honorary Fellows should be submitted to the Secretary of the Academy no later than October 1 of the year previous to the year of actual consideration. A nomination consists of 1) a cover letter from the primary nominator, 2) a second supporting letter from a co-sponsor, 3) a CV of the nominee, and 4) a concise document outlining the nominee's contribution to STEM in Arkansas. These accomplishments could be in any area of basic or applied science, engineering, math and technology as well as in STEM teaching or in service to STEM. The Executive Committee will determine if the nominee's qualifications are complete during the fall Executive Committee Meeting. A majority vote of the Executive Committee on any nominee will result in that nomination being presented to the Academy Membership during the following Spring Business Meeting for possible approval. The Secretary will distribute a list of nominees to the Academy Membership at least 10 days prior to the Spring Business Meeting. Honorary Fellows will receive a plaque at the subsequent year's business meeting. Fellows are appointed for life. There are no restrictions on the number of Honorary Fellows elected each year.

I currently have 7 boxes of back issues of the journal stored in my attic. I will continue to store them even after I retire as secretary.

Ed and Andy moved to approve the report and Todd seconded the motion. Approval was unanimous.

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

This report was the same as presented last December and it was approved by the executive committee at that time so is preapproved.

The end of 2021 AAS financial statement is presented at the end of these minutes.

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

The 2021 spring meeting of the Arkansas Academy of Science at the University of Arkansas at Fort Smith (UAFS), Arkansas was the 104th annual meeting of the Academy. This will mark the second time that UAFS has hosted the Academy having done so previously in 1996, when named Western College.

The 104th meeting was originally scheduled to take place on April 3-4th 2020 at UAFS. However, due to the global COVID-19 (Coronavirus) pandemic, UAFS leadership and AAS had no choice but to make the necessary decision to cancel the 2020 meeting in order to protect the health, safety, and well-being of its members and attendees. AAS decided to postpone its 104th meeting and host it at UAFS, possibly face-to-face. The 2020 membership dues were rolled forward and counted as 2021 dues. However, one year after the World Health Organization declared the covid-19 outbreak a Public Health Emergency of International Concern, the pandemic continued to rage on with infections reaching an all-time high rate during Fall 2020. During AAS executive meeting, which was conducted online via zoom on Dec 16, 2020, UAFS Jack L. Jackson II announced the meeting will be conducted virtually on April 9-10 2021. The 104th meeting marked the first time an annual meeting was held online.

The meeting was very efficiently managed by Jack Jackson, conference chair. Other local organizing committee members consisted of Ragupathy Kannan, Clint Brooks, Myron Rigsby, Tom Buchanan, Joshua Burns, Amy Skypala, Kristine Garner, and David McClellan of UAFS. The organizing committee did an outstanding job with hosting the meeting online; all research presentations were disseminated through prerecorded video lectures. This comes at a time where the online presence of the Academy was growing. Dr. Helen James, Research Zoologist and Curator of Birds at the Smithsonian Museum of Natural History, served as the keynote speaker and gave a presentation on Discovering a Lost World of Birds: Fossils in Hawaii's Lava Caves.

University of Arkansas – Fort Smith was founded in 1928 as Fort Smith Junior College. The name was changed to Westark Junior College in 1966, then Westark Community College in 1972, and Westark College in 1998 before becoming know as University of Arkansas – Fort Smith in 2002 as one of 21 academic institutions and affiliates governed by the University of Arkansas System board of trustees and administered by the president of the system.

No physical pictures were taken but screen shots of the virtual conference will be kept in the archives.

Collis moved approval of the report and Ivan seconded the motion. Approval was unanimous.

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

a) Volume 74

Sixteen manuscripts were submitted for consideration of publication in volume 74 (2020) of the JAAS. These manuscripts included 11 Articles, and 5 General notes, all being submitted by the electronic manuscript submission process on the Journal website. Numbers were obviously down due to the COVID19-induced issues resulting in the cancellation of the meeting last year.

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. I handled Physical Science papers, Invertebrate biology and some Vertebrate biology papers, while Doug Barron handled 2 Ecology/Environmental papers, and Cristina Blanco handled 2 Vertebrate Biology papers. The majority of manuscripts were sent out electronically for review by the beginning of June. I would like to thank the Associate Editors and reviewers for their help in the preparation of volume 74.

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. Two manuscripts required "major" corrections. Once reviews were returned to handling editors, control of manuscript processing was returned to me. Final revisions with page numbers were uploaded at the end of November: this does provide a final time for authors to check their manuscripts, for any errors arising during final editorial formatting (although not for changing content). The final completion of the Journal for general release is dependent on the December executive committee meeting, when Secretary and end of year Treasurer's report are available. Hence, the Journal was released electronically in the first week of January.

Volume 74 is 100 pages long, including cover pages. Printed copies have been sent out to members throughout March 2021 and should arrive to individuals over the next few weeks, depending on the USPS. I have "volunteered" individual members to receive the package of Journals at different institutions and to distribute Journals to members at their institutions. I would like to thank those individuals that performed that task in 2020 and those who have/will be doing the same for 2021. I have also attempted to find physical addresses for our Life Members for whom we have University/Institutional addresses, but who no longer appear on those Institutional websites. I sent out volume 74 to 10 such individuals. I specifically asked that they confirm by email to the jarksci@gmail.com (the Journal email) if they are still interested in remaining in contact with the Academy and still wish to receive their hardcopy of the Journal and the annual Academy newsletter in the future, as part of their Life membership. So far I have only received two responses: if I do not receive a response from the others, I plan to remove them from the mailing list for the Journal.

b) Leadership report

Download statistics for the on-line journal were: 4584 full-text downloads in the month of March 2021, 50,236 in year-to-date, and 160,319 total downloads since being on Scholarworks from a total of 2,361 manuscripts in the journal. For the past month, top countries for download are the USA (1909), followed by India (317) and then the UK (231). Top referrers: Google, then scholar.google.com.

c) Editorial Board and preparation for succession

I have recruited Dr. Amber Harrington as Associate Editor of Physical Science for Volume 75 onward. Dr. David Bowles (Missouri State University, *retired*) has volunteered to become the Invertebrate zoology Associate Editor this year too. I would like to express my gratitude to these new additions to the Editorial Board of the Journal.

