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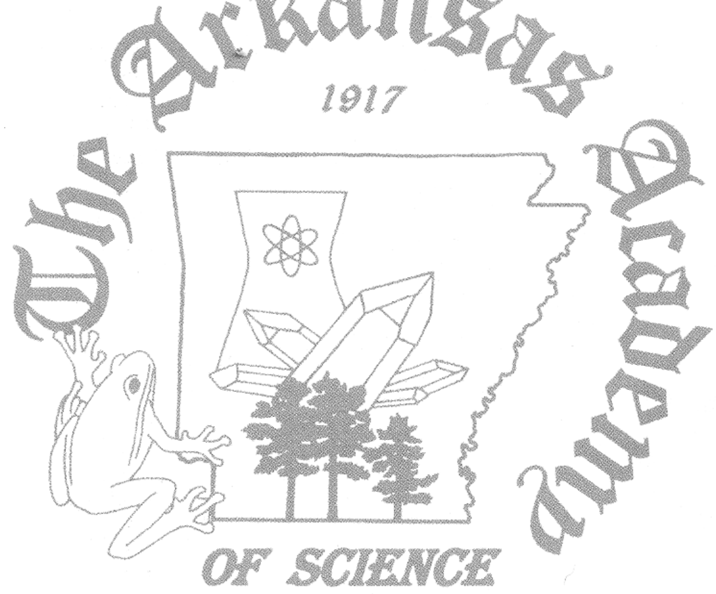
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COVER: Photograph of a Gray-headed Junco. *From: Second record of Gray-headed Junco (Junco hyemalis caniceps) in Arkansas by K.G. Smith and K. Garrett pp 167-168. Dr. Kimberly Smith passed away shortly after this year's meeting and an In Memorium for Dr. Smith is given on pp 218-220.*

ARKANSAS ACADEMY OF SCIENCE

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Germinal Epithelium Cytology during Spermatogenesis in the Alligator Snapping Turtle, *Macrochelys temminckii* (Reptilia: Chelydridae)

S.E. Trauth[1*] and K.M. Gribbins[2]

[1]*Department of Biological Sciences, Arkansas State University (Emeritus), State University, AR 72467*
[2]*Department of Biology, University of Indianapolis, Indianapolis, IN 46227*

*Correspondence: strauth@astate.edu

Running Title: Germinal Epithelium Cytology in *Macrochelys temminckii*

Abstract

We investigated the cytology of the seminiferous epithelia of the Alligator Snapping Turtle (*Macrochelys temminckii*). Spermatogenic and regressed testes were assessed from 2 adult individuals collected in Arkansas in May and September of 1993. Specifically, we focused on the cellular phases of germ cell development and maturation. The germ cell morphology and developmental strategy within the germinal epithelium of *M. temminckii* appear similar to that of other genera of turtles previously studied. Interestingly, mitotic, meiotic, and spermiogeneic cells are nearly identical to that of other turtles studied based on light microscopy. There are also 6 recognizable steps to spermiogenesis, which is slightly different than the 7 steps of most turtles (step 7 absent). Though this study only uses 2 individuals (because of its endangered status), *M. temminckii* appears to start spermatogenesis in the spring, and the climax of spermiogenesis occurs in the fall similar to that of other temperate turtles studied to date based on light microscopy. Peculiar to both turtles in this study were the regular appearance of very large germ cells in the basal compartment of the germinal epithelium. Based on previous research and our histological analysis these enlarged spermatogonia exhibit hypertrophic characteristics typical of cells undergoing apoptosis.

Introduction

Most key features of amniote spermatogenesis and histological architecture of the testis are present in all reptiles (Volsøe 1944; Pudney 1995; Gribbins 2011). The spermatogenic process includes the following events: 1) a proliferative phase (spermatocytogenesis) in which large numbers of germ cells are successively generated by numerous mitotic divisions of spermatogonial cells, 2) a meiotic phase that produces haploid secondary spermatocytes, 3) a spermiogenic phase in which spermatids transform into motile sperm, and 4) a regressive or quiescent phase with little or no germinal cell activity (indicative of seasonally breeding).

Most spermatogenic studies in turtles have described their testicular cycles in terms of seasonal changes in seminiferous tubule size and activity often with little to no histological data. Limited information, however, exists on the specific cytological events of spermatogenesis at the light microscopic level in a few chelonians (Pudney 1995; Gribbins *et al.* 2003; Miller and Dinkelacker 2008).

In temperate zone chelydrid turtles, such as the Snapping Turtle (C*helydra serpentina*), testes are flaccid and have a minimal mass during the spring and early summer months, whereas the testes are large during late summer and early fall (Mahmoud and Cyrus 1992). Other than a brief report by Dobie (1971), who noted the year-round presence of sperm in reproductive tracts, no documentation of the testicular cycle or spermatogenic activity in the Alligator Snapping Turtle (*Macrochelys temminckii*) has been reported. Furthermore, there are only two studies to date that present cytological data on the specific germ cell morphologies as they progress through spermatogenesis in turtles (Gribbins *et al.* 2003; Lancaster *et al.* 2014).

The present study describes the cytology of developing germs cells in the testes of the Alligator Snapping Turtle using light microscopy at the height of spermatogenic activity in a September specimen and during the quiescent phase in May. We also document the presence of severely enlarged germ cells in the basal compartment, which are most common in a September specimen at the climax of spermiogenesis and spermiation. Although these cells in the present study appear to be much larger than previously reported in other reptiles, they are morphologically indicative of apoptosis as defined by recent studies in other turtles (Zang *et al.* 2007) and lizards (Comitato *et* *al*. 2006).

Materials and Methods

Alligator Snapping Turtles were collected in Jackson and Ouachita counties on the 19th of May and on the 3rd of September, 1993, respectively. Both specimens were euthanized with an intra-pleuroperitoneal cavity injection of sodium pentobarbital. Samples of the right testis were extracted from both adult male Alligator Snapping Turtles prior to their deposition into the Arkansas State University Museum of Herpetology (ASUMZ 19010 and 19261; both turtles were of comparable size—the latter turtle measuring 507 mm in standard carapace length). Following testis fragment removal, each turtle was fixed using injections of 10% formalin. The whole turtles were then preserved in 70% ethanol.

For light microscopy (LM-plastic), we fixed testis fragments in 2% glutaraldehyde (GTA) for 2–4 hr. Segments were then dehydrated in a graded series of increasing ethanol solutions (50-100%), placed in a 50/50% acetone/plastic mixture for overnight infiltration, and were eventually placed in embedding molds using plastic resin, Mollenhauer's Epon-Araldite #2, as described by Dawes (1988). For thick sectioning of tissue blocks (approximately 1 µm in thickness) and staining, we used glass knives on an LKB Ultrotome (Type 4801A) with Ladd® multiple stain (LMS), respectively. For photomicroscopy, we used a Leica MC 120 HD camera atop a Leica DM 2000 LED compound light microscope.

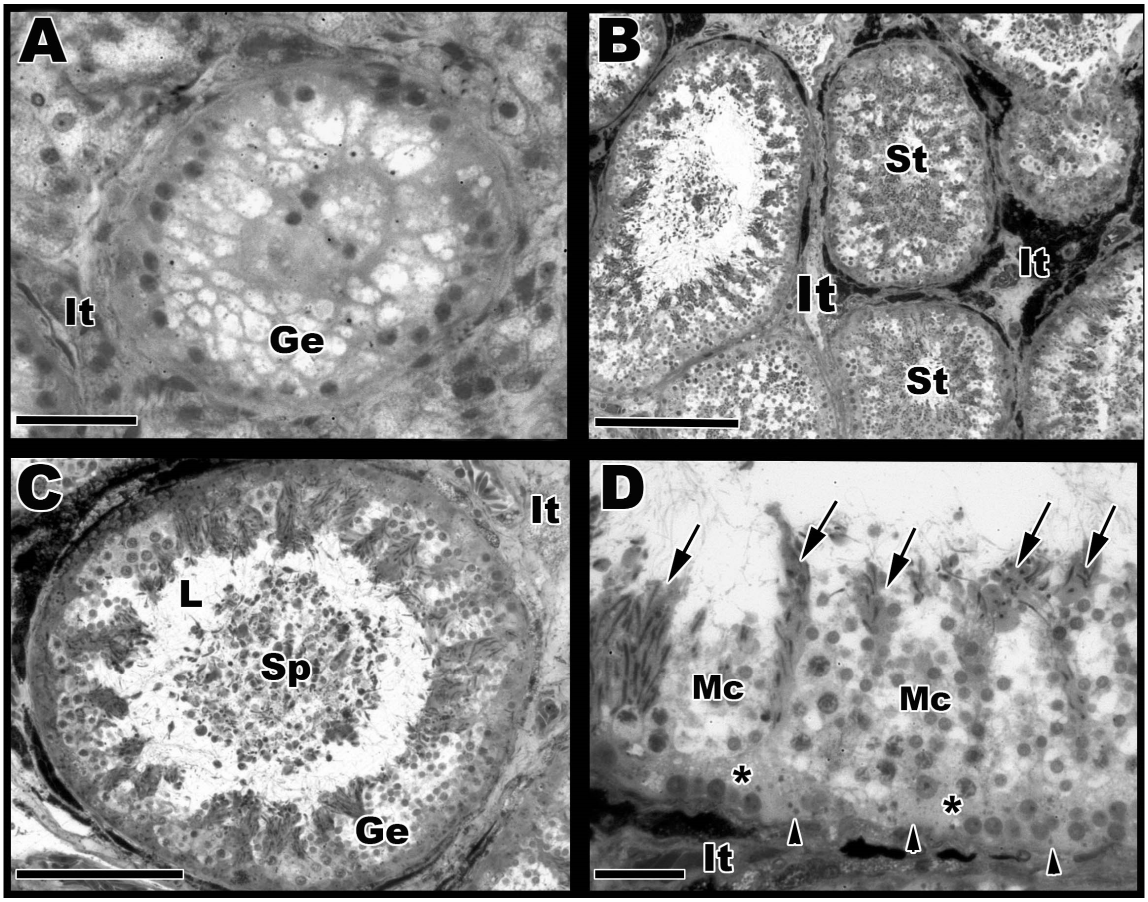

Figure 1. Light micrographs representing the organization of the testis in *Macrochelys temminckii* in May (A) and September (B, C, D) and. The Alligator Snapping Turtle exhibits the typical reptilian histological architecture with seminiferous tubules (St) interspersed with interstitial tissue (It) dominating the microscopic anatomy of the testis. The seminiferous tubules of the May testis are in quiescence with a heavily vacuolated germinal epithelium (Ge) with few germ cells, which are restricted to the basal compartment. In contrast, the September germinal epithelium shows spermatogenesis in full swing, with a thick epithelial lining exhibiting layers of different generations of developing germ cells. Sperm (Sp) are often located in the lumen (L) of each September seminiferous tubule along with shed Sertoli cell remnants. The thick epithelium is organized into columns (black arrows) containing cohorts of developing spermatids, intermediate meiotic cells (Mc), and basally located spermatogonia (*) resting on a prominent basement membrane (black arrowheads). Bars: A = 50 µm; B = 200 µm; C = 100 µm; D = 25 µm.

Results

The testis of the Alligator Snapping Turtle is comprised of seminiferous tubules that are lined with a continuous seminiferous epithelium, where germ cells develop in close association with Sertoli cells. The epithelium rests on a conspicuous basement membrane and the tubules are separated by interstitial space that is comprised of collagen-based connective tissue, blood vessels, and Leydig-like cells. The seminiferous epithelium in *Macrochelys temminckii* is quiescent in the month of May (Fig. 1A) within our samples, and the only dominant germ cells within the highly vacuolated epithelium are spermatogonia. The September seminiferous tubules (Fig. 1B, C, D), in contrast, are highly active in the process of spermatogenesis and multiple generations of germ cells with representative spermatogonia, spermatocytes, and spermatids, which are easily observed within the columns of seminiferous epithelia.

The most noteworthy germ cell morphology of the early and late seminiferous epithelia in *M. temminckii* is the presence of enlarged hypertrophic cells (Fig. 2A, C, Hp) near the basement membrane. These cells undergo a continual enlargement and can reach widths of over 30µm. These cells then undergo a complete breakdown of the cytoplasm and nucleus as seen in Fig, 2C. There are three types of spermatogonia found in the May seminiferous epithelium of the Alligator Snapping Turtle. The R type spermatogonia (Fig. 2A, SpR) have darkly staining nuclei and are inactive and not observed dividing within the epithelium. The type A and B spermatogonia (Fig, 2A, B, SpA, B) divide and then enter the start of meiosis within the basal compartment of the seminiferous tubules (Fig. 2A, B, Pl).

The September *Macrochelys temminckii* testis has seminiferous tubules that are dominated by developing generations of spermatids found in discernable columns of seminiferous epithelia (Fig. 1D). These fall turtles have started spermiation and tubular lumina often are occupied by spermatozoa (Fig. 1C). Meiotic spermatocytes (Fig. 3A–D, Pl, Pa, Zy, Di, Lp, M1, M2) are found near the basement membrane just above spermatogonia A and B, which have completed mitosis for the summer season. Interestingly, 4 to 7 generations of spermatids are observed within a cross section of a seminiferous tubule. These generations of maturing cell types are often in sequential generations, which appears to lead to waves of sperm release upon observation of multiple tubules in cross section.

Discussion

This qualitative study of spermatogenesis in spring and fall Alligator Snapping Turtles adds insight on germ cell development in turtles. The use of plastic embedded sections allowed acute visualization of the cellular events of spermiogenesis in these turtles, which has been noted as a problem in the past, particularly acrosome development (Gribbins *et al.* 2003). The acrosome vesicles are easily discernable in CS of the seminiferous tubules of the Alligator Snapping Turtle. Hypertrophic cells of great size are seen in *Macrochelys temminckii* basal compartments of the seminiferous epithelia. The incredible sizes of these

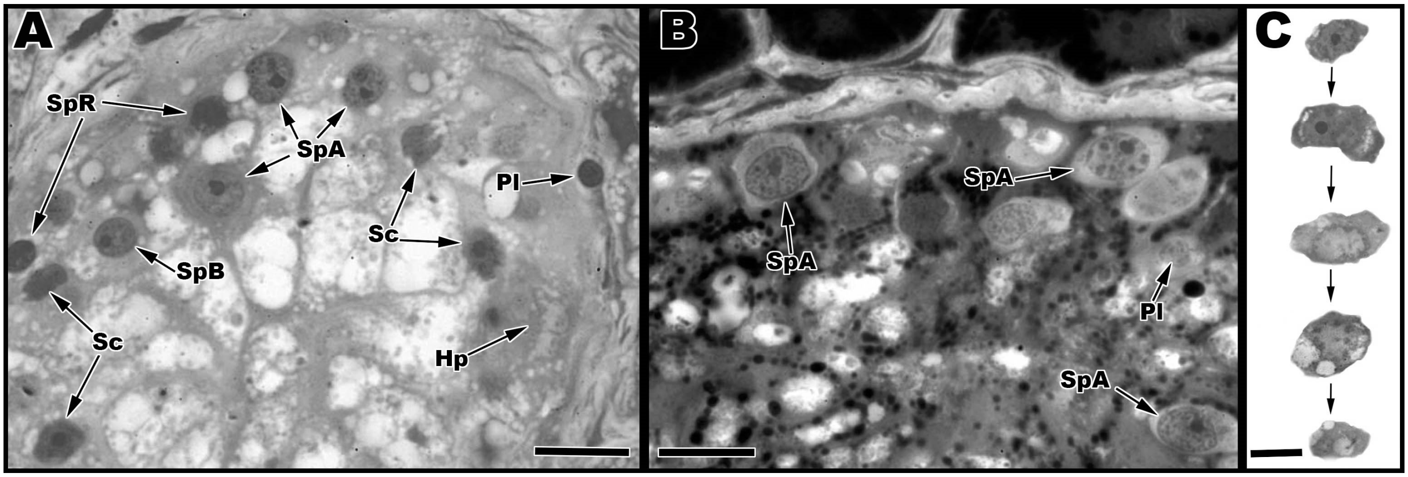

Figure 2. A and B (bars = 10 µm) light micrographs represent high magnification of the May germinal epithelium within the testis of *Macrochelys temminckii.* Note that only spermatogonia (SpR, SpA, SpB), Sertoli cell nucleus (Sc), and an occasional pre-leptotene (Pl) spermatocyte dominate the basal compartment of this epithelium. The only other cell type found in the seminiferous epithelium is sizeable hypertrophic cells (Hp) in various stages of enlargement or degradation (**C**) (bar = 5 µm).

cells are most likely an artifact of the infiltration and embedding process, which has been noted in basally located spermatogonia and apoptotic cells within other turtle testes (Hess 1990; Gribbins *et al*. 2003).

Nuclear elongation is enhanced in *M. temminckii* elongating spermatids and leads to linear sperm nuclei, which are bundled together within the seminiferous epithelium and most likely contributes to the column effect that is observed within the fully spermatogenic turtle in September. These spermatids in the elongation phase are very similar to avian spermatids (Sprando and Russell 1988, Kumar 1995) during late stage spermatogenesis, which has also been suggested in other species of turtles (Gribbins *et al*. 2003; Lancaster *et al*. 2014). The morphologies of spermatocytes and all three spermatogonia types in Alligator Snapping Turtles are similar to that of other turtles (e.g., *Chrysemys picta, Trachemys scripta,* and *Graptemys geographica*) as mentioned in Gribbins *et al*. (2003), Gribbins (2011), and Lancaster *et al.* (2014), respectively. Not only are these cell types similar in appearance, but also the mode and organization of the germ cells within the Alligator Snapping Turtle seminiferous epithelium corroborates what has been reported in these same species of turtles. Thus, it is reasonable to assume that *M. temminckii* follows the same postnuptial production of sperm that have been reported in other temperate turtles and snakes (Gibbons 1968; Moll and Legler 1971; Gribbins 2011). Also the arrangement of 4 to 7 generations of spermatids during late spermatogenesis in the Alligator Snapping Turtle, like that observed in other reptiles (Gribbins 2011; Gribbins and Rheubert 2015), suggests that these turtles follow a temporal germ cell development strategy rather than the spatial germ cell development strategy seen in mammals and birds (Rossen-Runge 1977; Russell *et al*. 1990).

The present cytological data on sperm development in the Alligator Snapping Turtle add needed information to what is known for spermatogenesis within Chelonia. Very few species have been studied to date, and though the data of the present study is from only two months of the year, our cytological results show similar trends to what is already known in turtles. These types of comparative histological data on the testis are important if inferences on the process of spermatogenesis within turtles are going to be robustly understood.

Acknowledgments

We thank Chris Thigpen for technical assistance. We are also appreciative of the continuing support by Kelly Irwin, herpetologist, of the Arkansas Game and Fish Commission (AGFC). Turtle collection was authorized by a scientific collection permit from the AGFC, and we also thank Anthony Holt and Patrick Daniel for assistance in collecting the turtles.

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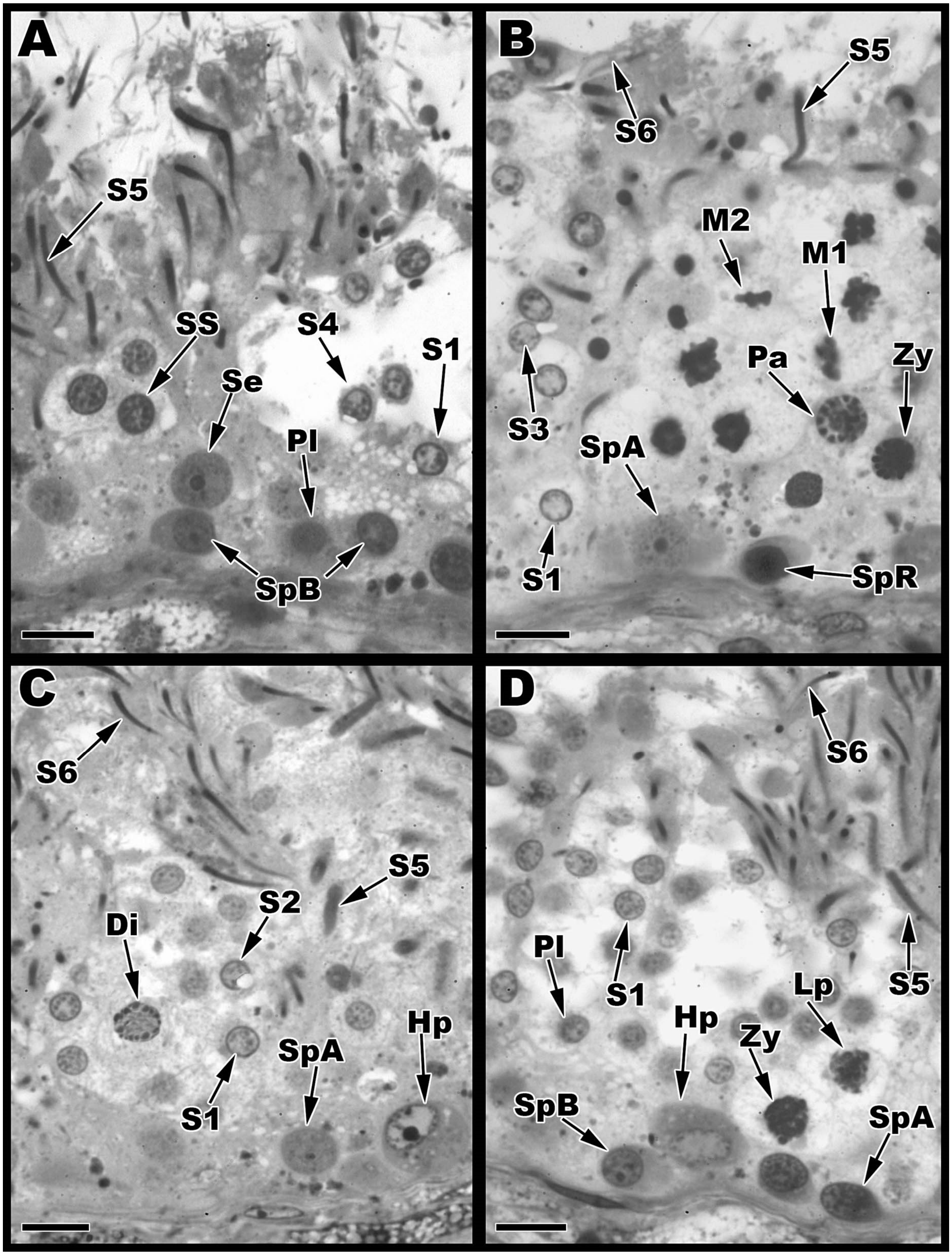

Figure 3. Light micrographs (A–D) representing various views of the September seminiferous epithelium at high magnification within the testis of *Macrochelys temminckii*. Notice that the fall testis of the Alligator Snapping Turtle is spermatogenically active with cell types of mitosis, meiosis, and spermiogenesis, and all are represented within the germinal epithelium. Se, Sertoli cell nucleus; SpR, resting spermatogonium; SpA, spermatogonium A; SpB, spermatogonium B; Hp, hypertrophic spermatogonium; Pl, pre-leptotene spermatocyte; Lp, leptotene spermatocyte; Pa, pachytene spermatocyte; Di, diplotene spermatocyte; M1, meiosis 1; SS, secondary spermatocyte; M2, meiosis 2; S1-S6, steps 1 through 6 spermatids. Bars = 10 µm.

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Distal Urogenital Anatomy of Male Southern Coal Skinks, *Plestiodon anthracinus pluvialis* (Reptilia: Scincidae)

S.E. Trauth

Department of Biological Sciences, Arkansas State University (Emeritus), State University, AR 72467-0599

Correspondence: strauth@astate.edu

Running Title: Distal Urogenital Anatomy of Male *Plestiodon anthracinus pluvialis*

Abstract

I investigated the morphology and histology of the distal urogenital anatomy of male southern coal skinks (*Plestiodon anthracinus pluvialis*) from reproductively active individuals collected in Arkansas in order to provide comparative information with recent studies on squamate urogenital anatomy. Specifically, I focused on the basic anatomy and positioning of posterior ducts in this skink, which included portions of the ductus deferens, the ampulla ductus deferentis, the sexual segment of the kidney, the ureter and collecting ducts, as well as aspects of the urodaeal chamber and urogenital papillae. I found a much reduced ampulla ductus deferentis comprising only 0.7 mm in length in the caudal region of the ductus deferens. The sexual segment of the kidney was well developed, being located in collecting ducts of the kidney proper, in walls of collecting ducts leading away from the kidney as well as within anterior portions of the ureter. The anterior dorsal recess of the urodaeum possessed epithelial crypts within a highly folded epithelium. Finally, a ductal triad (ductus deferens, ureter, and a single collecting duct) terminates at each orifice of the paired urogenital papillae. The distal urogenital anatomy of this scincid lizard revealed anatomical features similar to other species within the genus *Plestiodon*.

Introduction

Classical illustrations by Martin Saint Ange (1854), Brooks (1906), and Volsøe (1944) provided early macroscopic details of distal urogenital morphologies in male squamates (lizards and snakes). Although numerous studies have reported on male reproductive anatomy since these pioneer works (for comprehensive literature summaries, see Fox 1977; Trauth and Sever 2011; Rheubert *et al*. 2015), Gabe and Saint Girons (1965) provided the first detailed histological analysis of distal urogenital microanatomy. More recent investigations into the urogenital anatomy of male squamates, however, have launched a renewed interest in this region revealing several previously undescribed caudal micro-anatomical structures (Gribbins and Rheubert 2011; Trauth and Sever 2011; Rheubert *et al*. 2015). For example, Siegel *et al*. (2011) and Trauth and Sever (2011) recognized the uniqueness of a urogenital structure illustrated by Martin Saint Ange (1854)—the ampulla urogenital papilla (one of two complementary blind pouches)—which represents the terminal repository of products released from the distal urogenital tracts in North American colubrine snakes. Rheubert *et al.* (2015) also found these paired pouches in North American teiid lizards (family Teiidae) to the exclusion of all other lizards and families examined in their study. In addition, Trauth and Sever (2011) and Rheubert *et al*. (2015) were the first studies to document the broad range of variation in microstructure of the urogenital papillae in male squamates using scanning electron microscopy.

In an attempt to clarify nomenclature regarding the proximal testicular ducts (in snakes) for future studies, Sever (2010) synonymized all efferent ducts with those of higher amniotes. Included here were the rete testis, ductuli efferentes, ductus epididymis, and the ductus deferens (for these structures in a lizard, see Sever *et al*. 2013). Squamate distal urogenital ducts (i.e., ductus deferens, ampulla ductus deferentis, collecting ducts, ampulla urogenital papilla, and the ureter), on the other hand and with few exceptions, have received less histological attention compared to the proximal testicular ducts. Trauth and Sever (2011) and Rheubert *et al.* (2015) followed Sever's (2010) naming scheme in addressing urogenital structures in snakes and lizards, respectively.

The family Scincidae is a large, nearly cosmopolitan group of small-to-large lizards containing over 1,500 species (Vitt and Caldwell 2014). Skinks are widespread in Arkansas (Walley 1998; Trauth *et al*. 2004; Powell *et al*. 2016), and there are six species in two genera (*Plestiodon* [= *Eumeces*] and *Scincella*). Trauth *et al*. (1987) focused on female urogenital anatomy of

Plestiodon laticeps and only briefly described male cloacal anatomy. Trauth (1994) reported on the reproductive cycles of two skink species (*Plestiodon anthracinus pluvialis* and *Plestiodon fasciatus*) from Arkansas and provided light photomicrographs of the testicular and epididymal histology. Rheubert *et al.* (2015) described aspects of the male urogenital anatomy in *Plestiodon fasciatus* and *Scincella lateralis* and included brief comments and scanning electron micrographs of the urogenital papillae in *P. a. pluvialis* and *P. laticeps.*

In the present study, I describe the distal urogenital anatomy of male southern coal skinks (*Plestiodon anthracinus pluvialis*) using macrophotography and histology. I also compare these findings with those published on other scincid lizards reported in Rheubert *et al.* (2015).

Materials and Methods

For light microscopy (LM), I dissected distal urogenital tissues from two male southern coal skinks previously deposited in the Arkansas State University Museum of Herpetology (ASUMZ 8950 and 24012). The skinks had been collected in Arkansas by my students or me. I also utilized three additional male specimens (ASUMZ 12727, 16392, and 30659) for macroscopic analysis and macrophotography of urogenital structures. All five specimens had been euthanized with an intra-pleuroperitoneal injection of sodium pentobarbital, and internal organs were then fixed using abdominal injections of 10% formalin. Other skinks utilized (*Scincella lateralis*, ASUMZ 20335; *Plestiodon laticeps*, ASUMZ 31171) are museum specimens preserved in 70% ethanol.

I necropsied distal urogenital tissue segments (approximately, 10 mm in length) and placed these into vials of 70% ethanol. Then, I followed standard histological techniques to prepare tissues for LM following the paraffin embedding procedures outlined in Presnell and Schreibman (1997). In brief, these steps included dehydration using a graded series of ethanol solutions (70 to 100%), clearing to 100% xylene, infiltration overnight in a paraffin oven (56°C), embedding with paraffin into 23 mm square plastic molds (tissue positioned upright in a cranial-to-caudal axis), 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% formalin on a slide warmer), and staining using Pollak trichrome stain for the enhancement of connective tissues and muscle. Cover slips were adhered to microscope slides with Permount© (Fisher Scientific Products).

For slide photomicroscopy, I used a Leica MC 120 HD camera atop a Leica DM 2000 LED compound light microscope. For macrophotography, I used either a Canon T4i digital single lens reflex camera fitted with a macro lens or the aforementioned camera atop a Leica M 80 stereomicroscope.

All descriptions of urogenital structures follow the terminology found in either Trauth and Sever (2011) or Rheubert *et al.* (2015). Microscope slides are currently in my possession.

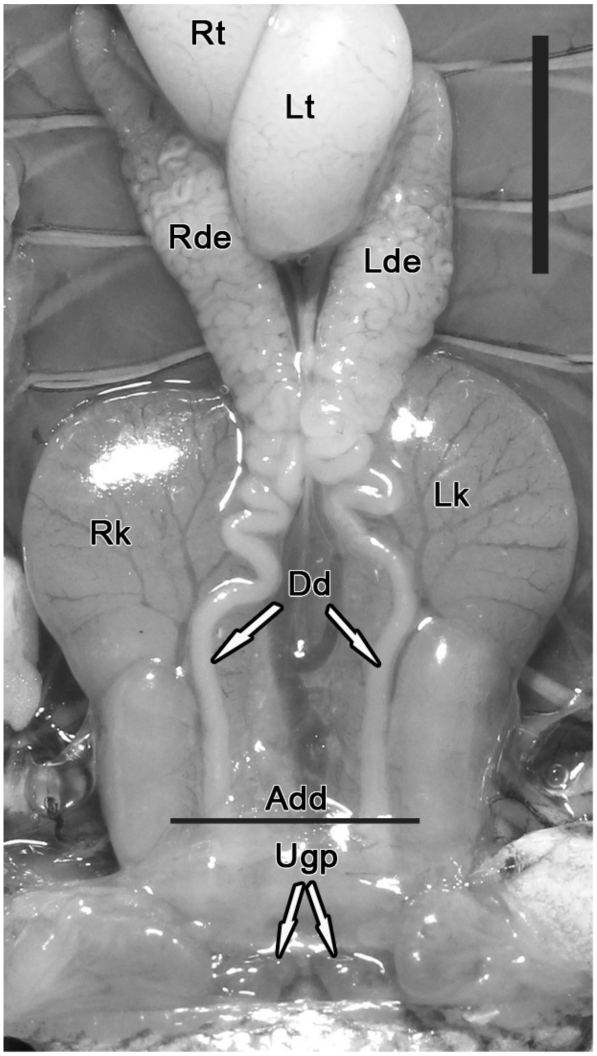

Figure 1. Urogenital system of a recently sacrificed scincid lizard (*Plestiodon fasciatus*, ASUMZ 32717) as adapted with modifications from Fig. 9.1D in Rheubert *et al.* (2015). Scale bar at upper right = 5 mm; Add, ampulla ductus deferentis (line below Add approximates the location of this structure within the ductus deferens); Dd, ductus deferens; Lde, left ductus epididymis; Lt, left testis; Rde, right ductus epididymis; Rt, right testis; Rk, right kidney; Lk, left kidney; Ugp, urogenital papilla.

Results

Gross Morphology

A representative scincid urogenital anatomy (ventral view) of a reproductively active male lizard (*Plestiodon fasciatus*) is shown in Fig. 1 and a brief description is provided here. The paired testes appear cream-to-white in color with the right testis lying more cranial compared to the left. Each testis is flanked laterally by an adrenal gland (best seen in figure as a thin line of tissue between the left testis and left ductus epididymis) and the convoluted efferent tubules of the ductuli efferentes (not identified in Fig. 1), which eventually converge into a common, highly looped, ductus epididymis. The ductus epididymis becomes less looped and straightens along the anterior ventromedial surface of the kidney as it becomes the anterior ductus deferens. Anterior urogenital structures of *Plestiodon anthracinus pluvialis* (starting with the right ductus epididymis) are shown in Fig. 2. The posterior ductus deferens continues as a straight duct on both sides (Figs. 1, 2). Not pictured in Figs. 1 and 2 is the ureter, which lies dorsomedial to the posterior ductus deferens.

Light Microscopy

The distal urogenital anatomy of *Plestiodon anthracinus pluvialis* is shown in Figs. 3 – 5 and is illustrated histologically from approximately 2 mm of tissue (see black box region in Fig. 2). For most of its length, the ductus deferens (Dd) is a circular-to-oblong linear duct and is uniformly lined with either a short or tall pseudostratified columnar epithelium (Fig. 5A); however, as the Dd leads caudally and nears the cloacal region, a short enlargement (approximately 0.7 mm in length) of the duct, the ampulla ductus deferentis (Add), becomes apparent (Figs. 3A; 4A, B; 5B). This expansion is characterized by a slight increase in the diameter of the duct and by a circumferential folding of its epithelial lining. These diagnostic histological and morphological features of the Add soon disappear, and the Dd regains its caudal structural appearance as observed in the more anterior regions of the duct (e.g., Fig. 5A).

Initially, the ureter (Ur) varies little with respect to its anatomical position lying ventral to the kidney and adhering closely with the dorsomedial surface of the Dd (Figs. 3A, B; 4A, B). In addition, its pseudostratified epithelium may appear folded (Fig. 5D) throughout much of its length. Caudally, however, the Ur moves to reside laterally (Fig. 3C) and then ventromedially to the Dd (Fig. 3D; 4D, E). As the Ur reaches the urodaeal

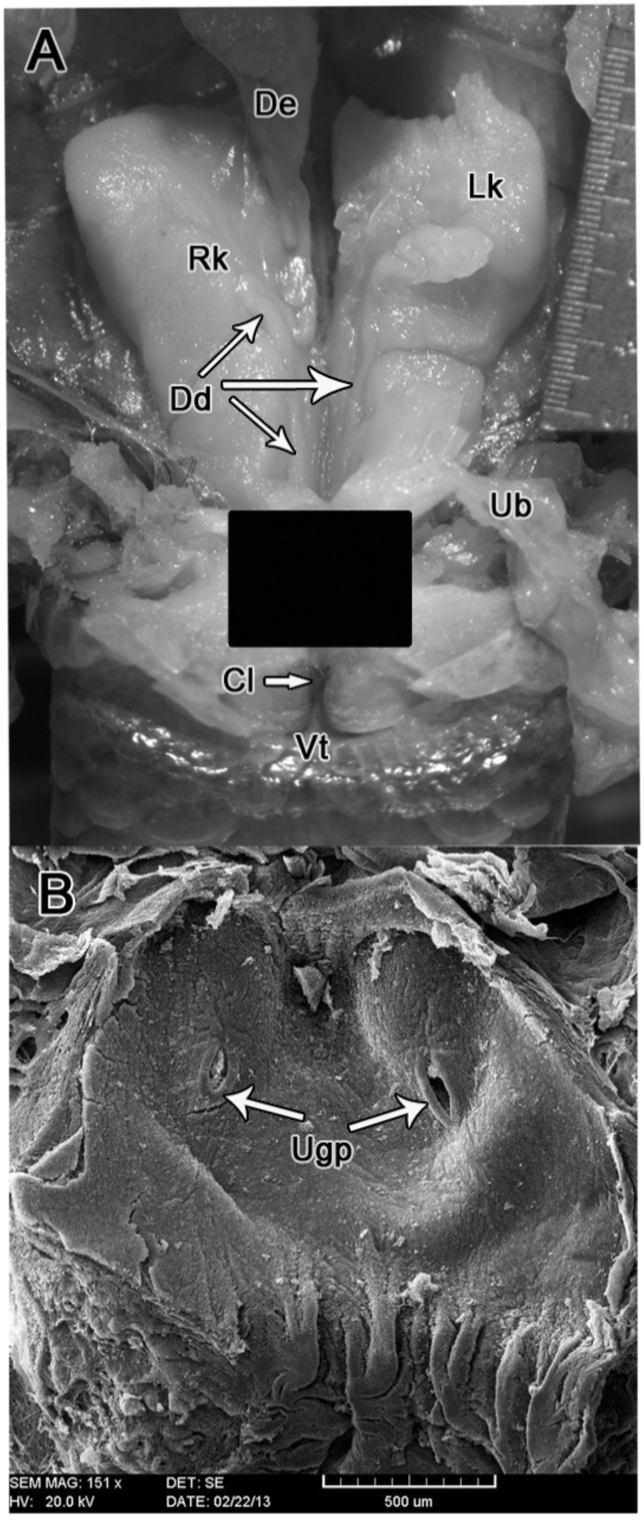

Figure 2. Macroanatomy (A) and scanning electron micrograph (B) of distal urogenital structures in *Plestiodon anthracinus pluvialis* (A, ASUMZ 12727; B, ASUMZ 16392; image adapted from Fig. 9.26F in Rheubert *et al*. 2015). Abbreviations same as in Fig. 1. Scale bar in upper right corner of A in mm; Ub, urinary bladder; Ugp, urogenital papilla; Cl, cloaca. Black box of A incorporates the portion of the distal urogenital anatomy illustrated in Figs. 3, 4.

region of the cloaca (Fig. 4F), it briefly receives sperm from the Dd and immediately redirects all urogenital products found within the distal urogenital ducts into the cloaca through the orifice of a urogenital papilla (Figs. 2B; 3F; 4F).

The sexual segment of the kidney (Ssk) is clearly evident at all levels of the kidney (Figs. 3–5). The Ssk is variable in structure, but is distributed throughout each kidney primarily as part of the collecting ducts of nephrons. Several collecting ducts (Cd) lead away from the kidney and can be seen residing dorsal to the Dd and Ur (e.g., Fig. 3A – C; 4B). They initially appear oblong or flattened (Figs. 3A, B; 4B) but will eventually enlarge and merge into a single, spherical duct lying dorsal to the Dd (Fig. 3C). This duct-like channel can be seen adjacent to the Dd along its dorsal and lateral surfaces (Fig. 3D, E). All three ducts (Dd, Cd, and Ur) are encased by a common sheath of connective tissue (Figs. 3C–E; 4E; 5E, F) and can be termed the urogenital ductal triad.

At approximately the level of the initial clustering of Cd, Dd, and Ur, two additional cavities appear (Figs. 3C; 4E). These cavities are the anterior dorsal recesses (Adr), which are anterior extensions of the urodaeum. These cavities are initially paired (Fig. 3C), but will eventually unite medially (Fig. 4E) and merge with the intestine.

Discussion

Ductus deferens

Rheubert *et al.* (2015) examined the distal urogenital anatomy in 7 species of lizards; 2 of these were skinks (*Plestiodon fasciatus* and *Scincella lateralis*). The pseudostratified epithelium of the Dd of *Plestiodon anthracinus pluvialis* was similar to that found in both species. Unlike *Scincella lateralis*, however, the Ur and Dd in *P. a. pluvialis* and *P. fasciatus* unite near the orifice of the urogenital papilla (see ***Urogenital papillae***).

Ampulla ductus deferentis

Only recently have researchers identified an Add within the Dd of squamates. Sever (2004) and Akbarsha *et al.* (2005) were the first studies to describe the Add in snakes and lizards, respectively. Trauth and Sever (2011) provided diagnostic morphological features of the micro-anatomies of the Add in seven colubrine snakes and noted the variable complexity of the epithelial folding of the ampulla. One species of watersnake (*Nerodia cyclopion*) exhibited a diverse branching pattern of the ductal epithelium. Rheubert *et al.* (2015) reported on the presence of an Add in additional lizards, including *Scincella lateralis* and four phrynosomatids (*Cophosaurus texanus*, *Sceloporus consobrinus*, *Holbrookia propinqua*, and *Phrynosoma cornutum*). The Add of *Plestiodon anthracinus pluvialis* is similar to that exhibited by these four phrynosomatids (presence of varying degrees of folding micro-anatomies of the epithelium). Thus far, however, the most complex Add epithelial morphology for any lizard was found by Rheubert *et al.* (2015) in *S. lateralis* (Fig. 4C).

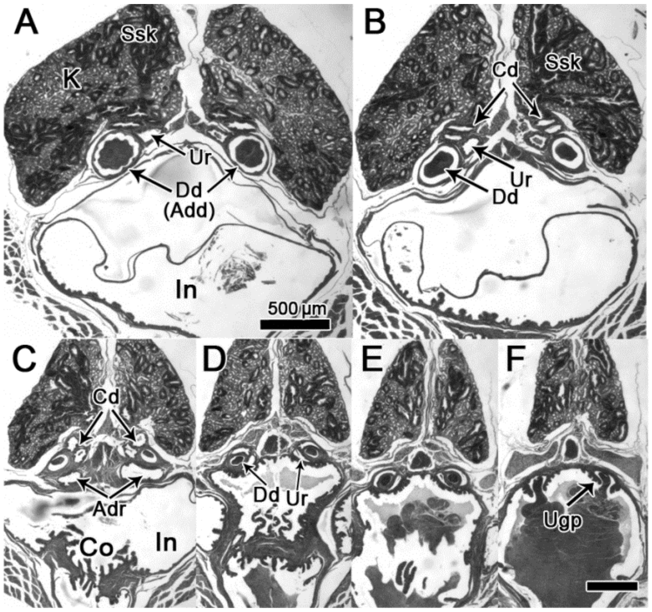

Figure 3. Light micrographs of the distal urogenital anatomy in *Plestiodon anthracinus pluvialis* (ASUMZ 8950) as revealed by a cranial-to-caudal series of histological sections (A–F). A. Section through the urogenital complex at the level of the ampulla ductus deferentis (Add). B. Section posterior to A showing sexual segment of the kidney (Ssk), collecting ducts (Cd), the ureter (Ur), and the ductus deferens (Dd). C. Section showing all structures in B, but also including the anterior dorsal recess of the urodaeum (Adr), the intestine (In), and the beginning of the coprodaeum (Co). D. Section just anterior to the cloacal chamber showing the relative size and position of the Cd, Dd, and Ur (see also Fig. 4E). E. Section just anterior to the urogenital papillae (Ugp). F. Section through the orifices of the paired Ugp showing release of sperm and other urogenital products; scale bar = 500 µm. Co, coprodaeum. Other abbreviations as in previous figures.

Sexual segment of the kidney

The morphology of the Ssk in lizards has been well illustrated (Prasad and Reddy 1972) and well documented in many snake and lizard families (Rheubert *et al*. 2015). Rheubert *et al.* (2015) also provided a table and a phylogenetic character map summarizing the morphological region of the Ssk within the nephron in squamates. In Scincidae, the Ssk is distributed within the Cd and in the Ur. The Ssk is characterized by tall columnar epithelial cells with basal nuclei (Fig. 5A, C). Large secretory granules are released as a merocrine secretion into the lumina of these ducts. In *Plestiodon anthracinus pluvialis*, the Ssk is similar to other skinks and to squamates, in general.