As of this meeting, we are still in need of an Editor-in-Chief and Managing Editor for Volume 76 onward, when I step down with the completion and any mailing of Volume 75 in 2022 (annual meeting). We have had a recruitment notification for Managing Editor in the Newsletter since 2018, and once Dr. Hemmati stepped down and I also took on the Editor-in-Chief position, for the Editor-in-Chief position. Duties for both positions were posted in the 2020 and 2021 Newsletter, and I included a one pager on the duties in Volume 74. I have been compiling a set of templates and where necessary additions to the "how to manual" for performing the Editorial and publishing process to be handed on to my successor. Dr. AlRoobi and I have streamlined the process of uploading changes to the Editorial Board and Instructions to Authors on the Journal webpage (the latter will also make it easier for authors as these are now in pdf format).

Approval of the report was unanimous.

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

Rami was unable to attend the meeting.

8. <u>Newsletter: Panneer Selvam</u>

The spring newsletter was completed in a timely fashion and contained the appropriate information for the meeting.

9. <u>Committee Reports:</u>

<u>Nominations Committee</u>

Jeff Shaver of UAFS was nominated for the position of Vice President while Steve Addison was nominated for the position of Secretary. No nominations from the floor occurred. Jeff and Steve were elected by a unanimous voice vote.

<u>Undergraduate Research Awards: Stephen Addison</u>

Four quality proposals were received and approved. They are summarized below.

Sara V. Vue, Henderson State, Mentor: Martin J. Campbell, Ionic liquid polymers for space environments, $1,000.

Lara I. Kockaya, Henderson State, Mentor: Martin J. Campbell, Air filtration in space environments, $1,000.

Cori Clower, Ouachita Baptist University, Mentor: Sharon Hamilton, Test and compare drug inclusion methods on polymer-based collagen mats, $1,000.

Aiiryel McCoy, Ouachita Baptist University, Mentor: Sara E. Hubbard, Investigation of damage to polycarbonate lamps stored at the Indianapolis Museum of Art (Chemistry investigation), $1,000.

<u>Outreach Committee Report- Edmond Wilson Chair</u>

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

We will work with various people and committees to see that our new Honorary Fellows get recognition for their outstanding contributions to this State.

We will be developing materials for our website to direct learning activities for K-12.

We will not necessarily develop the materials ourselves but rather gather information and place it on our website in a way to allow for hands-on learning experiences. AAS members will be encouraged to participate in development of these materials.

We are planning to develop a presentation to be made at public libraries, which can travel, to generate interest and knowledge about the great State of Arkansas.

With the hopeful demise of the severity of the COVID-19 Pandemic, we plan to participate in Thunder Over Arkansas and Tinkerfest this fall.

Help us find volunteers for our committee from each AAS college campus!

10. Related Organization Reports:

i. Arkansas Junior Academy of Science - Charles Mebi

I wish to thank all Participants and presenters for choosing to present your research work at the 2021 Arkansas Junior Academy of Science competition. The event took place on April 3, from 9 am to 11 am.

This year’s competition was virtual. All presenters received an email with the WebEx link for the presentations. The event started at 9 am with category presentations. The presentations were automatically recorded.

The judges selected the top three presenters for each category. The results were posted on the ArJAS website and emailed to all the presenters by Monday, April 5. Since the presentations were recorded, presentations by the 1st place category winners were reviewed later by the judges to determine overall winners. Therefore, the 1st place category winners were not required to present again.

The awards were mailed to the winners after the competition.

Awards and Recognitions:

1st place overall: $150 and plaque
2nd place overall: $100 and plaque
3rd place overall: $50 and plaque
1st place category: plague
2nd and 3rd place category: Certificates

1st place chemistry and biochemistry winners will receive recognition from the Central Arkansas Section of the American Chemical Society during its annual awards ceremony. Recipients will be contacted later.

Overall winners will be given the opportunity to represent the State of Arkansas at the 2022 American Junior Academy of Science (AJAS) Conference.

2021 Arkansas Junior Academy of Science Competition winners:

Overall Winners

1. Kevin D. Durden
   Little Rock Central High School
2. Amna Khan
   Little Rock Central High School
3. Dishant A. Sharma
   Little Rock Central High School

Subject Area Winners

Chemistry

1. Amna Khan
   Little Rock Central High School
2. Fatima Majid Pulaski Academy

Biochemistry

1. Jana H. Eid Pulaski Academy
2. Bhavana Sridharan
   Little Rock Central High School

Environmental Science

1. llie L. Thomas Pulaski Academy
2. Tarini Eswaran
   Little Rock Central High School
3. Aimee H. Bae
   Little Rock Central High School

Engineering

1. Landon P. Runion-Driskel
   Little Rock Central High School
2. Maya F. Uwaydat Pulaski Academy
3. Andrew A. Parson Pulaski Academy

Zoology

1. Gautami J. Lohakare
   Little Rock Central High School

Microbiology

1. Dishant A. Sharma — Little Rock Central High School
2. Andre E. Fiser — Pulaski Academy

Computer Science

1. Kevin D. Durden — Little Rock Central High School
2. Anu Iyer — Little Rock Central High School
3. Sofia Roman — Pulaski Academy

Behavioral and Social Science

1. Sydney C. Crary — Pulaski Academy
2. Akul Shrivastava — Little Rock Central High School
3. Audrey I. Gardner — Pulaski Academy

11. Business Old and New:

- **i.** 2022 meeting at OBU is confirmed as is the 2023 meeting at UAPB.
- **ii.** Order of Program for the Business Meeting will follow the same format as the executive committee meeting.
- **iii.** Collis reported that votes are coming in on the fellow nominees and will continue to be received through April 14.
- ***iv.*** Matters relating to the Journal: Ivan moved that the price of old hard copies of the journal be reduced to $35. This was approved unanimously. The general opinion of the committee is that a box to indicate whether or not a participant wanted a hard copy of the journal be included on the meeting registration form. John Jiang presented his credentials and expressed interest in the position of Managing Editor of the Journal.

12. Meeting was adjourned

Steve passed the hammer to Andy Sustich who is now our President. Andy thanked UAFS for an excellent meeting under difficult circumstances. He congratulated Doug's daughter for an excellent presentation as well as an excellent career. He is planning on a "regular meeting" in 2022.