Collecting ducts

The Cd are conspicuous ducts that reside near or adjacent to the Ur and Dd. Together, these three structures become a ductal triad as they reach their termination point at the orifice of the urogenital papilla in all skinks studied thus far. The Cd in *Plestiodon fasciatus*, *Plestiodon anthracinus pluvialis* (Fig. 5A), and *Scincella lateralis* may exhibit epithelial folding and cytoplasmic features characteristic of the Ssk. In *P. a. pluvialis*, however, the folding diminishes as the duct nears the cloaca (Fig. 5E, F).

Anterior dorsal recess of the urodaeum

Within squamates, the Adr was first described in snakes as anterior projecting cavities of the urodaeum (Trauth and Sever, 2011); these cavities were then described in lizards (Rheubert *et al.* 2015). In *Plestiodon fasciatus*, the epithelium of the Adr is highly convoluted and contains numerous primary and secondary crypts. An electron microsocopic analysis of these crypts revealed an orderly arrangement of sperm clustered within these spaces (Rheubert *et al.*, 2015). Although *Plestiodon anthracinus pluvialis* also possesses crypts (Fig. 5F), no sperm were detected in them during the present study. No Adr was observed in *Scincella lateralis*.

Ureter

The Ur lies medially to the Dd throughout most of its length in *Plestiodon anthracinus pluvialis*. Also, a highly folded epithelium was found within the anteriormost regions of the duct (Fig. 5D); this was attributed to Ssk development. In more caudal regions, the Ur loses most of its Ssk features (Fig. 5E, F), becomes flattened, and moves to reside in its terminal position ventromedial to the Dd (Fig. 4E). In contrast, *Plestiodon fasciatus* exhibited a relatively large Ur (called a secondary Cd by Rheubert *et al.* 2015), and it retained Ssk morphological features. Unlike both of these skinks, the Ur in *Scincella lateralis* opened separately into the cloaca.

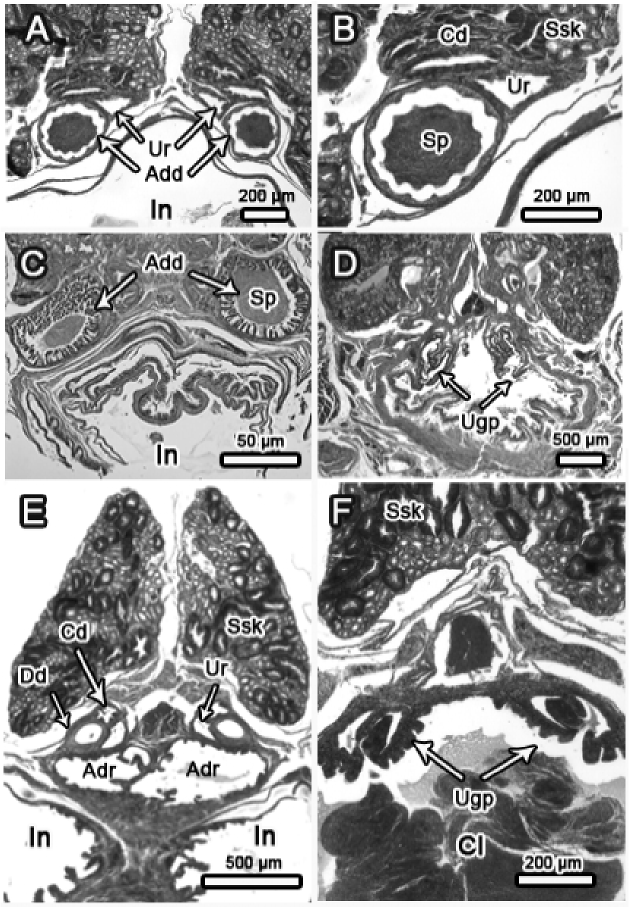

Figure 4. Light micrographs of urogenital structures in *Plestiodon anthracinus pluvialis* (A, B, E, and F; ASUMZ 8950), *Scincella lateralis* (C, ASUMZ 20335), and *Plestiodon laticeps* (D, ASUMZ 31171). Sp, spermatozoa; Cl, cloaca. Other abbreviations as in previous figures.

Urogenital papillae

The termination point for release of all ductal components in male squamates is the urogenital papilla (Ugp), which may be either a single medial structure or paired bilateral structures along the dorsal wall of the urodaeum of the cloaca (Trauth and Sever 2011; Rheubert *et al*. 2015). A detailed description and illustration of a generalized Ugp morphology (and its surrounding tissues) is found in Trauth and Sever (2011). In addition, the highly variable external micro-anatomy of the Ugp is best viewed using scanning electron microscopy as depicted in Trauth and Sever (2011) for snakes and Rheubert *et al*. (2015) for lizards. In all skinks studied thus far, there are two Ugp. In *Plestiodon anthracinus pluvialis* the Ugp are much reduced in size and complexity, and they are little more than small ridges or lips surrounding an orifice (Figs. 2B; 3D – F; 5E, F). In comparison, other skinks, like

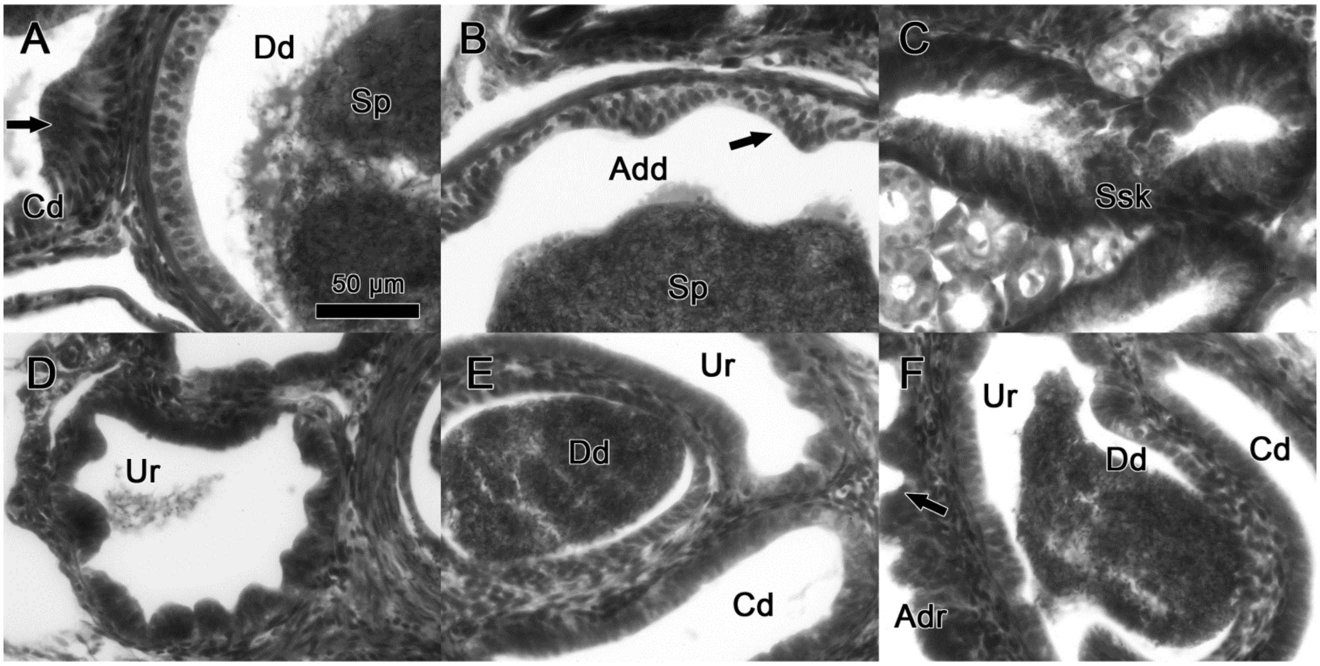

Figure 5. Light micrographs of epithelia of urogenital structures in *Plestiodon anthracinus pluvialis* (ASUMZ 8950). A. Section through the ductus deferens (Dd) and an adjacent collecting duct (Cd) showing epithelial pseudostratification; the Cd possesses tall irregular cells containing secretory granules (arrow) characteristic of the sexual segment of the kidney (Ssk). B. Section through the Dd posterior to A in the region of the ampulla ductus deferentis (Add). C. Section of kidney tubules showing a region of the Ssk. D. Section through a variable epithelium of the ureter (Ur). E. Section encompassing the Dd, Cd, and Ur nearing the urodaeal region (see Fig. 4E) revealing low heights of the epithelia. F. Section posterior to E through the Dd, Cd, and Ur. Scale bar in A the same for B–F; Sp, spermatozoa.

Plestiodon fasciatus (Fig. 1), *P. laticeps* (Fig. 5D), and *Scincella lateralis* (Rheubert *et al*. 2015), have Ugp that are more bulbous (as seen in reproductively active individuals), and they occupy more physical space within the cloaca.

The internal anatomy of the Ugp in *Plestiodon anthracinus pluvialis* reveals a ductal triad that varies little from the morphology found in *Plestiodon fasciatus*. In these two skinks, the Dd dumps sperm directly into the Ur (Fig. 5F), which then opens immediately into the Cd (Fig. 4F). All ductal materials are then released into the cloaca through a single common chamber (Fig 3F).

Acknowledgments

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Generation of Generalized-Gauss Laser Beams via a Spatial Light Modulator

S. Dix and J.P.C. Young*

Department of Physical Science, Arkansas Tech University, Russellville, AR 72802

*Correspondence: jyoung35@atu.edu

Running title: Generalized-Gauss Laser Beams via a Spatial Light Modulator

Abstract

Generalized-Gauss laser beams can be described as a continuous transition between the well-known Hermite-Gauss (HG) and Laguerre-Gauss (LG) laser beams. A spatial light modulator (SLM) was made by removing the liquid crystal display (LCD) from an overhead projector. The homemade SLM, encoded with a computer-generated hologram, was then used to convert a fundamental Gaussian beam from a small frame Helium-Neon (HeNe) laser into several different orders of Generalized-Gauss (GG) beams. The experimentally generated GG beam profiles matched the theoretically expected profiles.

Introduction

Controlling the intensity profiles and polarization of laser beams are of interest in applications such as material processing, optical communication, optical computing, and optical tweezing (Dickey *et al.* 2005). Laser beams are light waves and therefore can be described in terms of solutions to the Paraxial Wave Equation (PWE). In Cartesian coordinates, the solution to the wave equation leads to the HG modes (Siegman 1986):

$$\psi_{nm}^{HG}(x,y,z) = A_{nm}^{HG}\frac{w_o}{w(z)}e^{-(x^2+y^2)\left(\frac{1}{w^2(z)}+\frac{ik}{2R(z)}\right)}\,e^{-ikz} \times H_n\left(\frac{\sqrt{2}x}{w(z)}\right)H_m\left(\frac{\sqrt{2}y}{w(z)}\right)e^{-i(n+m+1)\theta(z)}, \quad (1)$$

where A_{nm} is a normalization constant, H_m is a Hermite polynomial of order m, $w(z)$ is the radius of the beam, w_o is the radius of the beam at its focus, and $k = 2\pi/\lambda$ is the wave number for wavelength λ. $R(z)$ is the radius of curvature of the wavefront and given by

$$R(z) = z + \left(\frac{z_R}{z}\right)^2, \quad (2)$$

where

$$z_R = \frac{\pi w_o}{\lambda}, \quad (3)$$

is the Rayleigh range, and

$$\theta(z) = \arctan\left(\frac{z}{z_R}\right), \quad (4)$$

is the Gouy phase. The indices n and m determine the number of lobes in the x and y directions, respectively. Figure 1a shows the beam profile of an HG_{20} beam, note n+1 nodes in the x-direction (horizontal) and m+1 nodes in the y-direction (vertical). In cylindrical coordinates, the solution to the wave equation leads to LG laser modes (Siegman 1986),

$$\psi_{nm}^{LG}(r,\phi,z) = A_{nm}^{LG}\frac{w_o}{w(z)}e^{-r^2\left(\frac{1}{w^2(z)}+\frac{ik}{2R(z)}\right)}\,e^{-ikz} \times L_{\min(\mathrm{n,m})}^{|n-m|}\left(\frac{2r^2}{w^2(z)}\right)(-1)^{\min(n.m)}\,e^{-i(n-m)\phi} \times \left(\frac{r\sqrt{2}}{w}\right)^{|n-m|}e^{-i(n+m+1)\theta(z)}, \quad (5)$$

where L_μ^ν is the generalized Laguerre polynomial. The smaller of n and m plus one ($\min(n,m)+1$) gives the number of rings; this is demonstrated in Figure 1c which shows the intensity distribution of an LG_{20} beam.

HG and LG modes are complete and orthogonal solutions to the wave equation; therefore, they can be written in terms of each other (Beijersbergen *et al.* 1993),

$$\psi_{nm}^{LG}(x,y,z) = \sum_{k=0}^{N=n+m} i^k b_k^{nm}\psi_{N-k,k}^{HG}\,, \quad (6)$$

where the coefficients

$$b_k^{nm} = \sqrt{\left(\frac{(n+m-k)!\,k!}{2^{n+m}\,n!\,m!}\right)}\frac{1}{k!}\frac{d^k}{dt^k}\left[(1-t)^n(1+t)^m\right]_{t=0}. \quad (7)$$

Note that the i^k term in the sum of Eq. (6) provides a $\pi/2$ phase difference between each term in the series.

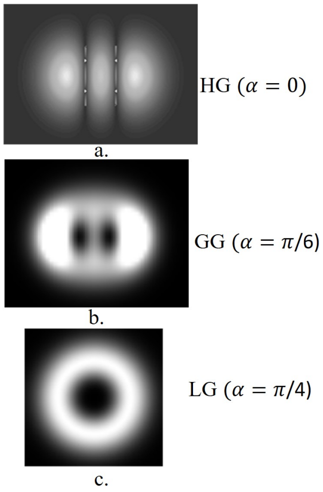

Figure 1: Intensity profiles for a a) Hermite-Gauss (HG), b) Generalized-Gauss (GG), and c) Laguerre-Gauss (LG) beam of order (n,m)=(2,0). A GG beam becomes an HG beam for $\alpha = 0$, and it becomes an LG beam for $\alpha = \pi/4$.

Additionally, an HG beam rotated $\pi/4$ (45°) to the axis can be written as the same sum as the LG beams but the successive terms are in phase (no i^k term):

$$H_{nm}\left(\frac{x+y}{\sqrt{2}},\frac{x-y}{\sqrt{2}},z\right)=\sum_{k=0}^{N=n+m} b_k^{nm}\psi_{N-k,k}^{HG}. \qquad (8)$$

Figure 2 pictorially demonstrates Eqs. (6) and (8) for the mode composition of HG_{10} rotated 45° (Fig 2a) and LG_{10} (Fig 2b). Note that LG_{10} and HG_{10} rotated 45° are composed of the same HG modes, but LG_{10} has a phase difference of $\pi/2$ between the terms.

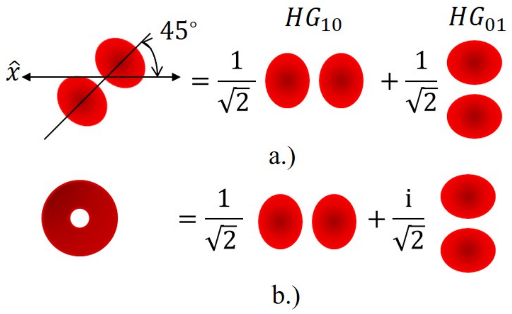

Figure 2: Mode composition of a) HG_{10} rotated 45° and b) LG_{10}.

Laguerre-Gauss beams can be generated from HG beams by exploiting this phase difference. An astigmatic mode converter (AMC) can introduce the i^k phase difference between each term by introducing an astigmatism that will force the Gouy phase to differ by $\pi/2$ in each sequential term (Beijersbergen *et al.* 1993). Figure 3 shows an AMC that consists of two cylindrical lenses of focal length f, separated by a distance $d = \sqrt{2}\, f$ and placed so that the beam waist location is at the midpoint between the two cylindrical lenses. An HG beam, incident on an AMC, will be converted to an LG beam of the same order provided the HG mode is rotated 45° with respect to the axis of the cylindrical lens.

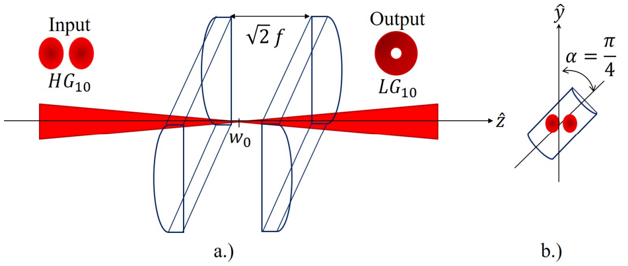

Figure 3: a) An AMC consisting of two cylindrical lenses of focal length f and separated by a distance $2f$. b) The axis of the input (HG) mode must be 45° with respect to the axis of the cylindrical lens for conversion from HG to an LG mode.

A more general solution to the PWE is developed if the angle between the AMC and the input HG mode is at an arbitrary angle, α. This leads to a third solution to the PWE that produces GG beams (sometimes referred to as Hermite-Laguerre-Gauss beams). This third family of solutions can be written in terms of HG beams and the angle α (Abramochkin and Volostnikov 2004):

$$\begin{aligned}\psi_{nm}^{GG}(x,y,\alpha) = \sum i^k \cos^{n-k}\alpha \sin^{m-k}\alpha \\ \times P_k^{n-k,m-k}(-\cos 2\alpha) \\ \times \psi_{n+m-k,k}^{HG}(x,y,z)\end{aligned} \qquad (9)$$

where

$$\begin{aligned}P_k^{ab}(\mu) = \frac{(-1)^k}{2^k k!}(1-\mu)^{-a}(1+\mu)^{-b} \\ \times \frac{d^k}{d\mu^k}[(1-\mu)^{k+a}(1+\mu)^k,\end{aligned} \qquad (10)$$

are Jacobi Polynomials. It is important to note here that that $0 \le \alpha \le \pi/4$ and when α equals zero the GG beam becomes an HG beam and when α is $\pi/4$ the beam becomes an LG beam. The GG beams can be thought of as a continuous transition between HG and LG beams.

Figure 1 illustrates this transition for $HG \rightarrow GG \rightarrow LG$ beams of order $n = 2, m = 0$.

The solution to the wave equation in elliptical coordinates leads to Ince-Gauss (IG) beams (Bandres and Gutiérrez-Vega 2004). IG beams are also a continuous transition between HG and LG beams in that when the ellipticity of the beam is zero (the elliptic coordinates become circular coordinates) the beam is an LG mode and as the ellipticity of the beam goes to infinity (the elliptic coordinates become Cartesian coordinates) the beam is an HG mode. There is no reported direct relationship between IG and GG beams.

Astigmatic mode converters produce high mode purity beams but are tedious to align and are sensitive to slight disturbances. Additionally, higher order HG beams are typically created by inserting a fiber inside the laser cavity (Beijersbergen *et al.* 1993). Therefore, the mode order is limited by the diameter of the cavity, and it is not often practical to have access to the inside of a laser cavity. In this work, spatial light modulation was used as an alternative method to generate Generalized-Gauss laser beams. Beam shaping using a spatial light modulator only requires that a fundamental (lowest order) Gaussian beam be produced by the laser cavity to externally generate any order GG beam with arbitrary α.

Beam shaping and experimental setup

A transmission spatial light modulator (SLM) can alter the phase, amplitude, and polarization of a wavefront by allowing light to either pass through each pixel or not depending on the input light's polarization (Dickey *et al.* 2005). In this work, a transmission SLM was made by removing the liquid crystal displays (LCDs) and light bulb from a surplus overhead projector (Epson Powerlite 83C), after overriding the missing hardware warnings, one LCD was reconnected to the projector using a flexible printed circuit (FPC) extension board and cable (Huang *et al.* 2012). Figure 4 is a photograph of the homemade SLM and the FPC extension board used to increase the cable length so the LCD could be used outside the projector casing. The SLM was addressed by the computer by treating the SLM as an additional monitor, replicating the computer screen on the SLM.

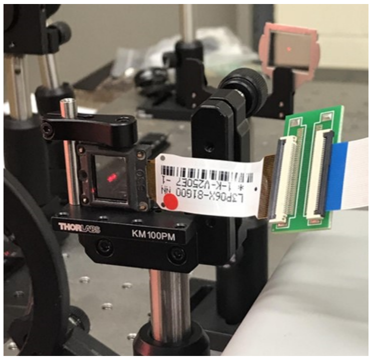

Figure 4: Photograph of the spatial light modulator and the flexible printed circuit extension board and cable.

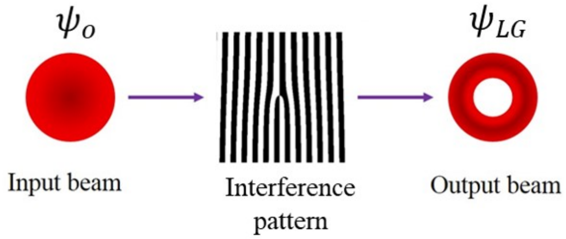

Figure 5: The interference pattern resulting from a fundamental Gaussian (ψ_o) and a Laguerre-Gauss (ψ_{LG}) beam interfering with each other.

Acting as a hologram, the SLM can, in theory, convert the intensity distribution of a beam into a beam with any desired profile provided the intensity distribution of the input beam is known. The hologram encoded on the SLM must be the interference pattern created when the input beam and desired output beam interfere. As an example, consider the fundamental Gaussian input beam and a desired donut shaped LG output beam shown, as shown in Figure 5. The pattern shown at the center of the figure is a result of the fundamental Gaussian beam interfering with the LG beam. Mathematically, the inference pattern is the intensity of the superposition of the two waves,

$$I = |\psi_o + \psi_{LG}|^2, \tag{11}$$

where ψ_o is the wave function of the fundamental Gaussian beam and ψ_{LG} is the wave function of the LG beam. When the interference pattern shown in Figure 5 is encoded on the SLM, and a fundamental Gaussian beam is passed through, the desired LG beam will be produced as an output. The program written to produce the holograms required to generate the GG beams was written in the open source science programing language, GNU Octave and based on code published by Rosales-Guzmán and Forbes (Rosales-Guzmán *et al.* 2017)

The experimental setup used to generate GG beams is shown in Figure 6. A HeNe laser operating in a fundamental Gaussian mode was first sent through a linear polarizer with its transmission axis aligned with the SLM, then the beam was sent through the SLM, a second polarizer, and then to the charged couple device (CCD) camera. The purpose of the second polarizer was to decrease the intensity of the GG beam so the CCD would not be saturated. The SLM was addressed by one laptop computer with a VGA output and the signal from the CCD was read via a USB cable by a second laptop computer. The use of two computers was necessary for this arrangement because it was not possible to have real-time observation of the beam profile acquired by the CCD on the computer screen and have the screen (and SLM) display only the hologram at the same time.

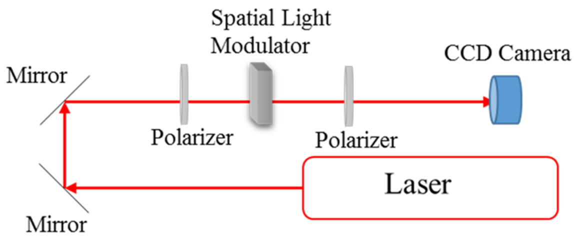

Figure 6: Diagram of the experimental setup consisting of a HeNe, laser, two polarizers, a CCD camera, and two laptop computers.

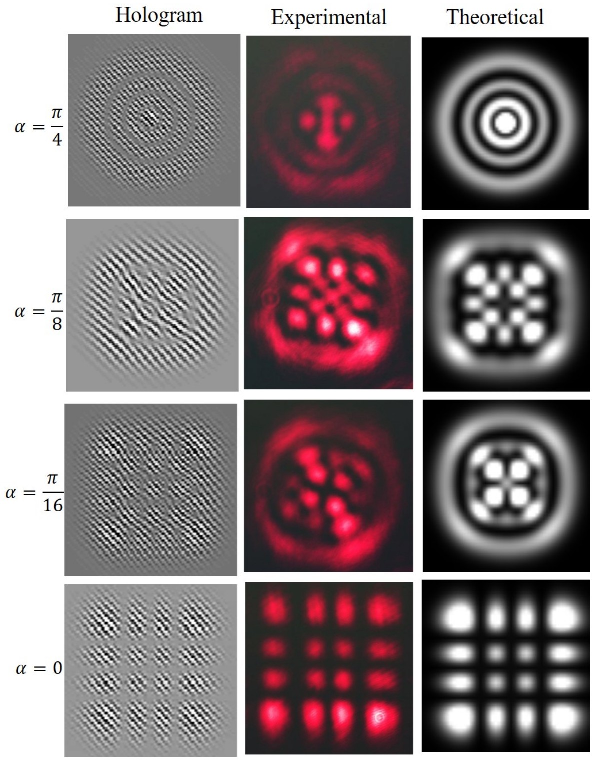

Figure 7: The holograms (first column) used to produce Generalized-Gauss beams of order GG_{33}. The second and third columns are the experimental and theoretically expected beam profiles for the GG_{33} mode, respectively for $\alpha = \pi/4,\ \pi/8, \pi/16,$ and 0.

Results

The beam profiles of the GG_{33} beam acquired by CCD are shown in Figure 7. The first column of Figure 7 are the holograms that were encoded on the SLM to generate the corresponding beams. While profiles for one mode order for a few values of α are shown here, we were able to generate any order GG beam, with reasonable quality, up to $(n, m) = (5,5)$ with the setup described in the previous section. The mode order is only limited by the resolution of the SLM. Using a lens to change the beam size will effectively increase the usable number of pixels on the SLM, and higher order modes could be generated. It is important to note that, as expected, as α goes to $\pi/4$ the GG beam transitions to an LG beam with cylindrical symmetry and as α goes to zero the GG beam becomes an HG beam with Cartesian symmetry. The experimental and theoretical beam profiles shown in Figure 7 are in reasonable agreement. Some difference between the experimental and theoretical profiles are observed such as a four lobed pattern at the center of the experimental profile for $\alpha = \pi/4$ compared to the ring in the theoretical profile. The primary reason for this and other observed differences is that the SLM is programmed assuming that that input beam was a pure fundamental Gaussian mode. The laser used does not have the ideal perfectly Gaussian intensity profile. Improving the quality of the input beam or compensating for the slightly non-Gaussian profile when programming the SLM would improve the correlation between the theoretical and experimental beam profiles.

Conclusion

We have shown that Generalized-Gauss laser beams of several orders can be generated using a computer-generated holograms encoded on a homemade SLM. The experimentally generated beams match well with the theoretically expected profiles.

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Vertebrate Natural History Notes from Arkansas, 2018

R. Tumlison[1*], D.B. Sasse[2], H.W. Robison[3], M.B. Connior[4], C.T. McAllister[5], K. Jobe[6] and M. Anderson[7]

[1]*Department of Biology, Henderson State University, Arkadelphia, AR 71999*
[2]*Arkansas Game and Fish Commission, 213A Highway 89 South, Mayflower, AR 72106*
[3]*9717 Wild Mountain Drive, Sherwood, AR 72120*
[4]*Life Sciences, Northwest Arkansas Community College, One College Drive, Bentonville, AR 72712*
[5]*Division of Science and Mathematics, Eastern Oklahoma State College, Idabel, OK 74745*
[6]*24615 Ross Hollow Rd, Roland, AR 72135*
[7]*Forest Service, Ozark-St. Francis National Forests, 605 West Main, Russellville, AR 72801*

*Correspondence: tumlison@hsu.edu

Running Title: Vertebrate Natural History Notes, 2018

Abstract

Because meaningful observations of natural history are not always part of larger studies, important pieces of information often are unreported. Small details, however, can fill gaps in understanding and lead to interesting questions about ecological relationships or environmental change. We have compiled recent important observations of distribution, deformities, and foods of various vertebrates, observations of winter activity of a Groundhog (*Marmota monax*) and winter torpor of a Hoary Bat (*Lasiurus cinereus*), and also report a very unusual case of bilateral gynandromorphism in a Northern Cardinal (*Cardinalis cardinalis*). These unique observations continue to add to the growth of knowledge of the biology of vertebrates in Arkansas.

Introduction

Human alteration of environments and introduction of non-native species constantly alters relationships and life history parameters of species studied by vertebrate field biologists. Distribution and natural history of many species within Arkansas is becoming better documented, but much remains to be discovered and reported. We continue a series of papers to update the state of knowledge of the natural history of Arkansas's vertebrates (see Tumlison *et al*. 2016 and references therein, Tumlison *et al*. 2017). Herein, we include previously unreported records of distribution, food habits, disease, and observations of behavior in vertebrates from Arkansas. Of particular interest, a genetically peculiar bird is documented and described.

Methods

Fishes were collected during June 2001 and March 2003, and again between April 2017 and February 2018 with 3.1 × 1.4 m, 3.1 × 1.8 m, and 6.1 × 1.8 m seines (all 3.175 mm mesh) or with gill and hoop nets. All fish specimens documented herein are housed either in the Southern Arkansas University Vertebrate Collection (SAU) in Magnolia, Arkansas or in the Henderson State University Collection (HSU) in Arkadelphia, Arkansas. Unvouchered fishes were identified by HWR. New records of bats sent to the Arkansas Department of Health to be tested for rabies were identified by D. Saugey (no physical vouchers remain). Other voucher specimens or photo-vouchers are deposited in the vertebrate collections at Henderson State University (HSU) unless otherwise noted.

Results and Discussion

CLASS ACTINOPTERYGII

Lepisosteidae - Gars

***Atractosteus spathula* (Lacépède) – Alligator Gar**. Robison and Buchanan (1988) listed only one record of *A. spathula* from the Red River (Lafayette Co.) in southwestern Arkansas. On 1 July 2017, HWR found a decomposing specimen (ca. 1.2 m [4 ft.] long) at the boat launch on the Little River just downstream of Millwood Dam on Millwood Lake, Little River Co. (Sec. 26, T12S, R28W). This specimen represents the first published record of the Alligator Gar from the Little River, and second record of this gar from the Red River drainage of southwestern Arkansas.

***Lepisosteus osseus* Winchell – Longnose Gar**. Robison and Buchanan (1988) reported only 2 records of *L. osseus* from the mainstream Red River in

Arkansas. On 9 February 2018, Jim Cunningham, a professional fisherman, caught a large (1,129 mm TL) specimen of this gar in a 91.4 m (300 ft.) gill net placed 0.4 km (0.25 mi.) downstream of the confluence of the Little and Red rivers (33.6129767°N, 93.8217663°W) in Little River Co. This is the third record of this gar from the mainstream Red River in Arkansas.

Hiodontidae – Mooneyes and Goldeyes

***Hiodon alosoides* (Rafinesque) – Goldeye.** On 18 March 2017, R. Morphew collected a Goldeye from the White River in Prairie Co., 6.5 km (4 mi.) downstream of DeVall's Bluff Bridge, 34.77415°N, 91.40902°W (HSU 3621). This is a new county record (Fig. 1) and extends the documented range about 50 km (32 mi.) south in the White River (Robison and Buchanan 1988).

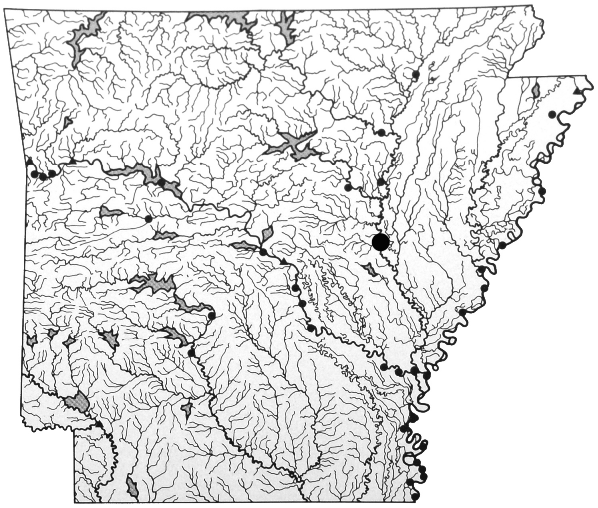

Figure 1. Arkansas distribution of *Hiodon alosoides*. Large dot in the White River represents new record for Prairie Co. (modified from Robison and Buchanan 1988).

Cyprinidae – Minnows and Carps

***Luxilus chrysocephalus* Rafinesque – Striped Shiner.** We found an anomalous 89 mm TL female *L. c. isolepis* in a sample of 18 specimens that was collected by CTM on 21 December 2017 from Big Fork Creek, Polk County (34.468144°N, 93.947978°W). The specimen had a deviation of the spine on the coronal or frontal plane (scoliosis; Fig. 2). Fish affected by this deformity usually do not swim efficiently, are less capable of acquiring food, are at a greater risk of predation, as well as being more susceptible to physiological imbalances (Silverstone and Hammel 2002). This is the first time, to our knowledge, that this deformity has been documented in a Striped Shiner.

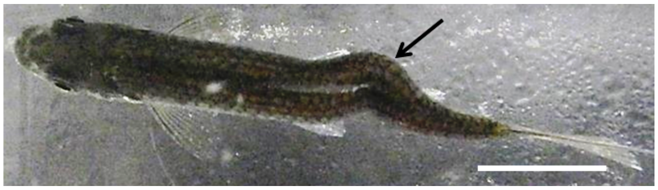

Figure 2. Striped Shiner (*Luxilus chrysocephalus isolepis*) with skeletal deformity showing deviation of caudal spine (scoliosis, arrow). Scale bar = 20 mm.

Catostomidae – Suckers

***Cycleptus elongatus* (Lesueur) – Blue Sucker**. Blue Suckers are difficult to capture, resulting in few voucher specimens from Arkansas. Robison and Buchanan (1988) reported a single Blue Sucker record from the Red River in southwestern Arkansas. We report the collection of 6 specimens of *C. elongatus* captured between 8 and 10 February 2018, representing the second record from the Red River in southwestern Arkansas. All 6 individuals of *C. elongatus* (2 adult females [590 and 660 mm TL] and 4 adult males [480-510 mm TL]) were taken in 6 hoop nets set along the bank of the Red River in Little River Co., about 2.4 km (1.5 mi.) upstream of the confluence of Little and Red rivers, between coordinates of 33.6047260°N 93.8418315°W and 33.6069381°N 93.8446241°W.

Blue Suckers feed primarily on trichopteran larvae and pupae, hellgrammites, fingernail clams and filamentous algae (Rupprecht and Jahn 1980, Moss *et al*. 1983). Guts of our 6 specimens contained primarily chironomid larvae, but also one water mite was recovered.

***Ictiobus bubalus* Rafinesque – Smallmouth Buffalo**. Although *I. bubalus* has been collected previously in the Red River in southwestern Arkansas (Robison and Buchanan 1988), little is known about its abundance in the Red River system. Jim Cunningham collected 27 individuals of this catostomid in just 30 min. using hoop nets in Little River Co. on 9 February 2018. Two Bigmouth Buffalo (*I. cyprinellus*) were taken in the same net. The locality was the same as for the *C. elongatus* already mentioned.

Aphredoderidae – Pirate Perches

***Aphredoderus sayanus* Gilliams – Pirate Perch**. Pirate Perch are rarely encountered in the Arkansas

River Valley upstream of Morrilton (Robison and Buchanan 1988). We report a single specimen of *A. sayanus* collected from a tributary to Baker's Creek, approximately 3.2 km (2 mi.) East of AR St. Hwy. 7 (Sec. 22, T8N, R20W) by C. Gagen and Limnology class on 26 March 2003. This collection marks the first county record of *A. sayanus* from Pope Co., and its first published occurrence upstream of Morrilton.

Fundulidae – Topminnows

***Fundulus chrysotus* (Gunther) – Golden Topminnow**. Robison and Buchanan (1988) did not report *F. chrysotus* from the L'Anguille River drainage in Arkansas. On 21 April 2017, we (HWR, Dave Neely, CTM, and Uland Thomas) collected 20 specimens of *F. chrysotus* from 3 localities in the L'Anguille River Drainage of St. Francis Co., as follows: (1) roadside ditch in Horton, northwest of Forrest City at jct. of AR St. Hwy 261 and Saint Francis Co. (SFC) road 255 (Sec. 10, T5N, R2E), 21 April 2017 (8 specimens). The tannin stained water was heavily vegetated with Floating Primrose-Willow (*Ludwigia peploides*), duckweed (mixed species), and Lizardtail (*Saururus cernuus*); (2) unnamed tributary of L'Anguille River at SFC road 255 (Sec. 20, T5N, R2E), 21 April 2017 (3 specimens); (3) backwater swamp of Cypress Creek at the edge of SFC road 255, ca. 4 km (2.5 mi.) North of the jct. of SFC road 255 and I-40 at Palestine, (Sec. 29, T5N, R2E), 22 April 2017 (9 specimens). These specimens are the first record of *F. chrysotus* in the L'Anguille River drainage (Fig. 3).

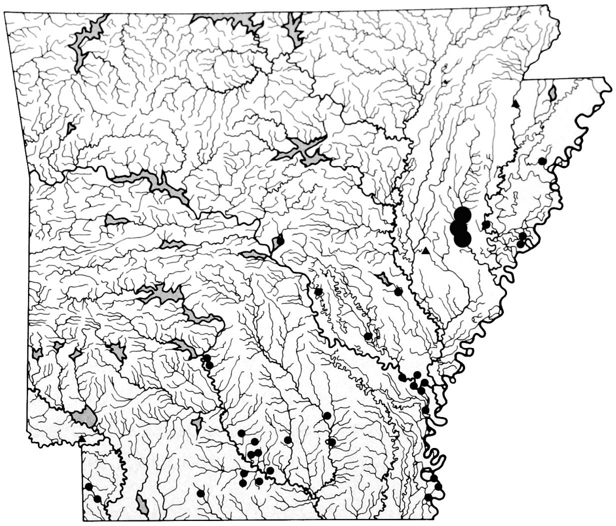

Figure 3. Arkansas distribution of *Fundulus chrysotus*. Large dots represent new records for the L'Anguille River drainage (modified from Robison and Buchanan 1988).

Centrarchidae - Sunfishes

***Morone mississippiensis* Jordan and Eigenmann – Yellow Bass**. This bass is a small moronid species with scattered populations in Arkansas (Robison and Buchanan 1988). On 13 June 2001, 2 juvenile specimens collected by Arkansas Tech University biologists (no county recorded), mark the first published record of this bass from Lake Dardanelle and from this far west in the Arkansas River Valley. Records extending the known range up the Ouachita River include specimens from Lake DeGray, Hot Spring Co. (HSU 1980); Caddo River, Clark Co. (HSU 3360); and Lake Ouachita, Garland Co. (HSU 3427; Fig. 4).

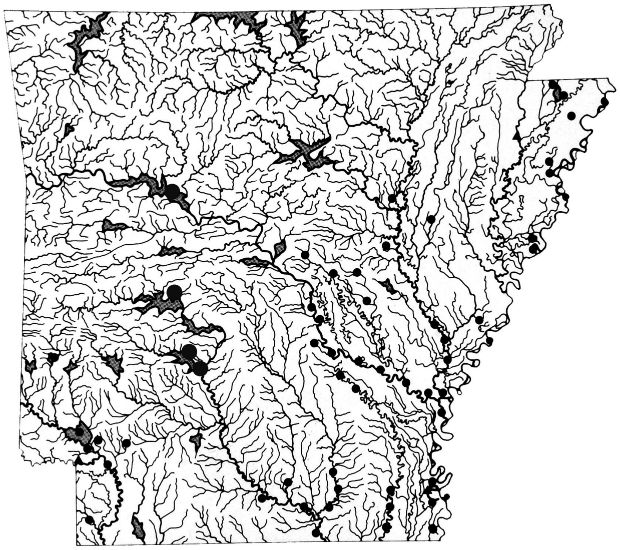

Figure 4. Arkansas distribution of *Morone mississippiensis*. Large dots represents new records (modified from Robison and Buchanan 1988). Placement of dot in Lake Dardanelle is arbitrary due to lack of specific locality data.

***Lepomis symmetricus* Forbes – Bantam Sunfish**. This small sunfish is uncommon in eastern Arkansas (Robison and Buchanan 1988). Five specimens were collected in St. Francis Co. from a backwater swamp of Cypress Creek at the edge of SFC road 255, ca. 4 km (2.5 mi.) North of the jct. of SFC road 255 and I-40 at Palestine, (Sec. 29, T5N, R2E), 22 April 2017 (collectors were HWR, Dave Neely, CTM, and Uland Thomas). This is only the third record of *L. symmetricus* from the L'Anguille River drainage.

Percidae – Perches

***Etheostoma fusiforme* (Girard) – Swamp Darter**. The Swamp Darter is uncommon and never abundant in Arkansas (Robison and Buchanan 1988). We collected a single specimen from Cypress Creek, St. Francis Co., at the edge of SFC road 255, ca. 4 km (2.5 mi.) North of the jct. of SFC road 255 and I-40 at Palestine, (Sec. 29, T5N, R2E), 22 April 2017 (collectors were HWR, Dave Neely, CTM, and Uland Thomas). This specimen represents the first record of this species from the L'Anguille River drainage (Fig. 5).

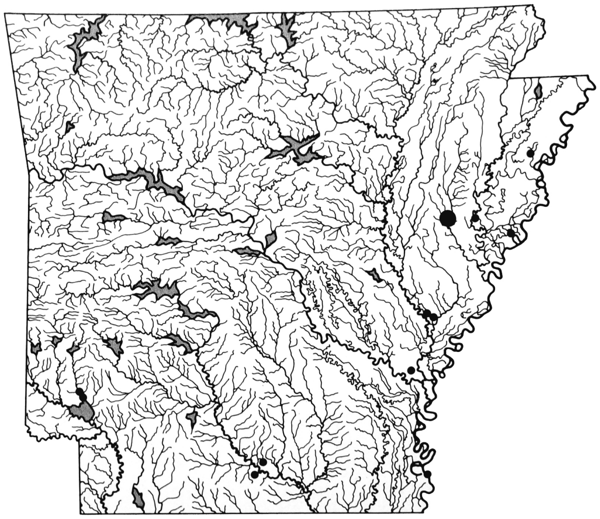

Figure 5. Arkansas distribution of *Etheostoma fusiforme*. Large dot represents the first record for the L'Anguille River drainage (modified from Robison and Buchanan 1988).

CLASS REPTILIA

Gekkonidae – Geckos

***Hemidactylus turcicus* (L.)** – **Mediterranean Gecko**. A juvenile specimen was collected on 2 September 2017 on the outside of an industrial building in downtown Conway (Faulkner Co., 35.092755°N, 92.441469°W). This specimen represents a new county record of an exotic species with a patchy distribution throughout Arkansas in metropolitan areas. The nearest published record for this species in Arkansas is from adjacent Pulaski Co. (Trauth *et al*. 2004).

CLASS AVES

Cardinalidae – Cardinals

***Cardinalis cardinalis* (L.)** – **Northern Cardinal**. Northern cardinals are sexually dimorphic birds in which the male usually has uniform bright red coloration whereas the female is more drab with tinges of red. Images of yellow specimens (usually males) have appeared on the web and in some literature recently (Winstead 2017), which is caused by a mutation that prevents affected birds from metabolizing the usual red pigments from dietary carotenoids (McGraw *et al*. 2003).

Genetic aberrations cause other unusual color patterns that rarely appear on Northern Cardinals. On 11 February 2018, KJ observed several cardinals eating sunflower seeds at bird feeders about 5 km NW of Roland, Pulaski Co. (GPS 34.939°N, 92.526°W). The left side of one individual presented the coloration of a female whereas the right side of the same bird was bright red like a male (Fig. 6).

This bird exhibited a very rare condition known as bilateral gynandromorphism. Such birds are bilateral sex chimeras, presenting male characters on one side of the body and female characters on the other (Major and Smith 2016). Analyses of physical specimens show mostly male chromosomes in cells on the male side and mostly female chromosomes on the female side. Typically, only the left ovary is functional in birds, although both testes are functional in males. Dissection of a gynandromorph specimen from Ohio revealed an ovary on the left side but small testis on the right side of the bird, and some feathers common to specimens of each sex were present on the opposite sides (Jones and Bartlett 2017).

Figure 6. Image of bilateral gynandromorph Northern Cardinal (*Cardinalis cardinalis*) taken in Pulaski County, 11 February 2018. Note the lighter female coloration on the left side and the dark red male coloration on the right side of the bird. Photo by KJ.

It is generally thought that sexual differentiation is determined by genes in the gonads, but Major and Smith (2016) argued that sexual differentiation may depend partly on genes active in various parts of an embryo. Thus, hormonal as well as genetic factors may be involved in determining sex (Clinton *et al*. 2012).

This appears to be only the fourth report of bilateral gynandromorphism in the Northern Cardinal presented in peer-reviewed literature, and the first for Arkansas. Previous records were from Tennessee (Laskey 1969; Peer and Motz 2014), and Ohio (Jones and Bartlett 2017). Photographs of gynandromorphic Northern Cardinals reported to have been taken in Illinois, New Jersey, Texas, and Virginia were located on internet websites.

CLASS MAMMALIA

ORDER RODENTIA

Sciuridae – Squirrels

***Marmot monax* (L.)** – **Groundhog**. Groundhogs hibernate during the fall and winter months in some regions, but the likelihood of hibernation is not known in Arkansas. Absence of specimens collected during winter months was argued to reveal that they hibernate in Oklahoma (Caire *et al*. 1989). Sealander and Heidt (1990) suggested that groundhogs in northern Arkansas likely have a short hibernation period with entry in November and emergence in January or February. A groundhog photo-captured on a game camera by MBC in Marion Co. (36.08404°N, 92.59949°W) on 3 January 2018 supports the view that hibernation can be short or intermittent in Arkansas.

Cricetidae – New World Mice

***Reithrodontomys humulis* – (Audubon and Bachman) Eastern Harvest Mouse**. On 14 and 17 April 1979, 2 male specimens of *R. humulis* were collected from Franklin Co., near exit 35 along I-40 (35.52048°N, 93.86439°W). Specimens are housed in the Museum of Texas Tech University (TTU 38689, 38691). Previously, only a record from Sebastian Co. was known near this new county record in west-central Arkansas. This small mouse had been reported from only 9 counties in Arkansas, mostly located in the northeastern and southwestern portions of the state (Connior *et al*. 2012).

Although this mouse is found only sporadically, it appears to be common at local sites and times. Tumlison *et al*. (1988) reported the capture of 32 specimens from the campus of Southern Arkansas University (Columbia Co.) and Connior *et al*. (2017) reported 9 taken at Grandview Prairie (Hempstead Co.). A search of museum records on VertNet produced accounts of 9 specimens from the area of Fort Chaffee (Sebastian Co.), housed in the collections of the Sam Noble Oklahoma Museum of Natural History (SNOMNH).

Another specimen of considerable interest located via VertNet is a male housed in SNOMNH (59947), with collection data of Stone Co., 3 mi. N of Fifty-Six, Ozark National Forest, (35.994901°N, 92.213266°W), 20 January 1972. Standard measurements provided via VertNet are 138 mm total length, 69 mm tail length, 19 mm hind foot length, and 16 mm ear length. Skin measurements mostly are in the upper range for this species, but only a skull is available in the collection. Evaluation of the skull by RT resulted in re-identification as *R. fulvescens*.

ORDER CHIROPTERA

Vespertilionidae – Vesper Bats

***Lasiurus cinereus* (Palisot de Beauvois) – Hoary Bat**. A roosting Hoary Bat, found alive but believed to be in torpor, was photo-vouchered at 1520 h on 2 January 2018, hanging approximately 6 m (20 ft.) off the ground on a rock bluff in the vicinity of Gustafson Cave, Stone Co. The site is on a southeasterly-facing bluff north of Sugarloaf Creek, 36.05404°N, 92.19694°W. Some sunlight was on the bat, but the maximum temperature that day reached only -7.2°C (19°F). There were numerous small karst openings in the bluff and elsewhere in the vicinity. The site was in a mature forest dominated by hardwoods, adjacent to a mature pine stand that evidenced a recent burn. This observation is very unusual because this migratory bat is usually not found in the middle of winter in Arkansas, and it is associated with trees and not with bluffs and caves. It has been reported once previously in an Arkansas cave (Saugey *et al*. 1978) and an individual was caught by G. O'Hagan in a mist net in front of that cave (Rowland Cave) on 4 September 1979.

An adult female Hoary Bat collected 4 October 2017 from Russellville, Pope Co., and submitted to the Arkansas Department of Health for rabies testing (positive) is a new county record for this bat.

Molossidae – Free-tailed Bats

***Tadarida brasiliensis* (I. Geoffroy) – Brazilian Free-tailed Bat**. A male specimen collected 28 March 2017 from Solgohachia, Conway Co., and submitted to the Arkansas Department of Health for rabies testing (negative) is a new county record for this bat.

Acknowledgments

The Arkansas Game and Fish Commission (AGFC) issued Scientific Collecting Permits to RT, HWR, MBC, and CTM, and the USDA Forest Service (Ouachita and Ozark/St. Francis National Forests) issued a Scientific Collecting Permit to CTM. Information and observations about the Hoary Bat were provided by T. Inebnit. Nikolas H. McAllister assisted with collecting in Polk County. Eric Brinkman and Noah Moses, AGFC-Hope Regional Office, and Jim Cunningham, commercial fisherman, Fulton, AR, provided fishes from the Red River in February 2018. Dr. Dave Neely (Chattanooga, TN) and Uland Thomas (Chicago, IL) assisted in collecting fishes on Crowley's Ridge and vicinity. Susan Weinstein and David Saugey provided new records of bats and rabies. Loan of the specimen of *Reithrodontomys* from the SNOMN was provided by Janet Braun and Brandi Coyner.

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Wave Profile for Current Bearing Lightning Return Strokes

B. Landers[1], M. Hemmati[1]*, and A. Alzhrani[1]

[1]*Department of Physical Sciences, Arkansas Tech University, Russellville, Arkansas 72801, USA*

Correspondence: mhemmati@atu.edu

Running Title: Wave Profile for Current Bearing Lightning Return Strokes

Abstract

The propagation of breakdown waves in a gas, which is primarily driven by electron gas pressure, is described by a one-dimensional, steady-state, three-component (electrons, ions, and neutral particles) fluid model. This study will involve waves propagating in the opposite direction of the electric field force on electrons (anti-force waves—lightning return stroke) only. We consider the electron gas partial pressure to be much larger than that of the other species and the waves to have a shock front. Our set of equations consists of the equations of conservation of the flux of mass, momentum, and energy coupled with the Poisson's equation. The set of equations is referred to as the electron fluid dynamical equations.

For breakdown waves with a significant current behind the shock front, the set of electron fluid dynamical equations and also the boundary condition on electron temperature need to be modified. For a range of experimentally measured current values and a range of possible wave speeds, we will present the method of solution of the set of electron fluid dynamical equations and also the wave profile for electric field, electron velocity, electron temperature, and number density, as well as the ionization rate within the dynamical transition region of the wave.

Introduction

Electron shock waves are the propagating processes that convert an ion-less gas into a neutral plasma. A small amount of gas is ionized near the discharge electrode, and the resulting high-temperature electron gas expands rapidly, which yields an electron shock wave. Also, free electrons are accelerated by the electric field until they have enough energy to ionize the neutral gas through collisions. There are two types of breakdown waves: proforce waves and antiforce waves. Proforce waves are waves for which the electron velocity is in the same direction as the direction of the propagation of the wave. Antiforce waves are waves for which the electron velocity is in the opposite direction as the direction of the propagation of the wave; however, we assume the electron gas partial pressure to be large enough to provide the required force for the propagation of the wave. Antiforce waves are the lightning return strokes, which are the subject of our investigation. The wave has two distinct regions: A thin dynamical transition region, referred to as the sheath region of the wave, directly following the shock front, and a relatively thicker thermal region (referred to as the quasi-neutral region). In the thin dynamical transition region, electric field, starting from its maximum value at the shock front, reduces to zero at the end of the sheath, and the electrons, starting from an initial speed at the shock front slow down to speeds comparable to the speed of the ions and heavy particles. In the thermal region, electrons cool down to approximately room temperature by further ionization of the heavy particles.

Model

To develop the model for current-bearing antiforce waves, one must start with the basic equations for proforce waves. These equations, developed by Shelton and Fowler (1968) and completed by Fowler et al. (1984), are a one-dimensional fluid-dynamical model. The set of equations is comprised of the equations for conservation of mass, momentum, and energy along with Maxwell's equations, which reduces to Poisson's equation only and are given as

$$\frac{d(nv)}{dx} = n\beta \tag{1}$$

$$\frac{d}{dx}\left[mnv(v-V) + nkT_e\right] = -enE - Kmn(v-V) \tag{2}$$

$$\frac{d}{dx}[nmv(v-V)^2 + nkT_e(5v-2V) + 2env\phi + \varepsilon_0 VE^2 -$$

$$\frac{5nk^2T_e}{mk}\frac{dT_e}{dx}] = -(\frac{m}{M})[3nkKT_e + nmK(v-V)^2] \quad (3)$$

$$\frac{dE}{dx} = \frac{en}{\varepsilon_0}(\frac{v}{V}-1) \quad (4)$$

Variables *E, x, β, K, V, M, E_o*, and *φ* represent the electric field, position within the wave, ionization frequency, elastic collision frequency, wave velocity, neutral particle mass, electric field at the wave front, and ionization potential respectively. The remaining variables, *e, T_e, n, v,* and *m*, are the charge, temperature, concentration, velocity, and mass for electrons.

Next, a set of dimensionless variables developed by Fowler *et al.* (1984) are used to reduce Equations (1-4) to their non-dimensional form. These variables are

$$\eta = \frac{E}{E_o}, \nu = (\frac{2e\phi}{\varepsilon_o E_o^2})n, \psi = \frac{v}{V}, \theta = \frac{T_e k}{2e\phi}, \xi = \frac{eE_o x}{mV^2},$$

$$\alpha = \frac{2e\phi}{mV^2}, \kappa = \frac{mV}{eE_o}K, \mu = \frac{\beta}{K}, \omega = \frac{2m}{M},$$

Here, *ν, ψ*, and *θ* are the dimensionless electron concentration, velocity and temperature, while *μ, η,* and ξ are the dimensionless ionization rate, electric field, and position inside the wave. Equations (1-4) in their new, non-dimensional form are

$$\frac{d(\nu\psi)}{d\xi} = \kappa\mu\nu, \quad (5)$$

$$\frac{d}{d\xi}[\nu\psi(\psi-1) + \alpha\nu\theta] = -\nu\eta - \kappa\nu(\psi-1), \quad (6)$$

$$\frac{d}{d\xi}[\nu\psi(\psi-1)^2 + \alpha\nu\theta(5\psi-2) + \alpha\nu\psi + \alpha\eta^2 -$$

$$\frac{5\alpha^2\nu\theta}{\kappa}\frac{d\theta}{d\xi}] = -\omega\kappa\nu[3\alpha\theta + (\psi-1)^2], \quad (7)$$

$$\frac{d\eta}{d\xi} = \frac{\nu}{\alpha}(\psi-1). \quad (8)$$

To change these equations for proforce waves such that they apply to the antiforce case, one must define a set of variables similar to the ones used above. These dimensionless variables, introduced by Hemmati (1999), are as follows:

$$\eta = \frac{E}{E_o}, \nu = (\frac{2e\phi}{\varepsilon_o E_o^2})n, \psi = \frac{v}{V}, \theta = \frac{T_e k}{2e\phi}, \xi = -\frac{eE_o x}{mV^2},$$

$$\alpha = \frac{2e\phi}{mV^2}, \kappa = -\frac{mV}{eE_o}K, \mu = \frac{\beta}{K}, \omega = \frac{2m}{M}.$$

where again *ν, ψ*, and *θ* are the dimensionless electron concentration, velocity and temperature, while *μ, η,* and ξ are the dimensionless ionization rate, electric field, and position inside the wave.

By implementing this second set of variables into the original, one-dimensional proforce equations, Equations (1-4), one reduces them to a dimensionless form while also converting them for use in the antiforce case. The non-dimensional equations describing antiforce waves are

$$\frac{d}{d\xi}[\nu\psi] = \kappa\mu\nu, \quad (9)$$

$$\frac{d}{d\xi}[\nu\psi(\psi-1) + \alpha\nu\theta] = \nu\eta - \kappa\nu(\psi-1), \quad (10)$$

$$\frac{d}{d\xi}[\nu\psi(\psi-1)^2 + \alpha\nu\theta(5\psi-2) + \alpha\nu\psi - \frac{5\alpha^2\nu\theta}{\kappa}\frac{d\theta}{d\xi}$$

$$+ \alpha\eta^2] = -\omega\kappa\nu[3\alpha\theta + (\psi-1)^2], \quad (11)$$

$$\frac{d\eta}{d\xi} = -\frac{\nu}{\alpha}(\psi-1). \quad (12)$$

For current bearing antiforce waves, Hemmati *et al.* (2011) gives the current as

$$I_1 = eN_iV_i - env$$

where N_i and V_i are the ion number density and velocity behind the wave front respectively.

Solving this for N_i and then substituting it into the Poisson's equation gives the following.

$$\frac{dE}{dx} = \frac{e}{\varepsilon_0}(\frac{I_1}{eV} + \frac{nv}{V} - n)$$

Substituting in the dimensionless variables for antiforce waves as well as introducing dimensionless current, given by

$$\iota = \frac{I_1}{\varepsilon_0 K E_0}$$

reduces the Poisson's equation to the following.

$$\frac{d\eta}{d\xi} = \kappa\iota - \frac{\nu}{\alpha}(\psi-1).$$

Solving this for *ν(ψ-1)* and substituting it into the equation for conservation of energy gives the full set of non-dimensional equations for current bearing antiforce waves, which are

$$\frac{d}{d\xi}[\nu\psi]=\kappa\mu\nu, \qquad (13)$$

$$\frac{d}{d\xi}[\nu\psi(\psi-1)+\alpha\nu\theta]=\nu\eta-\kappa\nu(\psi-1), \qquad (14)$$

$$\frac{d}{d\xi}[\nu\psi(\psi-1)^2+\alpha\nu\theta(5\psi-2)+\alpha\nu\psi-\frac{5\alpha^2\nu\theta}{\kappa}\frac{d\theta}{d\xi}$$
$$+\alpha\eta^2]=2\eta\kappa\iota\alpha-\omega\kappa\nu[3\alpha\theta+(\psi-1)^2], \qquad (15)$$

$$\frac{d\eta}{d\xi}=\kappa\iota-\frac{\nu}{\alpha}(\psi-1). \qquad (16)$$

Results and Discussion

Since antiforce waves are observed in nature as lightning return strokes, values measured from real lightning strokes can be used to assess the fluid model. For the average speeds of lightning return strokes, Idone *et al*. (1987) have reported values in the range of $0.9\text{x}10^8$ to $1.6\text{x}10^8$ m/s. Mach and Rust (1992) reported an average of $0.8\text{x}10^8$ to $1.7\text{x}10^8$ m/s, while Nakano *et al.* (1987) measured a wide range of speeds from $0.3\text{x}10^8$ to $2\text{x}10^8$ m/s. In our analysis of antiforce waves, we have been able to find solutions to the model for speeds as low as $2\text{x}10^6$ m/s.

For the currents measured in lighting return strokes, averages ranging from less than 10kA to more than 40kA were reported for different regions of the US by Orville and Huffines (1999). In Japan, meanwhile, currents up to 340kA were reported from direct measurements by Goto and Narita (1995).

As for the temperatures reported, Jurenka and Barreto (1985) estimate the neutral air in a lightning channel to be at a range of 4000 to 8000K. For the temperature of the electrons inside the wave, they estimate a value of around 20,000K.

Electron number density was also reported by Jurenka and Barreto (1985). In their paper they give a range of 10^{10} to 10^{12} el./cm^3 and greater.

We have integrated equations (13-16) through the sheath region using a trial and error method. First, values are selected for dimensionless wave speed, α, and current, ι. We then adjusted values for electron velocity, ψ_1, electron number density, ν_1, at the wave front and the wave constant, κ, and integrated through the sheath region and repeated this process until the solutions met the excepted physical conditions at the trailing edge of the wave ($\eta_2 \rightarrow 0$ as $\psi_2 \rightarrow 1$). For a relatively large speed, α=0.001, we have been able to integrate our set of electron fluid dynamical equations for a set of six different dimensionless current values—ι=0.5, 0.7, 1, 2, 5, and 10. α=0.001 represents an actual wave speed value of $9.37\text{x}10^7$ m/s.

The set of initial conditions which resulted in successful integration of the set of equations is as follows:

$\alpha=0.001$, $\iota=0.5$, $\kappa=0.144$, $\psi_1=0.51721$, $\nu_1=0.1750$
$\alpha=0.001$, $\iota=0.7$, $\kappa=0.144$, $\psi_1=0.5142$, $\nu_1=0.167$
$\alpha=0.001$, $\iota=1$, $\kappa=1.44$, $\psi_1=0.514$, $\nu_1=0.1816$
$\alpha=0.001$, $\iota=2$, $\kappa=1.44$, $\psi_1=0.5093$, $\nu_1=0.1857$
$\alpha=0.001$, $\iota=5$, $\kappa=0.144$, $\psi_1=0.4855$, $\nu_1=0.1953$
$\alpha=0.001$, $\iota=10$, $\kappa=0.144$, $\psi_1=0.442$, $\nu_1=0.199$

Figure 1 represents dimensionless electric field, η, as a function of dimensionless electron velocity, ψ, within the sheath region of the wave. Note that the graphs for all values of dimensionless currents, except for ι=10, meet the expected conditions at the end of the sheath region. The largest value of current that gave a proper solution was ι=7.

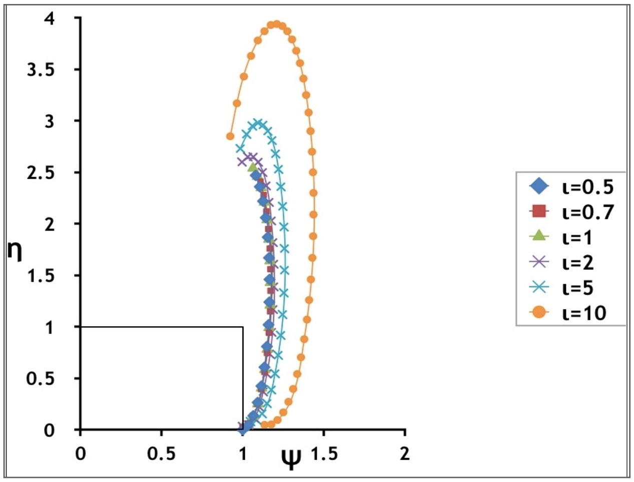

Figure 1. Electric field, η, as a function of electron velocity, ψ, within the sheath region of current bearing antiforce waves for a wave speed value of α=.001 and for current values of 0.5, 0.7, 1, 2, 5, and 10.

Figure 2 represents dimensionless electron velocity, ψ, as a function of dimensionless position, ξ, within the sheath region of the wave. The graphs show that as current increases, sheath thickness increases.

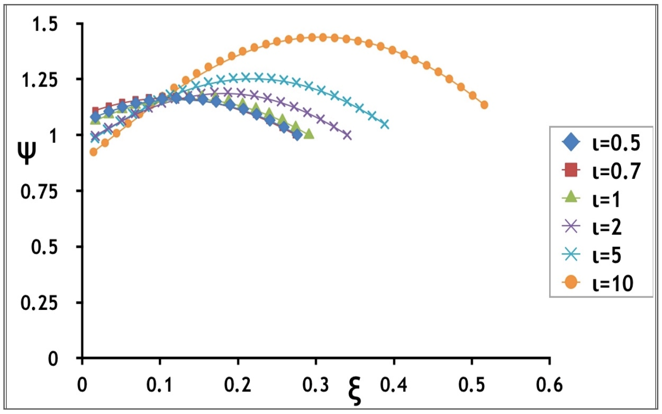

Figure 2. Electron velocity, ψ, as a function of position, ξ, within the sheath region of the current bearing antiforce waves for a wave speed value of α=0.001 and for current values of 0.5, 0.7, 1, 2, 5, and 10.

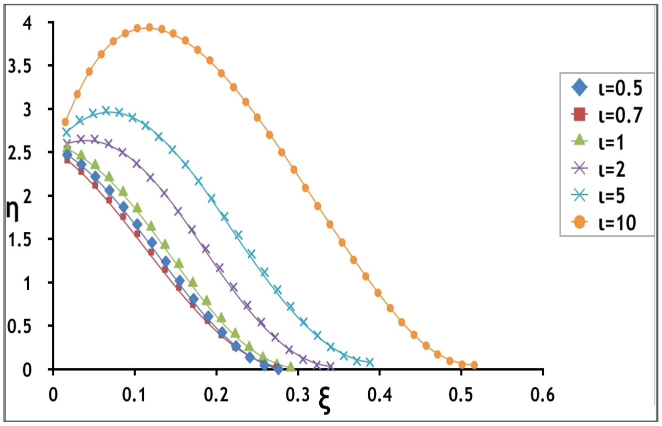

Figure 3. Electric field, η, as a function of position, ξ, within the sheath region of the current bearing antiforce waves for a wave speed value of α=0.001 and for current values of 0.5, 0.7, 1, 2, 5, and 10.

Figure 3 represents dimensionless electric field, η, as a function of dimensionless position, ξ, within the sheath region of the wave. It is seen that, for all values of ι, the expected conditions at the trailing edge of the wave are met, that is at the end of the sheath region η goes to zero.

Figure 4 represents dimensionless electron temperature, θ, as a function of dimensionless position, ξ, within the sheath region of the wave. As one traverses through the sheath region of the wave, the electron temperatures increases.

Figure 5 represents dimensionless electron number density, ν, as a function of dimensionless position, ξ, within the sheath region of the wave. For all ι values, ν initially decreases and then increases again to almost the initial values.

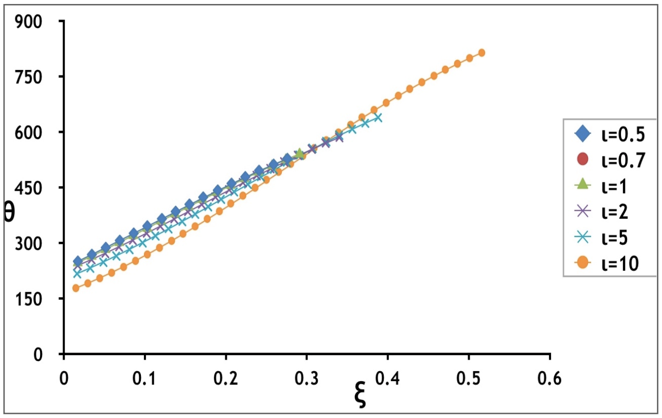

Figure 4. Electron temperature, θ, as a function of position, ξ, within the sheath region of the current bearing antiforce waves for a wave speed value of α=0.001 and for current values of 0.5, 0.7, 1, 2, 5, and 10.

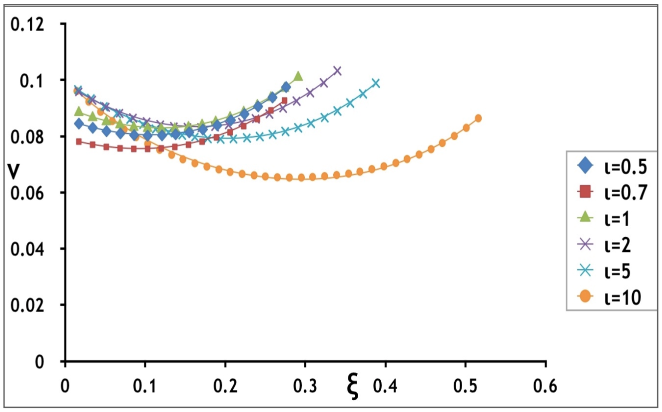

Figure 5. Electron number density, ν, as a function of position, ξ, within the sheath region of the current bearing antiforce waves for a wave speed value of α=0.001 and for current values of 0.5, 0.7, 1, 2, 5, and 10

As we increase the wave speed, it can support higher currents. ι = 1 approximately represents an actual current value of 10kA, and α = 0.001 represents an actual wave speed value of 9.37x10^7 m/s. For α=0.001, we have been able to solve our set of electron fluid dynamical equations for dimensionless current as high as ι = 7. Rakov (2000) reports a speed range of 0.3x10^8 to 2x10^8 m/s and a peak current of up to 300kA. Therefore, we should be able to solve the equations for much higher currents than the 5 to 35kA generally reported. For antiforce waves, Rakov (2000) gives the lower end of the speed range as 0.3x10^8 m/s.

We have been able to integrate our set of equations for considerably lower wave speeds as well. This means that slower antiforce waves than reported are possible to observe.

In addition to the six dimensionless current values for α=0.001, we have been able to integrate our set of equations for dimensionless wave speed values of α=1, which is equivalent to an actual wave speed as low as 2.96x10^{6} m/s and a dimensionless current value as high as ι = 0.25, as well as for α=0.1, which represents an actual wave speed of 9.37x10^{6} m/s, and a dimensionless current as high as ι = 1.

Figure 6 represents the dimensionless electric field, η, as a function of dimensionless electron velocity, ψ, within the sheath region of the wave. Both graphs meet the expected conditions at the end of the sheath region.

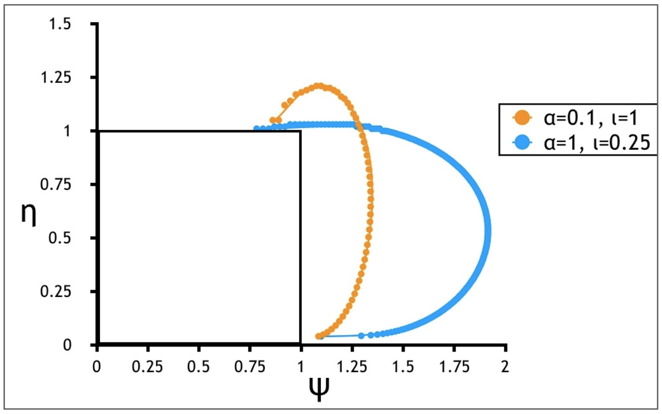

Figure 6. Electric field, η, as a function of electron velocity, ψ, within the sheath region of current bearing antiforce waves for wave speed values of α=0.1 and 1 and for current values of ι= 1 and 0.25 respectively.

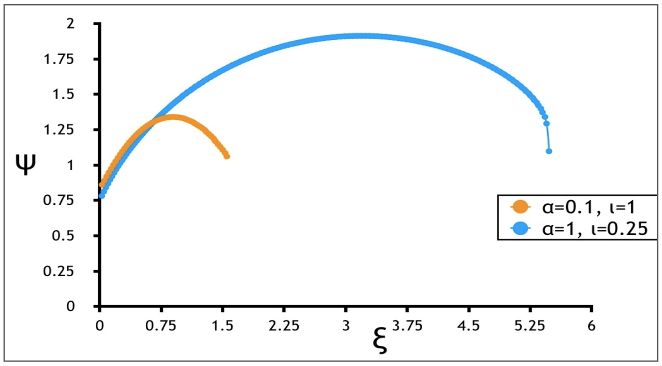

Figure 7. Electron velocity, ψ, as a function of position, ξ, within the sheath region of current bearing antiforce waves for wave speed values of α=0.1 and 1 and for current values of ι= 1 and 0.25 respectively.

Figure 7 represents dimensionless electron velocity, ψ, as a function of dimensionless position, ξ, within the sheath region of the wave. As wave speed decreases, sheath thickness increases considerably. For reference, the dimensionless sheath thickness ξ=1.5 represents an actual sheath thickness of 1.2x10^{-2}m.

Figure 8 represents dimensionless electron temperature, θ, as a function of dimensionless position, ξ, within the sheath region of the wave. As wave speed decreases, electron gas temperature decreases considerably. For comparison with electron temperature reported by Jurenka and Barreto (1985), a dimensionless electron temperature value of θ = 6 represents an actual electron gas temperature of $3.48x10^{6}$ K. Our number represents the temperature at the core of the lightning return stroke.

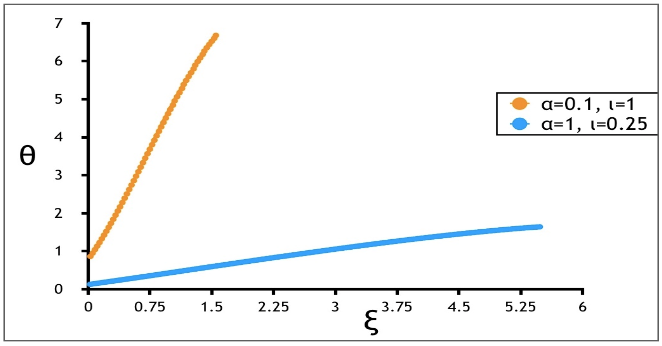

Figure 8. Electron temperature, θ, as a function of position, ξ, within the sheath region of current bearing antiforce waves for wave speed values of α=0.1 and 1 and for current values of ι= 1 and 0.25 respectively.

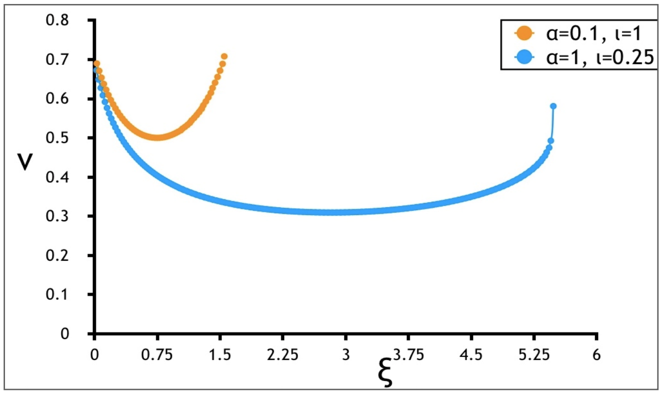

Figure 9. Electron number density, ν, as a function of position, ξ, within the sheath region of current bearing antiforce waves for wave speed values of α=0.1 and 1 and for current values of ι= 1 and 0.25 respectively.

Figure 9 represents electron number density, ν, as a function of dimensionless position, ξ, within the sheath region of the wave. The non-dimensional electron number density value of ν=0.7 represents an actual electron number density value of 7.7x10^{15} el./m^{3}, and this almost agrees with the electron number density reported by Jurenka and Barreto (1985)

Figure 10 represents dimensionless ionization rate, μ, as a function of dimensionless position, ξ, within the sheath region of the wave. For the higher wave speed, ionization rate increases slightly, whereas for the lower wave speed, μ slowly decreases. For higher dimensionless current values and lower waves speeds, the integration of our set of electron fluid dynamical equations become difficult and very time consuming.

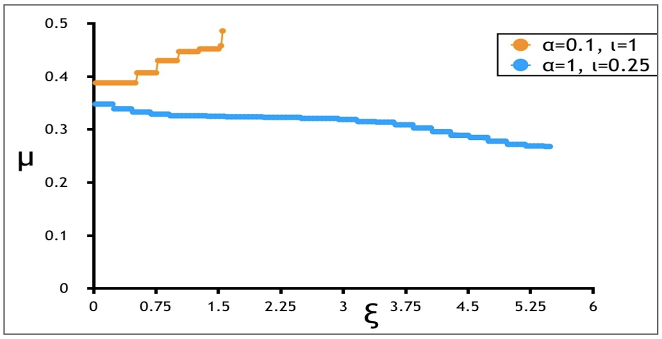

Figure 10. Ionization rate, μ, as a function of position, ξ, within the sheath region of current bearing antiforce waves for wave speed values of α=0.1 and 1 and for current values of ι= 1 and 0.25 respectively

Conclusion

We have been able to solve our set of electron fluid dynamical equations for a range of current values and also for a range of wave speed values. For the most part, the results of our integration agree with experimental data reported by others; however, we are able to integrate our set of equations for much lower wave speeds than reported experimentally.

For lightning return strokes, some investigators have suggested the existence of a relationship between the peak current values and wave speed values (Wagner (1963)). In other words, as the wave speed increases, the current that it can support increases as well. Some others (Willett *et. al* (1989)) disagree with the existence of such a relationship. We also confirm the existence of such a relationship.

Acknowledgments

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Microbial Diversity in the Thermal Springs within Hot Springs National Park

E.T. Stone[1], R. Murray, and M.D. Moran

Department of Biology, Hendrix College, 1600 Washington Ave., Conway, AR 72032

[1]Corresponding author

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Running Title: Microbial Diversity in Arkansas Hot Springs

Abstract

The thermal water systems of Hot Springs National Park (HSNP) in Hot Springs, Arkansas exist in relative isolation from other North American thermal systems. The HSNP waters could therefore serve as a unique center of thermophilic microbial biodiversity. However, these springs remain largely unexplored using culture-independent next generation sequencing techniques to classify species of thermophilic organisms. Additionally, HSNP has been the focus of anthropogenic development, capping and diverting the springs for use in recreational bathhouse facilities. Human modification of these springs may have impacted the structure of these bacterial communities compared to springs left in a relative natural state. The goal of this study was to compare the community structure in two capped springs and two uncapped springs in HSNP, as well as broadly survey the microbial diversity of the springs. We used Illumina 16S rRNA sequencing of water samples from each spring, the QIIME workflow for sequence analysis, and generated measures of genera and phyla richness, diversity, and evenness. In total, over 700 genera were detected and most individual samples had more than 100 genera. There were also several uncharacterized sequences that could not be placed in known taxa, indicating the sampled springs contain undescribed bacteria. There was great variation both between sites and within samples, so no significant differences were detected in community structure between sites. Our results suggest that these springs, regardless of their human modification, contain a considerable amount of biodiversity, some of it potentially unique to the study site.

Key Words: Hot Springs National Park, Microbial Diversity, Next Generation Sequencing, Thermophiles

Introduction

Constraints governing the biochemical processes at work in thermophilic bacteria have yielded discoveries with far-reaching implications, ranging from biogeochemistry to biotechnology (Leis *et al.* 2015; Shrestha *et al.* 2018; Undsworth and Koutsopoulos 2007). Evolving in relative isolation from other extremophiles provides opportunities for unique communities to develop, resulting in community structures that vary tremendously, even among similar study sites (Amin *et al.* 2017). Investigation of individual microbes in extreme environments has yielded the discovery of thermostable enzymes critical to biotechnological advances such as PCR (Brock 1969), treatment of industrial waste (Shrestha *et al.* 2018), and industrial chemistry (Leis *et al.*2015).

The in-depth characterization these communities warrant cannot be accomplished with culture-dependent methods alone, as it has been hypothesized that less than 1% of environmental bacteria are likely to be cultured in laboratory settings (Staley and Konopka 1985). Given the stringent growth requirements for extremophiles, and the observation that some thermophiles appear to be co-culture dependent (Stewart 2012), the proportion of culturable thermophiles is likely smaller. The limitations of culture-dependent characterization render these methods insufficient for improving understanding of these microbial communities. However, with the increasing accessibility of modern high-throughput sequencing techniques, researchers have improved understanding of high-temperature microbial community structure, allowing the development of metagenomic libraries specific to thermophiles (Mirete *et al.* 2016). In spite of these advances, many isolated regions of thermophilic activity remain unexplored, limiting knowledge of prospective biodiversity.

Hot Springs National Park is an isolated thermophilic environment located in the Ouachita Mountains of Arkansas, and is the only hot water thermal spring complex with average water temperatures above 50°C in the central U.S. (NOAA NCEI 2018). Given its geographic isolation, opportunities for biological exchange with other North American thermophile communities are likely very limited. While the hot water springs have been used for human recreation for thousands of years, intense development of the area began in the 1830s (Hanor 1980). Modern development has included heavy landscaping and the capping and diversion of the springs to recreational facilities, such as bath houses. Today, only ten spring complexes are uncapped and open to the environment (Yeatts 2006). In the open springs, a presumably more complex ecological community exists with thermophilic algae (Smith 2010) and crustaceans (Meg O'Connor, personal correspondence). By contrast, the capped springs, which lack a light source, are likely to contain a simpler community consisting mostly of chemotrophs (probably chemolithotrophs). Our goal in this study was to characterize the microbial communities in both capped and uncapped hot springs within Hot Springs National Park. We hypothesized that the microbial diversity would be lower and the community structure distinctive in capped springs compared to the uncapped springs. While some novel thermophiles have been identified in this system via traditional laboratory culture and 16S rRNA sequencing at specific sites within the park (Marks *et al*. 2012), the community structures of these different springs have not been broadly compared with culture-independent methods. Therefore, our study may provide insight into the biodiversity in this isolated system, detect human impacts on the communities, and assess the conservation value of the capped and uncapped springs.

Materials and Methods

Sampling Sites

All sampling sites were located within Hot Springs National Park (HSNP) (34°30'53'' N, 93°03'12'' W). While the park contains 43 thermal springs located in a 5.6 Ha section of the park (Yeatts, 2006), all sampling sites were located on Bath House Row, where over the last 150 years, 33 of the thermal springs have been highly modified by human activity. In this system, rain water seeps slowly into cracks in Hot Springs rock formations, where it is heated before being forced back to the surface. Temperatures of the springs average 61.4°C. The rock in the area is largely shale, chert, novaculite, and sandstone, of which only shale impedes the ground water movement in the area (Yeatts 2006).

Sites within HSNP were sampled between 10:30-12:00am CST on 26 Sept 2017. Of the 43 individual springs where heated water rises to the surface, four individual springs were selected for sampling. Of these four springs, two sites were capped springs, where bathhouse buildings were constructed on top of the individual flows and previously used for recreation and tourism. The two capped sites, the Fordyce Bath House Spring (F) and the Hale Bath House Spring (H), have been decommissioned for public use. The remaining sites, the Lamar Display Spring (L) and the Tunnel Display Spring (T), are open-air springs on display to the public.

Sample Collection and Preparation

Duplicate water samples from each site were collected aseptically at the surface level using sterile 50 mL conical tubes and stored immediately on ice in transit to the laboratory for further processing. Samples were processed within four hours of collection for DNA extraction. All water samples were filtered via a Millipore® filtration apparatus containing a filter with a pore size of 0.45 μm. DNA extraction was performed using the Zymo Research® ZR Fecal DNA Miniprep Kit (Catalog No: D6010) according to manufacturer specifications. Purified product was quantified by spectrophotometry and stored at -80°C until preparation for Illumina Sequencing.

Sequencing and Analysis

PCR-amplification targeted variable regions V3 & V4 of the 16S ribosomal subunit and amplified from 515bp to 806 bp within the gene using the Illumina 515FB/806RB primer pair (Caporaso *et al*. 2011; Parada *et al*. 2016) for a total of 291bp. This PCR-amplification, as well as barcoding and high-throughput sequencing on an Illumina platform, was performed by Wright Labs (Huntingdon, PA 16652) using primers utilized in previous studies. All sequence data was analyzed with access to computing cluster located at Juniata College using Quantitative Insights Into Microbial Ecology (QIIME version 1.9.1). Before using this program, sequences were trimmed to 253bp and discarded if sequence overlap was less than 200 bp. The USEARCH version 7 algorithm filtered sequences with an expected error rate of <1%, and with a minimum of 5,000 reads required for retention. This resulted in a total of 646, 651 quality reads which were then analyzed using QIIME program (v1.9.1). Operational taxonomic units (OTU's) were selected using the open reference OTU UCLUST algorithm (Edgar 2010), and were defined by 97% sequence similarity. Taxonomy was assigned using the RDP Classifier and Greengenes 16S rRNA gene database (DeSantis *et al*. 2006, 13-8 release).

For alpha diversity measurements, we counted the number of genera present in each sample within each site. To determine the general similarity between sites, we calculated genera richness (S), the Shannon diversity index (H'), and evenness (J'). To examine general community structure, we performed a nonmetric multidimensional scaling (NMDS, Kruskal 1964) analysis and plotted the results on a two-dimensional graph. We utilized the Bray-Curtis method to measure beta diversity, or dissimilarity between samples.

Results

We detected 646,651 quality-reads for the eight samples after removal of low-quality and chimeric sequences (Table 1). These data resulted in a total of 46 bacterial phyla and 3 archaeal phyla across all sampling sites (Table 2). Only one phylum of Archaea (Crenarchaeota) and 12 phyla of Bacteria were found within all of the sites sampled. Although there were some phyla found only in a single sampling site, there was no indication of groups exclusively present in one type of sample (i.e. capped or uncapped). The 12 phyla of bacteria found in all sites, in order of abundance were Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes, Cyanobacteria, Bacteriodetes, Planktomycetes, Chloroflexi, Verrucomicrobia, Nitrospirae, Chlorobi, and Elusimicrobia. The proportion of unassigned taxa in our samples ranged from 0.5 – 19.9% of sequences, indicating a considerable amount of new unknown biodiversity. We detected a total of 706 genera with OTU's >97% similarity to previously described microbes (see supplemental data, Moran 2018).

Table 1. Number of reads returned from next generation sequencing of 16S rRNA variable regions V3 & V4 after quality filtering. L = Lamar Display Spring, F = Fordyce Bathhouse, T = Tunnel Spring, and H = Hale Bathhouse.

NGS Reads within HSNP	
Sample	Number of reads
H1	136748
H2	22666
F1	206444
F2	18165
L1	49789
L2	86089
T1	37401
T2	89349
Total	646651

There was high variation in genus richness between sites and between samples within sites (Table 3). With the exception of one sample (F2), all samples contained over 100 genera, while some had over 400 genera represented. H' and J' values were relatively high in most samples, indicating a general lack of dominance by one particular group. However, the various genera within the Proteobacteria tended to make up over 50% of sequence abundance. The NMDS results at the genus level showed clustering of sites L, H, and T, while both F samples were dramatically different in community structure. (Stress = 0.12, Fig. 1).

When examined at the site level, each location had over 250 genera and each had unique genera not found in other springs (Table 4). However, the proportion of unique genera varied greatly from 4% (Fordyce) to 34% (Hale).

Discussion

Based on our assessment of the four thermal springs in HSNP, the area contains considerable biodiversity. Our sequence data indicates that no single genus of bacteria or archaea are dominant within the springs, but instead a variety of genera thrive in these habitats, ranging from 90 to over 400 across sample sites. The phylum Proteobacteria, however, did typically comprise ~50% of sequences within a given sample. These biodiversity results generally agree with other studies that have examined thermal communities using the 16S rRNA sequence method (Amin *et al.* 2017; Chan *et al.* 2015; De León *et al.* 2013; Vick *et al.* 2010).

Our results contrast with a previous study of the Hale Bathhouse in HSNP, which found high abundance of the phylum Nitrospira (Bacteria) and Thaumarchaeota (Archaea, Marks *et al*. 2012). In our samples, however, Nitrospira was relatively rare (less than 2%) and Thaumarchaeota was absent. However, this study examined biofilms on submerged glass slides, while our study sampled the water column. This difference shows that thermophilic microbial communities may vary greatly within sites, depending on the location and method of collection. It should be noted that the number of reads and community structures in our study vary dramatically, as much as ten-fold, across replicates of the same site, indicating possible sampling error (Table 1). For example, in the low flow spring of Fordyce Bathhouse, one sample (F2) visibly contained more sediment, which may have changed the biodiversity profile for that sample (Dalu *et al.* 2017; Smolders *et al.* 2003) and may explain the large difference in community structure seen in the

Table 2. Phyla diversity and proportional representation based on 16S rRNA sequencing data across four sampled sites in Hot Springs National Park. L = Lamar Display Spring, F = Fordyce Bathhouse, T = Tunnel Spring, and H = Hale Bathhouse.