The meeting was adjourned shortly before 11:00 to allow transition to the general business meeting.

Minutes prepared by Secretary Collis Geren, April 12 2021. Approved by Exec. Com. December 17 2021

Treasurer's Report

ARKANSAS ACADEMY OF SCIENCE
2021 FINANCIAL STATEMENT
December 9, 2021

Balance – December 9, 2021	**$161,517.27**
Balance – December 9, 2020	**$164,264.61**
Net Gain	**-$2,747.43**

DISTRIBUTION OF FUNDS

Checking Account Dec. 9, 2021 Arvest Bank	$12,994.18
PayPal Account: Available funds on Dec. 9, 2021	$37.81
Certificate of Deposit Dec. 9, 2021 Includes Phoebe and George Harp Endowment Arvest Bank	$53,531.04
Certificate of Deposit Dec. 9, 2021 Arvest Bank	$53,531.04
Certificate of Deposit Dec. 9, 2021 Arvest Bank	$41,423.11
TOTAL	**$161,517.18**

INCOME

1. INTEREST (Interest Earned Year to Date December 9, 2021)

a. Checking Account, Arvest Bank	$0
b. CD1 (Arvest Bank)	$80.22
c. CD2 (Arvest Bank)	$80.22
d. CD3 (Arvest Bank)	$62.08
All interest was added to the CDs	**$222.52**

2. JOURNAL

a. Page Charges (includes $150 from UAFS for rejected manuscript)	$4,800.00
b. Subscriptions, University of Arkansas	$300.00
Total	**$5,100.00**

3. MEMBERSHIP

a. Individual/Associate	$955.00
b. Individual collected at the meeting	$0
c. Institutional	$1,000.00
Total	**$1920.00**

4. MEETING INCOME	**$0**

5. MISCELLANEOUS INCOME

a.	Unspent/returned UG awards	$0
b.	Gifts: PayPal Charitable Giving Fund	$13.00

Total	**$13.00**

TOTAL INCOME	**$7,290.52**

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. Four from 2020, four from this year	$7,805.00
Total	**$7,805.00**

4. JOURNAL

a. Volume 74 Printing Cost	$1,724.38
b. Journal Mailing Cost	$255.37
Total	**$1,979.75**

5. MISCELLANOUS EXPENSES

a. Reimburse Rami for Website registration	$163.49
b. Reimburse Andy for Quicken subscription	$62.99
c. Dues: National Assoc. of Academies of Science	$0
d. PayPal fees	$26.72
Total	**$253.20**

6. MEETING EXPENSES	**$0**

TOTAL EXPENSES	**$10,037.95**

ARKANSAS ACADEMY OF SCIENCE
COST OF JOURNAL

VOLUME	COPIES	PAGES	PRINTER CHARGE	TOT. VOL. COST	COST/ COPY	COST/ PAGE
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	$2,915.12	$2,963.03	$14.82	$15.27

VOLUME	COPIES	PAGES	PRINTER CHARGE	TOT. VOL. COST	COST/ COPY	COST/ PAGE
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	$2,848.28	$15.82	$17.16
74 (2020)	170	100	$1,724.38	$1,979.75	$11.65	$19.80

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

- 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.

APPENDIX A

AWARD WINNERS FROM THE 104th ANNIVERSARY AKANSAS ACADEMY OF SCIENCE (awardees are underlined)

UNDERGRADUATE AWARDS

BIOCHEMISTRY/MOLECULAR BIOLOGY

Sydney Du, Cladie B. White, University of Arkansas - Fort Smith
Faculty Mentors: Archana Mishra, Mohammad A. Halim, University of Arkansas - Fort Smith
Designing Antimicrobial Peptides Against the Main Protease of SARS CoV-2: A Molecular Modelling Approach

VERTEBRATE BIOLOGY/ZOOLOGY

Willow Newman, Chance Garrett, Matthew Gifford, University of Central Arkansas
Faculty Mentors: Ginny Adams, Reid Adams, University of Central Arkansas
Turning Up the Heat: Thermal Tolerances of Fishes in the Kings River, Arkansas

PLANT BIOLOGY/BIOMEDICAL STUDIES

Ashlyn Estes, University of Central Arkansas
Faculty Mentor: Erin Wiley, University of Central Arkansas
Effects of Late Winter/Early Spring Flooding on Pin Oak Saplings

MUSEUM SCIENCE/BIODIVERSITY

Grace Capooth, Hendrix College
Faculty Mentor: Adam C. Schneider, Hendrix College
Curating and Digitizing the Bryophyte Collections of the Hendrix College Herbarium

CHEMISTRY I

Paul Gambill, University of Arkansas - Fort Smith
Faculty Mentor: Mohammad A. Halim, University of Arkansas - Fort Smith
Menthol: Acetic Acid Based Low Viscosity Deep Eutectic Solvents

CHEMISTRY II

Evan Wittig, University of Arkansas – Fort Smith,
Faculty Mentor: Charuksha Walgama, University of Arkansas - Fort Smith
Electrochemical Biofilms for Drug Metabolite Synthesis

PHYSICS

Joe Coker, Hendrix College
Faculty Mentor: Julie Gunderson
The Fluorino: A Low-Cost, Arduino-Controlled Fluorometer

ENGINEERING

Grace Zimmerman, University of Central Arkansas,
Faculty Mentors: Jessica S. Friz, Nathan T. Perreau, NASA Langley Research Center
Generic Robotic Guidance, Navigation, and Control Classes for the OSAM Architecture Simulation System (OASiS)

GEOSCIENCES

Matthew Neal, Emily Blitz, Andrew Edmonds, Ian Hattabaugh, Preston Liles, Julia Mathews, Jake Thompson, Jonathan Turco, University of Arkansas - Fort Smith
Faculty Mentors: Maurice Testa, Jordan Mader, University of Arkansas - Fort Smith
Development of a Standardized Methodology for the Collection and Quantification of Microplastics in Fort Smith Regional Waterway

MEDICINE & PARASITOLOGY

Samantha Jones, Southern Arkansas University
Faculty Mentor: James Hyde, Southern Arkansas University
Testing the Long Term Viability of Organotypic Cultures of the Anterior Mouse Pituitary

GRADUATE AWARDS

BIOCHEMISTRY/MOLECULAR BIOLOGY/ VERTEBRATE ZOOLOGY/MEDICINE

Billy Huggins, University of Central Arkansas
Faculty Mentor: Mindy Farris, University of Central Arkansas
Vitamin D: A Longevity Vitamin?