Phylum	L1	L2	T1	T2	F1	F2	H1	H2
Unassigned;Other	0.0249	0.0192	0.0053	0.0080	0.1987	0.0147	0.0297	0.0494
Kingdom Archaea								
Crenarchaeota	0.0010	0.0011	0.0121	0.0074	0.3460	0.0087	0.0042	0.0066
Euryarchaeota	< 0.0001	0.0001	0.0022	0.0031	0.0050	0.0003	0.0002	NA
Parvarchaeota	NA	< 0.0001	NA	< 0.0001	NA	NA	0.0004	NA
Kingdom Bacteria								
Other	NA	NA	NA	NA	< 0.0001	NA	NA	NA
k__Bacteria;p__	NA	NA	0.0010	0.0011	0.0002	NA	0.0019	0.0026
AD3	NA	< 0.0001	NA	0.0106	NA	NA	0.0515	0.0014
Acidobacteria	0.0613	0.0399	0.0223	0.2349	0.0543	0.0216	0.1436	0.0498
tinobacteria	0.0147	0.0301	0.1652	0.1624	0.0442	0.0236	0.1053	0.1088
Aquificae	NA	NA	0.0003	NA	NA	NA	NA	NA
Armatimonadetes	0.0019	0.0022	0.0006	0.0071	0.0026	NA	0.0152	0.0114
BHI80-139	NA	NA	NA	< 0.0001	NA	NA	0.0002	NA
BRC1	0.0007	< 0.0001	NA	0.0002	NA	NA	0.0017	NA
Bacteroidetes	0.0115	0.0306	0.0479	0.0160	0.0036	0.1497	0.0168	0.0321
Chlamydiae	0.0035	0.0015	0.0005	< 0.0001	< 0.0001	NA	0.0007	< 0.0001
Chlorobi	0.0031	0.0058	0.0003	0.0007	0.0110	0.0012	0.0024	0.0101
Chloroflexi	0.0058	0.0417	0.0014	0.0865	0.0445	0.0012	0.0695	0.0334
Cyanobacteria	0.0688	0.1764	0.0188	0.0058	0.0012	0.0171	0.0105	0.0230
Deferribacteres	NA	NA	NA	NA	NA	NA	0.0001	0.0003
Elusimicrobia	0.0002	< 0.0001	0.0041	0.0023	0.0017	0.0052	0.0007	0.0008
FBP	NA	0.0002	NA	NA	NA	NA	NA	NA
FCPU426	< 0.0001	< 0.0001	NA	NA	NA	NA	< 0.0001	0.0037
Fibrobacteres	< 0.0001	0.0002	NA	0.0002	NA	NA	< 0.0001	NA
Firmicutes	0.0160	0.0101	0.0309	0.0113	0.0059	0.2833	0.0296	0.0397
GAL15	< 0.0001	< 0.0001	NA	0.0001	0.0211	0.0701	0.0003	NA
GN02	NA	0.0001	NA	< 0.0001	NA	NA	NA	NA
Gemmatimonadetes	0.0044	0.0028	0.0001	0.0090	< 0.0001	NA	0.0038	0.0004
MVP-21	NA	NA	NA	0.0001	NA	NA	0.0002	NA
NC10	NA	NA	NA	NA	0.0152	NA	0.0002	< 0.0001
NKB19	0.0001	NA	NA	< 0.0001	NA	NA	< 0.0001	NA
Nitrospirae	0.0009	0.0004	0.0012	0.0258	0.1083	0.0197	0.0052	0.0153
OD1	< 0.0001	0.0001	NA	NA	NA	NA	< 0.0001	NA
OP1	0.0001	< 0.0001	0.0004	NA	0.0413	0.0076	0.0014	NA
OP11	NA	NA	NA	NA	NA	NA	< 0.0001	NA

Table 2. *continued.*

OP3	0.0011	< 0.0001	NA	< 0.0001	0.0005	NA	0.0005	0.0006
OP8	NA	NA	NA	NA	NA	NA	< 0.0001	NA
Planctomycetes	0.0283	0.0307	0.0328	0.0653	0.0047	< 0.0001	0.1123	0.0285
Proteobacteria	0.7331	0.5875	0.6471	0.2858	0.0815	0.3686	0.2287	0.5041
SBR1093	< 0.0001	0.0002	NA	NA	0.0073	NA	0.0002	NA
Spirochaetes	0.0013	0.0059	NA	NA	NA	NA	0.0052	0.0118
TM6	0.0041	0.0020	NA	< 0.0001	NA	NA	< 0.0001	0.0003
TM7	NA	NA	NA	0.0001	NA	NA	0.0011	NA
Tenericutes	NA	NA	NA	< 0.0001	NA	0.0004	< 0.0001	NA
Thermotogae	NA	NA	NA	NA	NA	NA	< 0.0001	0.0004
Verrucomicrobia	0.0095	0.0081	0.0029	0.0523	0.0001	0.0069	0.1247	0.0146
WPS-2	0.0022	0.0006	0.0018	0.0006	0.0005	NA	0.0167	0.0022
WS2	NA	NA	NA	0.0006	NA	NA	0.0002	NA
WS3	0.0001	NA	NA	0.0022	NA	NA	0.0003	NA
WWE1	NA	NA	NA	NA	0.0003	NA	NA	NA
Thermi	0.0013	0.0022	0.0007	< 0.0001	< 0.0001	NA	0.0147	0.0488

NMDS results. Testing both the water column and the sediment in each spring may help elucidate the impact of substrate and microhabitats on microbial biodiversity within the springs. With the exception of Fordyce Bathhouse spring however, community structure is relatively similar between sites.

The community structure of these thermophilic environments is complex, perhaps reflecting the diversity of energy sources present in these waters, which includes iron, sulfur compounds, ammonia, and methane (Marks *et al.* 2012). Some of our identified phyla contain species that are known to use chemoautotrophic metabolism, including Crenarchaeota (sulfur, Woese 1984), Nitrospira (ammonia, Marks *et al.* 2012), Crenarchaeota (iron, Kozubal *et al.* 2008), and a variety of methane metabolizing Archaea (Evans *et al.* 2015; Ozuolmez *et al.* 2015; Yang *et al.* 2017). We find it interesting that even the capped springs had high biodiversity, showing that complex bacterial communities are surviving without external energy sources (i.e., light). Therefore, it appears that a large amount of the biodiversity has been maintained in these sites, even with the high degree of human modification.

Table 3. Genera richness (S), Shannon diversity index (H'), and evenness index (J') for each thermophilic sample. L = Lamar Display Spring, F = Fordyce Bathhouse, T = Tunnel Spring, and H = Hale Bathhouse.

Sample	S	H'	J'
L1	345	3.20	0.55
L2	420	3.94	0.65
F1	240	2.59	0.47
F2	90	3.61	0.80
T1	134	2.98	0.61
T2	420	4.18	0.69
H1	466	4.46	0.73
H2	224	4.05	0.75

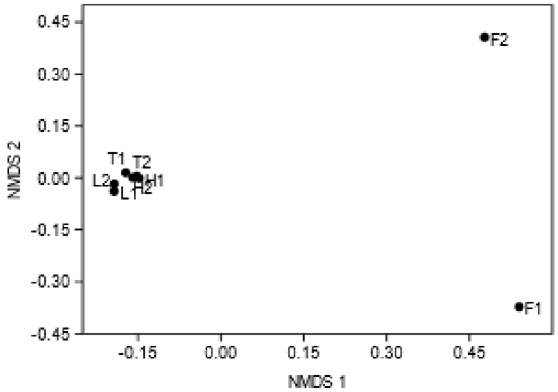

Figure 1. Results of the NMDS analysis showing the similarity between samples. L = Lamar Display Spring, F = Fordyce Bathhouse, T = Tunnel Spring, and H = Hale Bathhouse.

Table 4. Number of unique genera, total genera, and proportional unique at each site. L = Lamar Display Spring, F = Fordyce Bathhouse, T = Tunnel Spring, and H = Hale Bathhouse

	L	H	F	T
Unique Genera	89	171	18	42
Total Genera	468	504	452	272
Proportion Unique	0.19	0.34	0.04	0.15

This study, although limited to a small subset of springs in HSNP, found a high diversity of thermophilic microbes. It is likely that further sampling of the spring system would yield additional genera. We recommend a thorough sampling of microhabitats within each spring (e.g., substrate, water column, and different distances from spring source) to fully document the microbe biodiversity. These springs have been recognized for decades for their unique geological and cultural value, while their biological value has been less well-understood. Our analysis shows that the microbial biodiversity remains rich in HSNP despite continual anthropogenic modification, and should remain a focus of ongoing conservation efforts, as this diversity could be of considerable scientific value.

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Avian Frugivory in a Fruiting Mulberry Tree (*Morus rubra*) in Arkansas

J.L. Jackson, II[1] and R. Kannan[2*]

[1]*Department of Mathematics, University of Arkansas - Fort Smith, Fort Smith, AR 72913*
[2]*Department of Biology, University of Arkansas - Fort Smith, Fort Smith, AR 72913*

[*]Correspondence: ragupathy.kannan@uafs.edu

Running title: Avian Frugivory in a Fruiting Mulberry Tree

Abstract

A fruiting Mulberry tree (*Morus rubra*) was observed for 67 hours in the spring of 2016 and 2017 in Fort Smith, Arkansas. A total of 172 five-minute scans were performed, during which the following parameters were recorded: species visited, number of individuals of each species, time of visitations, and foraging tier. Between each scan, the foraging rate (number of fruits consumed/min) and inter- and intra- specific aggressive interactions were recorded. A total of 3465 observations of individual birds from 32 species was recorded. Species diversity index was higher in the upper half of the tree. The mean foraging rates for the 6 most commonly observed species ranged from 1.2-2.3 fruits/min. A total of 346 aggressive interactions was observed of which 68% were intraspecific.

Introduction

Many studies have characterized bird communities in tropical (e.g. Eshiamwata *et al*. 2006; Coates-Estrada and Estrada 1986) and temperate (e.g. Snow and Snow 1988; Herrera 1998) fruiting trees. In temperate latitudes, fruits constitute an important food resource for birds, especially in the fall (Rybczynski and Riker 1981; Logan 1987; Smith and Riley 1990) and spring (Martin *et al*. 1951; Stapanian 1982). There have been two avian frugivory studies from Arkansas, both from the northwestern part of the state. Prather *et al.* (2000) studied characteristics of some fall fruiting tree species and their avian assemblages. Smith and Riley (1990) quantified avian removal of fruits from a pokeweed (*Phytolacca americana*) in late summer and fall. Neither of these studies investigated spring fruiting plants.

The Red Mulberry (*Morus rubra*) is a common deciduous tree native to eastern North America (Flora of North America 2018). In Arkansas, it is commonly found both in gardens as a planted ornamental, and in the wild, especially in fencerows and disturbed early or mid-successional habitats. When the tree fruits in the spring (April-May), it attracts hordes of migratory and resident birds. The fleshy aggregate fruit of the mulberry tree is synchronously produced, turning from pink to blackish during the approximately three weeks of fruiting. These fruits are consumed even by birds like Swainson's Thrush *Catharus ustulatus* (Wiley *et al*. 2015) and Eastern Kingbird *Tyrannus tyrannus* (Murphy 1996) that are usually insectivorous in their breeding range. Many rare or declining neotropical migrants like Eastern Kingbird, Scarlet Tanager *Piranga olivacea*, and Swainson's Thrush, eat mulberry fruits during spring when they are in migration or after they have arrived at their breeding grounds (Murphy 1996; Mowbray 1999; Mack and Yong 2000). Despite this apparent importance of the tree as a food source for birds, only one systematic study has been conducted to study avian frugivore assemblages in a mulberry tree in North America (Robbins *et al*. 1975), and this was in Michigan. Stapanian (1982) studied the effectiveness of fruiting displays of mulberry trees for seed dispersal by birds in Kansas.

We observed a fruiting Mulberry tree for 67 hours in the spring of 2016 and 2017 in Fort Smith, Arkansas (Sebastian Co.). The 15-year old, 12-meter (40-feet) tall tree stood by itself in a suburban lawn. This study had three main objectives: 1) to characterize the species composition and abundance of birds visiting the fruiting tree in the spring, 2) to quantify the foraging behavior of bird species in terms of foraging rate and foraging tier in the canopy, and 3) to study inter- and intra-specific aggressive interactions in the avian frugivore community.

Materials and Methods

In both years, we started formal observations immediately after commencement of significant bird frugivory activity, and observations ceased when bird activity declined. Observations were conducted from a porch about 20 meters from the tree in the early morning

(0700-0900 hrs) or late evening (1800-2000 hrs) using 10x42 binoculars and Olympus WS-852 digital voice recorders. We conducted five-minute scans (after Athreya 1997), during which the following parameters were recorded: species visited, number of individuals of each species, and foraging tier (upper half of canopy, lower half of canopy, and ground). Scans were done three times an hour, the first at the top of the hour, the second starting 20 minutes past the hour, and third starting 40 minutes past the hour. Every effort was made to avoid double counts. In instances where there was a lot of influx and egress of flocks, only the maximum number in the tree at any one time during that scan period was recorded. A total of 172 five-minute scans of the tree was performed, with 56 scans in 2016 and 116 scans in 2017. Of these, 105 scans were in the morning and 67 in the evening. These scans give snapshots of which birds were present together. In the 15-minute intervals between the scans, observations of aggressive interactions and foraging behaviors were recorded. Aggressive interactions were recognized when one bird chased or attacked another. Both the aggressor and supplanted species were recorded.

Foraging rate was recorded by following individual birds in the tree canopy. Each bird was followed for a maximum of 5 observations of fruit consumed, to avoid the dependency bias associated with sequential observations (Morrison 1984; Wiley *et al*. 2015). The number of fruits consumed was divided by the number of seconds the individual bird was tracked and then converted to fruits per minute. Because the act of plucking a fruit often drew the observer's attention to the bird, it produced a bias when the observation period was small, by overestimating foraging rates. To correct for this bias, 132 observations lasting less than 30 seconds were discarded from the analysis, leaving 349 viable observations. Qualitative information was noted regarding fruit procuring behavior.

Excel, Minitab, and SPSS were used in the statistical analysis. Quartiles were computed using the Minitab/SPSS method. The letter *s* represents the standard deviation of the number of individual birds in the sample. Numbers listed in brackets represent 95% confidence intervals. All hypothesis tests used an alpha level of 0.05. Because of large sample sizes, sample means are approximately normal, so hypothesis tests of means are two-tailed, two independent sample *t*-tests. Tests of proportions used a binomial distribution to compute two-tailed *p*-values. The Shannon-Weiner Diversity Index (Molles 2016) was used as a measure of species diversity.

Results

Species Composition

During the 172 scans a total of 3325 observations of individual birds of 30 species was recorded (Table 1). Two additional bird species were observed between scans. A complete list of all 32 species, along with number of observations, mean number per scan, and the percentage of scans with at least one bird of the species, is presented in Table 1.

Nearly 77% of observed birds represented just two species: Cedar Waxwings (51.4%) and American Robins (25.4%). Most of the data analysis here pertains to the 9 most abundant species. Collectively, the 23 species which are not part of this top 9 accounted for only 4% of the total observations of individual birds, with each of the 23 species making up less than 1% of the total observations and occurring in fewer than 14% of the scans.

Number of Birds

The tree often teemed with bird activity. The total number of birds per scan ranged from 2 to 66 with a mean of 19.3 ([17.8, 20.9], *s* = 10.1) and median of 18 [16, 19]. The distribution of total number of birds per scan is approximately normal, with most of the scans having 8-29 total birds and few scans having either fewer or more birds (Fig. 1).

In contrast, when disaggregated by species, the distributions of birds per scan are highly skewed to the right (Fig. 2). For all the species, the most frequent number of birds per scan was 0 with the number of scans decreasing as the number of birds per scan increased.

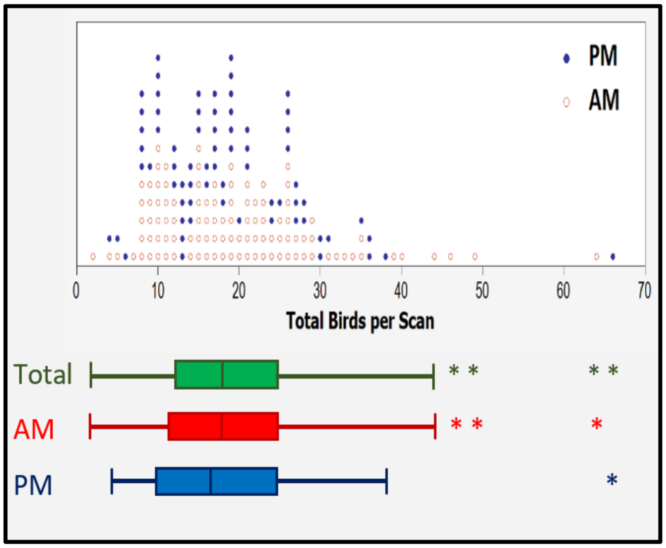

Fig. 1. Total birds per scan (all species combined, one dot per scan).

Table 1. Frequency of observations of birds in the tree, by species, and their method(s) of feeding.

Rank	Common Name	Scientific Name	Number observed during scans	Mean number per scan	Percentage of scans present	Fruit Procuring Method*
1	Cedar Waxwing	*Bombycilla cedrorum*	1709	9.94	76	a, e
2	American Robin	*Turdus migratorius*	843	4.90	87	a, c, e
3	Northern Mockingbird	*Mimus polyglottos*	177	1.03	57	a, c, e
4	Rose-breasted Grosbeak	*Pheucticus ludovicianus*	131	0.76	44	b
5	Swainson's Thrush	*Catharus ustulatus*	90	0.52	42	a, e
6	Gray Catbird	*Dumetella carolinensis*	73	0.42	31	a
7	House Finch	*Haemorhous mexicanus*	67	0.39	23	a
8	Eurasian Starling	*Sturnus vulgaris*	54	0.31	17	a, c
9	Baltimore Oriole	*Icterus galbula*	41	0.24	14	a
10	Mourning Dove	*Zenaida macroura*	22	0.13	11	
11	Northern Cardinal	*Cardinalis cardinalis*	22	0.13	10	a
12	Red-bellied Woodpecker	*Melanerpes carolinus*	17	0.10	9	a
13	House Sparrow	*Passer domesticus*	14	0.08	6	
14	Eastern Kingbird	*Tyrannus tyrannus*	11	0.06	3	d
15	Tennessee Warbler	*Oreothlypis peregrina*	11	0.06	6	
16	Brown Thrasher	*Toxostoma rufum*	7	0.04	4	a
17	Nashville Warbler	*Oreothlypis ruficapilla*	7	0.04	3	
18	Brown-headed Cowbird	*Molothrus ater*	5	0.03	2	
19	Painted Bunting	*Passerina ciris*	5	0.03	3	a
20	White-crowned Sparrow	*Zonotrichia leucophrys*	4	0.02	2	a
21	Summer Tanager	*Piranga rubra*	3	0.02	2	a
22	American Goldfinch	*Spinus tristis*	2	0.01	1	a
23	Pine Siskin	*Spinus pinus*	2	0.01	1	
24	White-throated Sparrow	*Zonotrichia albicollis*	2	0.01	1	
25	Chipping Sparrow	*Spizella passerina*	1	0.01	1	
26	Indigo Bunting	*Passerina cyanea*	1	0.01	1	
27	Lincoln's Sparrow	*Melospiza lincolnii*	1	0.01	1	
28	Magnolia Warbler	*Setophaga magnolia*	1	0.01	1	
29	Yellow-bellied Sapsucker	*Sphyrapicus varius*	1	0.01	1	
30	American Crow	*Corvus brachyrhynchos*	1	0.01	1	
31	Downy Woodpecker	*Picoides pubescens*	0	0	0	
32	Scarlet Tanager	*Piranga olivacea*	0	0	0	

*a: berry plucked, then swallowed whole
b: bits of berry plucked and eaten, without removing from branch
c: berry plucked and taken away from tree
d: berry removed from branch while bird in flight
e: fallen berries eaten from ground
blank: species that may have visited the tree for arthropod prey, or whose frugivory method was unclear

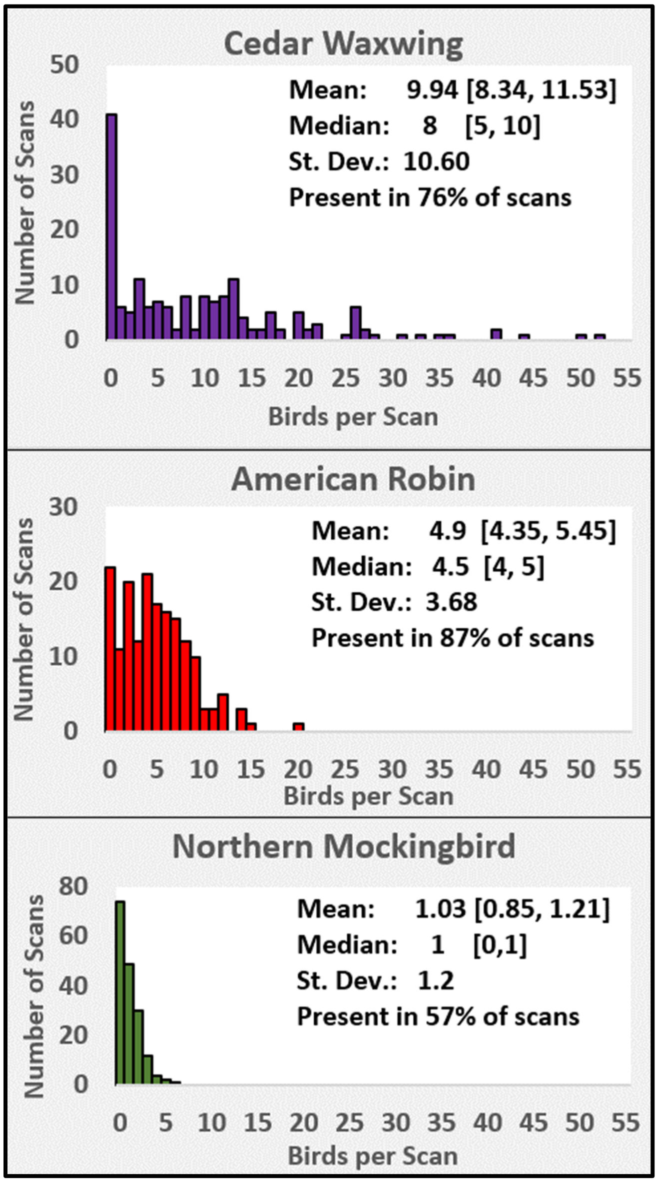

Fig. 2: Birds per scan for the 3 most common species. See Appendix for sample sizes.

Northern Mockingbirds exhibited this typical pattern, being absent in 43% of the scans and typically appearing with 1-3 birds per scan when present (Fig. 2). American Robins were present in the most scans, but almostalways appeared fewer than 12 at a time (Fig. 2). The most observed species, Cedar Waxwings, appeared in fewer scans than American Robins, but often appeared in larger groups of 15 or more at a time. All the outliers in the total birds per scan (Fig. 1) are explained by the presence of large flocks of up to 52 Cedar Waxwings (Fig. 2), whose numbers per scan was the most variable of all species. They typically appeared in groups of 2-20 birds, with an approximately uniform distribution within this common range (Fig. 2). The rest of the species each appeared in fewer than 45% of the scans and rarely showed more than 2 at a time when present, making histograms uninformative (Table 1).

Temporal Pattern of Visitations

Unlike the studies of Athreya (1997) and Stapanian (1982), which found a significant peak in bird activity in the morning hours, our data showed no significant difference (p = 0.615) in total birds per scan between morning (AM) and evening (PM) hours (Fig. 1). The overall mean number of birds per scan was 19.6 AM and 18.9 PM.

There was no clear pattern to either the number of birds per scan or number of species per scan through the various days of observation (Fig. 3). Apparently, the abundant and synchronously produced fruit attracted a random assortment of frugivorous bird species present in the area. The variation in the number of birds per scans was influenced largely by the nomadic movements of Cedar Waxwings (Fig. 4).

The mean total number of birds per scan was not significantly different for the two years (20.6 in 2016 and 18.7 in 2017, p = 0.26). However, except for House Finches, the number of birds/scan between the two years was significantly different for each of the 9 common species (Table 2). Cedar Waxwings were more abundant in 2016 (mean 16.5 [13.6, 19.5]) than in 2017 (mean 6.8 [5.2, 8.5]), with all the outliers occurring in

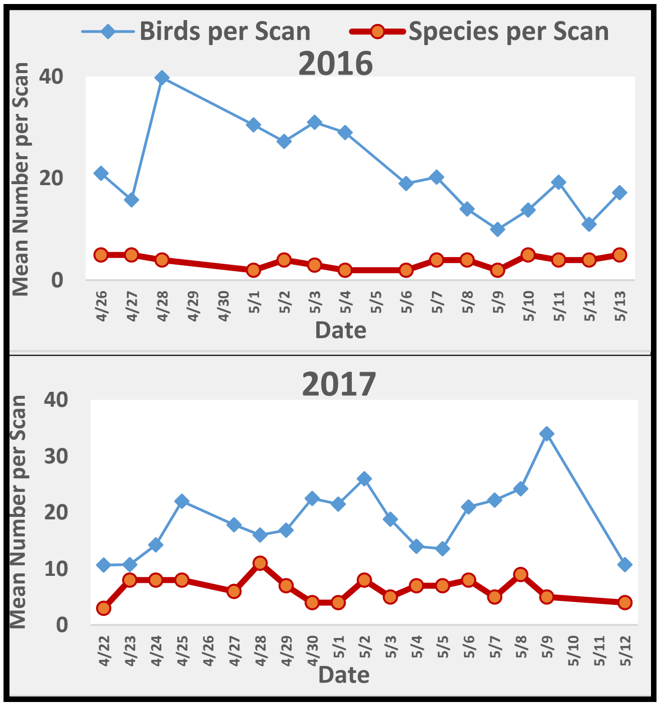

Fig. 3. Mean number of birds per scan and mean number of species per scan by date.

Table 2. Mean number of birds per scan by year*.

Species	2016	2017	*p*-value
Cedar Waxwing	**16.53**	6.84	0.000
American Robin	1.93	**6.30**	0.000
Northern Mockingbird	0.36	**1.34**	0.000
Rose-breasted Grosbeak	0.33	**0.97**	0.000
Swainson's Thrush	0.09	**0.73**	0.000
Gray Catbird	0.11	**0.57**	0.000
House Finch	0.33	0.42	0.472
Eurasian Starling	0.00	**0.46**	0.000
Baltimore Oriole	0.04	**0.33**	0.000

*Numbers in bold indicate significant difference between the years.

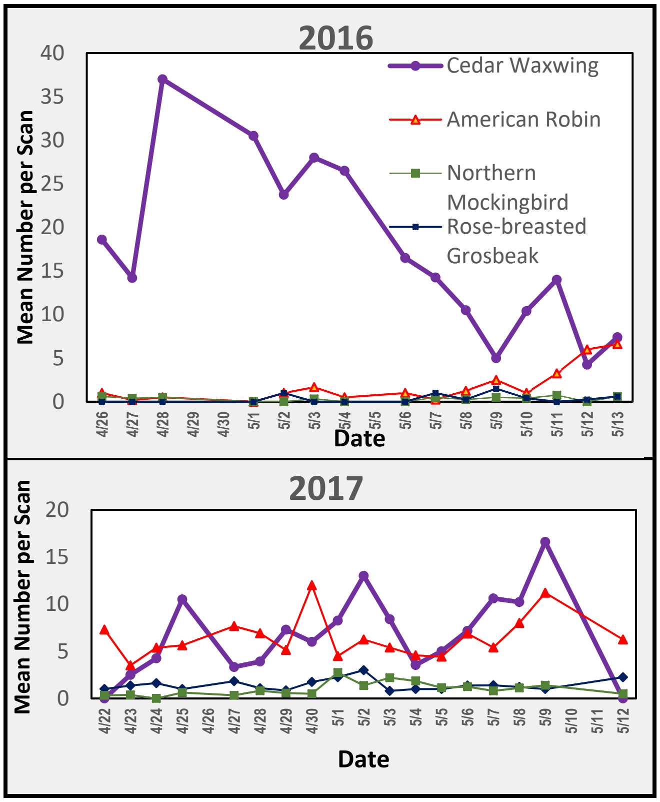

Fig. 4. Mean number of birds per scan by species and date.

2016 (Figs. 1, 2, 4, Table 2). They were also considerably more abundant than the next most abundant species in 2016, but they were similar in numbers with American Robins in 2017 (Fig. 4). The 7 other prevalent species showed little variation in the mean number of birds per scan for each day in 2016 and 2017. Since the *p*-values for the comparisons for these species are all less than 0.0005, even if applying a Bonferroni-Holm correction for multiple tests, these species were significantly more abundant in 2017 than in 2016 (Fig. 4, Table 2). Note that Figure 4 shows only the top four species to reduce clutter.

Table 3. Vertical separation of species within the tree.

Species	% Ground	% Upper	*p*-value*
Significantly more in the upper half of the tree			
Brown Thrasher**		100	0.016
House Finch		96	0.000
Red-bellied Woodpecker**		94	0.000
Baltimore Oriole		90	0.000
Rose-breasted Grosbeak		87	0.000
House Sparrow**		86	0.013
Eastern Kingbird**		82	0.012
Tennessee Warbler**		82	0.012
Cedar Waxwing		63	0.000
Significantly less in the upper half of the tree			
Northern Mockingbird	18	40	0.010
American Robin	43	35	0.000
Swainson's Thrush	8	31	0.000
Mourning Dove	91	9	0.000

*The alternate hypothesis is that the percentage in the upper tier is not equal to 50%.
**Species appeared in less than 10% of scans.

Foraging Tier of Tree

Based upon anecdotal observations from 2015 and earlier we knew that there were many birds of many species foraging in the tree simultaneously. We suspected that this might be facilitated in part by different species using different parts of this mature tree. To test this hypothesis and to gain information about preferred foraging habits of different species, we investigated if there were any spatial differences in bird usage among various tiers of the tree canopy. Among the 4 most prevalent species, Cedar Waxwings and Rose-breasted Grosbeaks were significantly more often in upper half of tree than lower. American Robins and Northern Mockingbirds were significantly more often in lower half or on the ground (Table 3). There were high percentages of observations of American Robins and Mourning Doves on the ground (Table 3), where they often forage. Even if applying a Bonferroni-Holm correction for multiple tests, the 8 species in Table 3 which appeared in more than 10% of the scans show significant vertical separation within the tree. Because of the smaller sample size, the conclusions for the 5 species appearing in fewer than 10% of the scans is less conclusive. The 18 species not listed in Table 3 showed no significant vertical separation.

Species Diversity

The number of species per scan (Fig. 3) varied from 1 to 9 with a mean of 4.6 ([4.3, 4.8], *s* = 1.8) and median 5 [4, 5]. The mean number of species per scan was significantly higher in 2017 than in 2016 (*p* = 0.00) (Table 4).

The species diversity increased with height of foraging tier (Table 4). The mean number of species per scan was significantly higher in upper half of canopy than lower (*p* = 0.00), and mean number of species per scan was significantly higher in lower half of tree than ground (*p* = 0.00). There was no significant difference in mean number of species per scan by time of day (*p* = 0.27), even though there was a higher diversity index in morning (Table 4).

Aggressive Interactions

All 346 recorded aggressive interactions are portrayed in the weighted directed graph (Fig. 5). To rank species by aggressiveness (Table 5), we adjusted for number of birds recorded. While aggressive interactions were primarily observed between the 5-minute scans, the total number of times that a bird of that species was observed in aggressive action against another bird was divided by the total number of times birds of that species was observed during scans. These percentages (column 2 of Table 5), were the basis by which we ranked aggressiveness.

Of the 16 species observed in aggressive interactions, Northern Mockingbird was the most aggressive (Table 5). American Robins ranked third in aggression largely due to their aggressive behavior toward other American Robins. This species was the victim of interspecific aggression more than it was the instigator. Cedar Waxwing, Swainson's Thrush, Gray Catbird, and House Finch were among the least aggressive species (Fig. 5, Table 5). We once even observed a Cedar Waxwing feeding another Cedar Waxwing.

The proportion of intraspecific aggression (68%) was significantly higher than that of interspecific aggression (*p* = 0.00) (Fig. 5, Table 5).

Table 4. Species Diversity Measures.

	Total number of species	**Mean number of species per scan**	**Shannon-Weiner Diversity Index**
Upper half	27	3.3	1.21
Lower half	20	2.1	0.76
Ground	10	1.2	0.46
AM	26	4.4	1.24
PM	22	4.8	1.00
2016	21	3.2	0.67
2017	25	5.2	1.45

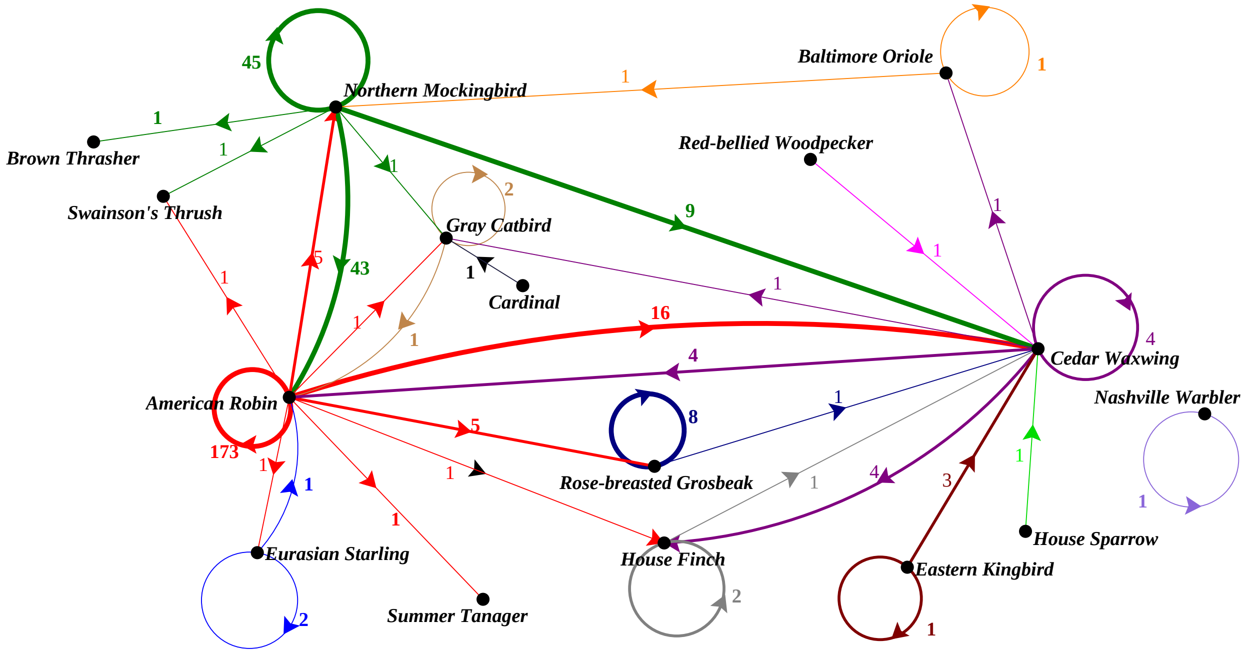

Fig. 5. Weighted directed graph of aggressive interactions. Arrows point from aggressive species to supplanted species. Weights indicate number of observations. Thicker lines indicate higher number of observations. Loops (circles) are intraspecific aggressions and other edges are interspecific aggressions.

Table 5. Aggressive interactions by species.

Species (most aggressive to least aggressive)	Percentage of aggressions per number scanned	Total times aggressor	Intra-specific aggressions	Inter-specific aggressions	Number of species aggressed	Number of times supplanted by another species	Number of species supplanted by
Northern Mockingbird	56	100	45	55	6	6	5
Eastern Kingbird	36	4	1	3	2	0	3
American Robin	24	204	173	31	9	49	8
Nashville Warbler	14	1	1	0	1	0	3
House Sparrow	7	1	0	1	1	0	1
Rose-breasted Grosbeak	7	9	8	1	2	5	3
Red-bellied Woodpecker	6	1	0	1	1	0	1
Eurasian Starling	6	3	2	1	2	1	3
Baltimore Oriole	5	2	1	1	2	1	3
Cardinal	5	1	0	1	1	0	2
House Finch	4	3	2	1	2	5	3
Gray Catbird	4	3	2	1	2	4	3
Cedar Waxwing	1	14	4	10	5	32	5
Swainson's Thrush	0	0	0	0	0	2	0
Brown Thrasher	0	0	0	0	0	1	0
Summer Tanager	0	0	0	0	0	1	0
Total (16 species)		**346**	**234 (68%)**	**112 (32%)**	**13**		

Foraging Rates

Of the six species with at least 20 observed foraging sequences of at least 30 seconds, American Robins were the fastest foragers (mean 2.3 fruits/minute [2.0, 2.6], *s* = 1.27) and Rose-breasted Grosbeaks were the slowest (mean 1.2 fruits/minute [1.0, 1.3], *s* = 0.61) (Fig. 6). See Fig. 8 for sample sizes.

American Robins, the fastest foragers, were tracked for the second least time (mean sequence of 43 sec.) (Fig. 7). Rose-breasted Grosbeaks were not only the slowest foragers, they were also tracked the longest (mean observed foraging sequence of 178 seconds). The true foraging sequence length for Rose-breasted Grosbeaks is bound to be much higher, because in 10 of the 54

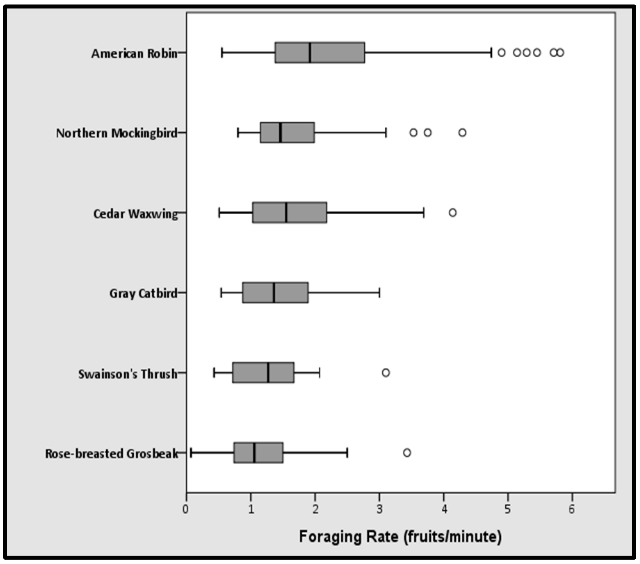

Fig. 6. Foraging rates of the top 6 species.

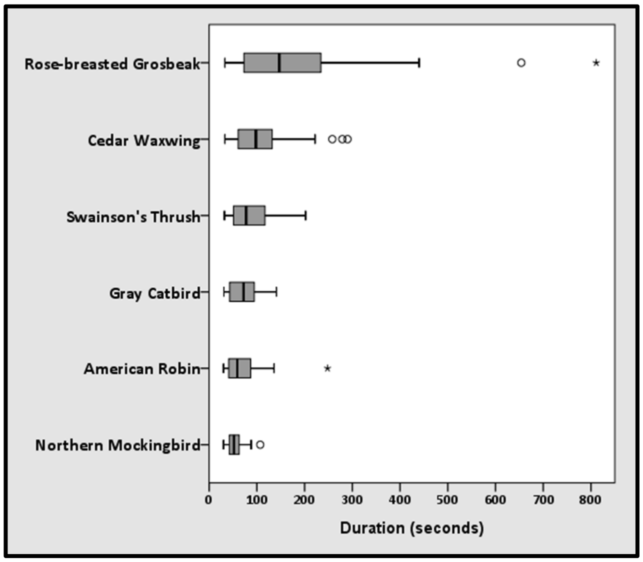

Fig. 7. Length of foraging sequence observed (up to 5 fruits).

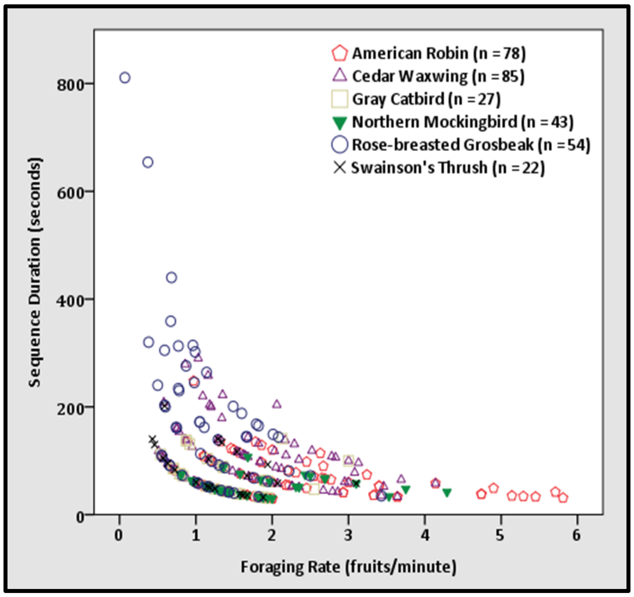

Fig. 8. Relationship between foraging rate and observed foraging sequence for the top 6 species. Each symbol represents one observation.

observed foraging sequences we ceased observations of this species when the self-imposed maximum of 5 fruits was reached. Overall, birds that ate fruits at a slower rate tended to be observed for longer periods of time, and those that foraged faster tended to disappear in the foliage faster and thus could not be tracked longer (Fig. 8).

Fruit Procuring Behavior

Five distinct types of fruit obtaining maneuvers were recognized: 1. berry plucked, then swallowed whole, 2. bits of berry plucked and eaten, without removing from branch, 3. berry plucked and taken away from tree, 4. berry removed from branch while bird in flight, and 5. fallen berries eaten from ground (after Robbins *et al*. 1975). These qualitative notes are given in Table 1.

Discussion

The synchronously produced abundance of fruit on the mulberry tree attracted many birds. In particular, Cedar Waxwings and American Robins were attracted to the tree in large numbers. Many other local and migratory species were also attracted. Despite some aggressive interactions among the birds, the fruit was abundant enough to support multiple species simultaneously.

Most of the aggressive behavior was between birds of the same species, suggesting that territoriality, rather than interspecific competition, shapes these behaviors in synchronously fruiting trees. Some degree of species coexistence was facilitated by species foraging in different vertical zones of the canopy. The two most abundant species, Cedar Waxwings and American Robins, were typically separated in this manner, with American Robins often feeding on fruit which had fallen to the ground, and most Cedar Waxwings foraging near the top of the tree. Even though the presence of the more aggressive American Robins tended to decrease the presence of Cedar Waxwings, their interactions were typically limited to the middle of the tree, allowing both species to appear in most of the scans.

There were few strong temporal patterns to the visitations. This suggests that birds take advantage of the easy and abundant source of food, with little need for temporal separation to minimize competition. This general lack of interspecific exclusion from the tree was also observed in a fruiting mulberry tree in Michigan (Robbins *et al*. 1975) and in a fruiting *Ficus* in India (Athreya 1997). The presence of predominantly insectivorous birds in the tree, such as Eastern Kingbird and three species of warblers, indicates that mulberry fruits may be consumed opportunistically by some migrants (Murphy 1996; Mowbray 1999; Mack and Yong 2000; Wiley *et al*. 2015). The unpredictability in the number of birds present at different times can be largely explained by the nomadic behavior of flocks of Cedar Waxwings, which account for over half of the data. This also explains the low species diversity index in 2016 when Cedar Waxwings dominated the community.

This study was limited to a single tree in a suburban location. Care should be taken to not overgeneralize the results reported here. Factors such as location (urban, suburban, forest, as well as geographic location within the migratory path of certain species), presence of other fruiting trees of the same or different species, size of the tree, and other factors which we did not investigate would likely vary the makeup of species foraging in a mulberry tree.

Furthermore, there were significant differences in species composition foraging in the tree between the two years of study. Given this marked interannual variation, we suggest that future such studies should ideally be conducted over multiple years to get a better picture of the composition of these bird assemblages. Most studies cited in this paper were conducted over a single fruiting season. Although much of the annual variation in this study can be explained by the presence of larger flocks of Cedar Waxwings in 2016, more significant differences could occur if (as in 2018) the time of fruiting is significantly delayed due to late cold weather, so that the fruiting phenology may interact differently with migratory patterns.

The 32 species we recorded from the tree is similar to some tropical bird species richness in fruiting *Ficus* (Athreya 1997; Tello 2003) and *Cecropia* trees (Estrada *et al*. 1984). The data we presented in this paper clearly showed that the abundant fruit of the mulberry is a popular and easy source of fuel for many species passing through on migration, as well as for summer residents in the area. Given that this tree was such a magnet for birds, it is ironic that more research has not been done to document this spectacular annual phenomenon of avian frugivory in North America.

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Lindsey Rice and Ashley Cooper helped with data collection as part of the Undergraduate Research course at the University of Arkansas - Fort Smith (UAFS). Lindsey also assisted with some data entry and analysis. The UAFS Department of Biology provided equipment and financial support for conference travel. This paper is dedicated to the memory of the late Dr. Kimberly G. Smith, who served as an inspiration, mentor, and valued colleague to the authors and many others throughout his career.

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Deep Learning-based framework for Autism functional MRI Image Classification

X. Yang[1*], S. Sarraf[2,3], and N. Zhang[4]

[1]*Department of Math & Computer Science, Southern Arkansas University, USA*
[2]*Department of Electrical and Computer Engineering, McMaster University, Hamilton, Ontario, Canada*
[3]*The Institute of Electrical and Electronics Engineers, IEEE*
[4]*Computational Science Ph.D. Program, Middle Tennessee State University, USA*

[*]Correspondence: XinYang@saumag.edu

Running Title: Deep Learning for Autism fMRI Image Classification

Abstract

The purpose of this paper is to introduce the deep learning-based framework LeNet-5 architecture and implement experiments for functional MRI image classification of Autism spectrum disorder. We implement our experiments under the NVIDIA deep learning GPU Training Systems (DIGITS). By using the Convolutional Neural Network (CNN) LeNet-5 architecture, we successfully classified functional MRI image of Autism spectrum disorder from normal controls. The results show that we obtained satisfactory results for both sensitivity and specificity.

Introduction

The human brain is the most complex organ of human beings, which could include 100 billion neurons with more than a trillion connections. Although technology has been developing, humans are constantly exploring the mysteries of the brain, we still cannot prevent or treat brain disorders such as Autism Spectrum Disorder (ASD), Alzheimer's disease, stroke, and so on. In order to make breakthroughs in brain disease treatment and prevention, many academic institutions and scientists have conducted a lot of research studies in these fields (Olivito *et al.* 2017; Igelström *et al.* 2016; Dajani and Uddin 2016). Among all these research studies, neuroimaging technique has become the most commonly used imaging technique for the study of the human brain. The most commonly used neuroimaging techniques include Magnetic Resonance Imaging (MRI), functional Magnetic Resonance Imaging (fMRI), Positron Emission Tomography (PET) and so on (Poldrack *et al.* 2011). These neuroimaging techniques can provide us insights into the neural characteristics of the human brain and also help the diagnosis and prevention of many diseases. However, the analysis of neuroimaging data is extremely complicated, which requires the raw image preprocessing and efficient statistical analysis. Therefore, the main purpose of this paper is to implement the deep-learning based framework for the functional MRI image classification. The deep-learning framework is a very efficient learning framework for image analysis.

During the past decade, autism spectrum disorder (ASD) prevalence rate has increased dramatically. ASD is a neutrally based psychiatric disorder which is characterized by the impaired development of social interactions and communication skills. Although strong genetic factors are suspected, ASD continues to be diagnosed using symptom-based clinical criteria, and its etiology remains unestablished. Social and communicative impairments are the core symptoms of ASD, and a lot of research indicates that these impairments are associated with functioning and connectivity of cortical networks (Olivito *et al.* 2017; Igelström *et al.* 2016; Dajani and Uddin 2016). Recent epidemiological studies have shown that the incidence of autism is increasing. Although many researchers are currently studying ASD, no one has applied deep learning to classify ASD functional MRI images. In this work, we implement a deep-learning based framework for Autism Spectrum disorder fMRI image classification. Further research in this area could provide helpful information in gaining a better understanding of the neuronal pathology of autism in children.

Problem Statement and Related Work

Artificial Neural Networks have been introduced since the 1940s (Heaton 2015). However, because of limited computing power, people were not aware of the advantages of Artificial Neural Networks. With the development of advanced computing power, people began to recognize its superiority. Because Artificial Neural Networks can efficiently recognize patterns, they have been widely applied in many fields, such as speech recognition (Lippmann 1987; Lang *et al.* 1990; Fels and Hinton 1993), image classification (Rowley *et al.* 1998; Lawrence *et al.* 1997), and disease diagnosis (Khan *et al.* 2001; Kato *et al.* 2007; Petrosian *et al.* 2001). The accuracy of these works is highly dependent upon the feature extraction stage, which is a traditional analysis step for pattern analysis and classification (Rawat and Wang 2017).

CNN was proposed by LeCun *et al.* (1989**)**. They used backpropagation (LeCun *et al.* 1999) to train the architecture of the networks, and it has been successfully applied to the recognition of handwritten zip code digits provided by the US Postal Service. Their work has demonstrated that the Convolutional Neural Networks can directly deal with large low-level information rather than feature vector. This has overcome the traditional pattern analysis and classification challenges. Although the Convolutional Neural Networks achieved its initial successful application, it is difficult to implement, and it is really slow. As a result, it was not widely used until 20 years later due to the limitation of computing power.

In recent decades, the computational power of computers has increased dramatically, and neural networks have once again received widespread attention. In 2006, Chellapilla *et al.* first introduced GPUs (Graphics Processing Units) implementation of Convolutional Neural Networks. The GPUs contain parallel pipelines which speed up the computations (Chellapilla *et al.* 2006). In addition to improving computing power, some researchers have made some improvements to the original Convolutional Neural Network algorithm. The first application of maximum pooling was proposed by Huang *et al.* (2007). In 2012, Alex Krizhevsky *et al.* introduced deep convolutional neural networks to large-scale images recognition in the paper (Krizhevsky *et al.* 2012). They classified 1.3 million images in the LSVRC-2010 ImageNet training set into 1000 different classes, which yielded more accurate results than any previous algorithms. Since then, deep Convolutional Neural Networks have been applied to various fields with large-scale datasets (Oquab *et al.* 2014; Karpathy *et al.* 2014; Ji *et al.* 2013).

Because of the powerful analysis capabilities, deep neural networks have become one of the hottest topics in Pattern Recognition and Artificial Intelligence (Schmidhuber 2015). Large corporations and many research institutions are all paying attention to these fields. For a long time, people have always believed that computers are not intuitive at all. However, after a long period of research by scientists, this understanding has been challenged. In 2015, Google DeepMind developed a computer program called AlphaGo which plays the board game go. To capture the intuitive of the game, the AlphaGo combines advanced tree search with deep neural networks (Silver *et al.* 2016). In 2016, AlphaGo becomes the first computer program to beat a 9-dan professional Go player.

Although many researchers are currently studying ASD, no one has applied deep learning framework to classify ASD functional MRI images. The purpose of this paper is to introduce deep learning-based framework with LeNet-5 architecture and implement the experiments for functional MRI image classification of ASD. This efficient deep learning process will provide us some insights into the neuronal pathology of autistic children, and also help the diagnosis of the early stage of autism in children.

Overview of the LeNet-5 Architecture

Neural Networks consist of three types of layers: a convolution layer, a pooling layer, and fully connected layer. The convolution layer extracts feature from the original input images. It detects the same feature at all locations on the input image. The different feature detector in the layer can extract different types of local features (LeCun *et al.* 1989). The output from this layer is called a feature map. Once the features have been detected, the exact location is not so important. The pooling layer is used to reduce the spatial resolution of the feature maps. The pooling layer reduces the dimensions of the feature maps in the convolution layer.

In LeNet-5, the pooling layer is called the subsampling layer. The fully connected layer is the hidden layer of Artificial Neural Networks, which fully connects the features from convolution layer and pooling layer to produce the output for image classification. A typical convolutional neural network of LeNet-5 is shown in Figure 1. LeNet-5 has a total of 7 layers, excluding the input layer (LeCun *et al.* 1989). In our experiments, the input is a 64*64 color image. Layer C1 is a convolution layer with 20 feature maps. Each unit in each feature map is a convolution between a 5 by 5 neighborhood in the input image and a feature detector. 20 different feature detectors produce 20 feature maps. Layer P1 is a pooling layer with a stride of 2. The feature map from the C1 layer is connected to a 2 by 2 pooling filter; max pooling is implemented to reduce the spatial resolution of the feature maps. The max pooling reduces the dimension of the feature maps. Layer C2 is a convolution layer with 50 feature maps. Each unit in each feature map is a convolution between a 5 by 5 neighborhood in the P1's feature maps and a feature

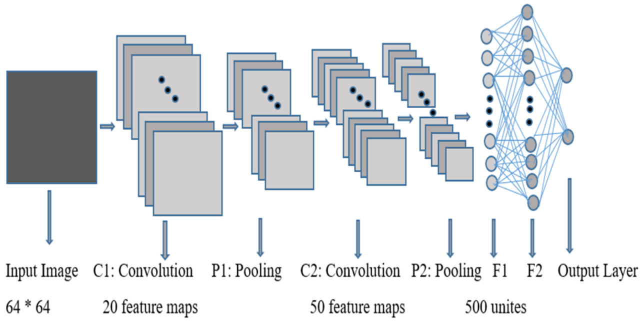


Figure 1: LetNet-5 Architecture

detector. Layer P2 is a pooling layer with a stride of 2. The feature map from the C2 layer is connected to a 2 by 2 pooling filter; max pooling is implemented to reduce the spatial resolution of the feature maps. Layer F1 contains 500 units and is fully connected to P2. As in artificial neural networks, units in F1 layer compute a dot product between their input vector and the weight vector, then pass through a rectifier activation function to produce the inputs for Layer F2. Units in Layer F2 also compute a dot product between their input vector and the weight vector (LeCun *et al.* 1989). Finally, the output layer passes through a soft-max activation function to get probabilities between 0 and 1 for each class. The class label with the highest probability is assigned to the corresponding input image.

Experiments Data Set

In this work, we implement a deep learning-based framework on a NVIDIA GPU platform. By using the CNN LeNet-5 architecture, we successfully classified functional MRI image of Autism Spectrum Disorder as being distinct from Normal Controls.

In our work, all fMRI data set are downloaded from an Image & Data Archive of ABIDE (Autism Brain Imaging Data Exchange) (https://ida.loni.usc.edu/). Initially, we downloaded 100 subjects in total from the Image & Data Archive: 50 controls and 50 autisms. However, in order to provide more meaningful analysis results, only 16 controls and 11 autisms from the 100 subjects group are qualified for our analysis after screening. The subjects were removed who has the wrong image information. And all subjects we used in this paper are collected under the same scanning protocol and parameters. The chosen subjects include both female and male, whose age is between 9 and 20.

The analysis of fMRI data is extremely slow due to its high dimensionality. For each subject, the scanner collects 64*64*29*210 images. There are 29 slices for each subject, the dimension of each slice is 64*64, and in total there are 210 time courses. To improve the efficiency of the data analysis process, we implement the NVIDIA Deep Learning GPU Training System (DIGITS). DIGITS can be used to train the deep neural network (DNNs) for image classification with high accuracy (https://developer.nvidia.com/digits). To train DNNs on GPU systems, we installed DIGITS in Ubuntu 16.0 under NVIDIA GeForce GTX 1060 and used LeNet-5 under deep learning framework Caffe.

Image Preprocessing

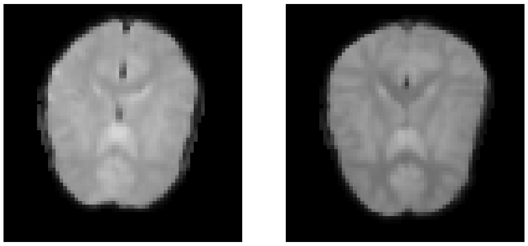
The analysis of fMRI data is incredibly complicated. First, it has particularly high dimensionality. Second, the data is interfered by other factors such as head movement, variability between individuals, and variability through time within individuals. Therefore, applying imaging preprocessing to fMRI data will provide more meaningful interpretation of the analysis results. The most common image preprocessing packages include SPM, FSL, AFNI, Brain and Voyager (Poldrack *et al.* 2011).

In our experiments, we use FMRIB Software Library (FSL) for the image preprocessing. The first step is brain extraction, to do this, we need to remove the skull from the anatomical scans (structural MRI) by using FSL BET brain extraction. The second step is fMRI data preprocessing; and this step is done by using FSL FEAT fMRI analysis. This step will register the fMRI data by using the extracted brain image from step 1, and the standard space we used here is MNI152_T1_2mm_brain. After the fMRI data have been preprocessed, the third step is to convert NII files to PNG images. The third step is done under OpenCV library in Python. When all NII files have been converted to PNG images, we can apply deep learning framework to these preprocessed PNG images (Sarraf and Tofighi 2016).

Results

To make the experiments more meaningful, first, we combine both Autism and normal controls images into one folder. Then we randomly split all images to 4 folders. 110376 images (3-fold) are taken as the training dataset; there are 65100 NC images and 45276 AT images among them. 36793 images (1-fold) are taken as the testing dataset; there are 21700 NC images and 15093 AT images among them. We repeat this process five times with different random seed. Figure 2 shows image slice examples for both AT and NC. Table 1 and Table 2 summarize the training and testing results. The sensitivity and specificity for test data are listed in Table

2. Here NC means normal controls, AT means autistic subjects. We have applied 30 epochs for each training process, with a batch size of 100. The training loss results for training 1 are displayed in Figure 3. From Figure 4, we can see that the test data accuracy rate converges to 100% after 2 epochs. The training loss and test data accuracy rate figures for the other 4 training sets are similar to training 1.



AT slice NC slice

Figure 2: Autistic subject and Normal Control fMRI image slice

Table 1: Training data

Training Data	NC Count	AT Count	Training Time
Train1	65100	45276	10 min 1 sec
Train2	65100	45276	10 min 1 sec
Train3	65100	45276	10 min 7 sec
Train4	65100	45276	10 min 1 sec
Train5	65100	45276	9 min 56 sec

Table 2: Testing Results

Testing Data	NC Count	AT Count	Sensitivity	Specificity
Test 1	15093	21700	99.99%	100%
Test 2	15093	21700	99.98%	100%
Test 3	15093	21700	99.99%	99.99%
Test 4	15093	21700	99.99%	100%
Test 5	15093	21700	99.99%	100%

By using the CNN LeNet-5 architecture, we successfully classified functional MRI image of ASD from Normal Controls. The results listed in Table 2 show that we obtained satisfactory results for both sensitivity and specificity. Because of the high speed of the GPU implementation, the training model can be trained in a concise time even though the training data are large.

Figure 3: Loss function for training data

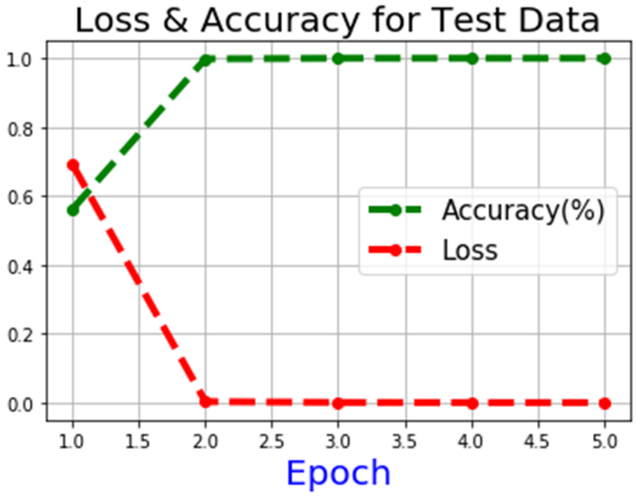


Figure 4: Loss function and classification accuracy rate for test data

Conclusion

Over the last several years, deep neural networks have played an increasingly important role in the field of pattern recognition and machine learning. In this paper, we implement CNN LetNet-5 architecture for autism fMRI image classification under the NVIDIA GPU platform. By using the CNN LeNet-5 architecture, we successfully classified functional MRI image of ASD from Normal Controls. The results in Table 2 demonstrate that we obtained satisfactory results for both sensitivity and specificity.

Although many researchers have studied functional connectivity of ASD, no one has applied deep learning framework to classify ASD functional MRI images. In this paper, we implemented slice-level image classification by using deep learning CNN. This study can help us the further research to locate the brain pathology of ASD subjects and identify the autism biomarkers, which will be our future work.

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Small Mammal Abundances in a Grassland and Forest Area at the Lake Fayetteville Environmental Center, Arkansas

D.A. James, S.A. Bartholomew, A. Deshwal, and P. Panwar

Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701

*Correspondence: djames@uark.edu

Running Title: Small Mammal Trapping at Fayetteville, Arkansas

Abstract

We examined changes in abundance of small mammals in forest and prairie-grassland habitat at Lake Fayetteville, Arkansas over a period of 32 years. We estimated the population size of small mammals using a mark-recapture method by capturing small mammals employing rat-sized Sherman live traps laid out in a grid with 8 rows of traps, 15 traps per row, 9.14 m (30 feet) between traps and rows covering an area of 1.01 ha (2.5 acres) in size. Six species of mammals were trapped in the prairie-grassland and three species were captured in the forest habitat. In the forest, the white-footed deermouse (*Peromuscus leucopus*) was greatest in 1998 and in 2006. In the prairie-grassland, the population of hispid cotton rats (*Sigmodon hispidus*) were greatest in 2004, 2008, 2010 and 2014 and have increased over the years with the change in grass composition. The prairie-grassland in 1962 was mainly a broomsedge bluestem (*Andropogon virginicus*) field but as time progressed more and more prairie grasses invaded helped by controlled burns and removal of the invading eastern red cedars (*Juniperus virginiana*). The population of *Sigmodon hispidus* was weakly correlated with the minimum winter temperatures from the previous year.

Introduction

Long term field studies of small mammals are essential for establishing general patterns of population abundance (Rehmeier *et al*. 2005). Small mammals play vital roles in ecosystems, serving as dispersers of fungal spores (Maser and Maser 1988; Pyare and Longland 2001) and seeds (Vander Wall 1993; Vander Wall *et al*. 2001); consumers of plants, seeds, and fruits (Carey *et al*. 1999); and as prey for mammalian and avian predators (Zielinski *et al*. 1983; Forsman *et al*. 1984; Carey *et al*. 1992). Their essential interactions with flora and fauna across multiple trophic levels (Forsman *et al*. 1984; Carey *et al*. 1992) implicates that land management should be based in part on an understanding of the ecology of small mammals.

Population density of small mammals can respond to habitat changes at different scales (Morris 1987). Variation in sizes of population of *Sigmodon hispidus* also appears to be regulated by minimum winter temperature of the previous year (Sealander and Walker 1955; Goertz 1964). The main objectives of this study were to 1) identify small mammal species in forest and prairie-grassland habitat, and 2) examine the population dynamics of small mammals over time.

Methods

The data for small mammal abundance were collected in oak-hickory forest and prairie-grassland at the Lake Fayetteville Environmental Center, Fayetteville, Arkansas. Although no detailed analysis of the vegetation was conducted throughout the years that trapping was conducted at the prairie-grassland area. D. James observed that throughout the years, the prairie-grassland's vegetation was transitioning from an old field mainly consisted of broomsedge bluestem (*Andropogon virginicus*) grass to a vegetation that consisted of diverse prairie grasses. The new grasses observed included little bluestem (*Schizachyyrium scoparium*), big bluestem (*Schizachyyrium gerardii*), switchgrass (*Panicum virgatum*) and indiangrass (*Sogastrum nutans*). This data was collected as part of Mammalogy class trapping experiences from 1983 to 2014.

The population of small mammals in the forest and prairie-grassland was estimated using mark-capture-recapture method (Nichols 1992). We used grid-trapping using rat-sized Sherman live traps laid out in a grid around 1.01 ha (2.5 acres). The grid consisted of eight rows with 15 traps per row. The distance between each trap and each row was 9.14 m (30 feet). The forest grid and prairie grassland grid were nearby each other separated approximately by more than 100 m. The traps were baited with oats and peanut butter paste and cotton (for nesting material). Students in the Mammalogy class checked the traps each morning for three days under the supervision of the instructor. We identified all small mammal species caught in the traps and recorded weight, sex and reproductive condition. Each trapped animal was marked with a unique identifying number using ear-tags and then released. The trap setting was interrupted by a heavy rain in 2014 causing the students to seek cover so that only 0.61 ha (1.5 acres) of the grid was set. In 2008, a hurricane forced wind passed through northwestern Arkansas that blew down many limbs and trees in the study area, making it impossible to establish the forest trapping grid thereafter. Thus, there were only six years of trapping in the forests compared to nine years in the prairie-grassland habitat. Table 1 lists the scientific and common names of the mammals trapped in both the forest and the prairie-grassland. Table 2 and Table 3 show the details of species captured for each year in prairie-grassland and forest respectively.

We estimated the total small mammal population (N) using the following formula

$$N = mn/x$$

where n is the total number of individuals captured on a given night, m is the number of individuals captured and tagged in previous trappings and x is marked individuals (recaptured) trapped on the subsequent night (Giles 1969).

In addition, we recorded the minimum winter temperature for the previous year. Using the software R 3.3.2 software (R Core Team 2016), a regression analysis was conducted for the minimum winter temperature of the previous year and *Sigmodon hispidus* populations in fall of the current year.

We analyzed the change in population size of small mammal population in both forest and prairie-grassland habitat using regression analysis in R. We also analyzed the change in abundance of each species to assess the trends in their population over the duration of the study. The abundance data for year 2014 was excluded from the all analysis because the grid was setup only for .61 hectare and the abundance was corrected to account for difference in trapping area.

Results

Six species of small mammals were trapped in the prairie-grassland habitat and three species were captured in the forest (Table 1). Table 2 and Table 3 show the number of individuals of each species captured for each year in prairie-grassland and forest respectively. The estimated small mammal population for each year in prairie-grassland and forest habitat is listed in Tables 4 and 5 respectively.

A regression analysis on *Sigmodon hispidus* abundance (column in Table 2) showed a significant relationship between abundance and time ($p = 0.0164$, $R^2 = 0.7479$). The regression formula was

$$y = 0.335x – 1999.03$$

where y is the number of *Sigmodon* captured, 0.335 is the slope of the regression line, x is the year and -1999.03 is the intercept of the regression line. This was the only significant relationship found between abundance of each species and time in Table 2.

Even though there was no significant relationship between abundance of *Reithrodontomys fulvescens* and year, the population was greatest in 2002 followed by 2004 (Table 2). There were six species trapped in the prairie-grassland (Table 2). Only in 1983, the grassland subspecies of *Peromyscus maniculatus bairdi* was captured in prairie-grassland.

Regression analysis on the total population in the prairie-grassland habitat (N) in Table 4, showed that N

Table 1. Scientific and common names of mammals captured.

Scientific Name	Common Name
Sigmodon hispidus	Hispid cotton rat
Reithrodontomys fulvescens	Fulvous harvest mouse
Cryptotis parva	Least shrew
Peromyscus leucopus	White-footed deermouse
Mus musculus	House mouse
Peromyscus maniculatus	North American deermouse
Tamias striatus	Eastern chipmunk
Glaucomys volans	Southern flying squirrel

Table 2. Species captured in prairie-grassland trappings.

Year	*Sigmodon hispidus*	*Reithrodontomys fulvescens*	*Cryptotis parva*	*Peromyscus leucopus*	*Mus musculus*	*Peromyscus maniculatus*
2014	33	0	0	0	0	0
2010	36	5	0	0	0	0
2008	17	3	0	0	0	0
2006	2	1	1	1	0	0
2004	15	8	0	0	3	0
2002	4	16	1	0	0	0
2000	9	3	0	0	0	0
1998	2	1	0	0	0	0
1983	5	5	0	0	0	5

and year was significantly correlated. The formula for regression was

$$y = 0.253x - 1996.03$$

The coefficient of determination for N in Table 4 was 0.7556 (=R^2). The value for F-statistic was 16.46 (1 and 4 *df*, *p*=0.01539).

There was a weak correlation ($p = 0.609$, R squared = 0.0393) between minimum winter temperature of the previous year and population of *Sigmodon hispidus* of the current year.

There were only 3 species of small mammals trapped in forest in 6 years of trapping as compared to 6 species in the prairie-grassland trapped in 9 years of trapping in the prairie-grassland. The wind storm in the forest produced so much downed timber that it was impossible thereafter to establish the trapping grid. The average population of small mammals captured in forest was estimated by averaging N for each night (Table 5).

Discussion

Sigmodon hispidus showed a cyclical population dynamic where abundance was greatest in certain years and declined in intervening years at our study site from year 1998-2008 (Table 2). However, the population has been increasing steadily since 2008, this might be attributed to the change in vegetation composition of the prairie-grassland habitat. We trapped nothing but *Sigmodon hispidus* in 2014 (Table 2) this has happened before in northwestern Arkansas (Gipson 1968). The cyclical nature of hispid cotton rats is found extensively in literature (Smith 1964; Fleharty *et al.* 1972; Martin and Huffman 1980; Doonan and Slade 1995; Calisher *et al.* 2005).

Table 3. Species captured in forest trappings.

Year	*Peromyscus leucopus*	*Glaucomys volans*	*Tamias striatus*
2008	2	0	1
2006	5	0	0
2004	1	0	1
2002	2	1	0
2000	1	0	0
1998	4	1	0

Table 4. Prairie-grassland small mammal population estimate (N).

Year	N	N/ha
2014+	105.6	104.3772
2010	49	48.4326
2008	34	33.6063
2006	*	*
2004	39	38.5484
2002	31	30.6410
2000	15	14.8263
1998	4	3.9536
1983	18.8	18.5823

*Indicates no calculations due to no recaptures

+The population estimate of year 2014 is corrected, given smaller acreage of trapping due to rain.

Table 5. Forest small mammal population estimate (N)

Year	AVG N	N/ha
2008	2.5	2.4691
2006	6	5.9259
2004	*	*
2002	3.5	3.4567
2000	0	0
1998	4	3.9506

* One unknown organism set off numerous traps without being captured which made it impossible to interpret the trapping results.

Although no detailed analysis of the vegetation was conducted throughout the years that trapping was conducted at the prairie-grassland area. D. James observed that throughout the years, the prairie-grassland's vegetation was transitioning from mainly broomsedge bluestem (*Andropogon virginicus*) grass to vegetation that consisted of diverse prairie grasses. The new grasses observed included little bluestem (*Schizachyyrium scoparium*), big bluestem (*Schizachyyrium gerardii*), switchgrass (*Panicum virgatum*) and indiangrass (*Sogastrum nutans*). This change was aided by controlled burns and removal of invading eastern red cedars (*Juniperus virginiana*). Change in grass composition might have caused an increase in abundance of *Sigmodon hispidus* in the grassland as depicted by the data (Table 4).

We did not observe any strong correlation between population of *Sigmodon hispidus* and the lowest recorded temperature of previous year in Arkansas. In higher latitudes at the northern limits of geographic range of *S. hispidus*, temperature is the major driving factor in controlling the population (Sealander and Walker 1955; Goertz 1964). However, as Arkansas is far below the northern limit for *S. hispidus* range, the lowest recorded temperature does not play a major role here.

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Travel to Extraterrestrial Destinations Over Time: Some Exploratory Analyses of Mission Data

V. Kodali[1], R. Duggirala[1], R. Segall[2*], H. Aboudja[3], and D. Berleant[1]

[1]*Dept. of Information Science, University of Arkansas at Little Rock*
[2]*Dept. of Computer & Information Technology, Arkansas State University, Jonesboro*
[3]*Dept. of Computer Science, Oklahoma City University*

*Correspondence: rsegall@astate.edu

Running title: Exploration of Extraterrestrial Mission Data

Abstract

This paper discusses data pertaining to space missions to astronomical bodies beyond earth. The analyses provide summarizing facts and graphs obtained by mining data about (1) missions launched by all countries that go to the moon and planets, and (2) Earth satellites obtained from a Union of Concerned Scientists (UCS) dataset and lists of publically available satellite data.

Introduction

The ultimate goal of this research project is to search for quantitative trends that describe humankind's advancement in the physical exploration of extraterrestrial bodies. The objective of the research described here is to perform preliminary, yet critical, steps toward that ultimate goal. Specifically, we seek to identify sources of data about space exploration missions, extract pertinent facts about the great majority of missions rather than a sampling of missions, and perform exploratory analyses on the extracted facts to better understand the data and domain. We seek data on all missions rather than just a representative subset because the total number of missions is manageable – in the hundreds for missions to extraterrestrial bodies and in the thousands for missions into orbit around Earth.

There are a number of aspects to space exploration. One is the science – how the universe works and what is happening in distant stars and galaxies, and even the far reaches of the observable universe. One traditional approach to measuring scientific progress is paper and citation counts. While such counts clearly measure activity, they don't necessarily accurately reflect the amount of advancement in human knowledge, and indeed, it is not clear how to accurately do this in a quantitative way. Advances in knowledge are often unique and differ in important ways, and thus are hard to compare in terms of quantitative amount of progress. Which is a bigger advance, for example, special relativity or Newtonian mechanics, and by how great a percentage? From the standpoint of actual travel to extraterrestrial bodies, the science – perhaps in some ways unfortunately – becomes secondary to the fact of getting there. A mission that lands on the moon for the primary purpose of competition with other countries (as was the case between the US and the Soviet Union during the "space race" period) lands on the moon as much as one that successfully executes a mission intended to squeeze every ounce of science possible out of a moon landing.

To summarize, the four main objectives of this research, including both overarching and specific to this paper, are as follows:

1. identify quantitative trend lines that permit extrapolations predictive of future human space exploration activity;
2. identify sources of data to support item 1 above;
3. develop a data set that covers much of or most relevant activities rather than relying on a sampling strategy; and
4. do exploratory mining of the dataset.

Background

The performance of space exploration technology must be understood, first, by collecting data from which the performance can be extracted. Data collection and analysis is an intrinsic part of the space exploration endeavor in multiple ways. Big data has become a high profile term as well as field of both research and practice in recent years. Space science and technology is no exception. NASA, for example, has numerous projects that relate to handling and analyzing big data (Savaram 2017). One of the high profile missions, the Pluto contact of 2016, provided a special challenge in getting all the data that was acquired downloaded to Earth over the several light hours of distance required (Stockton

2016). Space, after all, does not support high bandwidth commercial data trunks. Stefano *et al.* (2017) address the data storage and management part of the problem with an IT platform, Eodataservice.org, designed for space mission data needs.

Focusing on technical performance over time, one approach is to focus on "bang for the buck," that is, amount of the technology per dollar. This is the "Carlson curve" approach commonly used in measuring technical performance over time of biotechnology (NHGRI 2017). Wright's (1936) law used this approach. For space exploration, costs tend to be high and cost-per-performance is generally an important engineering issue. For example Cordova and Gonzalez (2017) analyze NASA's "Faster, Better, Cheaper" program, which focused on this aspect but was deemed not successful. Thus, for a country to have a space program, it must be willing to spend the necessary funds. Luxton (2016) presents lists of countries that spend significant money on space exploration. Unfortunately the figures are just for one year. Expenditures are not necessarily easily available for all years, thus enhancing the interest of non-monetary measures for which the data might be easier to obtain. Multi-year data on space related financials is provided by Bryce (2017). However the data is limited to startup investment, which while it captures an important part of the space funding picture that is often proposed as auguring a transition to commercial development of space related industry, does not capture the overall picture of space funding in general or, more specifically, space exploration per dollar.

Due in part to the difficulties associated with obtaining the data to measure technical performance per dollar for space exploration, pursuing measures that rely on performance without reference to costs is a natural strategy. With respect to space exploration data, Eshbach and Hathaway (2014) provide an online service showing how many people are in space at the current time. As of the moment of this writing, the web page lists six names, their mission roles, and how many days each has been in space. Duffy (2015) assesses 2015 as a banner year for space exploration.

To get at trends over time it is necessary to obtain data and provide assessments spanning time. Hicks (2015), focusing on crewed missions, concludes that "It's sad that human space exploration has stalled." Oukaci (2017) argues that space exploration is slowing. Bardi (2015) is even more pessimistic, suggesting that "… human spaceflight is coming to an end" and providing a graph of human space flights 1960–2014 to illustrate his fears. Technologies often develop in an exponential fashion. Although it is early enough that the data regarding space exploration does not yet lead to definitive conclusions, Adams (2015) provides a graph and blames the lack of commercial use of space as a specific reason for why space exploration has not yet demonstrated a clear case of exponential development.

Arguing that a pessimistic assessment of advancement in space exploration is not warranted, Roberts (2011) describes how progress is proceeding in multiple ways that, while genuine, do not jump out from much commonly tabulated data. Flo422 [sic] (2017) charts the number of people launched into space per year, 1961–2016. Elliott (2014) plots the population of space over time; the "collapsed view" mode clearly suggests a trend of increasing human population of space over time. In previous work in our lab we identified a model for measuring space exploration activity. This model was tested only on NASA data, and with that limitation in mind the model nevertheless suggests generally increasing technical performance (Hall *et al*. 2017).

To understand human space exploration, it is necessary to analyze the data available on space missions. Data from NASA (2018) is an integral part of a larger whole that incorporates data about the space missions of all countries. McDowell (2017) accounts for satellite launches worldwide. The Union of Concerned Scientists (UCS 2017) maintains a catalog of currently active satellites. Space exploration seems to appeal to enough Wikipedians (mostly volunteer editors) that Wikipedia's information is kept up to date and has reasonable coverage of satellite activity (Category 2018), general space mission lists broken out by year (Timeline 2018), and many related listings. The dream of human exploration of extraterrestrial bodies remains before our eyes, tantalizing the imagination with its potential (Berleant 2017).

Results

Missions to extraterrestrial bodies

For the next several figures, missions in which spacecraft were sent to extraterrestrial bodies were recorded. Missions were scored according to the type of contact made with the destination body. The scoring assigned 2 points to a launch failure, 3 to a distant flyby, 6 to a close flyby, 9 to orbiting the destination, 12 to a hard (destructive) landing, 12 to a return to Earth, 15 to a soft landing, 17 to a crewed mission, and 18 to a mission with a robotic rover. This represents an adjustment to the values used in Hall *et al*. (2017). Many missions qualified for multiple categories, and were assigned to the score of the highest-scoring category for which it qualified. The scores of the missions launched in a particular year were summed, and the % of the sum that was attributable to various countries was calculated and graphed from year to year.

Figure 1 shows the % of the summed scores of missions for each year attributable to US launched missions.

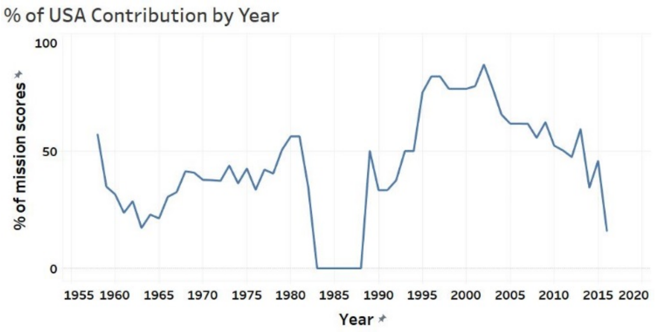

Figure 1. Percentage of missions launched by USA.

Figure 1 was plotted by using moving averages to smooth out yearly fluctuations in the data. The value for a given year was calculated by averaging the raw values of that year and the four previous years. The graph reached its maximum in the year 2003.

Figure 2 shows the data for the Soviet Union and its successor state Russia. From the graph we can observe that there is a major contribution from the USSR/Russia in the initial years. Later the contribution falls rapidly and remains low thereafter.

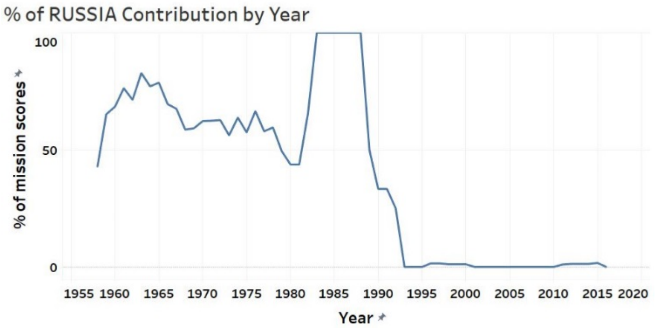

Figure 2. Percentage of missions launched by USSR/Russia.

Figure 3 shows the situation for Japan. Compared to the US and the USSR/Russia, Japan has zero contribution initially, then ramps up a space program that makes significant contributions to the world's exploration of extraterrestrial bodies.

Figure 4 shows the relative activity of China. We can observe that China started exploring astronomical bodies relatively late but has been increasing its share of missions on a generally increasing trajectory.

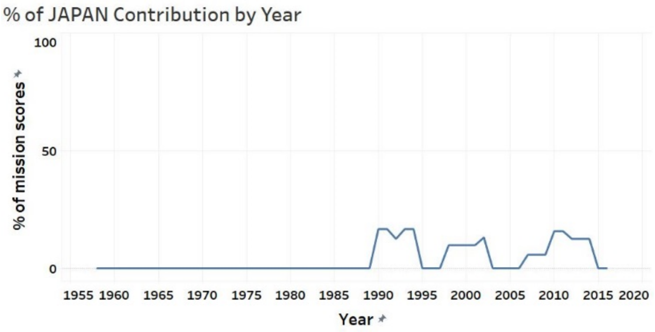

Figure 3. Percentage of missions launched by Japan.

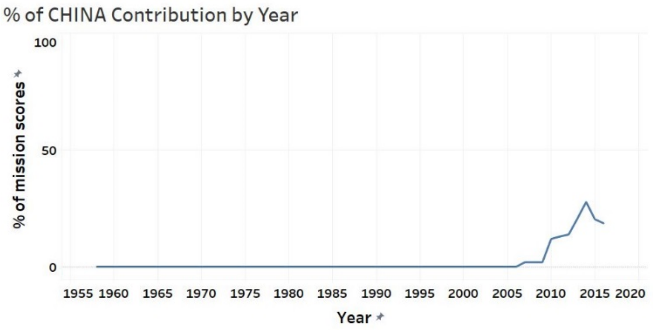

Figure 4. Percentage of missions launched by China.

Figure 5 shows the situation for European-launched missions. Europe did not contribute to launching missions to astronomical bodies at first. More recently Europe has been a significant contributor to such missions.

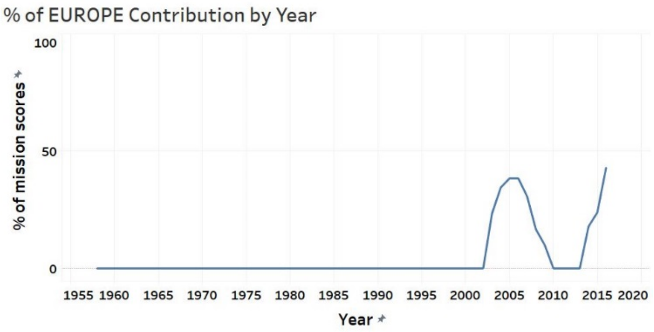

Figure 5. Percentage of missions launched by Europe.

The next graph, Figure 6, shows the smoothed percentages for India.

It is possible that with efforts such as SpaceX, the costs of lifting mass into space will be reduced and this might lead to more launches in the coming years.

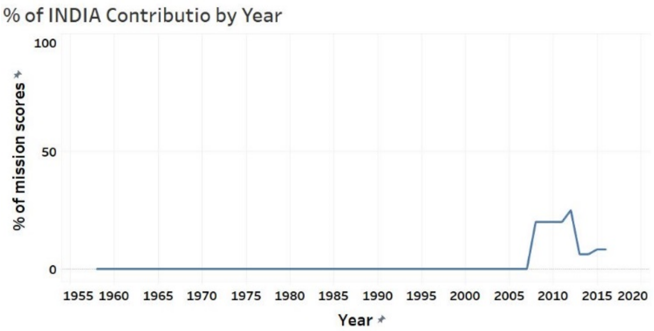

Figure 6. Percentage of missions launched by India.

Satellites

Extraterrestrial bodies are only one category of space mission. Another category is satellites, of which the vast majority are around the Earth. These satellites are one variety of humankind's expansion into outer space. Satellites gather data that is otherwise unobtainable, provide services such as GPS and radio transmission that are not otherwise possible or economic, and form an important portion of our space exploration activity. This subsection provides some analyses of satellite data.

Purpose is a key column in the Earth satellite dataset used by the Union of Concerned Scientists (UCS 2017) that we downloaded for analysis. Figure 7 shows a facet chart that we developed to show the satellite launches by year, differentiated by the purpose of the satellite, for satellites that were operational as of 9/1/2017. The chart shows growth in communication satellites over time, and growth in Earth observation satellites starting a bit later. Following that, there was a gradual increase in technology development satellites. Space science shows steady growth in recent years. Technology development satellites launches very late in the timeline compared to Communication satellites or Earth observation satellites. The differences in the timing of the increases in these categories is of interest as it permits comparing the categories over time. Overall, communication satellites have constituted the largest category of Earth satellite launches. What are the implications of these observations? One may hypothesize that these trends reflect trends in underlying need by society for satellites with those purposes.

Another data element in Earth satellite dataset we analyzed (UCS 2017) is the launch site. A histogram chart was developed for active Earth satellite counts by launch site. Figure 8 shows that the highest number of currently active Earth satellites were launched from the Baikonur Cosmodrome.