COMPUTER SCIENCE/ENGINEERING

Etee Kawna Roy, Arkansas State University
Faculty Mentor: Brandon Kemp, Arkansas State University
Dynamic Modeling of Multiparticle Electrostatic Self-assembly Toward Tunable Surfaces in Inverted Dielectric System

APPENDIX B: RESOLUTIONS

Arkansas Academy of Science

104th Annual Meeting, 2021 Resolutions

Be it resolved that we, the membership of the Arkansas Academy of Science (AAS) offer our sincere appreciation to The University of Arkansas – Fort Smith for hosting the 104th annual meeting of the Academy. We thank the local arrangements committee: Myron Rigsby, Tom Buchanan, Joshua Burns, Amy Skypala, Kristine Garner, David McClellan, Julie Martin, Jason McGee, Matt Hartstein, Rhonda Boyd, Linus Yu, and especially Associate Conference Chairs, Ragupathy Kannan and Clint Brooks, and Conference Chair, Jack Jackson who supported the program and meeting arrangements listed in the AAS proceedings. We sincerely thank University of Arkansas- Fort Smith for providing its services during this, the first virtual meeting of the Academy.

We especially thank our keynote speaker, Dr. Helen James, for her informative keynote address: *Discovering a Lost World of Birds: Fossils in Hawaii's Lava Caves*.

Appreciation and sincere gratitude is extended to our dedicated judges for the student presentations including Chiraz Amrine, Douglas Barron, Tom Buchanan, Joshua Burns, John Chamberlin, Puskar Chapagain, Shannon Clardy, Nawa Dahal, Lisa DuBose, Joel Funk, Mariusz Gajewski, Kristine Garner, Jacob Grosskopf, Julie Gunderson, Sharon Hamilton, Lionel Hewavitharana, Sara Hubbard, Nick Huisman, James Hyde, Naga Lakkaniga, Roger Lightner, David Mayo, David McClellan, Archana Mishra, Janet Renwick, Blake Sasse, Jeff Shaver, Ashokkumar Sharma, Amy Skypala, Ryan Stork, Suresh Kumar Thallapuranam, Candice Thomas, Susanne Wache, Charuksha Walgama, Edmond Wilson. We thank Dr. Ragupathy Kannan for coordinating the judging.

We congratulate our student researchers, scientists, and engineers who presented papers and posters whose efforts contribute directly to the future success of the Academy and the improvement of advancement of science in Arkansas. We congratulate the student award winners listed in the minutes.

The Academy recognizes its leadership and offers its thanks to this year's set of executive officers including Stephen Addison (President), Andrew Sustich (President Elect and Treasurer), Todd Tinsley (Vice President), Franklin Hardcastle (Past President), Ivan Still (Journal Editor-in-Chief and Journal Managing Editor), Panneer Selvam (Newsletter Editor), Rami Alroobi (Webmaster), Abdel Bachri (Historian), and Collis Geren (Secretary).

Respectfully submitted on this 10th day of April, 2021. Resolutions Committee.

2021 ARKANSAS ACADEMY OF SCIENCE MEMBERSHIP

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Steven	Addison	University of Central Arkansas
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Floyd	Beckford	Duke Kunshan University
Don	Bragg	USDA Forest Service
Calvin	Cotton	Geographics Printing Company
Betty	Crump	Ouachita National Forest
James	Daly	UAMS (retired)
Leo	Davis	Southern Arkansas University (ret.)
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Jim	Edson	University of Arkansas-Monticello(ret.)
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Collis	Geren	University of Arkansas-Fayetteville
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Philip	Hyatt	Arkansas Tech University (retired)
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Suresh	Thallapuranam	University of Arkansas-Fayetteville
Stanley	Trauth	Arkansas State University (retired)
Gary	Tucker	FTN Associates
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Sandhya	Baviskar	University of Arkansas-Ft. Smith
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David	Bowles	US. National Park Service
Tom	Buchanan	University of Arkansas-Ft. Smith
Joshua	Burns	University of Arkansas-Ft. Smith
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Gary	Graves	Smithsonian Institute
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Hamed	Shojaeo	Arkansas Tech University
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Matthew	Young	Arkansas Tech University
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Grace	Hoss	Arkansas College of Osteopathic Medicine
Olivia	Loudermilk	Harding University
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Brandon	Parker	Mensch Wold Lab
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Audrey	Thomas	University of Arkansas-Ft. Smith
Kyla	Wilson	John Brown University

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Bryan	Lockhart	
Edmond	Wilson	Harding University

KEYNOTE ADDRESS

Discovering a Lost World of Birds: Fossils in Hawaii’s Lava Caves

Helen F. James, Ph.D.

Presented 7:00 PM on Friday, April 9

We are extremely happy to welcome Dr. Helen F. James as our keynote speaker. Dr. James is a leading paleo-ornithologist, a fellow of the American Association for the Advancement of Science, and the Curator of Birds for the Smithsonian's National Museum of Natural History where she oversees one of the largest museum collections of birds in the world. She has led or participated in over thirty field expeditions, most of them to collect fossils for the Smithsonian Institution in Washington, DC, and the Bernice P. Bishop Museum in Honolulu. During several decades of research, she and her collaborators discovered over sixty species of extinct fossil birds in the Hawaiian Islands, many of them with odd traits like the inability to fly. She will share the story of these astounding discoveries and how they changed what we think about extinction, as well as how to prevent it.

Born in Hot Springs and raised on Kessler Mountain, just outside of Fayetteville, Arkansas, Dr. James is an alumna of University of Arkansas. As a child, she was captivated by visits to the Smithsonian's National Museum of Natural History in Washington, DC, and while still in her teens, she began volunteering to work behind the scenes in the museum's Paleobiology Department. She was soon drawn into a quest to discover the fossil birds of the Hawaiian Islands, which were all but unknown at the time. Her research has combined stratigraphic excavations of paleontological sites, comparative osteology to interpret fossils, and laboratory analyses of the ancient biomolecules in excavated bones to gain further insight into the past. The lecture will touch on the famous adaptive radiation of Hawaiian Honeycreepers, the wondrous lives of wide-ranging seabirds, and the many extinct island birds that were unable to fly. It will highlight the value of reconstructing ecological histories to help understand and conserve modern ecosystems.