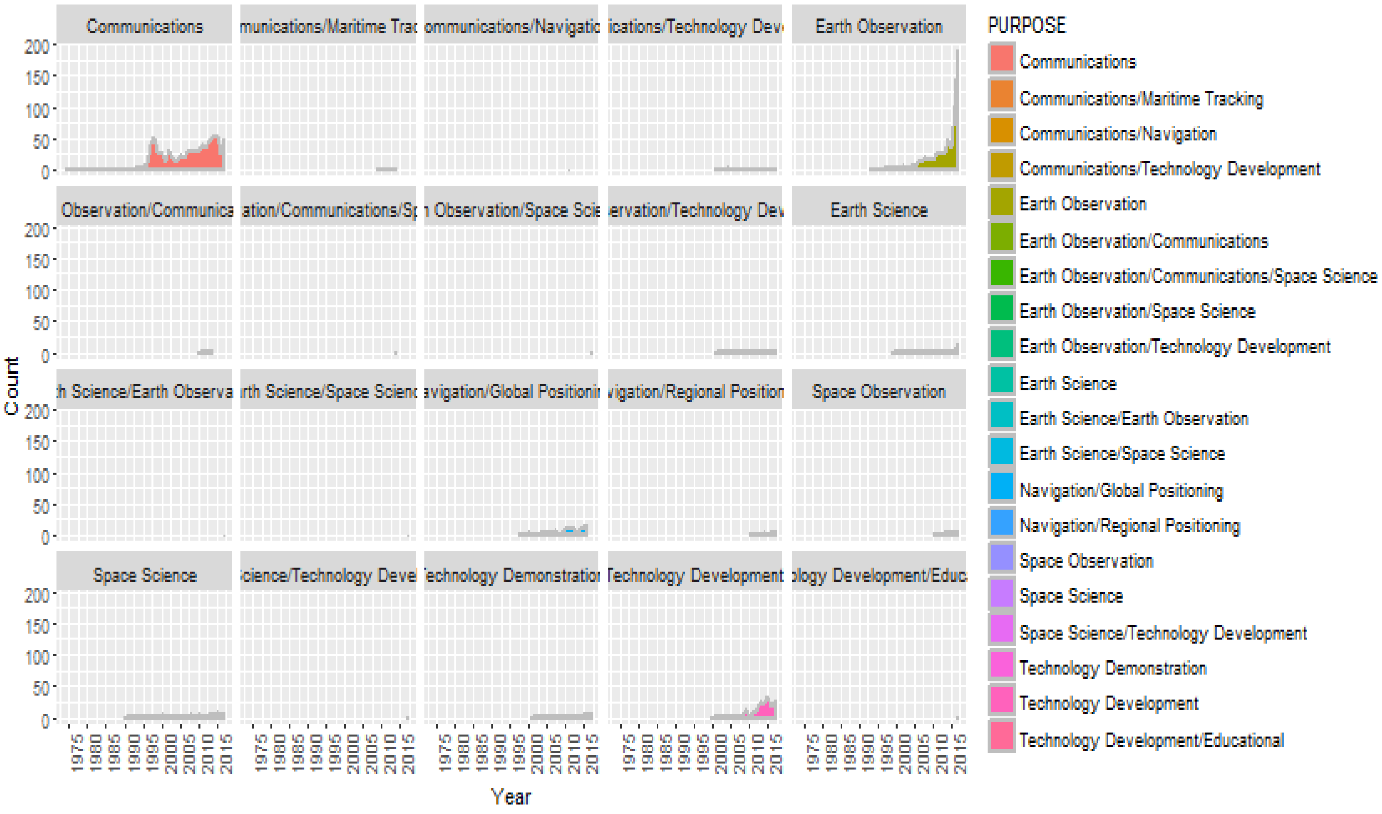

Figure 7. Purpose categories of artificial satellites.

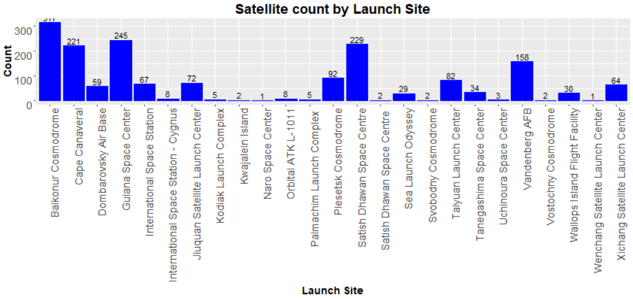

Figure 8. Active satellites by launch site.

Obviously, the Baikonur Cosmodrome is one of the world's largest and most active space facilities. The chart shows that Baikonur Cosmodrome leads with 254 launches of active (as of 9/1/2017) satellites, followed by other launch sites, notably Guiana Space Center, Cape Canaveral, and Vandenberg Air Force Base. The Guiana Space Center is in French Guiana and began operating in 1968. Reasons for the location include being near the equator, so that the spinning Earth gives launches a faster boost thus minimizing the energy needed to launch into space. Also, sea east of the spaceport provides a measure of safety in that launch debris has a place to fall without endangering people on land. French Guiana is in South America and is a part of France.

Apogee and perigee distances are two of the data elements regarding satellites in the McDowell (2018) dataset. A scatter plot of average apogee vs. average perigee for Earth satellites was developed using that data. Note that apogee and perigee (peri- is from Greek and means near) may be defined as follows.

- **Apogee**: that point in an orbit at which the orbiting body is furthest from the center of the orbit.
- **Perigee**: that point in an orbit at which the orbiting body is closest to the center of the orbit.

Figure 9 shows that the average apogee and perigee of satellites in orbit has increased over time. Most of the satellites have an apogee significantly greater than the perigee. Furthermore, the larger apogees and perigees tend to be associated with newer satellites.

Patterns in data on perigee and apogee were further studied using the k-means clustering algorithm on the UCS (2017) dataset. Figure 10 uses 2 clusters to identify similarities in the data. Color depicts the purpose of the satellite. The graph shows that all the earth observation satellites are grouped at a relatively close distance and communication satellites are grouped at further distances. The graph shows a pattern of most of the Earth/Space Science, Navigation/Global Position and Earth science satellites located centrally on the chart. Cluster 2 does not contain any the Earth/Space Science, Navigation/Global Position or Earth science satellites.

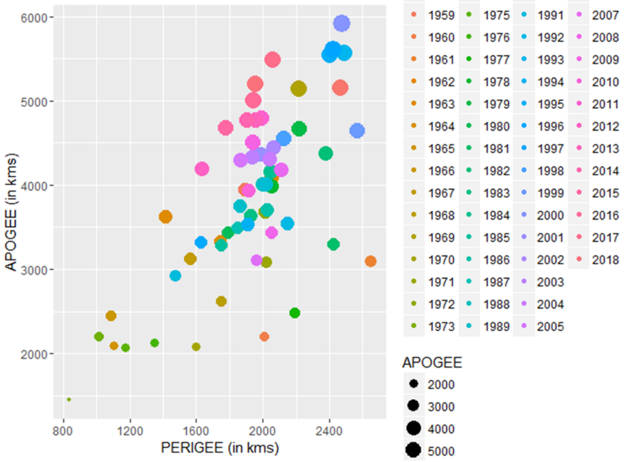

Figure 9. Apogee vs. perigee for Earth satellites. (Color coding is viewable on a computer display.)

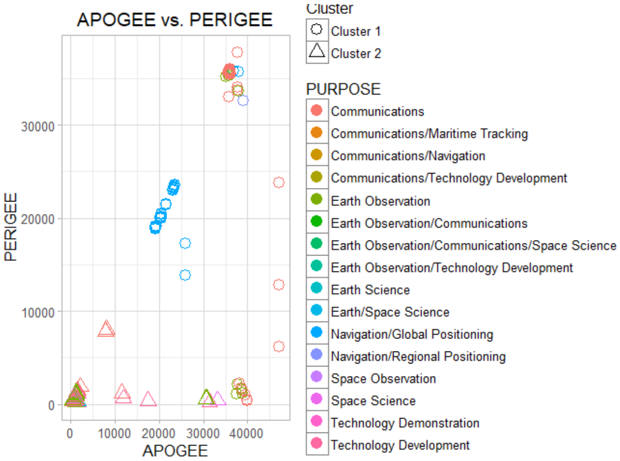

Figure 10. Clustering of satellites on the basis of their orbital distances.

McDowell (2018) provides a lengthy compendium of basic data on satellite launches worldwide. Satellite launch data were extracted from the web site, cleaned, and analyzed. Figure 11 shows some of these results. These data indicate that the fraction of satellites that are successfully launched into orbit, out of all satellite launches, has tended to improve over time. This is a positive trend.

Figure 11 shows a spike around 1998. The Union of Concerned Scientists (UCS 2017) dataset was analyzed to better understand it. Figure 12 resulted, showing a corresponding spike. A closer investigation of the data revealed that the spike was due to communication satellites launched between 1997 and 1999. While the data do not explain why, these visualizations do highlight the fact that it occurred, thus suggesting an exploration of the "why?" question.

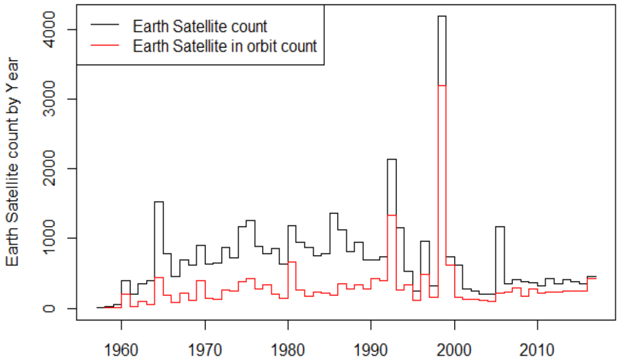

Figure 11. Total satellite launches and successful satellite launches.

Another analysis is shown in Figure 13. Different countries have different numbers of satellites in orbit. A comparison of operating (as of 9/1/2017) Earth satellites owned by different countries was done using the Union of Concerned Scientists (UCS 2017) dataset. A histogram chart was developed showing country vs. number of operating satellites. Figure 13 shows that the highest number of Earth satellites in orbit are owned by the US with a count of 786, followed by China at 203. Russia has a count of 138. The chart shows total 81 countries involved in the satellite launches. Overall, it seems like many countries have satellites but still have a long way to go to catch up with the US.

Discussion

There are several possible goals for mining space mission data. These fit into two broad categories: understanding what has been accomplished (the past), and understanding what is possible (the future).

Goals related to the past focus on historical understanding. These include the following.

- *The reasons, results, and social effects of the space race between the US and USSR decades ago*. For example, there was a burst of early space exploration activity early on known as the "Space Race." The degree of activity was higher than expected given the longer term trajectory of more gradual progress in space exploration. That level of activity was not sustained, because once people landed on the Moon the race seemed to have been "won," as though space exploration was akin to a sports contest, and having been "won" it lost some of its attraction and thus US government funding decreased.
- *The satellite infrastructure, its effects on communication, and the effects of the communication thus enabled on the evolution of the current world order*. Unlike most space related activities, satellites have a lot of commercial and other practical applications. This has driven much of the satellite construction and launching activity, a force that does not apply to other space exploration mission types such as those to distant planets.

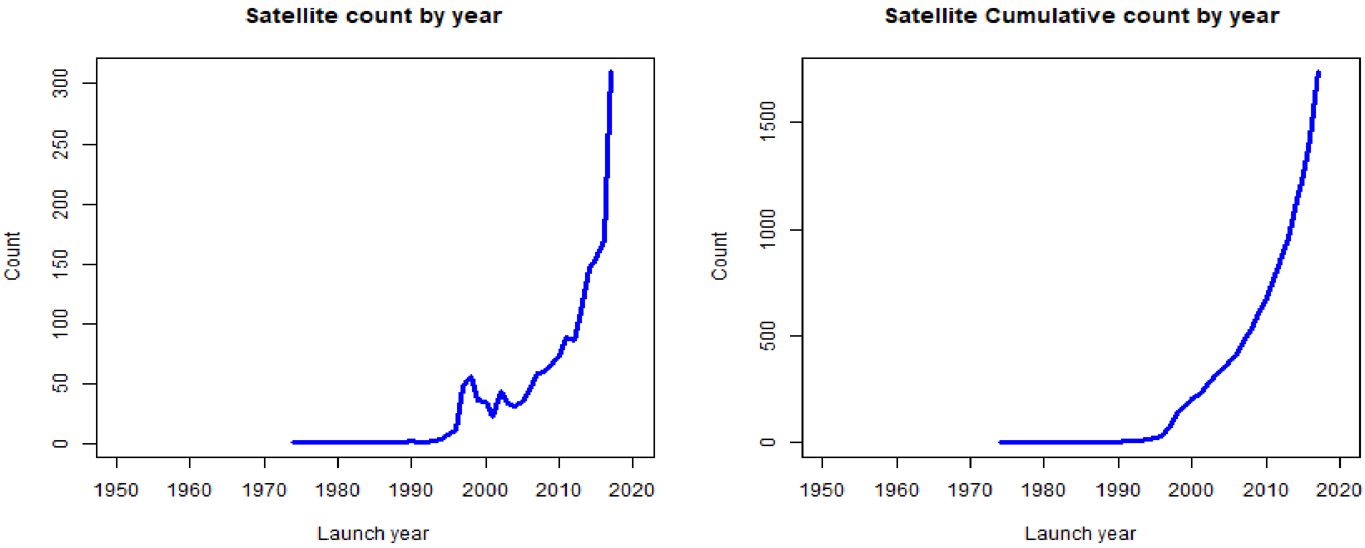

Figure 12. Satellites that were operational on 9/1/2017. (Left) Number launched in a given year. (Right) Number launched in or before a given year.

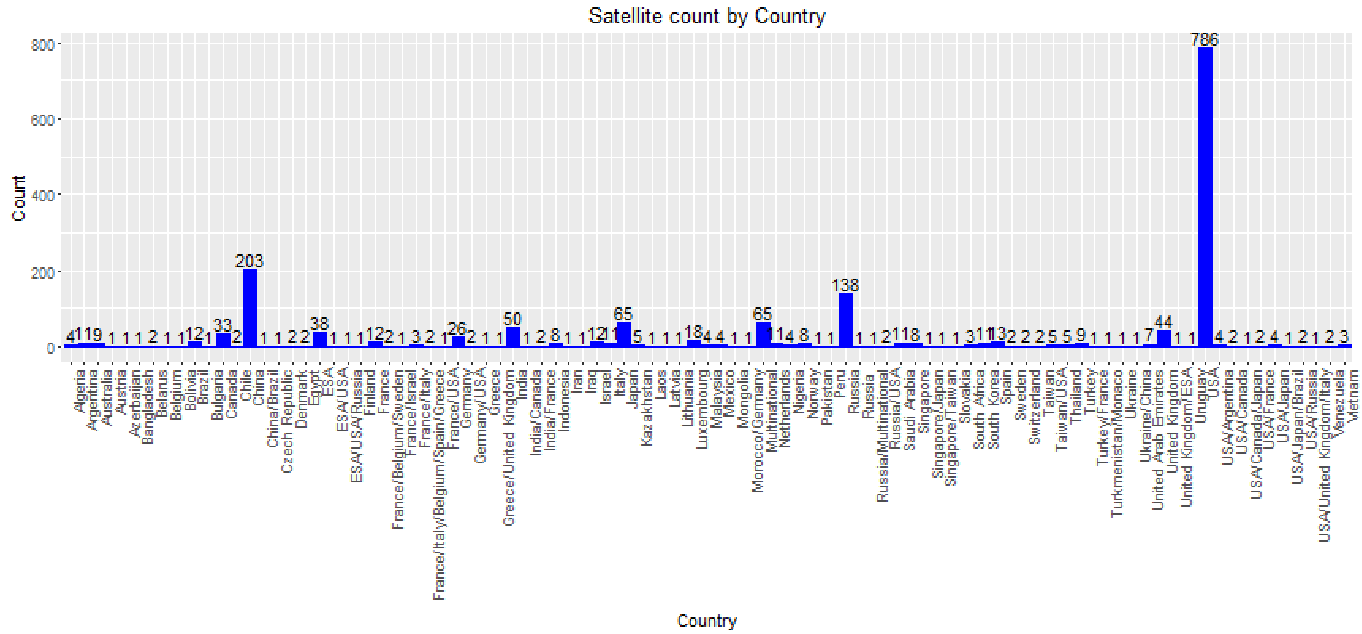

Figure 13. Number of satellites in operation by country

- *The effects of long-standing popular cultural interests on large national science and engineering efforts*. This is evidenced by the differences in efforts made to travel to extraterrestrial bodies with historically strong ties to popular consciousness and imagination like Mars (Crossley 2011), compared with efforts to travel to bodies with weaker such ties.
- *The experimental testing of laws that govern and describe trends in technological performance*. Exponential and similar trends are examples. Less quantitative examples include effects of population, societal wealth, and previous technologies on technological advancement. Historical data can be used to test if such a proposed law has held in the past.

In contrast to understanding the past, goals related to understanding the future use data about the past for predictive purposes. These purposes include the following.

- *Technology forecasting of future space exploration.* If a trend can be quantified based on historical data, it could be extrapolated to make predictions about future levels of space exploration activity.
- *Technology foresight of likely scenarios of future space exploration.* The future will always be fraught with uncertainty. The concept of foresight is distinguished from forecasting in that it is about determining future possibilities rather than predicting which one will occur. Because of the inherent uncertainty about the future, foresight is a reasonable approach to understanding the future of space exploration.
- *Insight into effective national or corporate policy options for future space exploration efforts.* Interest in the future of space exploration (or other technologies) is not just about what will eventually happen. There is instead the possibility of affecting that future. There are always reasons to pursue certain futures over others. Understanding and evaluating different scenarios leads naturally to efforts to make the most desirable ones happen.

Conclusions and Future Work

We have collected data and performed exploratory analyses. The ultimate goal, however, is to see if we can extract an overarching trend that will permit understanding of likely future levels of space exploration. Such a trend would be analogous to Moore’s law for computer chips and other exponential curves describing technical performance over time for various technologies.

A trend curve, by definition, can be extrapolated to make predictions about the future because it shows a trend. Such predictions are testable by checking if the predictions hold when the data finally becomes available. On the other hand, a curve from which no extrapolatable trend can be determined provides little basis for projection and thus does not support technology forecasting. Thus for forecasting purposes a curve that shows a trend is better.

How can a trend curve be found? First, there must be an underlying trend in order for a valid trend curve to exist and be found. This is a modeling problem. Satellite launches and visits to extraterrestrial bodies, which we analyzed herein, are obvious candidates for model components. It is not clear however if those components are sufficient. Other components that might need to be accounted for are the following.

- *The International Space Station*. The space station is not an astronautical body and, while a satellite, is a much bigger part of the whole space exploration picture than an ordinary satellite. Most astronauts currently go there, for example. If it were not for the existence of the International Space Station (ISS), perhaps other exploration activity would be done instead. Activities related to the ISS have constituted a significant portion of space exploration during the period of existence of the ISS.
- *The US Space Shuttle program*. Like the ISS, the US Space Shuttle program made up a significant fraction of the overall space exploration effort, but is not represented adequately when focusing on satellites or interplanetary missions. Data (e.g. Catlett 2004) exists that may support including the Space Shuttle program in a model of space exploration.

To properly model space exploration activity, various parameters must be defined. For example, does a trip to the moon count less than a trip to Mars? By how much? Also different types of contact with the destination need to be distinguished. For example, a soft landing with a rover and a return trip should presumably count more than a flyby. We have done this in an ad hoc manner as described earlier. A principled approach to inferring these numbers would be better, but it is not clear what is the best way to do it.

One approach to the parameter tuning problem posed by the foregoing paragraph is to seek parameter values that result in a space exploration trend curve that is relatively smooth and extrapolatable. But is determining parameter values that way fair? Here, in a nutshell, are the “no” and “yes” arguments.

- *No, it is not fair*. The counterargument to choosing parameters that result in an extrapolatable curve is simply that it looks too much like an attempt to force fit the data to a curve. It appears to be the dual of the overfitting problem in machine learning: instead of finding an overly complicated curve that fits data that might in fact be more noise than signal, this approach involves finding a complicated transformation of the data values to fit a simple curve.
- *Yes, it is fair*. A set of parameter values that results in an extrapolatable curve is not, in itself, a claim that the curve is a valid model for space exploration activity over time. As just discussed it might be no more than a force fit of the data with no predictive value for future years. On the other hand, it might turn out to have the desired predictive ability, and thus be part of a useful model. Only time can tell which possibility applies, but one thing that can be known immediately is that the parameter values form a hypothesis. This hypothesis will be tested by future events. If future events follow the resulting curve’s extrapolation, that is corroborating evidence for the hypothesis (i.e., the model and its parameter values). If future events do not comply with the extrapolation, that is evidence against the hypothesis.

Different sets of parameter weights would provide different hypotheses, and these each can be tested against future events. If a set of parameter weights can be justified by domain facts and historical context, the hypothesis embodied by that set of weights gains explanatory heft as well. Ultimately a model of advancement in space exploration over time may be derived that, like the exponential and other laws shown to be useful with various other technologies, will be shown to predict future levels of space exploration activity.

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Aquatic Invertebrate Community Structure, Biological Condition, Habitat, and Water Quality at Ozark National Scenic Riverways, Missouri, 2005-2014

D.E. Bowles*, L.W. Morrison, and J.T. Cribbs

[1] National Park Service, Heartland Inventory & Monitoring Network, 6424 West Farm Road 182, Republic, Missouri 65738

*Correspondence: david_bowles@nps.gov

Running title: Aquatic Invertebrate Community Structure

Abstract

Ozark National Scenic Riverways (OZAR) was established to protect the corridor of the Current River and its major tributary, the Jacks Fork. The Current River is one of the few remaining free-flowing rivers in the U.S., with much of its base flow coming from several large springs. To assess the biological condition of these rivers, aquatic invertebrate community structure was monitored from 2005 to 2014. Benthic invertebrate samples and associated habitat and water quality data were collected from each of nine sampling sites using a Slack-Surber sampler. The Stream Condition Index (SCI), a multimetric index that incorporates taxa richness, EPT (Ephemeroptera, Plecoptera, Trichoptera) richness, Shannon's diversity index, and Hilsenhoff Biotic Index (HBI), was calculated. The benthic invertebrate fauna was diverse with 155 distinct taxa identified from all sites. Mean taxa richness was high, ranging from 22 to 30 among sites. The invertebrate taxa of the Current River and Jacks Fork are largely intolerant across all taxa represented (mean tolerance value= ~4.25). Mean HBI did not exceed 3.9 in the Current River or 4.4 for the Jacks Fork. Mean SCI scores across sampling sites generally were well above 16, indicating they are not impaired. Habitat and water quality data were summarized, but they were poorly correlated with individual invertebrate metrics. Sørenson's similarity index was used to assess community similarity among sites, and similarity scores were then analyzed using ascendant hierarchical cluster analysis. Similarity among sites was 72% or greater. Cluster analysis showed that Current River and Jacks Fork sites clustered separately and in a downstream progression. The uppermost collection site on the Current River was most unlike the other sites, which probably relates to the distinct physical features of that site compared to the others. Nonmetric Multidimensional Scaling (NMDS) was used to evaluate the relationship of invertebrate metrics to habitat and water quality. The NMDS model was found to be a good fit (stress=0.04) and specific conductance, temperature, discharge, filamentous algae and aquatic vegetation were among the most important habitat variables in defining the relationship among sampling sites. The three lower Current River and Jacks Fork sites each were closely grouped in ordination space, but the three upper Current River sites were farther apart from each other. The influence of several large volume springs near those sites is suspected of producing such disparity through press type disturbances. Although the invertebrate communities and water quality in the Current River and Jacks Fork are largely sound and have high biological condition, ongoing and projected threats to these resources remain, and those threats largely originate outside park jurisdictional boundaries. Inherent variability of invertebrate community diversity across sites and years highlights the importance of using multi-metric assessments and multiyear monitoring to support management decisions.

Introduction

Aquatic invertebrates are useful for understanding and detecting changes in biological condition because they reflect cumulative impacts not typically detected through traditional water quality monitoring (Barbour *et al.* 1999; Moulton *et al.* 2000, 2002). The occurrence of pollution sensitive taxa, dominance by a particular taxon combined with low overall taxa richness, or appreciable shifts in community composition relative to a reference condition are all ways that invertebrates are useful for assessing stream biological condition (Lazorchak *et al.* 1998; Barbour *et al.* 1999; Bonada *et al.* 2006).

Short-term, single event invertebrate monitoring is a strategy commonly used by resource and regulatory agencies for assessing stream stressors such as habitat disturbance, and chemical and biological pollution (Bonada *et al.* 2006). While short-term invertebrate monitoring serves a valuable purpose, evaluation of long-term variability helps researchers and managers better understand and gage chronic alterations in stream condition relative to climatic variability and change, as well as other anthropogenic disturbances (Bruce 2002; Jackson and Füreder 2006; Mazor *et al.* 2009; Vaughan and Ormerod 2012; Bowles *et al.* 2013a, 2013b).

Study area

Ozark National Scenic Riverways (OZAR), located in southeastern Missouri, was established in 1964 to protect the corridor of the Current River, its tributaries (including the Jacks Fork), and springs. The Current River is one of the few remaining large, free-flowing streams in the U.S. The extensive karst topography of the region results in formation of springs, of which there are more than 425 in the Current River basin (Bowles and Dodd 2015). Several of these springs are 1st and 2nd magnitude (Meinzer 1927; Bowles and Dodd 2015) and they provide the bulk of the baseflow for these rivers. The boundary of OZAR encompasses only 4% of the watershed, leaving much of it unprotected from human activities (e.g., agriculture, urbanization, and logging), which could result in alteration of water quantity and quality. Protecting and maintaining the integrity of the natural resources at OZAR is a high priority because it also serves as a major economic contributor to the region (Cui *et al.* 2013; Cullinane *et al.* 2014; NPS 2014).

Past disturbances and current threats

Although wadeable streams in the Ozark region, including those at OZAR, are generally considered to be in good condition, multiple stressors threaten their integrity (Davis and Richards 2002; Petersen and Femmer 2002; Huggins *et al.* 2005; USEPA 2006; Heth *et al.* 2016). Due to the karst topography, interbasin groundwater connections make these streams vulnerable to contamination that may originate from adjacent watersheds (Adamski *et al.* 1995; Mugel *et al.* 2009). Stressors such as deforestation and other land management practices in the watershed are particularly problematic because they tend to overwhelm localized protection of stream corridors at the watershed level (Roth *et al.* 1996; Heino *et al.* 2003; Zumberge *et al.* 2003). For example, increases in bank erosion rates and changes in channel morphology through time have been correlated with increased land clearing of steep uplands within a stream basin, as well as historical riparian land clearing (Jacobson and Primm 1997, Panfil and Jacobson 2001).

Previous aquatic invertebrate studies

Several previous studies have been conducted on stream invertebrate communities at OZAR to assess water quality impacts and biological condition. They include Clifford (1966), Duchrow (1977), Doisy *et al.* (1997, 2002), Rabeni *et al.* (1997), Doisy and Rabeni (1999, 2001), Sarver *et al.* (2002), Heth (2015), and Heth *et al.* (2016). With the exception of Doisy *et al.* (1997), Doisy and Rabeni (2001) and Heth *et al.* (2016), all of these works exist as gray literature and have not been published. Additionally, these studies were based on either single season events, or multiple season events within the same year. We do not attempt to summarize those studies here.

Other aquatic invertebrate studies at OZAR have attempted to take a more comprehensive and long-term approach to assessing invertebrate community dynamics and stream biological condition. For example, the National Park Service's Heartland Inventory and Monitoring Network (HTLN) began monitoring invertebrates, habitat and water quality at OZAR in 2005. Bowles *et al.* (2016) presented a summary of the first few years of this monitoring program for mainstem river sampling locations.

The purpose of this paper is three fold. First, we describe patterns in selected characteristics of invertebrate community structure, habitat, and water quality at OZAR. Second, we assess the biological condition of those invertebrate communities relative to regional reference sites. Third, we determine the strength of relationships between invertebrate community metrics and environmental variables (habitat and water quality).

Methods and Materials

Site Selection

Sampling was conducted at six permanent mainstem river sites on the Current River and three sites on the Jacks Fork annually from 2005 to 2009, and again in 2012 and 2014 (Fig. 1). All samples were collected from riffles during November through early January.

Invertebrate Sampling

Three benthic invertebrate samples were collected from each of three successive riffles at each sampling site using a Slack-Surber sampler (500 μm mesh, 0.25 m^2, n=9; Moulton *et al.* 2002). The sample area was agitated for 2 minutes with a garden cultivation tool. Large pieces of substrate were scrubbed with a brush as necessary to remove attached invertebrates. Samples were placed in plastic jars and preserved with 99% isopropyl or 95% ethyl alcohol. Samples were sorted in the laboratory following a subsampling routine

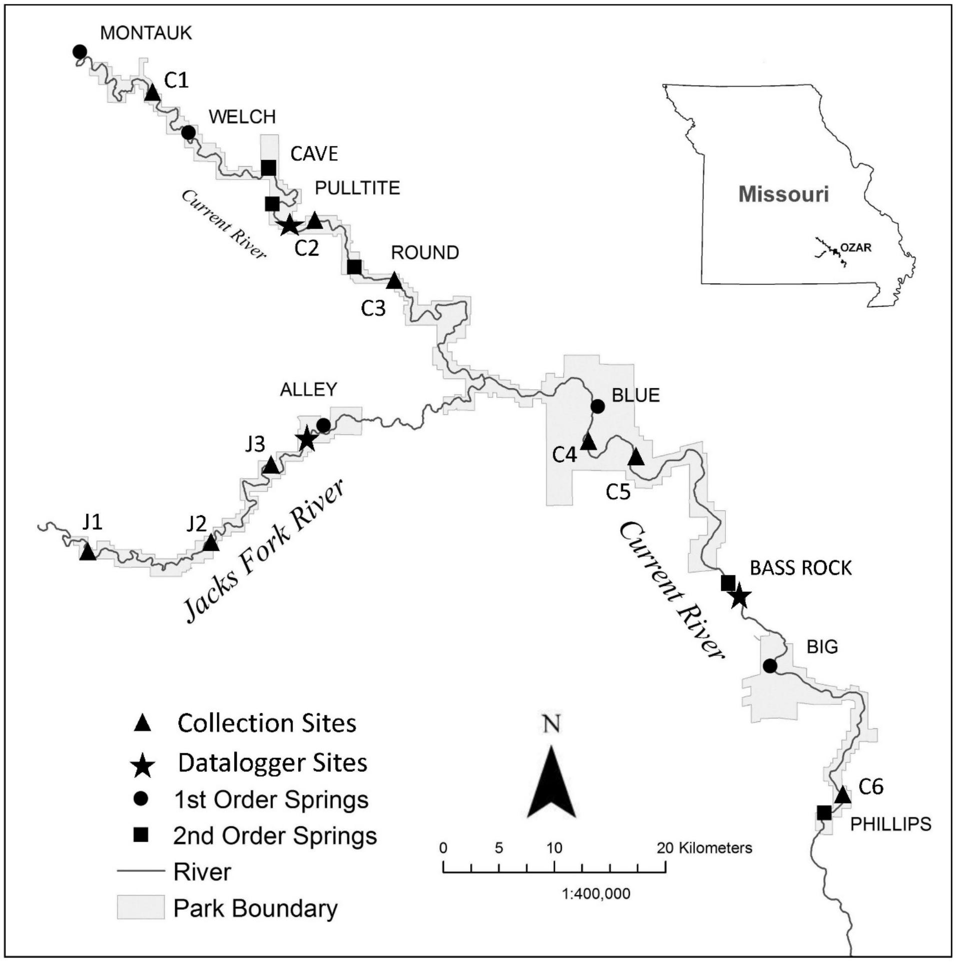


Figure 1. Location of water quality, habitat, and benthic invertebrate sampling sites at Ozark National Scenic Riverways, Missouri.

described in Bowles *et al.* (2007), and taxa were identified to the lowest practical taxonomic level (usually genus) and counted. We recognize that raw taxa richness estimates based on our subsampling routine (≥200 organisms, plus large and rare search) possibly may result in biased estimates of that metric, but as noted by Vinson and Hawkins (1996), taxa richness increases rapidly in samples up to 200 individuals but it increases at a much slower rate thereafter. So, we contend our data reasonably reflect richness in our samples without using rarefaction procedures.

Habitat and Water Quality

Qualitative habitat variables (percent substrate embeddedness, periphyton, filamentous green algae, and aquatic vegetation) were estimated within the sampling net frame as percentage categories (0, <10, 10-40, 40-75, >75). Habitat data were analyzed as midpoints of each category across years for each site to estimate the general condition of those resources. Dominant substrate size was visually estimated within the sampling net frame using the Wentworth scale (Wentworth 1922). Depth (cm) and current velocity (m/sec) were measured immediately in front of the sampling net frame using a top-setting wading rod fitted with a calibrated Marsh-McBirney Flow-Mate 2000 flow meter. Discharge was taken from appropriate USGS gages or measured by hand using the method of Carter and Davidian (1969). Discrete readings of water quality parameters (temperature, dissolved oxygen, specific conductance, and pH) were recorded at each riffle sampled with calibrated, hand-held instruments (YSI models 55, 63, ProPlus). In addition, hourly readings of water quality parameters (temperature, dissolved oxygen, specific conductance, pH, and turbidity) were recorded continuously at least 1 week prior to sampling using calibrated data loggers (YSI models 6600, 6920) at two fixed sites on the Current River and one site on the Jacks Fork (Fig. 1). Water quality data were summarized as means across years for each site to estimate the general condition of those resources. The water quality data collected for this study only describe the prevailing conditions that may influence the structure of invertebrate communities, and they represent only a small snapshot of the broader range of possible conditions over longer periods.

Statistical Analysis

On the recommendation of Reynoldson *et al.* (1997) we used both a multimetric index and multivariate statistical analyses to analyze our data to maximize their interpretive value.

Multimetric analysis

The Stream Condition Index (SCI), a multimetric index developed by Rabeni *et al.* (1997) for the state of Missouri, was used to assess biological condition of invertebrate community data. The SCI is founded on data collected from 26 reference streams in the Ozarks region (Rabeni *et al.* 1997). It is calculated using four metrics as measures of community structure and balance, including taxa richness, EPT (Ephemeroptera, Plecoptera, Trichoptera) richness, Shannon's diversity index, and Hilsenhoff Biotic Index (HBI; Hilsenhoff 1982, 1987, 1988). Procedures for calculating and scoring these four metrics and the SCI can be found in Bowles *et al.* (2007). For this study, we used only that portion of the index as it relates to single habitat, coarse substrates (i.e., riffles) during a fall index period (Rabeni *et al.* 1997).

High values are preferred for all metrics used in the index, except for HBI, where smaller values are the desired response. An increase in HBI values over time is undesired, because that would reflect the community's increasing tolerance to disturbance. See Bowles *et al.* (2007) for sources of assigned invertebrate tolerance values. The chosen metrics are sound measures of community structure and balance and are generally considered sufficiently sensitive to detect a variety of potential pollution problems in Ozark streams (Rabeni *et al.* 1997) (Table 1). The lower or upper quartile of the distribution for each metric is used as the minimum value representative of reference conditions (Table 1). Mean metric values were established by averaging the values for each of three samples per riffle and then averaging the means for the three riffles to establish a site mean. The SCI produces three possible levels of stream condition: 1) fully biologically supporting (unimpaired), 2) partially biologically supporting (impaired), and 3) non-biologically supporting (very impaired). Unimpaired or reference sites score ≥16 and have the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a taxa composition, diversity, and functional organization comparable to that of the natural habitat of the region. Both partially biologically supporting (SCI 10-14) and non-biologically supporting (SCI 4-8) categories indicate impaired streams that do not fully meet the beneficial use of protection of aquatic life.

Multivariate analyses

Pairwise correlation coefficients for each pair of invertebrate metrics and habitat and water quality variables were calculated using nonparametric Kendall's tau (Daniel 1990), because examination of histograms revealed lack of normality for many of the habitat variables. SPSS version 20.0 was used to calculate correlation coefficients (IBM Corp. 2011).

This analysis evaluated correlations between the four biological metrics calculated from aquatic invertebrate samples and 11 habitat variables. Data were grouped separately and analyzed by year and by site. When grouped by year, all riffles from all sites were included in the same analysis, and the analysis was repeated for each year (N= 7 years; n = 18 observations for each correlation: 3 riffles x 9 sites) (4 metrics x 11 habitat variables x 7 years = 308 total correlations). This approach provided the strongest level of independence among observations. When grouped by site, all years of data for all riffles of each site were included, and the analysis was repeated for each site (N= 9 sites; n = 21 observations for each correlation: 3 riffles x 7 years) (4 metrics x 11 habitat variables x 9 sites = 396 total correlations). Because these analyses produced many correlation coefficients and P-values, with an unknown actual type I error rate, a meta-analytic approach was applied to these data, and the number of "significant" (alpha = 0.05) correlations was summarized for each pair of metrics and habitat variables. The percentages of "significant" correlations for each pair of metrics and habitat variables were summarized over all metrics. Habitat variables with a greater percentage of "significant" correlations are likely to have, in general, greater potential to explain variability in these metrics.

Because we anticipated there would be differences in the invertebrate community structure along the river continuum, we used Sørenson's similarity index (presence/absence) to analyze similarity of taxa occurrences across years among the different sampling sites (Vannote *et al.* 1980; Southwood and Henderson 2000; Hammer *et al.* 2001). Similarity index scores

Table 1. Descriptive statistics, quartiles and scores for aquatic invertebrate metrics calculated using single habitat coarse substrate (riffle) data during a fall index period (from Rabeni *et al.* 1997). Summary statistics are from riffle habitat of reference streams (n=18) in the Ozark ecoregion during the fall index period.

Metric	Statistics				Quartiles			Scores		
	Mean	Standard Error	Minimum	Maximum	25%	50%	75%	5	3	1
Taxa Richness	28.3	3.3	23.5	41.0	21	26	29	>=21	20-11	<11
EPT Richness	13.1	0.7	11.5	15.0	9	11	12	>=9	8-5	<5
HBI	4.3	0.3	3.3	5.0	3.6	4.9	5.3	<=5.3	5.4-7.7	>7.7
Shannon's Diversity Index	2.4	0.1	2.1	2.7	2.29	2.44	2.61	>=2.29	2.28-1.15	<1.15

SCI Scoring: ≥16 not impaired, 10-14 impaired, 4-8 very impaired.

among sites were subsequently analyzed using ascendant hierarchical cluster analysis (Ward 1963) following the recommendation of Magurran (2004). Sørenson's similarity index and cluster analysis were conducted using PAST statistical software (Hammer *et al.* 2001).

Nonmetric multidimensional scaling (NMDS) with a Bray-Curtis distance measure was used to evaluate the relationship of invertebrate metrics (taxa and EPT richness, Shannon diversity index, HBI) and associated environmental variables among collection sites (PAST statistical software, Hammer *et al.* 2001). Variables were transformed prior to analysis using Log_{10} for water quality data and ArcSin Square Root for proportional data to reduce skew and increase interpretability. Data were averaged over all years for each site. Depth and current velocity were not included in this analysis due to their relative uniformity among samples.

Results and Discussion

Aquatic invertebrates

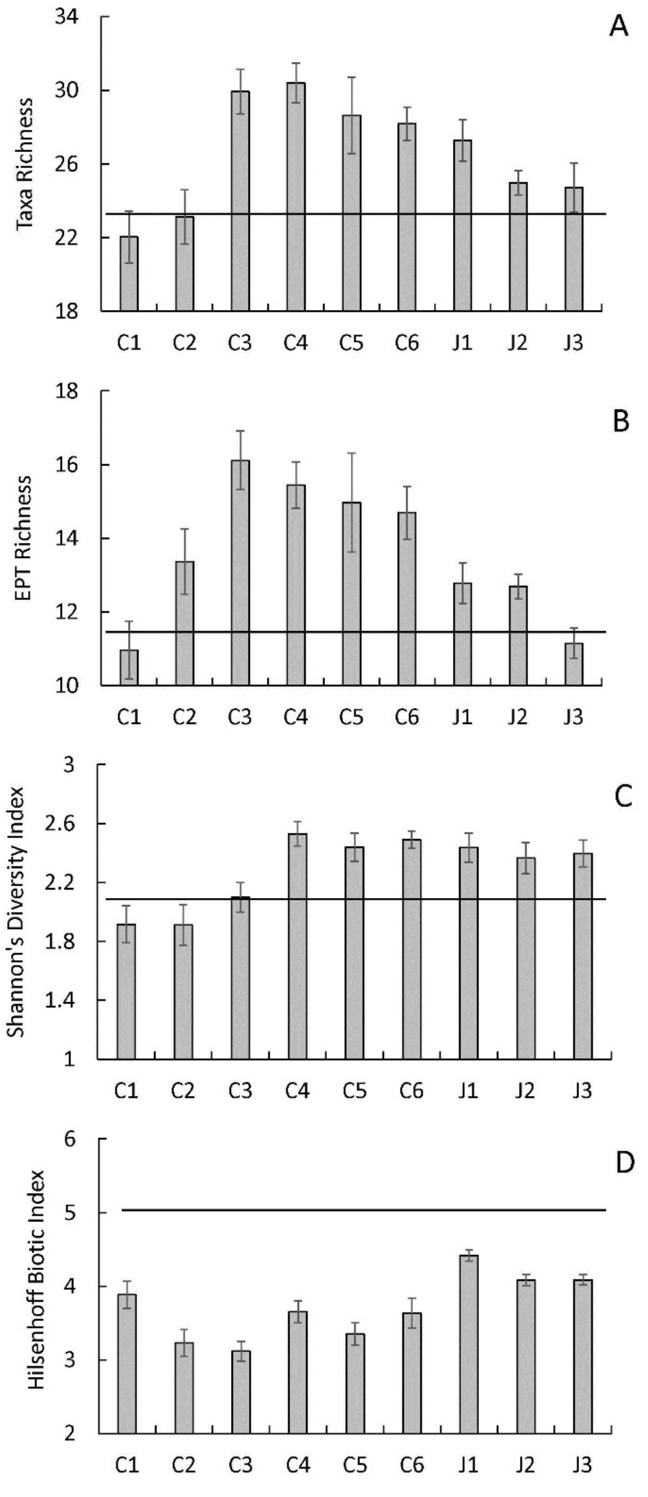
The aquatic invertebrate faunas of the Current River and Jacks Fork are diverse and many taxa are shared across sampling sites. Among all sites, 155 distinct taxa were identified with similarities ranging from 72% to 86% (Table 2). We identified Chironomidae (Diptera) only to the family level because doing so does not appreciably change the metrics used in this paper (Rabeni and Wang 2001). However, we recognize that by making this grouping the number of distinct taxa is likely much higher. A complete list of invertebrate taxa at each site, their abundances and associated environmental data are too voluminous to present here, but can be obtained from the authors.

The invertebrate metric values recorded among sites exceeded the minimum reference stream values (maximum for HBI) across years (Table 1, Figs. 2A-D).

Table 2. Sørensen similarity index for aquatic invertebrate taxa among river collecting sites on the Current River (C1-C6) and Jacks Fork (J1-J3), Missouri. Taxa compositions were accumulated over 7 years (2005-2009, 2012 and 2014).

	C2	C3	C4	C5	C6	J1	J2	J3
C1	0.76	0.76	0.73	0.75	0.72	0.72	0.73	0.73
C2		0.82	0.78	0.79	0.76	0.73	0.73	0.76
C3			0.85	0.81	0.80	0.81	0.77	0.79
C4				0.86	0.86	0.78	0.78	0.82
C5					0.83	0.77	0.79	0.81
C6						0.78	0.76	0.79
J1							0.79	0.80
J2								0.85

Individual metrics were highly variable among years and sites, although such variability is expected (Mazor *et al.* 2009). Mean taxa richness ranged from 22.0 to 30.4 among sites with the lowest richness values occurring at sites C1 and C2 (22.0 and 23.1, respectively) (Fig. 2A). It is particularly noteworthy that representatives of intolerant EPT taxa were abundant at all sampling sites with mean EPT richness values ranging from 10.9 to 16.1 among sites. Site C1 also had the lowest EPT richness among all sites (Fig. 2B). In contrast, taxa and EPT richness were highest at Current River sites 3 and 4. Taxa and EPT richness values for all three Jacks Fork sites were generally lower than those observed for the Current River. Mean Shannon's diversity index values ranged from 1.9 to 2.5 among sites, with the two upper Current River sites (C1,



Figures 2A-D. Aquatic invertebrate community metrics for 9 sites on the Current River and Jacks Fork, Missouri. Values are means averaged over 7 years (2005-2009, 2012 and 2014) and vertical bars are standard errors. The horizontal line conforms to the minimum reported value for Ozark reference streams, except for HBI, which is the maximum reported value (from Rabeni *et al.* 1997).

C2) consistently having values below 2 (Fig. 2C). For biological data, Shannon's diversity index ranges generally from 1.5 (low taxa richness and evenness) to 3.5 (high taxa evenness and richness) (McDonald 2003), but the actual value is contingent on the number of taxa in the community.