Sponsored by:

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SECTION PROGRAM

Due to the meeting going virtual this year, the program is separated into three main sections: undergraduate presentations followed by graduate student presentations, and the faculty presentations. Each section is then further divided by subject area. The presenter(s) is (are) underlined.

UNDERGRADUATE STUDENT PRESENTATIONS

BIOCHEMISTRY/MOLECULAR BIOLOGY

1. DETERMINATION OF MATING TYPE AND SPECIES OF A NATURAL ISOLATE OF DICTYOSTELIUM USING MOLECULAR TECHNIQUES AND SEXUAL CROSSES
Harrison Jenkins, University of Arkansas - Fort Smith
Faculty Mentor: Sandhya Baviskar, University of Arkansas - Fort Smith

2. SMALL ANTIVIRAL PEPTIDE INHIBITORS AGAINST SARS-COV-2
Riley Roper, University of Arkansas - Fort Smith
Faculty Mentor: Mohammad A. Halim, University of Arkansas - Fort Smith

3. DESIGNING ANTIMICROBIAL PEPTIDES AGAINST THE MAIN PROTEASE OF SARS-COV-2: AMOLECULAR MODELLING APPROACH
Sydney Du, Cladie B. White, University of Arkansas - Fort Smith
Faculty Mentors: Archana Mishra, Mohammad A. Halim, University of Arkansas - Fort Smith

4. ARGININE: GLYCERIN BASED DEEP EUTECTIC SOLVENT AND ITS IMPACT ON PROTEIN STRUCTURE AND DYNAMICS
Kairy Galvez, University of Arkansas - Fort Smith
Faculty Mentor: Mohammad A. Halim, University of Arkansas - Fort Smith

5. THERAPEUTIC APPLICATION OF SCORPION VENOM PEPTIDES AGAINST SARS-COV-2
Honey Matevia, Harmeet Chohan, University of Arkansas - Fort Smith
Faculty Mentors: Archana Mishra, Mohammad A. Halim, University of Arkansas - Fort Smith

VERTEBRATE BIOLOGY

6. TURNING UP THE HEAT: THERMAL TOLERANCES OF FISHES IN THE KINGS RIVER, ARKANSAS
Willow Newman, Chance Garrett, Matthew Gifford, University of Central Arkansas
Faculty Mentors: Ginny Adams, Reid Adams, University of Central Arkansas

7. DO FARM PONDS AND SMALL IMPOUNDMENTS INFLUENCE FISH ASSEMBLAGES IN THE BLACK RIVER WATERSHED IN ARKANSAS?
Grace Davenport, Jennifer Main, George Gavrielides, and Calvin Rezac, University of Central Arkansas
Faculty Mentors: Ginny Adams and Reid Adams, University of Central Arkansas

8. DOES GUT LENGTH OF DUSKYSTRIPE SHINER LUXILUS PILSBRYI VARY IN RESPONSE TO STREAM DRYING?
Jessica Rath, Reagan Spinelli, Chance Garrett, University of Central Arkansas
Faculty Mentors: Ginny Adams, Reid Adams, University of Central Arkansas

9. DISTRIBUTION AND REPRODUCTION BY THE SWALLOW-TAILED KITE (*ELANOIDES FORFICATUS*) IN ARKANSAS
Grace Wills, Henderson State University
Faculty Mentor: Renn Tumlison, Henderson State University

10. THE DECLINE OF BIRD SPECIES AT JACK MOUNTAIN WILDLIFE MANAGEMENT AREA
Madison Shankle, Alexis Summerford, Ouachita Baptist University
Faculty Mentor: Christin Pruett, Ouachita Baptist University

11. ENERGY CONTENT OF SEEDS OF PALMER'S PIGWEED (*AMARANTHUS PALMERI*) IN THE DIET OF SCALED QUAIL (*CALLIPEPLA SQUAMATA)* IN NEW MEXICO
Paige Eddington, University of Arkansas at Monticello
Faculty Mentors: John L. Hunt, University of Arkansas at Monticello; Matthew E. Grilliot, Auburn University at Montgomery; Troy L. Best, Auburn University; Isaac C. Castillo, Faith A. Johnson, University of Arkansas at Monticello

12. PINE FORESTS ARE AN IMPORTANT HABITAT FOR BREEDING BIRDS AT JACK MOUNTAIN WILDLIFE MANAGEMENT AREA
Alexis Summerford, Madison Shankle, Ouachita Baptist University
Faculty Mentor: Christin Pruett, Ouachita Baptist University

PLANT BIOLOGY/BIOMEDICAL STUDIES

13. ASSEMBLY, ANNOTATION, AND EVOLUTION OF THE PLASTOME OF PARASITIC APHYLLON PLANTS
Taylor Aishman, Hendrix College
Faculty Mentor: Adam C. Schneider, Hendrix College

14. EFFECTS OF LATE WINTER/EARLY SPRING FLOODING ON PIN OAK SAPLINGS
Ashlyn Estes, University of Central Arkansas
Faculty Mentor: Erin Wiley, University of Central Arkansas

15. EFFECT OF SOIL MICROBIOME SUCCESSION ON THE PREVALENCE OF ANTIBIOTIC RESISTANCE
Ethan Moore, Brandon Romero, Hannah Warrington, University of Arkansas - Fort Smith

Faculty Mentors: Roger Lightner, Jeffrey Shaver, University of Arkansas - Fort Smith

16. NATURE INSPIRED MATERIALS FOR BIOMEDICAL APPLICATIONS
Paula Najera-Diaz, Kenia Mendez, University of Arkansas - Fort Smith
Faculty Mentor: Charuksha Walgama, University of Arkansas - Fort Smith