Mean HBI values were low at all sites and well below that for Ozark reference streams (Fig. 2D) and other regional streams (Rabeni *et al.* 1997; Bowles *et al.* 2016). The invertebrate taxa of the Current River and Jacks Fork are largely intolerant (mean tolerance value=4.2, and HBI values generally were below 4.5 at all sites. Mean HBI across years for all sites ranged from 3.1 to 4.4, which reflects good conditions (Hilsenhoff 1982, 1988).

In general, SCI scores showed that the invertebrate communities in this study are indistinguishable from those of reference streams. All SCI scores indicated that our sampling sites are not impaired and are fully biologically-supporting (Fig. 3). Lower scores observed in some years are likely due to interannual variability of invertebrate communities coupled with instream flow dynamics (flood, drought) that occur at those sites rather than anthropogenic disturbances. These data also show the importance of collecting data during multiple years and at multiple sites so that low scores in any given year or location do not overly influence management decisions for corrective actions (Mazor *et al.* 2009). The data further illustrate the importance of using a multimetric index for stream assessment so that too much weight is not placed on the value of a single metric. Environmental stressors, such as extended drought and flooding, may impact invertebrate communities and influence assessment results in any given year.

Habitat and Water Quality

Only summary habitat data are presented here to generally characterize the conditions in which samples were collected. Exclusive of discharge, habitat conditions were generally consistent among sites and years (Figs. 4-7). Mean depth and current velocities where samples were collected were typical for Ozark stream riffles (depth range=25 to 33 cm, current velocities range=0.6 to 0.9 m/sec). Discharge predictably increased in a downstream progression for both the Current River and Jacks Fork (Fig. 4). Smallest mean substrate size for the Current River was at sites C1 and C6 (32.8 mm and 37.9 mm, respectively) (Fig. 5). Site C2 had the largest average substrate size (55.08 mm), while the remaining sites had smaller and more similar sized substrates (42-48 mm). Substrate size for

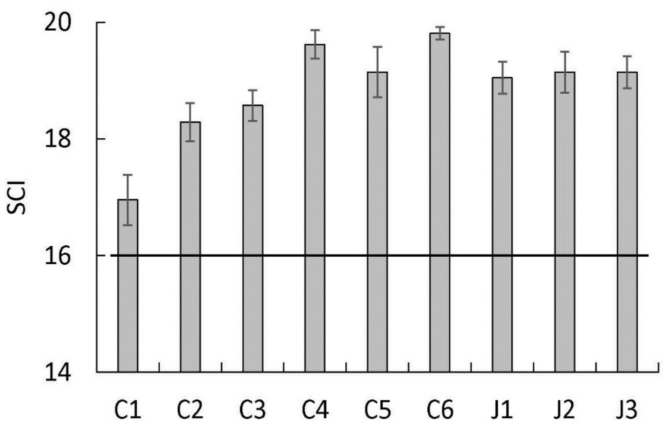


Figure 3. Mean SCI values averaged over 7 years (2005-2009, 2012 and 2014) and standard errors for 9 sites on the Current River and Jacks Fork. The horizontal line represents an SCI of 16, the lower limit for rating a site unimpaired.

the Jacks Fork was largest at site J1 (50.2 mm) and became increasingly smaller at the downstream sites (44.6 mm and 41.1 mm, respectively). Embeddedness was generally similar at all sites on both rivers (~26-29%), except site C6 on the Current River, which was about 38% (Fig. 5). Aquatic vegetation (mosses and various angiosperms) and filamentous green algae were poorly represented at all sampling sites (<11%) (Fig. 6). Periphyton densities growing on the rock substrates were generally consistent among sites, ranging from 26 to 34%.

Water quality met Missouri standards in all instances (Missouri Department of Natural Resources, 2014) (Fig. 7A-D). Temperature was variable among (means=8.7-11.9 °C) sampling sites and years, which is expected due to climatic variations among years sampled as well as location of sampling sites along the length of the river. Dissolved oxygen levels were high in all instances and were at or above saturation across years and sites (means=10.21-12.27 mg/liter). Specific conductance was generally consistent among sites, but slightly higher for the Jacks Fork as measured using the hand-held instruments. Overall values were highest for the three sites where dataloggers were deployed, which suggests differences in instrument sensitivities. In all instances, specific conductance ranged from 248 to 328 µm/cm. pH was consistent and similar among all sampling sites and years sampled (means=7.7-8.2). Turbidity, not shown here, was nearly always below 10 NTU. The water quality values we report are consistent with those summarized by Huggins *et al.* (2005), with the exception of temperature because their data were recorded during different seasons.

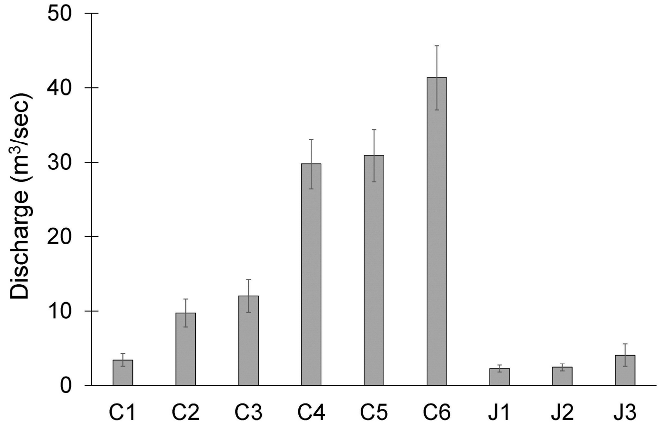


Figure 4. Mean discharge for the Current River and Jacks Fork, Missouri averaged over 7 years ((2005-2009, 2012 and 2014) with standard errors. See methods for site details.

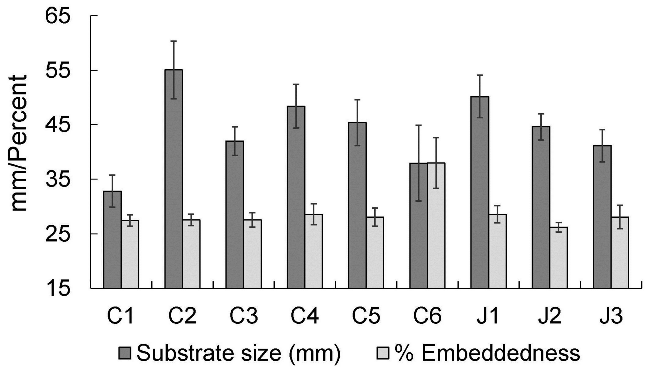


Figure 5. Mean substrate size (mm) and percent substrate embeddedness associated with benthic invertebrate samples from the Current River and Jacks Fork, Missouri. Values are means averaged over 7 years (2005-2009, 2012 and 2014) with standard errors. See methods for site details.

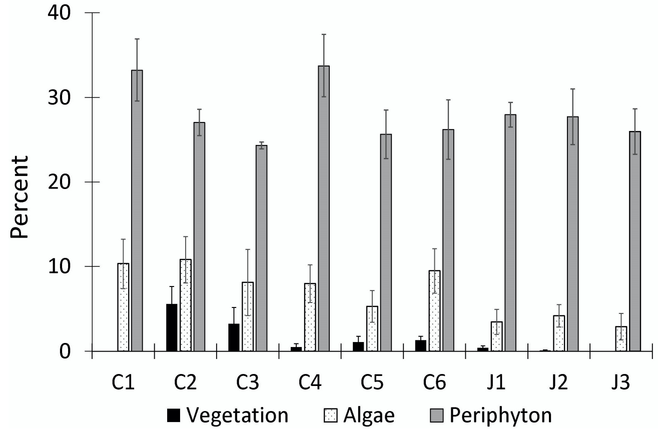
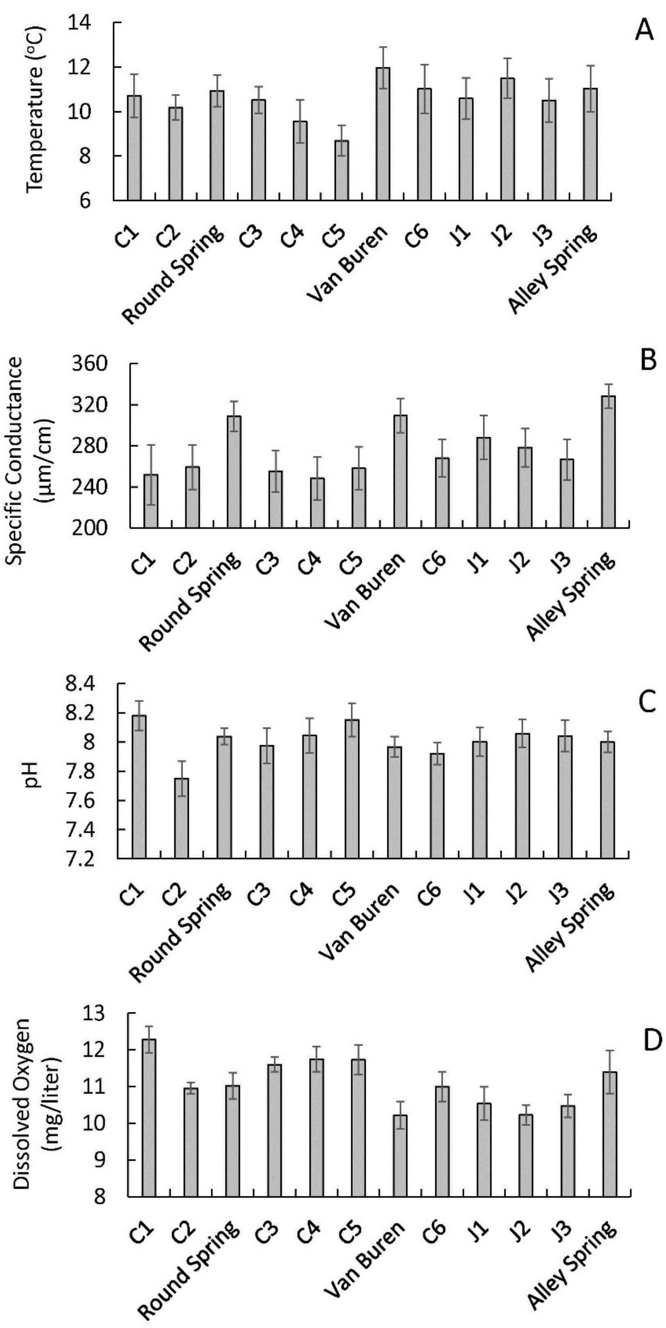


Figure 6. Percent vegetation, filamentous algae and periphyton occurring in samples from the Current River and Jacks Fork, Missouri. Values are means averaged over 7 years (2005-2009, 2012 and 2014) with standard errors. See methods for site details.



Figures 7A-D. Water physical-chemical data for sampling sites on the Current River and Jacks Fork, Missouri. Values are means averaged over 7 years (2005-2009, 2012 and 2014) with standard errors. Data were collected as discrete readings using hand-held meters at sampling sites 1-6, while data were collected continuously using dataloggers at fixed locations. See methods for site details and Fig. 1 for datalogger locations.

Overall, no habitat variables exhibited persistently strong correlations with any of the metrics, and the percentage of "significant" correlations was relatively low (<30%) in all cases (Table 3). In addition, a certain number of spurious correlations are expected (1 in 20 for alpha = 0.05) in analyses such as those conducted here. The number of expected spurious correlations ranged from 32 to 37% of the observed "significant" correlations (Table 3). Specific conductance, temperature, dissolved oxygen, substrate size, depth, periphyton, and filamentous algae usually had a greater percentage of "significant" correlations than the other variables, across all analyses, but some of these variables are autocorrelated, hence their biological significance may not be relevant. The low number of significant correlations for some habitat variables is likely due to the categorical scale used to assess some habitat data (see Methods), and the low variability among observed values. This analysis shows that the habitat data collected in relation to benthic invertebrate samples presently has limited value for correlating with community and diversity metrics, but that finding does not rule out further analyses with individual invertebrate taxa or groups of taxa (e.g., EPT), or assessing the collective relationships among habitat variables on the benthic communities.

Cluster analysis of Sørenson's similarity values showed that Current River and Jacks Fork sites clustered separately and in a downstream progression, with those sites closest to one another in linear distance generally being the most closely related (Fig. 8). The uppermost collection site on the Current River was most unlike the other sites, which probably relates to the distinct physical features of that site compared to the others. Our observations and collected data show the physical

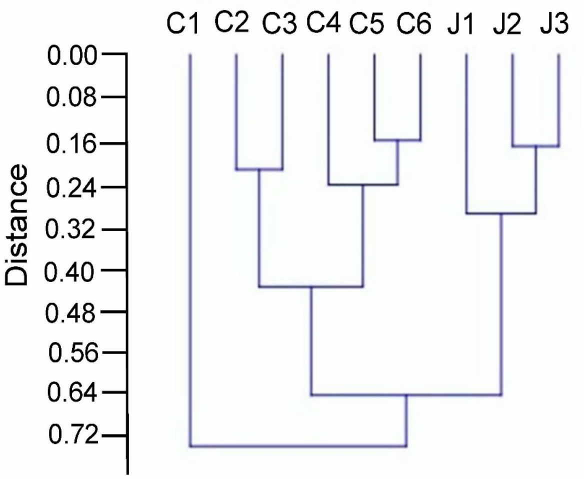


Figure 8. Dendrogram showing results for ascendant hierarchical cluster analysis and relative distance of Sørenson's similarity index scores of the aquatic invertebrate communities at sampling sites along the Current River (C1-C6) and Jacks Fork (J1-J3), Missouri. Taxa compositions were accumulated over 7 years (2005-2009, 2012 and 2014).

Table 3. Summary of OZAR pairwise correlations organized by site (i.e., correlations conducted among all years at each site, n=396) and by year (i.e., correlations conducted among all sites in each year, n=308). Values are number of significant correlations/percentage of significant correlations of total.

Variables	HBI	Taxa Richness	EPT Richness	Shannon Diversity Index	Total
			By Site		
Depth	3/0.33	2/0.22	5/0.55	0/0	10/0.28
Specific conductance	1/0.11	2/0.22	3/0.333	2/0.22	8/0.22
Current Velocity	2/0.22	1/0.11	1/0.11	3/0.33	7/0.19
Periphyton	3/0.33	2/0.22	1/0.11	1/0.11	7/0.19
Substrate size	1/0.11	2/0.22	2/0.22	2/0.22)	7/0.19
Dissolved oxygen	1/0.11	2/0.22	0/0	1/0.11	4/0.11
Filamentous algae	2/0.22	1/0.11	1/0.11	0/0	4/0.11
Vegetation	0/0	1/0.11	0/0	2/0.22	3/0.08
pH	2/0.22	0/0	0/0	0/0	2/0.06
Temperature	1/0.11	1/0.11	0/0	0/0	2/0.06
Substrate embeddedness	0/0	0/0	0/0	0/0	0/0
Total / %	16/0.16	14/0.14	13/0.13	11/0.11	54/0.14
			Expected number of spurious correlations =20		
			By Year		
Temperature	2/0.29	1/0.14	4/0.57	1/0.14	8/0.29
pH	1/0.14	3/0.43	0/0	4/0.57	8/0.29
Specific conductance	3/0.43	2/0.29	1/0.14	2/0.29	8/0.29
Filamentous Algae	1/0.14	2/0.29	2/0.29	2/0.29	7/0.25
Dissolved oxygen	3/0.43	1/0.14	2/0.29	0/0	6/0.21
Vegetation	0/0	2/0.29	3/0.43	0/0	5/0.18
Periphyton	2/0.29	0/0	1/0.14	0/0	3/0.11
Current Velocity	0/0	0/0	0/0	1/0.14	1/0.04
Substrate size	1/0.14	0/0	0/0	0/0	1/0.04
Substrate Embeddedness	0/0	0/0	0/0	0/0	0/0
Depth	0/0	0/0	0/0	0/0	0/0
Total / %	13/0.17	11/0.14	13/0.17	10/0.13	47/0.15
			Expected number of spurious correlations =15		

condition at the three upper Current River sites is more variable both within and among the sites. Site C1 had higher dissolved oxygen concentrations, lower specific conductance, and smaller substrate size compared to all other sites. In contrast, site C2 had the largest substrate, lowest pH, and greatest abundance of filamentous algae and aquatic vegetation among all sites.

The results of the cluster analysis were corroborated by a NMDS analysis (Fig. 9). The NMDS model for the diversity and environmental data was found to be a good fit (Shepard plot stress value =0.04; Axis 1=0.61, Axis 2=0.22). The three Jacks Fork sites grouped closely to one another as did the three lower Current River sites. In contrast, the three upper Current River sites were more widely separated in ordination space. Correlations of the habitat variables with the ordination axes indicate associations of the Jacks Fork sites with higher specific conductance and pH, and to a lesser extent higher temperature and periphyton density (Fig. 9, Table 4). In contrast, Current River sites 4 through 6 were associated with higher embeddedness and discharge (Fig. 9, Table 4). Current River sites 2 and 3 were associated with higher dissolved oxygen and greater abundance of filamentous algae and aquatic plants (Table 4, Fig. 9).

The relatively wider spacing of sites C1 through C3 may be due, in part, to the influences of two first magnitude springs (Montauk and Welch, ≥2,800 liter/sec) and three second magnitude springs (Cave, Pulltite and Round, ≥280 liter/sec) located in the upper river basin where those sites are located. The Current River is formed by Montauk Spring approximately 14 km upstream of site C1. Welch Spring, Cave Spring and Pulltite Spring are located approximately 17 km, 8 km, and 3.5 km, respectively, upstream of site C2. Round Spring is located approximately 0.5 km upstream of site C3.

Because these springs produce cold, thermally consistent flows and are environmentally stable and uniform, they exhibit strong localized influences on the structure and functioning of the three upper sampling sites, thus giving them their unique character. Inflows

Table 4. NMDS correlation coefficients for habitat variables. See methods for details.

Variable	Axis 1	Axis 2
Discharge	0.77	-0.20
Temperature	-0.46	0.14
Dissolved oxygen	0.21	-0.49
Specific conductance	-0.004	0.68
pH	-0.14	0.41
Filamentous algae	0.10	-0.72
Vegetation	0.30	-0.79
Periphyton	-0.29	0.11
Substrate size	0.29	-0.02
Substrate embeddedness	0.33	0.19

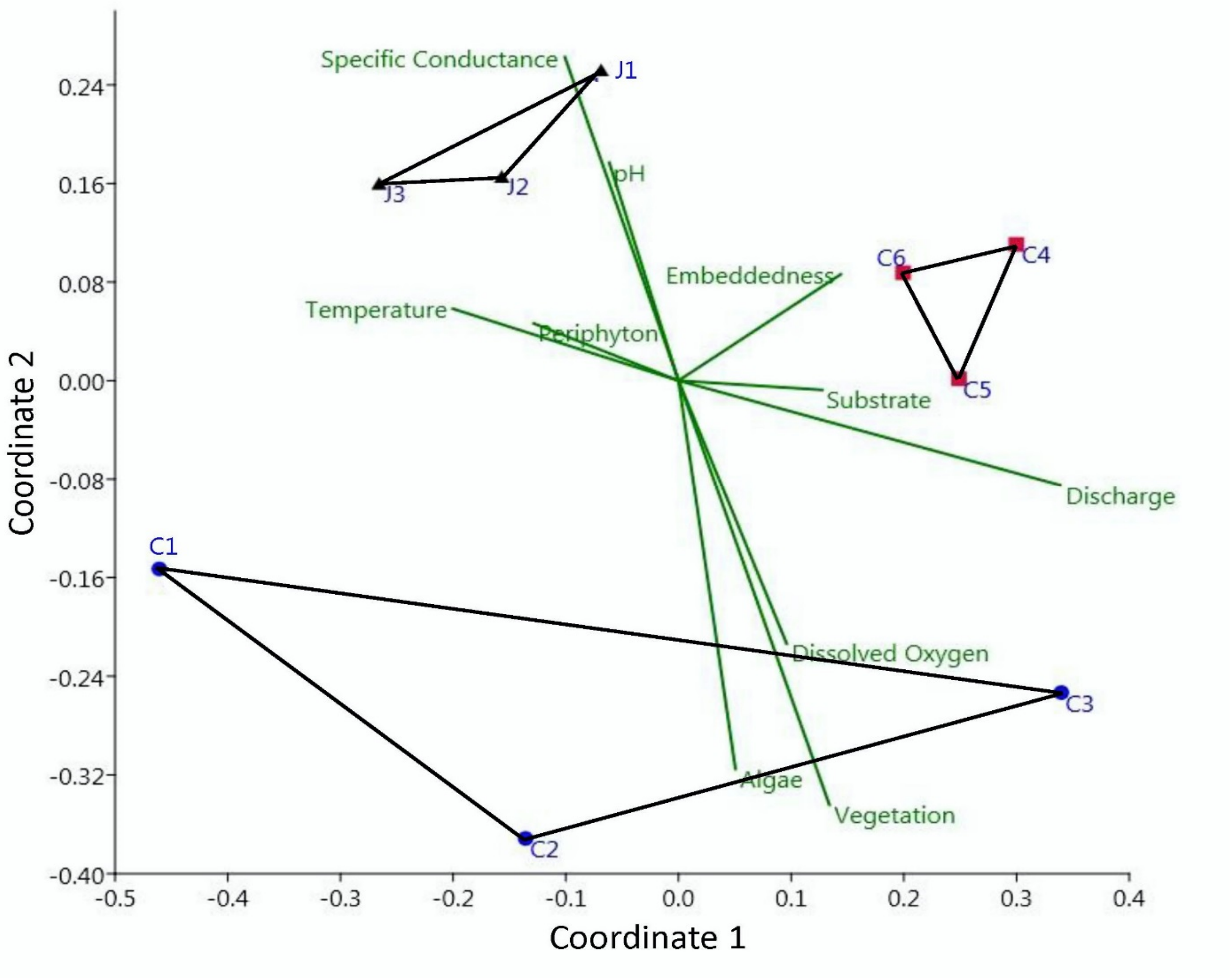


Figure 9. NMDS biplot with convex hulls for invertebrate diversity metrics by sampling sites and associated environment variables at Ozark National Scenic Riverways, Missouri. Triangles represent Jacks Fork sites (J1-J3), and circles (C1-C3) and squares (C4-C6) represent upper and lower Current River sites, respectively.

from these large springs influence surface stream character through thermal consistency (warmer in winter, colder in summer), higher dissolved calcium and specific conductance levels, lower dissolved oxygen concentrations, and potentially higher nutrient concentration (Smartt *et al.* 2013; Westhoff and Paukert 2014). Spring dominated streams also typically have lower faunal diversity and higher floral diversity in comparison to streams that receive most of their flow from surface sources because they generally have greater physical and chemical uniformity (Williams and Hogg 1988; Danks and Williams 1991; Varza and Covich 1995; Bowles and Dodd 2015). However, increased occurrence of aquatic vegetation and spring adapted aquatic invertebrates may occur in the mixing zone of springs and streams (Reiser *et al.* 2004; Barquín and Death 2011; Westhoff and Paukert 2014; Heth 2015).

Punctuated inflows of multiple large volume springs into the upper Current River effectively serve as predictable press type disturbances (Poff 1992; Lake 2000). Moreover, large spring inflows constantly reset or alter the predicted river continuum model (Vannote *et al.* 1980), and they mitigate patchiness associated with many surface fed streams (Resh *et al.* 1988; Lake 2000; Dornelas 2010). The uniformity and stability of the spring flows may also serve as a refugium for aquatic life from other disturbances (Lake 2000; Westhoff and Paukert 2014; Heth 2015), including floods, drought, and anthropogenic impacts. In contrast to the upper river, sampling sites on the lower Current River (C4-C6) have most of their baseflows originating from high magnitude springs (>90%, Mugel *et al.* 2009) so the punctuated disturbances from spring inflows observed upstream are not as pronounced. In addition, Blue Spring (first magnitude) is located approximately 8 km upstream of site C4 and Bass Rock Spring (second magnitude) and Big Spring (first magnitude) are located approximately 18 km and 10 km, respectively, upstream of site C6. The Jacks Fork is the major tributary of the Current River and most of its flows originate from surface flows. An additional first magnitude spring feeds the Jacks Fork downstream of our sampling sites with its confluence approximately 10 km upstream of Site C4. Finally, Current River basin tributaries located downstream of the confluence with Blue Spring have smaller drainage basins than those upstream, which may further increase the influence of springs in the lower river.

Conclusions

Invertebrate community structure in the Current River and Jacks Fork is diverse and reflects above average water quality. These two rivers are fully biologically supporting and meet Ozark reference stream criteria at all sites sampled. Inherent variability of invertebrate community diversity across sites and years highlights the importance of multiyear assessment and monitoring to support management decisions. Large volume springs likely serve as sustained and predictable sources of disturbance for the Current River, but this unique type of disturbance remains incompletely quantified. Although the condition of invertebrate communities and water quality at OZAR are largely sound and have high integrity, numerous ongoing and projected threats to these resources remain, and those threats largely originate outside of the park's jurisdictional boundaries.

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Winter surveys of *Cotinus obovatus* (American smoketree) in the Ozark Mountains

G.R. Graves

Department of Vertebrate Zoology, MRC-116, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, Washington, D.C. 20013
Center for Macroecology, Evolution and Climate, University of Copenhagen,2100 Copenhagen Ø, Denmark

Correspondence: gravesg@si.edu

Running Title: Winter Smoketree Surveys

Abstract

Cotinus obovatus (American smoketree) is a rare deciduous tree with a relictual distribution in southeastern North America. Efforts to map its fine-scale geographic distribution in the Ozark Mountains have been limited to the growing season when the distinctive blooming panicles and foliage facilitate detection in hardwood-cedar woodlands. I describe the physiognomic traits of leafless *C. obovatus* that permit effective population mapping in winter landscapes. Clumped growth and diagonally leaning stems facilitate detection at a distance. Bark texture, twig morphology, and sap odor confirm the identity of the tree at close range.

Introduction

Cotinus obovatus Raf. (Anacardiaceae: American smoketree), the sole member of the genus in the Western Hemisphere, has intrigued naturalists since Thomas Nuttall discovered it in 1819 on a bluff above the Grand River in eastern Oklahoma (Buckley 1881; Little 1942; Long 1970; Mohr 1882; Nuttall 1821; Palmer 1921; Sargent 1885, 1892; Weber 1990). Natural populations of this suffusively rare deciduous tree have been documented in 41 counties scattered in three disjunct regions: (1) Ozark Plateau in Arkansas and Missouri with a few scattered stations along bluffs of the Arkansas River and its tributaries in eastern Oklahoma, (2) southern Cumberland Plateau in northeastern Alabama and adjacent Tennessee and Georgia, and (3) the Edwards Plateau in west-central Texas. *Cotinus obovatus* is currently considered globally secure (G4) owing to protected populations on national forest lands in the Ozark Mountains of Arkansas and Missouri, but it is listed as threatened in Alabama (S2), Oklahoma (S2), and Tennessee (S2) and as critically imperiled (S1) in Georgia (Natureserve, 2017).

The center of abundance of *C. obovatus* on the Ozark Plateau occurs in four counties that border the Arkansas-Missouri state line (Baxter, Marion, Taney, Ozark). Factors that restrict the tree's geographic distribution are unknown, but all well-documented sites occur on soils derived from calcareous bedrock. An effort to map its fine-scale geographic distribution in relation to the soil chemistry and geology of the Ozark Plateau has been ongoing for the past decade. Population mapping has traditionally been conducted during the growing season when the distinctive blooming panicles (late April through mid-May) and foliage (April-September) are easily detectable in roadside surveys (Davis and Graves 2016). However, dense understory foliage may conceal isolated specimens of *C. obovatus* in the interior of hardwood-cedar woodlands. If the tree can be identified at modest distances (~10–40 m) in leafless winter landscapes, detection efficiency during walking surveys may actually increase relative to growing season efforts. Here I describe the physiognomic traits that facilitate winter surveys of *C. obovatus*. These can be grouped into characters that form the search image necessary to detect trees at distance (growth form) and those that confirm identity at close range (bark, twigs, sap).

Methods

Study area

Winter surveys of *C. obovatus* were conducted 28 February-14 March 2017 and 4-20 February 2018 in the Sylamore Ranger District of the Ozark National Forest, centered on the Norfork and Norfork SE topographic quadrangles (7.5-minute series, U.S. Geological Survey) in Baxter County, Arkansas. This region is topographically complex (100–429 m), dissected by deep valleys that drain into the White River and its major tributary, the Buffalo River. Current land cover in the national forest is a patchwork of secondary hardwood forest, *Juniperus virginiana* (eastern redcedar), and *Pinus echinata* (shortleaf pine). Glades are relatively

Figure 1. Typical growth form of *Cotinus obovatus* (American smoketree) in the Ozark National Forest, Baxter County, Arkansas. Multiple stems emerge from a single root crown in many specimens.

rare and those that do occur are relatively small. Old-growth trees and elevational bands of timber that exhibit no evidence of logging are found on steep, rocky slopes. Surface rocks over most of the study area are composed of interbedded limestone, dolostone, and sandy dolostone of the Everton Formation (middle Ordovician) which is overlain by thin beds of St. Peter sandstone. The highest ridges and knobs are capped by cherty limestones of the Boone formation, (Haley et al., 1993). Data reported in this study were obtained on off-road surveys aimed at documenting the geographic coordinates of witness specimens of *C. obovatus* at a 200 m grain size.

Data collection

I photographed specimens of *C. obovatus* (n = 177) to document growth form and stem count. Selected trees met the following criteria: (*i*) the base of the tree was unobscured by undergrowth; (*ii*) the number of stems (> 5 cm DBH) emerging from the root crown could be counted in the photograph; and (*iii*) the stems were mature enough to have attained the flaky bark characteristic of older trees. I walked around each tree until the principal axis of a focal stem, usually the thickest for multi-stemmed specimens, was perpendicular to the plane of the camera. From enlarged photographs, I measured the angle of the focal stem (from ground level) along its lower side with a protractor. Landmarks for angle measurement were stem base at ground level and a point on the stem ~1.5 m from the base. Only a single stem per specimen was measured. Summary statistics (mean ± standard deviation) are presented for stem angle and stem count.

Results

Growth form

Sargent (1892:3) offered a brief description of *C. obovatus* physiognomy: “A small tree, twenty-five to thirty-five feet in height, with a straight trunk occasionally twelve or fourteen inches in diameter, usually dividing, twelve or fourteen feet from the ground, into several erect stems which separate into wide-spreading, often slightly pendulous branches.” Individuals of this diameter are rare in the Ozark Mountains, but more importantly, a majority of *C. obovatus* trees are composed of multiple stems that emerge from the root crown (Fig. 1).

In the sample of photographed trees (Fig. 2), the number of stems per root crown varied from 1 to 15 ($\bar{x}$ = 2.6 ± 2.5). Stems have a tendency to lean or sprawl diagonally from the base. Stem angle (from horizontal) varied from 9° to 90° ($\bar{x}$ = 62.7 ± 18.6°; n = 177). Solitary stems tend to be more vertical ($\bar{x}$ = 68.2 ± 16.6°; n = 77) than stems randomly chosen from multi-stemmed specimens ($\bar{x}$ = 58.4 ± 18.9°; n =100). In any event, diagonally leaning stems and clumped growth are

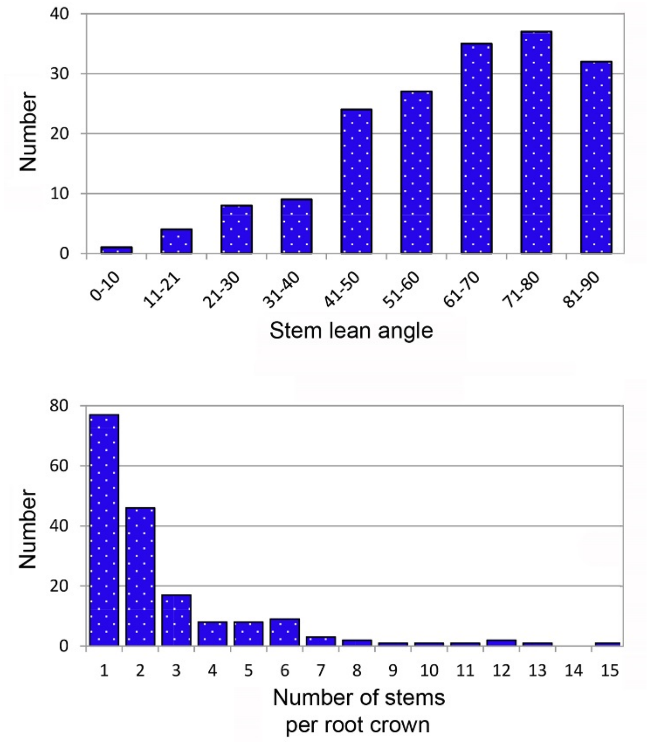

Figure 2. Histogram of stem lean angle (90º = vertical) and number of stems per root crown of *Cotinus obovatus* (American smoketree) in the Ozark National Forest, Baxter Country, Arkansas. Y-axis represents the number of specimens in each bin.

hallmarks of mature *C. obovatus* in open and closed canopy hardwood-cedar woodland in the Ozark Mountains.

Bark

Standard floras (Mohr 1901; Steyermark 1963) of states that harbor populations of *C. obovatus* omit descriptions of bark, and the accounts and illustrations in field guides (Leopold *et al.* 1998; Samuelson and Hogan 2003; Sibley 2009; Simpson 1988) are too brief or idiosyncratic to be useful for distinguishing the bark of *C. obovatus* from that of other small forest trees with rough bark. Buckley (1881: 125) observed, "…trunks and larger limbs [of *C. obovatus*] coated with light gray and deeply-furrowed bark resembling the bark of the larger trees of the common sassafras (*S. officinale*)." Perhaps the most accurate description was penned soon after the rediscovery of the tree in Alabama in 1881 (Mohr 1882: 218), "The bark is rough, covered with a whitish gray epidermis of a deep chestnut-brown underneath, and exfoliating in oblong square scales of uniform size."

In the Ozark Mountains, the smooth gray bark of young *C. obovatus* begins to crack and crenellate when the stems reach a diameter of 4–7 cm, although some sprouts as large as 10 cm have immature bark. The bark of mature stems is relatively thin and fractured into rounded rectangular or oval scales that are usually 1.8–3.5 times longer than wide (Fig. 3). Scale tips are frequently curled or curved away from the stem. Some specimens possess imbricated bark scales with curved tips pointing downward. Scale size and shape tend to be relatively uniform within individuals but vary significantly among trees. The bark is pale to medium gray when dry and dark gray to black when wet. The diagnostic combination of bark texture and color distinguishes *C. obovatus* from other small trees with craquelure bark in the Ozark Mountains (e.g., eastern redbud, *Cercis canadensis*; flowering dogwood, *Cornus florida*; sassafras, S*assafras albidum*; and gum bumelia, *Sideroxylon lanuginosum*). Large specimens of *C. obovatus* frequently exhibit cambial dieback, which can affect as much as 80% of the stem girth. Exposed wood weathers to grayish black. The crown foliage of many older specimens is nourished by a meandering band of cambium and phloem winding up the stem. The cause of cambial dieback is unknown.

Twigs

Sargent (1892: 3) provided a concise description of the twigs of *C. obovatus,* "The young shoots are purple at first, but soon become green; during the first winter they are bright red-brown and are covered with small white lenticular spots and marked by large prominent leaf-scars…The winter-buds are acuminate and an eighth of an inch long, and are covered with thin dark red-brown scales." During the late winter in the Ozark Mountains, the reddish-brown color, moderate diameter (3-5 mm), and absence of knobby or obliquely projecting buds give twigs a smooth silhouette and distinctive appearance (Fig. 4) that help confirm the identity of *C. obovatus* at moderate distances.

Sap

Buckley (1881:3) noted that broken branches of *C. obovatus*, "emitted a yellowish sap, the odor of which was highly aromatic; to me very unpleasant." Mohr (1882: 218) observed that the bark, when bruised, exuded "a resinous sap of a heavy, disagreeable terebinthinous odor."

I found the pungent odor to be reminiscent of thesap of *Rhus copallinum* (winged sumac) and *R. glabra* (smooth sumac), both of which are widespread in the Ozarks, and nearly identical to that of *Cotinus coggygria* (Eurasian smoketree), which is occasionally used in urban landscaping in the Ozark region. Broken twigs of *C. obovatus* are odorous, even in cool or cold weather (0–5° C). The odor persists for years in dead twigs. The aromatic constituents of *C. obovatus* sap have not been analyzed but intensive analyses of *C. coggygria* have revealed that the wood and bark are rich in monoterpenes, sesquiterpenes, and phenols *(Matić et al*. 2016). As a matter of practice, all witness trees encountered during winter surveys in areas where *C. obovatus* trees occurred at low density were confirmed through the examination of growth form, bark, twigs, and sap odor.

Figure 3. Bark of mature stems of *Cotinus obovatus* (American smoketree).

Figure 4. Twigs of the American smoketree (*Cotinus obovatus*).

Discussion

Winter survey efficiency has not been quantified although it certainly varies with topography, understory visibility, and the density of *Cotinus obovatus* trees. Once the search image has been formed, observers should be able to detect most if not all mature specimens within 20 m of the survey path at slow walking speed (1-3 km/hr). In relatively open understories, the clustered, leaning stems of *C. obovatus* may be apparent to the unaided eye at distances as great at 50 m. Binoculars are indispensable for scanning prospective specimens, and I never attempted a cross-country survey without them.

Acknowledgments

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Parasites of the Spotted Sucker, *Minytrema melanops* (Cypriniformes: Catostomidae) from Arkansas and Oklahoma

C.T. McAllister[1*], D.G. Cloutman[2], A. Choudhury[3], T. Scholz[4], S.E. Trauth[5], T.J. Fayton[6], and H.W. Robison[7]

[1]*Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745*
[2]*P. O. Box 197, Burdett, KS 67523*
[3]*Division of Natural Sciences, St. Norbert College, 100 Grant Street, DePere, WI 54115*
[4]*Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic*
[5]*Department of Biological Sciences, Arkansas State University (Emeritus), State University, AR 72467*
[6]*Lamar Fish Health Center, U.S. Fish and Wildlife Service, 400 Washington Avenue, Lamar, PA 16848*
[7]*9717 Wild Mountain Drive, Sherwood, AR 72120*

*Correspondence: cmcallister@se.edu

Running Title: Spotted Sucker Parasites

Abstract

During October 2015, March and April 2016 and again between March and April 2017, 15 Spotted Sucker (*Minytrema melanops*) were collected from sites in the Ouachita (*n* = 5), Red (*n* = 1), and St. Francis (*n* = 5) river drainages, Arkansas, and the Arkansas River drainage, Oklahoma (*n* = 4), and examined for protozoan and metazoan parasites. Found were *Calyptospora* sp., *Myxobolus* sp., *Pseudomurraytrema alabarrum*, *Biacetabulum banghami*, *Penarchigetes oklensis*, and *Acanthocephalus* sp. New host and distributional records are documented for these parasites.

Introduction

The Spotted Sucker, *Minytrema melanops* (Rafinesque) is a moderately slim-bodied nearly terete sucker that ranges from the Lower Great Lakes basin (lakes Erie, Huron, and Michigan) and Ontario, Canada, the upper Mississippi Valley south to the Gulf slope drainages from Texas to Florida and north on the Atlantic coast to Cape Fear drainage of North Carolina (Gilbert and Burgess 1980). In Arkansas, *M. melanops* is widespread and common but nearly absent from the upper White River drainage (Robison and Buchanan 1988). In Oklahoma, the Spotted Sucker is found in about the eastern half of the state (Miller and Robison 2004). It is adapted to slow-moving slightly-turbid waterways of hard-bottomed creeks and small rivers. They feed on detritus, zooplankton, and various organisms such as mollusks and other invertebrates (White and Haag 1977).

There is a moderate amount of information on parasites of *M. melanops* from various North American localities (Mackiewicz 1968, 1969, Chien and Rogers 1970, Bangham 1972, Dechtiar 1972, Combs *et al.* 1976, 1977, Williams 1976, Christensen *et al.* 1982, Williams and Rogers 1984, Hoffman 1999, Dutton and Barger 2010, Fayton and Kritsky 2013, Gale *et al.* 2014, McAllister *et al.* 2013, 2015, Oros *et al.* 2016, 2018). However, most reports involve fish collected east of the Mississippi River and nothing is known of specimens from Arkansas. Here we report some new host and distributional records for parasites of *M. melanops* from Arkansas and Oklahoma.

Materials and Methods

During October 2015, March and April 2016 and again between March and April 2017, 15 *M. melanops* (mean ± 1SD total length [TL] = 179.0 ± 58.6, range 92–243 mm) were collected with a boat electrofisher from **OKLAHOMA:** Arkansas River drainage of the Illinois River, ***Cherokee Co.*** (35° 57' 30.042"N, 94° 52' 10.0272"W) (*n* = 4); and with seines from **ARKANSAS:** St. Francis River drainage of Crow Creek at Madison, ***St. Francis Co.*** (35° 00' 45.4752”N, 90° 43' 08.3346”W) (*n* = 5), Red River drainage of the Saline River, ***Sevier Co.*** (34° 05' 46.3128"N, 94° 05' 5.0496"W) (*n* = 1), Ouachita River drainage at Calion Lake Spillway, ***Union Co.*** (33° 19' 32.2608"N, 92° 31' 35.1912"W) (*n* = 3), West Tulip Creek, ***Dallas Co.*** (34° 01' 13.026"N, 92° 44' 4.1136"W) (*n* = 1), and Caddo River, ***Montgomery Co.*** (34° 23' 52.7742"N, 93° 37' 17.6226"W) (*n* = 1). Specimens were placed in aerated habitat water and necropsied within 24 hr. We followed accepted guidelines for the use of fish in research (Use of Fishes in Research Committee 2014); specimens were overdosed with a concentrated chloretone solution and a mid–ventral incision was made to expose the gastrointestinal (GI) tract and viscera. The GI tract was split longitudinally from esophagus to anus and all internal organs were placed in Petri dishes containing 0.9% saline and examined under a stereomicroscope. A species of monogenean was picked with minuten nadeln directly from the gills of fish (*n* = 9) previously preserved in 10% formalin then mounted in Gray and Wess medium stained with Gomori's trichrome. Cestodes from the intestine were fixed in hot tap water without coverslip pressure, transferred to 70–95% (v/v) DNA grade ethanol, stained in Mayer's carmine, dehydrated in an ethanol series, cleared with eugenol, and mounted in Canada balsam (Scholz *et al.* 2015). Tissues with encapsulated parasites were fixed in 10% neutral buffered formalin and processed using standard histological methods (Presnell and Schreibman 1997), with sectioning at 8–10 µm followed by staining with hematoxylin and eosin. Voucher specimens of parasites were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska, or the helminthological collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS). Host voucher specimens were deposited in the Henderson State University (HSU) fish collection, Arkadelphia, Arkansas. Prevalence, mean intensity ± 1SD, and range of infection are provided in accordance with terminology given in Bush *et al.* (1997).