MUSEUM SCIENCE/BIODIVERSITY

17. CURATING AND DIGITIZING THE BRYOPHYTE COLLECTIONS OF THE HENDRIX COLLEGE HERBARIUM
Grace Capooth, Hendrix College
Faculty Mentor: Adam C. Schneider, Hendrix College

18. WILDLIFE AMONG US: PRELIMINARY RESULTS FROM THE FIRST TWO SEASONS OF THE CENTRAL ARKANSAS URBAN WILDLIFE PROJECT
Tristan Hoerschelmann, Lauren L. Berry, Jaclyn S. Reifeiss, Rebecca G. Burks, Hendrix College
Faculty Mentors: Kirsten Bartlow, Christopher R. Middaugh, Kimberly Sparks, Arkansas Game and Fish Commission

19. COMPARISON OF TICK COLLECTION METHODS
Ty Say, University of Arkansas - Monticello
Faculty Mentor: Keith Blount, University of Arkansas - Monticello

20. BIODIVERSITY OF ANTS IN THE OZARKS
Priscilla Hall, John Brown University
Faculty Mentor: Joel Funk, John Brown University

CHEMISTRY

21. A COMPACT METHOD FOR EASILY CREATING AND TESTING CZERNY-TURNER HIGH-RESOLUTION SPECTROGRAPHS AND ALLOWING QUICK INTERCHANGE OF COMPONENTS
Thomas Marshall, Harding University
Faculty Mentor: Edmond Wilson, Harding University

22. METAL OXIDE LEACHING FROM GLASS CULLET USED FOR SHORELINE RESTORATION
Emily Blitz, Kathryn Echevarria, Julia M. Mathews, University of Arkansas - Fort Smith
Faculty Mentors: Jordan A. Mader, Maurice P. Testa, University of Arkansas - Fort Smith

23. EXPLORING GREEN SYNTHESIS OF ALDOL REACTIONS USING DEEP EUTECTIC SOLVENTS
Michael Lee, Bobbi Evans, University of Arkansas - Fort Smith
Faculty Mentors: Jordan A. Mader, Souvik Banerjee, University of Arkansas - Fort Smith

24. SYNTHESIS, IR SPECTROSCOPY AND MASS SPECTROMETRY INVESTIGATIONS OF GLUTAMINE BASED DEEP EUTECTIC SOLVENT
Tailor Johnston, University of Arkansas - Fort Smith
Faculty Mentor: Mohammad A. Halim, University of Arkansas - Fort Smith

25. MENTHOL: ACETIC ACID BASED LOW VISCOSITY DEEP EUTECTIC SOLVENTS
Paul Gambill, University of Arkansas - Fort Smith
Faculty Mentor: Mohammad A. Halim, University of Arkansas - Fort Smith

26. IBUPROFEN-MENTHOL BASED THERAPEUTIC DEEP EUTECTIC SYSTEM
Mary-Kate Wewers, University of Arkansas - Fort Smith
Faculty Mentor: Mohammad A. Halim, University of Arkansas - Fort Smith

27. GLYCEROL: TYROSINE BASED NATURAL DEEP EUTECTIC SOLVENT: INSIGHTS FROM IRSPECTROSCOPY AND MASS SPECTROMETRY
Harmeet Kaur Chohan, University of Arkansas - Fort Smith
Faculty Mentor: Mohammad A. Halim, University of Arkansas - Fort Smith

28. ACIDIC AMINO ACID AND GLYCEROL BASED NATURAL DEEP EUTECTIC SOLVENTS
Cynthia Montoya, University of Arkansas - Fort Smith
Faculty Mentor: Mohammad A. Halim, University of Arkansas - Fort Smith

29. DETECTION OF ACTIVE PHARMACEUTICAL INGREDIENTS (APIS) IN DRUGS USING SCREEN PRINTED ELECTRODES
Matt Boston, University of Arkansas – Fort Smith
Faculty Mentor: Charuksha Walgama, University of Arkansas - Fort Smith

30. ELECTROCHEMICAL BIOFILMS FOR DRUG METABOLITE SYNTHESIS
Evan Wittig, University of Arkansas – Fort Smith
Faculty Mentor: Charuksha Walgama, University of Arkansas - Fort Smith

31. N-IMIDAZOLYL BENZAMIDES FOR THE FIGHT AGAINST MALARIA
Chloe Cline, Henderson State University
Faculty Mentor: Martin Campbell

32. PROBING THE INTERACTION OF PESTICIDES WITH THE HUMAN SERUM ALBUMIN USING MOLECULAR DOCKING AND MD SIMULATION
Jesse Scott, Joseph B. Stubblefield, University of Arkansas - Fort Smith
Faculty Mentors: Sayo O. Fakayode and Archana Mishra, University of Arkansas- Fort Smith

33. ELECTROCHEMICAL TECHNIQUE TO FABRICATE GLUCOSE BIOSENSOR USING ENZYME EXTRACT FROM CORN
Kayleigh Amber Rodríguez, Arkansas State University
Faculty Mentor: Anahita Izadyar, Arkansas State University

ENGINEERING

34. LASER BEAM PROFILER
Gage Miller, Harding University
Faculty Mentor: Edmond Wilson, Harding University

35. OPTICAL TRACKING OF HIGH ALTITUDE BALLOONS
Wade Lamberson, Harding University
Faculty Mentor: Edmond Wilson, Harding University

36. GENERIC ROBOTIC GUIDANCE, NAVIGATION, AND CONTROL CLASSES FOR THE OSAM ARCHITECTURE SIMULATION SYSTEM (OASIS)
Grace Zimmerman, University of Central Arkansas
Faculty Mentors: Jessica S. Friz, Nathan T. Perreau, NASA Langley Research Center

37. ROBOT - AUTONOMOUS NAVIGATION
Ilya Busaev, University of Central Arkansas
Faculty Mentor: William Slaton, University of Central Arkansas

38. AUTOMATED COLOR-DISTINGUISHING ROBOTIC ARM
Ashley Strohmeyer, University of Arkansas - Little Rock
Faculty Mentor: Steve Menhart, University of Arkansas - Little Rock

39. STRENGTHENING BEHAVIOR OF 7075 ALUMINUM ALLOY AFTER STRAIN HARDENING VERSUS THAT OF PRECIPITATION HARDENING
John Graham, University of Arkansas - Little Rock
Faculty Mentors: Ashokkumar M. Sharma, Srikanth B. Pidugu, University of Arkansas -Little Rock

GEOSCIENCES

40. USING HIGH-RESOLUTION FIELD PHOTOGRAMMETRY FOR 3D RENDERS AND MODELS OF SILURIAN REEFS IN MICHIGAN'S HIAWATHA NATIONAL FOREST
Preston Liles, Logan Pearson, Samantha Barnett, University of Arkansas - Fort Smith
Faculty Mentors: Maurice P. Testa, University of Arkansas Fort Smith, Erik B. Larson, Shawnee State University

41. PETROGRAPHIC AND BIOSTRATIGRAPHIC ANALYSIS OF A MCKAY BAY MEMBER MID-SILURIAN KNOLL REEF IN MICHIGAN'S UPPER PENINSULA
Logan Pearson, Samantha Barnett, Preston Liles, University of Arkansas Fort Smith
Faculty Mentors: Maurice P. Testa, University of Arkansas - Fort Smith, Erik B. Larson, Shawnee State University

42. DEVELOPMENT OF A STANDARDIZED METHODOLOGY FOR THE COLLECTION AND QUANTIFICATION OF MICROPLASTICS IN FORT SMITH REGIONAL WATERWAY
Matthew Neal, Emily Blitz, Andrew Edmonds, Ian Hattabaugh, Preston Liles, Julia Mathews, Jake Thompson, Jonathan Turco, University of Arkansas - Fort Smith
Faculty Mentors: Maurice Testa, Jordan Mader, University of Arkansas - Fort Smith

MEDICINE & PARASITOLOGY

43. A NUTRITIONAL INTERVENTION TO ATTENUATE UPPER RESPIRATORY TRACT INFECTIONS IN COLLEGIATE DISTANCE RUNNERS
Emily Newberry, University of Central Arkansas
Faculty Mentor: Candice Thomas, University of Central Arkansas

44. IDENTIFICATION OF POTENTIAL SMALL MOLECULE INHIBITORS OF SARS-COV-2 MAIN PROTEASE
Joshua Thammathong, Olivia Coulter, Ramiro Cruz, Ryan Whatcott, University of Arkansas- Fort Smith
Faculty Mentor: Souvik Banerjee, University of Arkansas - Fort Smith

45. DEVELOPMENT OF ER-ß SELECTIVE AGONISTS FOR THE TREATMENT OF OBESITY AND NON-ALCOHOLIC STEATOHEPATITIS (NASH)
Olivia Coulter, University of Arkansas - Fort Smith
Faculty Mentor: Souvik Banerjee, University of Arkansas - Fort Smith

46. TESTING THE LONG TERM VIABILITY OF ORGANOTYPIC CULTURES OF THE ANTERIOR MOUSE PITUITARY
Samantha Jones, Southern Arkansas University
Faculty Mentor: James Hyde, Southern Arkansas University

47. MAPPING SPATIAL AND TEMPORAL VARIATION IN *AEDES ALBOPICTUS* PREVALENCE ACROSS ARKANSAS
Alyssa N Jones, Arkansas Tech University
Faculty Mentor: Douglas G Barron, Eric C Lovely, Arkansas Tech University

PHYSICS

48. THE FLUORINO: A LOW-COST, ARDUINO-CONTROLLED FLUOROMETER
Joe Coker, Hendrix College
Faculty Mentor: Julie Gunderson

49. USING DIFFERENTIAL PHOTOMETRY TO STUDY VARIABILITY IN BINARY STAR SYSTEM XZ TAURI
Briana Budnick, University of Central Arkansas
Faculty Mentor: Scott Austin, University of Central Arkansas

50. COMPROMISED FUNCTIONALITY DUE TO CRANIAL RADIATION TO TREAT TUMORS EXPLAINED THROUGH 3D MODELING OF DENDRITIC SNIPPING
Simeon Simmons, University of Central Arkansas
Faculty Mentor: Azida Walker, University of Central Arkansas

51. EFFECTS OF RADIATION ON THE KV1.2 ION CHANNEL
Austin Hall, University of Central Arkansas
Faculty Mentor: Azida Walker, University of Central Arkansas

52. EFFECT OF LOW DOSE RADIATION ON THE STRENGTH OF RAT HINDLIMB BONES
Manling Cheng, University of Central Arkansas
Faculty Mentors: Rahul Mehta, Brent Hill, University of Central Arkansas

53. FINDING PULSARS – USING COMPUTATIONAL PHYSICS TO EXPLORE UNTAPPED ASTRONOMY DATABASES
John Singel, University of Central Arkansas
Faculty Mentor: Jeremy Lusk, University of Central Arkansas

54. A VERSATILE FIBER-FED COMPACT SPECTROGRAPH
Justin Hajicek, Harding University
Faculty Mentor: Edmond W. Wilson, Jr., Harding University

GRADUATE STUDENT PRESENTATIONS

BIOCHEMISTRY/MOLECULAR BIOLOGY

55. INVESTIGATING THE MOLECULAR RESPONSES IN RICE ROOTS DURING INTERACTION WITH PLANT GROWTH-PROMOTING BACTERIA, *BURKHOLDERIA UNAMAE*
John Cook, University of Central Arkansas
Faculty Mentor: Arijit Mukherjee, University of Central Arkansas

56. INVESTIGATE THE MOLECULAR MECHANISMS BY WHICH PLANT GROWTH-PROMOTING BACTERIA, *AZOSPIRILLUM BRASILENSE*, MEDIATE SALT STRESS
Zachariah Degon, University of Central Arkansas
Faculty Mentor: Arijit Mukherjee, University of Central Arkansas

VERTEBRATE ZOOLOGY

57. DETERMINATION OF BAT SPECIES' USE OF ARTIFICIAL BARK ENHANCED HABITAT AT SELECT SITES IN NORTH AND CENTRAL ARKANSAS
Sarah Martin, University of Central Arkansas
Faculty Mentor: M. Victoria McDonald

MEDICINE

58. VITAMIN D: A LONGEVITY VITAMIN?
Billy Huggins, University of Central Arkansas
Faculty Mentor: Mindy Farris, University of Central Arkansas

COMPUTER SCIENCE

59. HOW PREDICTABLE IS SPACE EXPLORATION?
Michael Howell, Daniel Berleant, University of Arkansas - Little Rock; Richard Segall, Arkansas State University; Hyacinthe Aboudja, Oklahoma City University; Peng-Hung Tsai -University of Arkansas - Little Rock

ENGINEERING

60. EFFECT OF POWDER ACTIVATED CARBON (PAC) ON CLASS C FLY ASH MODIFIED CONCRETE PROPERTIES
Sumon Roy, Arkansas State University
Faculty Mentor: Zahid Hossain, Arkansas State University

61. A STUDY ON FEASIBILITY OF WARM MIX ASPHALT IN ARKANSAS
Mohammad Najmush, Sakib Oyan, Arkansas State University
Faculty Mentor: Zahid Hossain, Arkansas State University

62. SEISMIC SITE RESPONSE ANALYSIS IN PERSPECTIVE VIEW OF NORTH-EAST ARKANSAS (NEA)
MD Rafiue Islam, Arkansas State University
Faculty Mentor: Zahid Hossain, Arkansas State University

63. EFFECT OF POWDERED ACTIVATED CARBON (PAC) IN FLY ASH ON ALKALI-SILICA REACTIVITY AND SCALING RESISTANCE OF CONCRETE
Raiyan Chowdhury, Arkansas State University
Faculty Mentor: Zahid Hossain, Arkansas State University

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Etee Kawna Roy, Arkansas State University
Faculty Mentor: Brandon Kemp, Arkansas State University

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Mohammad Halim, University of Arkansas - Fort Smith

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Renn Tumlison, Grace Wills, Henderson State University

70. BIRD USAGE OF BLACK MARASMIUS FIBERS AS NEST MATERIAL – TESTING TWO HYPOTHESES
Ragupathy Kannan, Shayla Smithson, Haris Rana, Jack Jackson, University of Arkansas -Fort Smith

71. VIRTUAL ORNITHOLOGY—STUDYING BERMUDA PETRELS FROM REMOTE VIA WEBCAMS
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Chris McAllister, Eastern Oklahoma State College; H.W. Robison, Sherwood, AR; E.T. Woodyard, T.G. Rosser, Mississippi State University; T.J. Fayton, Cornell University

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75. SEMI-SYNTHETIC MODIFICATION OF EPIPOLYTHIODIOXOPIPERAZINE ALKALOID ANALOGUES
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81. *EIMERIA LANCASTERENSIS* (APICOMPLEXA: EIMERIIDAE), THREE NEMATODES (HELIGMOSOMOIDEA: BOEHMIELLIDAE, HELIGMONELLIDAE), AND FLEA (ORCHOPEAS HOWARDI) FROM AN EASTERN FOX SQUIRREL (*SCIURUS NIGER*)
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82. NEW DISTRIBUTIONAL RECORDS FOR ECTOPARASITES (ACARI: LAELAPIDAE, MYOCOPTIDAE) OF THE WOODLAND VOLE, *MICROTUS PINETORUM* (RODENTIA)
Chris McAllister, Eastern Oklahoma State College; L.A. Durden, Georgia Southern University

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Chris McAllister, Eastern Oklahoma State College; H.W. Robison, Southern Arkansas University (Emeritus); T.G. Rosser, E.T. Woodyard, Mississippi State University

Journal Acknowledgements and Editorial Board

The Arkansas Academy of Science gratefully acknowledges the Editorial board for volume 75 of the Journal during 2021.

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The Editorial Board 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.

The Editorial Board and the Academy also extends our thanks to Cedar Middleton and Melody Herr of the Office of Scholarly Communication, University of Arkansas – Fayetteville, and the University of Arkansas – Fayetteville for webhosting the Journal of the Arkansas Academy of Science.

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. If you are interested in either of these positions, please contact: Dr. Still (before April 1 2022) (jarksci@gmail.com) and/or Dr. Addison (saddison@uca.edu) 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).

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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. Formatting of page size and margins for every page should be carefully checked at each stage of submission as this will impact number of pages that the manuscript occupies. Incorrect formatting will affect the turn-around time of the article and may incur extra page charges to be added.

Instructions to Authors

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. The Running Title must be the same as the Short Title entered into the electronic submission system
    - 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.
11. **Digital Object Identifiers** (DOIs): The Journal repository staff (at UARK) will assign DOIs via CrossRef to published JAAS manuscripts. Authors will see a DOI addition box in the submission page. **This box is for repository staff use only.**

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: (must be the same as the Short Title entered into the electronic submission system) (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. This **Running Title** MUST be the same as the **Short Title** the author entered into the electronic submission system.

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". 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.

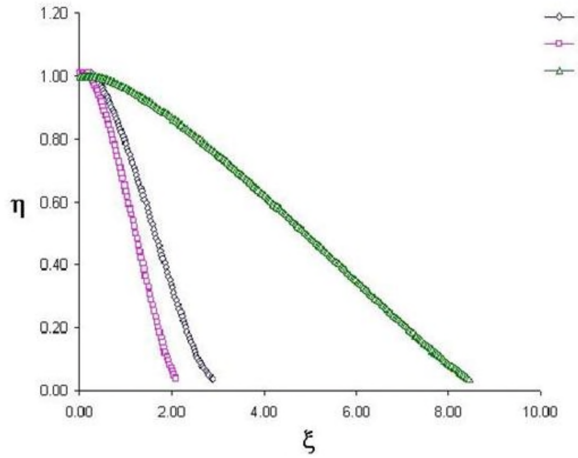

Figure 2. Electric field, η, as a function of position ξ, within the sheath region for three different wave speeds, α.

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. Novel nucleotide sequence data must be submitted to GenBank, and accession numbers presented within the manuscript. Sequence data may also be attached as Supplementary data in the electronic submission to the *JOURNAL.*

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. In order for the Journal to use CrossRef for assigning DOIs to JAAS manuscripts, authors of manuscripts must include DOIs for articles that they cite in the Literature Cited sections of the manuscript.

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*".

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Cited in text as: (Lauffart *et al*. 2002)

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. DOI: https://doi.org/10.1115/1.3153041

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.

Cited in text as: (Singleton and Bennett 1997)

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.

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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.

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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

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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).

9. 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.

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