Results and Discussion

The following is an annotated list of collection and parasite data as follows:

PROTISTA: APICOMPLEXA: CALYPTOSPORIDAE

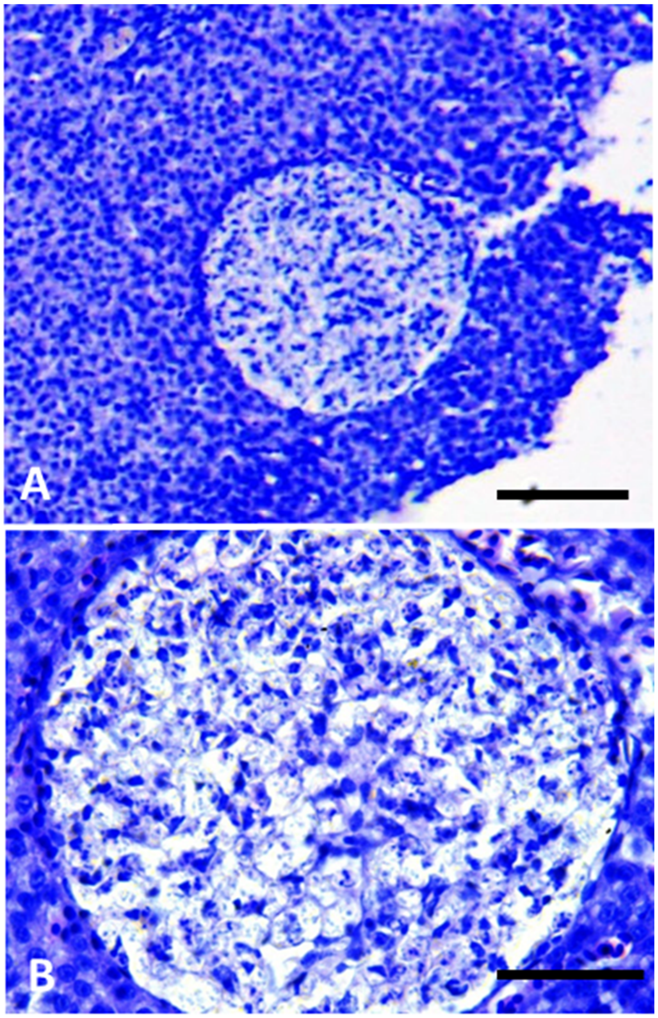
Calyptospora sp. (Figs. 1A–B)

Host and locality: 1 *M. melanops* (213 mm TL) collected on 21 Apr. 2017 from Crow Creek at Madison, St. Francis Co., Arkansas (35° 00' 45.4752"N, 90° 43' 08.3346"W).

Prevalence: 1/15 (7%) overall; 1/5 (20%) St. Francis Co.

Intensity: Sporulated and unsporulated oocysts too numerous to count.

Site of infection: Oocysts in hepatocytes of liver encapsulated in connective tissue (Fig. 1).



Figures 1A–B. *Calyptospora* sp. from *Minytrema melanops*. (A) Low power magnification showing oocysts in encapsulation of liver tissue; scale bar = 250 µm. (B) High power magnification of encapsulation showing hundreds of oocysts; scale bar = 125 µm.

Type-host and type locality: Gulf Killifish (*Fundulus grandis*), Ocean Springs, Jackson Co., Mississippi (Duszynski *et al.* 1979).

Other reported hosts: Several species of fundulid fishes are known as natural hosts, as well as the Inland Silverside (*Menidia beryllina*) and Gulf Toadfish (*Opsanus beta*) (Solangi and Overstreet 1980, Upton and Duszynski 1982, Fournie and Overstreet 1983, 1993, Hawkins *et al.* 1983, 1984, Oliveira *et al.* 1993,Whipps *et al.* 2012).

Geographic range: Arkansas (this report); Florida (Whipps *et al.* 2012); Louisiana (Duszynski *et al.* 1979); Mississippi (Duszynski *et al.* 1979, Fournie and Overstreet, 1983, 1993, Oliveira *et al.* 1993, Fournie *et al.* 2000).

Additional Arkansas records: None.

Specimens deposited: HWML 139429 (slide).

Remarks: Infected liver tissue from our host appeared identical to those reported by Duszynski *et al.* (1979) for *Eimeria* (=*Calyptospora*) *funduli* (see their figs. 12–15). This coccidian requires palaemonid shrimp intermediate hosts (Fournie and Overstreet 1983, Overstreet *et al.* 1984, Fournie *et al.* 2000), and one, the Mississippi grass shrimp (*Palaemonetes kadiakensis*), occurs at our study site. Fournie *et al.* (1985) observed numerous motile sporozoites free in the intestinal contents of experimentally infected *P. kadiakensis* and suggested that benthic crustaceans are likely candidates for intermediate hosts in *Calyptospora empristica* Fournie, Hawkins and Overstreet, 1985 because the Starhead Topminnow (*Fundulus notti*) fish-host must apparently die before oocysts can be dispersed.

There are 2 eimerians previously reported from catostomid fishes, *E. catostomi* Molnár and Hanek, 1974 from the intestine of White Sucker (*Catostomus commersoni*) and Northern Hogsucker (*Hypentelium nigricans*) from Canada, and *E. fernandoae* Molnár and Hanek, 1974 from the same hosts and locality (see Hoffman 1999). To date, neither species have been determined to be *Calyptospora* sp. and more importantly, the site of infection was intestinal tissue, not liver. Therefore, the current report is the first time *Calyptospora* sp. has been reported from a catostomid fish and the initial report of this protist from Arkansas and west of the Mississippi River.

CNIDARIA: MYXOBOLIDAE

Myxobolus sp. (Figs. 2A–B)

Hosts and localities: 4 *M. melanops* (222.8 ± 17.1, 205–243 mm TL) collected on 21 Apr. 2017 from Crow Creek at Madison, St. Francis Co., Arkansas (35° 00' 45.4752"N, 90° 43' 08.3346"W); 2 *M. melanops* (116, 120 mm) collected on 13 Mar. 2017 from Illinois River, Cherokee Co., Oklahoma (35° 57' 30.042"N, 94° 52' 10.0272"W).

Prevalence: 6/9 (67%) overall; 4/5 (80%) St. Francis Co., Arkansas; 2/4 (50%) Cherokee Co., Oklahoma.

Intensity: Too numerous to count.

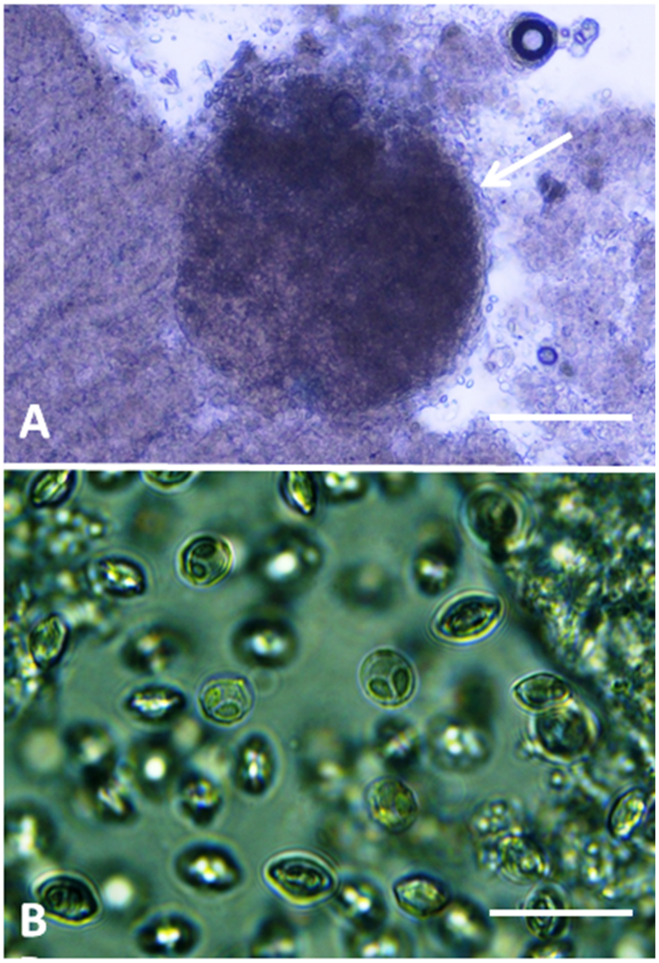
Site of infection: Gills.

Geographic range: The genus is cosmopolitan.

Additional Arkansas records: None.

Specimens deposited: HWML photovoucher 139414.

Remarks: Bangham (1972) reported an unidentified myxozoan from *M. melanops* from Lake Erie. However, a single described species of myxozoan was described 30 yr earlier from Spotted Sucker. Meglitsch (1942) described *M.* (syn. *Myxosoma*) *microthecum* from the mesenteries of *M. melanops* from Illinois. The spores of specimens found on the gills of *M. melanops* in this study are smaller on average (mean length 8.7, range 7.4–11.5 µm, *n* = 30) than those from the mesenteries in Illinois (11.7, 10.0–12.5 µm) (Meglitsch 1942). Although the ranges partially overlap, the sites of infection are clearly different. Of the other 10 species of *Myxobolus* reported from the gills of North American suckers (Eiras *et al.* 2005), *Myxobolus globosus* Gurley, 1893, from the Eastern Creek Chubsucker (*Erimyzon oblongus*) is most similar to *Myxobolus* sp. on the gills of *M. melanops* in size and shape of the spores. The spores of *M. globosus* are globose or subcircular, 7–8 µm long and 6–7 µm wide (Kudo 1920, Eiras *et al.* 2005). The cysts from these 2 species of *Myxobolus* differ in shape from elongate- ellipsoidal to rod-shaped in *M. globosus vs.* spheroidal in our *Myxobolus* sp. There appears to be considerable site specificity as well as host specificity among fish myxozoans. Therefore,



Figures 2A–B. *Myxobolus* sp. from gills of *Minytrema melanops*. (A) Cyst (arrow); scale bar = 100 µm. (B) Spores from cyst; scale bar = 20 µm.

our form is likely a new species that will require additional study.

MONOGENOIDEA: DACTYLOGYRIDA: PSEUDOMURRAYTREMATIDAE

Pseudomurraytrema alabarrum Rogers, 1966 (Figs. 3A–C)

Syn. *Murraytrema muelleri* Price, 1967

Hosts and localities: 2 *M. melanops* (205, 216 mm TL) collected on 21 Apr. 2017 from Crow Creek at Madison, St. Francis Co., Arkansas (35° 00' 45.4752”N, 90° 43' 08.3346”W).

Prevalence: 2/9 (22%) overall; 2/5 (40%) St. Francis Co., Arkansas.

Intensity: 2 and 3 worms.

Site of infection: Gills.

Type-host and type locality: M. melanops, Lee County, Alabama (Rogers 1966).

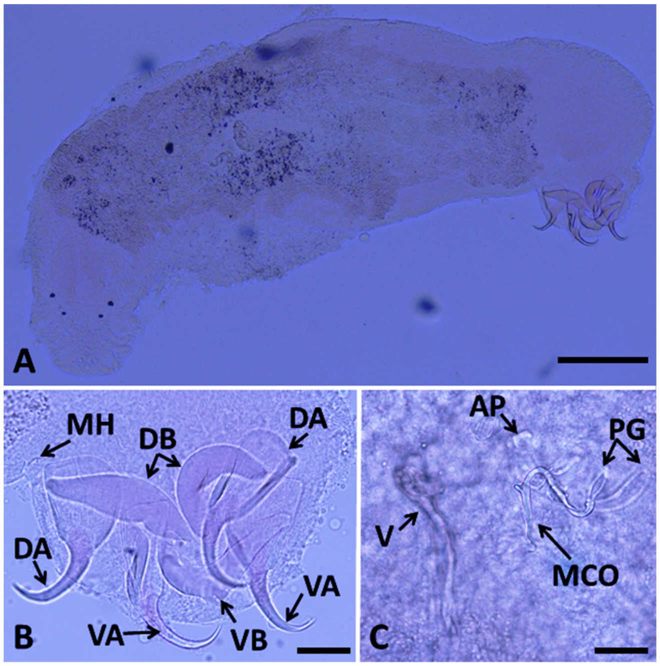
Other reported hosts: None.

Geographic range: Alabama (Rogers 1966); Arkansas (this report); Ohio (Mergo and White 1984).

Additional Arkansas records: None.

Specimens deposited: HWML 139418 (4 slides).

Remarks: In Alabama, *P. alabarrum* was described from *M. melanops* (Rogers 1966). For the first time we document *P. alabarrum* from Arkansas and west of the Mississippi River.



Figures 3A–C. *Pseudomurraytrema alabarrum* from the gills of *Minytrema melanops*. (A) Whole mount; scale bar = 150 µm. (B) Haptor showing marginal hook (MH), dorsal anchors (DA), dorsal bars (DB), ventral anchors (VA), and ventral bar (VB); scale bar = 25 µm. (C) Reproductive organs showing male copulatory organ (MCO), accessory piece (AP), prostatic glands (PG), and vagina (V); scale bar = 25 µm.

CESTODA: CARYOPHYLLIDEA: CARYOPHYLLAEIDAE

Biacetabulum banghami Mackiewicz, 1968

Hosts and localities: 1 *M. melanops* (230 mm TL) collected on 21 Apr. 2017 from Crow Creek at Madison, St. Francis Co., Arkansas (sample no. US 672) (35° 00' 45.4752”N, 90° 43' 08.3346”W); 1 *M. melanops* (190 mm) collected on 10 Apr. 2016 from Caddo River, Montgomery Co., Arkansas (sample no. US 593) (34° 23' 52.7742"N, 93° 37' 17.6226"W).

Prevalence: 2/15 (13%) overall; 1/5 (20%) St. Francis Co.; 1/1 (100%) Montgomery Co.

Intensity: 1 and 2 worms.

Site of infection: Intestinal tract.

Type-host and type locality: *M. melanops*; Opintalocca Creek, 3.2 km NE Tuskegee, Macon Co., Alabama (Mackiewicz 1968).

Other reported host: Golden Redhorse, *Moxostoma erythrurum*.

Geographic range: USA (Alabama (Mackiewicz 1968), Arkansas (this report), Oklahoma (Mackiewicz 1968).

Additional Arkansas records: None.

Specimens deposited: IPCAS C-000/1 (slide).

Remarks: *Biacetabulum banghami* was described by Mackiewicz (1968) from *M. melanops* (type host) and *M. erythrurum* from Alabama and Oklahoma. The species is characterized by the presence of 2 papilliform processes on each side of the scolex and vitelline follicles arranged in 2 lateral rows (Mackiewicz 1968). We document *B. banghami* from Arkansas for the first time.

Penarchigetes oklensis Mackiewicz, 1969

Host and locality: 1 *M. melanops* (213 mm TL) collected on 21 Apr. 2017 from Crow Creek at Madison, St. Francis Co., Arkansas (sample no. US 671) (35° 00' 45.4752”N, 90° 43' 08.3346”W).

Prevalence: 1/15 (7%) overall; 1/5 (20%) St. Francis Co.

Intensity: 1 worm.

Site of infection: Intestinal tract.

Type-host and type locality: M. melanops; Northeastern Outing Club Lake, 22 km NE of Tahlequah on OK St. Hwy. 10, Cherokee Co., Oklahoma (Mackiewicz 1969).

Other reported hosts: None.

Geographic range: Arkansas (this report); Oklahoma (Mackiewicz 1969).

Additional Arkansas records: None.

Specimens deposited: IPCAS C-000/1 (slide).

Remarks: *Penarchigetes* was erected by Mackiewicz (1969) to accommodate the new species, *P. oklensis* from *M. melanops* (type and only host) in Oklahoma. Since then, another 2 species were described from Lake Chubsucker, *Erimyzon sucetta*, namely *P. fessus* Williams, 1979 in southeastern USA and *P. macrorchis* Christensen and Calentine, 1983 in New York and Oklahoma (Hoffman 1999).

In addition to the 2 above-listed species, *M. melanops* has been reported to host another 4 species of caryophyllideans. *Promonobothrium ulmeri* (Calentine and Mackiewicz 1966) (syn. *Monobothrium ulmeri*) was described from Northern Hogsucker, *Hypentelium nigricans* in Iowa. The species has recently been reported from *M. melanops* in Mississippi by Oros *et al.* (2016). *Promonobothrium minytremi* Mackiewicz, 1968, was described from *M. melanops* in Oklahoma and then found in the same fish species from Mississippi, South Carolina and Wisconsin (Oros *et al.* 2016) and *Isoglaridacris agminis* sensu Williams and Rogers, 1972 was reported from *M. melanops* from Alabama (Williams 1976). A larval *Glaridacris* sp. was reported from *M. melanops* from Lake Erie by Bangham (1972).

ACANTHOCEPHALA: EOACANTHOCEPHALA: NEOECHINORHYNCHIDAE

Neoechinorhynchus sp.

Host and locality: 1 *M. melanops* (92 mm TL) collected on 22 Apr. 2016 from Calion Lake Spillway, Union Co., Arkansas (33° 19' 32.2608"N, 92° 31' 35.1912"W).

Prevalence: 1/15 (7%) overall; 1/3 (33%) Union Co.

Intensity: 2 male worms.

Site of infection: Intestinal tract.

Other reported hosts: Numerous fishes of various taxa (see Hoffman 1999).

Geographic range: Cosmopolitan.

Additional Arkansas records: Acanthocephalans of the genus *Neoechinorhynchus* have been reported from various Arkansas fishes (see McAllister *et al.* 2016, 2018).

Specimens deposited: None (specimens retained in senior author's collection).

Remarks: Three acanthocephalans have been previously reported from *M. melanops*, *Pomphorhynchus bulbocolli* from Lake Erie (Dechtiar 1972), *Leptorhynchoides thecatus* from Alabama (Williams and Rogers 1982) and *Pomphorhynchus lucyae* from Alabama and Florida (Williams and Rogers 1984). Since females were not collected in our study, it is not possible to provide a species designation. However, we document a new host record for *Neoechinorhynchus* sp.

In summary, we provide the first parasitological survey of *M. melanops* from Arkansas by documenting 2 new host and 4 new distributional records for some of its parasites. In an attempt to collect additional specimens, the senior author (CTM) revisited the St. Francis County site noted herein on 22 July 2018 and did not locate any *M. melanops*. Indeed, larger samples sizes are needed as well as collections of Spotted Suckers from other drainages in Arkansas and Oklahoma. Future studies will undoubtedly report additional records, including the possibility of description of new taxa.

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Additional Records of Acanthocephalan Parasites from Arkansas Fishes, with New Records from Missouri Fishes

C.T. McAllister[1*], M.A. Barger[2], and H.W. Robison[3]

[1]*Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745*
[2]*Department of Natural Science, Peru State College, Peru, NE 68421*
[3]*9717 Wild Mountain Drive, Sherwood, AR 72120*

*Correspondence: cmcallister@se.edu

Running Title: Acanthocephalans of Arkansas and Missouri Fishes

Abstract

Over the last decade, our research consortium has provided information on acanthocephalan parasites of Arkansas vertebrates, including records from some of the state's fishes. Here, we continue to provide data on new geographic and new host records of acanthocephalans from Arkansas fishes. In addition, for the first time, we report records of acanthocephalans for some Missouri fishes. We document 2 new state records as well as 10 new host records for some fish acanthocephalans.

Introduction

Acanthocephalans, as adults, are highly specialized, dioecious parasites of the intestinal tract of all groups of vertebrates, including a great variety of taxa from freshwater fishes. Recently, McAllister *et al.* (2016b) provided an overview of the acanthocephalans of Arkansas vertebrates which included information on those from some of the state's fishes. Here, we continue to document new host and geographic records for acanthocephalans from fishes of the state. In addition, there is little available on acanthocephalans from Missouri fishes (Banks and Ashley 2000, Day *et al.* 2014), so we report here, for the first time, on some acanthocephalans from fishes from the Ozark Faunal Region of the state (see Pflieger 1997).

Methods

Between March 2016 and June 2017, we collected fishes with backpack electroshockers (DC current), dipnets and seines. Fish were placed in habitat water and necropsied within 24 hr. We followed accepted guidelines for the use of fish in research (Use of Fishes in Research Committee 2014). Specimens were overdosed with a concentrated tricaine methanesulfonate solution and measured for total length (TL). A mid–ventral incision from cloaca up to the level of the stomach was made to expose the gastrointestinal tract as well as other internal viscera (including gallbladder and liver) which was removed and placed in a Petri dish containing 0.9% w/v saline. Acanthocephalans found were transferred to Petri dishes containing distilled water overnight to completely evert their proboscides. They were then fixed in 70–95% v/v DNA-grade ethanol, stained with acetocarmine and mounted entire with Canada balsam. Voucher specimens were deposited in the Harold W. Manter Laboratory of Parasitology Collection (HWML), Division of Parasitology, University of Nebraska-Lincoln. Host voucher specimens were deposited in the Henderson State University Museum (HSU), Arkadelphia, Arkansas. We follow Amin's (2013) classification of the Acanthocephala.

Our annotated list of data for fishes harboring acanthocephalans is as follows: host and TL (when available), collection site (latitude and longitude, WGS 84), collection date, HWML accession number (when available), prevalence, intensity, and remarks.

Results and Discussion

The following taxa of acanthocephalans were found in Arkansas and Missouri fishes from their river drainages/basin and counties as follows:

EOACANTHOCEPHALA: NEOECHINORHYNCHIDA: NEOECHINORHYNCHIDAE

***Neoechinorhynchus cylindratus* (Van Cleave, 1911) Van Cleave, 1919**

Hosts and locality: 2 (147, 152 TL mm) *Micropterus dolomieu* (Smallmouth Bass), Little Indian Creek (Meramec River Basin), ***Franklin Co.,*** Missouri (38° 12' 48.5958"N, 90° 54' 08.6862"W), 10 Jun. 2017. HWML 139434; 1 *M. dolomieu* (127 mm TL), Panther

Creek (Meramec River Basin), ***Webster* Co.,** Missouri (37° 28' 46.182"N, 92° 46' 57.7596"W), 12 Jun. 2017.

Prevalence and intensity: 2/2 (100%) Little Indian Creek, 1–2 worms; 1/1 (100%) Panther Creek, 1 worm.

Remarks: The first intermediate host in the life cycle is an ostracod, *Cypria* sp. (Ward 1940). Although *N. cylindratus* has been reported from a variety of fishes (see McAllister *et al.* 2016b), including *M. dolomieu* (Hoffman 1999), this is the first time it has been reported from Smallmouth Bass in Missouri. Banks and Ashley (2000) reported *N. cylindratus* was the most common helminth from Largemouth Bass, *M. salmoides* in Smithville Reservoir, northwest Missouri.

***Paulisentis missouriensis* (Keppner, 1974)**

Host and locality: 1 (95 mm TL) *Campostoma pullum* (Central Stoneroller), Panther Creek (Meramec River Basin), ***Webster* Co.,** Missouri (37° 28' 46.182"N, 92° 46' 57.7596"W), 12 Jun. 2017.

Prevalence and intensity: 1/1 (100%); 1 worm (retained for further study).

Remarks: In the life cycle, larval *P. missouriensis* develop in copepods (Keppner 1974). While this acanthocephalan has been previously reported in Missouri from Creek Chubs, *Semotilus atromaculatus* (Keppner 1974), this is the first report of its occurrence in *C. pullum*. McAllister *et al.* (2015) reported a *Paulisentis* sp. that could not be differentiated from *P. missouriensis* or *P. fractus* Van Cleave and Bangham, 1949 from *Campostoma anomalum* (Central Stoneroller) from Benton Co., Arkansas. It is quite possible that both of these acanthocephalans are synonymous and *P. fractus* would have priority; however, additional study is needed to arrive at that conclusion, including molecular analyses.

PALEOACANTHOCEPHALA: ECHINORHYNCHIDA: ECHINORHYNCHIDAE

***Acanthocephalus* sp.**

Host and locality: 1 (93 mm TL) *Fundulus catenatus* (Northern Studfish), Caddo River at Caddo Gap (Ouachita River drainage), ***Montgomery* Co.,** Arkansas (34° 23' 55.752"N, 93° 37' 17.3712"W), 21 Apr. 2016.

Prevalence and intensity: 1/3 (33%), 1 worm.

Remarks: McAllister *et al.* (2016a) reported cystacanths of an unknown acanthocephalan commonly in liver tissues of 17 of 25 (68%) *F. catenatus* from Crooked Creek, Marion Co., Arkansas. Therefore, we document the first adult acanthocephalan from this host.

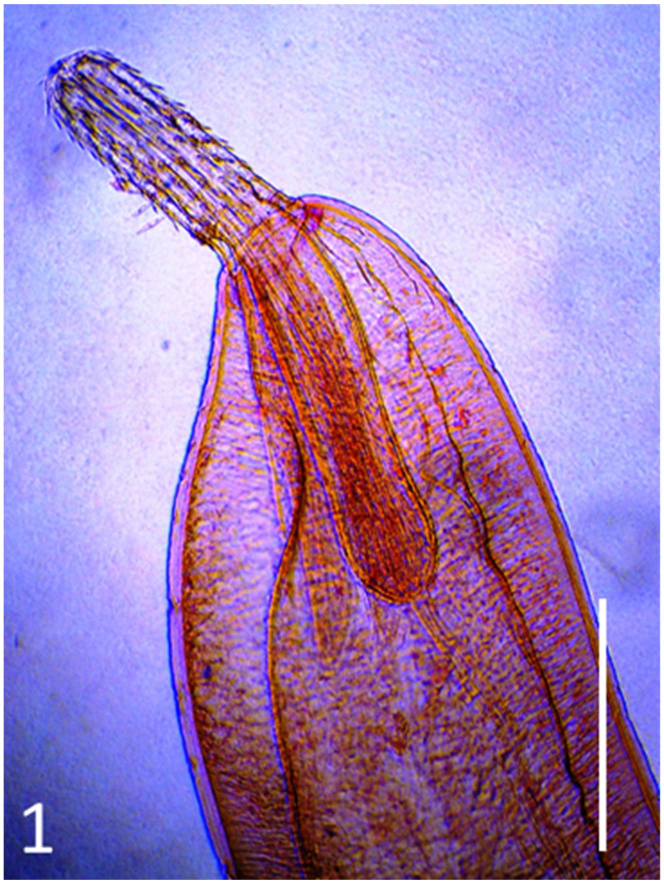

Figure 1. *Acanthocephalus dirus* (HWML 139439) from *Ambloplites constellatus*. Scale bar = 500 µm.

***Acanthocephalus dirus* (Van Cleave, 1931) Van Cleave and Townsend, 1936 (Fig. 1)**

Host and locality: 1 (191 mm TL) *Ambloplites constellatus* (Ozark Bass), Little Red River (White River drainage), ***Van Buren* Co.,** Arkansas (35° 39' 08.8992"N, 92° 19' 12.3774"W), 10 July 2016. HWML 139439.

Prevalence and intensity: 1/1 (100%), 1 worm.

Remarks: This acanthocephalan develops as larvae in isopod intermediate hosts, *Asellus* and *Lirceus* (Seidenberg 1973, Muzzall and Rabalais 1975). *Acanthocephalus dirus* has the widest distribution and diversity of hosts compared to its North American congeners (Amin 1985). McAllister *et al.* (2016b) reported this acanthocephalan from 2 darters, 1 sunfish, and 1 shiner from Arkansas for the first time. We document a new host record for *A. dirus*.

***Acanthocephalus tahlequahensis* Oetinger and Buckner, 1976 (Fig. 2)**

Hosts and localities: 1 (62 mm TL) *Etheostoma caeruleum* (Rainbow Darter), Smyrna Creek (White

River drainage), ***Madison* Co.,** Arkansas (35° 39' 04.8522"N, 92° 55' 13.6122"W), 31 Mar. 2016; 2 (60, 65 mm TL) *Etheostoma flabellare* (Fantail Darter), Panther Creek (Meramec River Basin), ***Webster* Co.,** Missouri (37° 28' 46.182"N, 92° 46' 57.7596"W), 10 Jun. 2017. HWML 139435; 1 (200 mm TL) *Hypentelium nigricans* (Northern Hogsucker), Little Indian Creek (Meramec River Basin), ***Franklin* Co.,** Missouri (38° 12' 48.5958"N, 90° 54' 08.6862"W); 1 (90 mm TL) *Cottus bairdi* (Mottled Sculpin), Bennett Spring (Osage River Basin), ***Laclede* Co.,** Missouri (37° 42' 6.5082"N. 92° 50' 24.8418"W), 11 Jun. 2017.

Prevalence and intensity: 1/1 (100%) *E. caeruleum*; 2/7 (29%) *E. flabellare*; 1/1 (100%) *H. nigricans*; 1/1 (100%) *C. bairdi*. All hosts with 1 worm each.

Remarks: Although the life cycle has not yet been demonstrated for this acanthocephalan, like other members of the genus, larval *A. tahlequahensis* are thought to occur in isopods (Hoffman 1999). This acanthocephalan was originally described from Oklahoma in the Illinois River drainage from 2 darters and 2 cyprinids (Oetinger and Buckner 1976). McAllister *et al.* (2014b, 2015a, 2016b) extended the host (family) range of *A. tahlequahensis* by reporting it in Arkansas from fishes of the families Centrarchidae, Cottidae (*C. carolinae*, Banded Sculpin) and Ictaluridae. We document additional hosts for *A. tahlequahensis*, including the first time from a catostomid fish, as well as a new state record for Missouri.

POMPHORHYNCHIDAE

Pomphorhynchus bulbocolli Linkins *in* Van Cleave, 1919 (Fig. 3)

Hosts: 2 (57, 95 mm TL) *Aphredoderus sayanus* (Pirate Perch), Spring Mill at Big Spring (White River drainage), ***Independence* Co.,** (35° 49' 41.6856"N, 91° 43' 27.3822"W), 23 Apr. 2016; 2 (58, 62 mm TL) and one (63 mm TL) *Etheostoma spectabile* "complex" (Ozark Darter n. sp.), same locality, 23 Apr. 2016 & 8 Jun. 2017. HWML 139436; 1 (110 mm TL) *Luxilus zonatus* (Bleeding Shiner), same locality, 8 Jun. 2017. HWML 139437–139438.

Prevalence and intensity: 2/11 (18%) *A. sayanus*, 1 worm each; 2/6 (33%) and 1/11 (9%) *E. spectabile* complex, 2 worms each, 1 worm; 1/1 (100%) *L. zonatus*, 1 worm.

Remarks: In the life cycle, larvae occur in amphipods (Jensen 1953). We (McAllister and Robison *unpubl.*) have observed that the *E. spectabile* complex darter noted above with *P. bulbocolli* also contained *Hyalella azteca* amphipods in their gut. This acanthocephalan is widely-distributed in North American freshwater fishes (Amin 1987, Hoffman 1999) and show little host specificity. However, McAllister *et al.* (2016b) was the first to report *P. bulbocolli* from Arkansas fishes (2 cyprinids) and we add 3 new host records. In addition, McAllister *et al.* (2015b) reported *P. bulbocolli* from a Midland watersnake (*Nerodia sipedon pleuralis*) from the same site noted herein that was considered an artifact of its piscivorous diet.

Figures 2–3. (2) *Acanthocephalus tahlequahensis* (HWML 139435) from *Etheostoma flabellare*. Scale bar = 250 µm. (3) *Pomphorhynchus bulbocolli* (HWML 139437) from *Luxilus zonatus*. Note distal bulb (arrows). Scale bar = 500 µm.

ILLIOSENTIDAE

Leptorhynchoides sp. (Fig. 4)

Host and locality: 5 (81.0 ± 5.6, 72–86 mm TL) *Lepomis marginatus* (Dollar Sunfish), Beard Lake (Red River drainage), ***Hempstead* Co.,** Arkansas (33° 41' 46.8996"N, 93° 56' 33.6336"W), 30 Jun. 2017. HWML 139433.

Prevalence and intensity: 5/7 (71%), 3.6 ± 2.2, 1–7 worms.

Remarks: Leptorhynchoides acanthidion Steinauer and Nickol, 2014 was reported from *L. marginatus* from Louisiana (Steinauer and Nickol 2014). Unfortunately, because ours were juvenile specimens, we were not able to provide a specific identity. However, this finding represents the first report of an acanthocephalan parasite from this host in Arkansas.

***Leptorhynchoides aphredoderi* Buckner and Buckner, 1976 (Fig. 5)**

Host and locality: 3 (41, 47, 52 mm TL) *A. sayanus*, Locust Bayou at US 278 (Ouachita River drainage), **Calhoun Co.,** Arkansas (33° 33' 27.5034"W, 92° 40' 32.9124"N), 10 Jul. 2016. HWML 139441.

Prevalence and intensity: 3/5 (60%), 1, 2, and 3 worms.

Remarks: Larval *L. aphredoderi* occur in amphipods and larvae less than 30 days old and become encysted in mesenteries of fish (Hoffman 1999). McAllister *et al.* (2014a) reported a *Leptorhynchoides* sp. similar to *L. aphredoderi* from *A. sayanus* from the Rolling Fork River, Sevier Co., Arkansas. However, we document the first definitive report of this acanthocephalan from the state (see McAllister *et al.* 2016b). Buckner and Buckner (1976) originally described this acanthocephalan from *A. sayanus*, Redbreast Sunfish (*Lepomis auritus*) and Spotted Sunfish (*Lepomis punctatus*) from Louisiana. In addition, Buckner (1991) reported *L. aphredoderi* from Warmouth (*Lepomis gulosus*) and *A. sayanus* from Alabama. We document the first report of *L. aphredoderi* from west of the Mississippi River.

Figures 4–5. (4) *Leptorhynchoides* sp. (HWML 139433) from *Lepomis marginatus*. Scale bar = 500 µm. (5) *Leptorhynchoides aphredoderi* (HWML 139441) from *Aphredoderus sayanus*. Scale bar = 250 µm.

Compared to what we know about the acanthocephalans of Arkansas fishes, information on those from Missouri fishes is mostly lacking (Hoffman 1999). Missouri supports more than 200 species of fishes (Pflieger 1997) and many have never been surveyed for acanthocephalans or even helminths in general. Here we have attempted to augment that with some new records but additional work is badly needed.

In summary, we document 10 new host records for acanthocephalans from fishes from the Ouachita, Red, and White river drainages in Arkansas and the Meramec and Osage river basins in Missouri. We also document a new state record for an acanthocephalan found in 3 fish from Missouri and report *L. aphredoderi* from Arkansas for the first time. Additional surveys will undoubtedly increase our knowledge of these parasites in Arkansas and Missouri.

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Comparison of Various Mean Field Formulations for Retrieving Refractive Indices of Aerosol Particles Containing Inclusions

K.S. Dooley* and J. DeYoung

Department of Chemistry, University of Central Arkansas, Conway, AR 72035

*Correspondence: kdooley@uca.edu

Running Title: Refractive Indices of Particles Containing Inclusions

Abstract

Application of effective medium approximation (EMA) methods to two-component systems are presented. Systems studied are composed of water, sulfate, soot, and dust as these are commonly encountered atmospheric aerosol components. Atmospheric models often employ EMAs to include internally mixed aerosols without the computational burden of exact theory. In the current work, several types of mixing rules (Maxwell-Garnet, Bruggeman, and coherent potential approximation) have been applied to various two-component internally mixed particles at 550 nm using volume fractions of the minor component below 0.1. As expected, results show that the formulations tested produce very similar effective refractive indices indicating that electric field interactions between inclusions is negligible at the tested volume fractions. This indicates that the differences in component refractive index has only a minor effect on the validity of the EMA at the tested volume fractions. In all cases considered, the linear average of the refractive indices of the two components provides an upper limit for the EMAs.

Introduction

The optical properties of heterogeneous particles are of considerable interest in determining their role in climate change. Correctly modelling their effect on the global radiation energy budget hinges on the accurate computation of the particle's associated scattering and absorbing optical properties (Ackerman and Toon 1981; Lesins *et al.* 2002). A few scenarios where this type of internal mixing model is appropriate are when a hygroscopic component becomes solvated in a high humidity environment; cases where dust or soot particles contain numerous scattering inclusions due to adsorbed water or a mineral inclusion; or instances where particles are formed through various coagulation processes.

Mie scattering theory is used to computationally describe the scattering of radiation by particles which have a diameter similar to or larger than the wavelength of incident light. It can be used to retrieve information such as the single scattering albedo of the particle, when the particle's shape (in simple cases, particles are assumed to be spherical), size and refractive index of the particle are known (Bohren and Huffman 1983).

In more complex cases when particles are composed of multiple components in a random orientation, the particle does not have a homogeneous index of refraction and exact solutions of Maxwell's equations with appropriate boundary conditions are needed in order to solve the scattering problem. Solutions to this problem do exist in the literature (Chýlek and Videen 1998; Videen *et al.* 1995; Ioannidou and Chrissoulidis 2002). Unfortunately, the treatment is a multiple scattering problem resulting in solutions that are impractical for use in climate models due to their computational complexity. In atmospheric applications, the computational cost of exact solutions is unwarranted given that other data such as particle shape, and size distribution also contribute to the errors in the computed radiation budget. Consequently, prediction of the optical properties to within a few percent is often deemed sufficient for geophysical applications (Chýlek *et al.* 2000).

Therefore, it is appealing in the case of climate models to approximate the effective refractive index (n_{eff}) of a particle containing inclusions using various computational mixing formulations known as effective medium approximations (EMAs) (Chýlek *et al.* 2000). EMAs are based on *ad hoc* assumptions that lead to a simplified, more easily solvable model of the real particle. They allow for the computation of n_{eff} of the mixture based on the refractive indices of the individual components and their relative volume fractions as illustrated in Fig.1. Effective medium approximation formulations have been shown to be effective up to a volume fraction of inclusions (f) of 0.1 or smaller, although they are often used outside of this range

(Bohren and Huffman 1983). These formulations are also valid only when the size of the inclusions is much smaller than the wavelength of the incident light.

Figure 1: The diagram illustrates how effective medium approximations (EMAs) simplify the treatment of a particle containing randomly placed inclusions. By inputting the permittivities of the components with their respective volume fractions, the EMA computes an effective permittivity, ϵ_{eff}, of the homogenized particle for use in Mie scattering calculations.

There are numerous EMA formulations in the scientific literature, with various and sometimes conflicting merits and limitations (Sihvola 1999). In the present work, the Maxwell-Garnet, Bruggeman, and coherent potential approximations are compared. These formulations all assume a dilute suspension of small inclusions (<0.01μm diameter) inside a single-component matrix. Although they have all been expanded to include ellipsoids and other types of mixtures, only the original formulations for spherical inclusions are considered here. The main difference in these formulations is the amount of coupling between the electric fields of the inclusions (Sihvola 1999).

For small volume fractions of inclusions, the three formulations should predict the same results for n_{eff}. The formulations differ at larger volume fractions of inclusions, but the point and degree of the difference is dependent on the permittivities of the components (Sihvola 1999). In the current work, the approximate formulations have been applied to mixtures composed of common atmospheric aerosol components (water, sulfate, soot, and dust).

Materials and Methods

All computational work was done using Mathcad 15 (PTC).

Computational parameters of mixtures

The effective refractive indices of two-component mixtures are computed at an incident wavelength of 550 nm as this is relevant to climate models. Because they are common materials in atmospheric aerosols and provide a range of relevant visible refractive indices, water, sulfate, dust, and soot are chosen as the major components of the mixtures while one of the other four materials is chosen as the low volume fraction inclusions.

At 550 nm, the refractive indices of the components used are: $n_{water} = 1.333 + i1.96 \times 10^{-9}$, $n_{sulfate} = 1.470 + i1.00 \times 10^{-7}$, $n_{dust} = 1.530 + i5.50 \times 10^{-3}$, and $n_{soot} = 1.750 + i0.440$ (Hale and Querry 1973; Weast *et al.* 1986; Toon *et al.* 1976; d'Almeida *et al.* 1991). It should be pointed out that the refractive index of soot and dust are somewhat variable. The refractive index of soot will be dependent on the combustion conditions in which the particles are generated, and the specific mineral components will affect the refractive index of dust. As the point of the study is to explore the EMAs themselves, the use of these representative refractive indices is acceptable.

One significant advantage of using these parameters is that a small portion of the effective refractive indices computed in this study can be verified via data published previously (Erlick 2006). That work included only combinations of a non-absorbing particle with absorbing inclusions at low volume fractions corresponding to data reported in our Tables 5 and 6. The current values are in very good agreement with those previously reported. The current work expands that study to include all combinations of materials and volume fractions to provide a clearer understanding of the differences in the EMA formulations when used in instances where the indices of refraction of the major component and inclusion are quite similar and when they are relatively different.

As the incident wavelength is at least four times the size of the inclusions, it is more appropriate to apply the mixing rules to the dielectric constants of the materials rather than to the refractive indices themselves. The relationship between refractive index, n, and dielectric constant, ϵ, is $n = \epsilon^{1/2}$. The real and imaginary terms of the dielectric constant (corresponding to capacitance and loss, respectively) for a material are related to the real and imaginary terms of the index of refraction through $\epsilon_{real} = n_{real}^2$ and $\epsilon_{imag} = 2n_{real}n_{imag}$ (Bohren and Huffman 1983).

The effective dielectric constants (ϵ_{eff}) of the mixtures are computed using the mixing rules described in the following subsection. The effective dielectric constant may then be transformed to an effective refractive index (n_{eff}) by taking the complex square root of ϵ_{eff} (Bohren and Huffman 1983). These are given as:

$$n_{real} = \left[\frac{\left(\epsilon_{real}^2 + \epsilon_{imag}^2\right)^{1/2} + \epsilon_{real}}{2}\right]^{1/2} \quad (1)$$

$$n_{imag} = \left[\frac{\left(\epsilon_{real}^2 + \epsilon_{imag}^2\right)^{1/2} - \epsilon_{real}}{2}\right]^{1/2} \quad (2)$$

Mean field formulations used to compute ϵ_{eff}

The Maxwell-Garnet, Bruggeman, and coherent potential approximations (CPA) are used for computation of ϵ_{eff}. In these equations, the permittivities of the inclusion and the major component are designated as ϵ_{incl} and ϵ_{major}, respectively. The value f refers to the volume fraction of the inclusion in the mixture. These results are also compared with a simple volume weighted linear average of the component refractive indices. A linear average has often been found to overestimate the imaginary term of the refractive index of a particle with inclusions; thus, it will provide an upper bound for this value (Sihvola 1996).

The most commonly used mixing rule is the Maxwell-Garnet formulation. This model depends only on the volume fraction (f) of the inclusion and assumes that second-order effects due to neighboring inclusions may be neglected (Bohren and Huffman 1983). The formulation for ϵ_{eff} is given in Equation 3.

$$\epsilon_{eff} = \epsilon_{major} + \frac{3f\epsilon_{major}(\epsilon_{incl} - \epsilon_{major})}{\epsilon_{incl} + 2\epsilon_{major} - f(\epsilon_{incl} - \epsilon_{major})} \quad (3)$$

The Bruggeman approximation makes many of the same assumptions as Maxwell-Garnett, but increases the influence of the inclusions to be symmetric to that of the background medium (Bohren and Huffman 1983). This formulation is shown in Equation 4:

$$(1-f)\frac{\epsilon_{major} - \epsilon_{eff}}{\epsilon_{major} + 2\epsilon_{eff}} + f\frac{\epsilon_{incl} - \epsilon_{eff}}{\epsilon_{incl} + 2\epsilon_{eff}} = 0 \quad (4)$$

The model known as the coherent potential approximation (CPA) assumes full coupling between neighboring inclusions. The formulation is shown as Equation 5:

$$\epsilon_{eff} = \epsilon_{major} + \frac{3f\epsilon_{eff}(\epsilon_{incl} - \epsilon_{major})}{3\epsilon_{eff} + (1-f)(\epsilon_{incl} - \epsilon_{major})} \quad (5)$$

These formulations can be combined into a unified mixing rule (Equation 6) where the formulation contains a unitless parameter υ (Sihvola 1999).

$$\frac{\epsilon_{eff} - \epsilon_{major}}{\epsilon_{eff} + 2\epsilon_{major} - \upsilon(\epsilon_{eff} - \epsilon_{major})} = \frac{f(\epsilon_{incl} - \epsilon_{major})}{\epsilon_{incl} + 2\epsilon_{major} + \upsilon(\epsilon_{incl} - \epsilon_{major})} \quad (6)$$

The value of υ produces the three previous formulations: $\upsilon = 1$ produces the Maxwell-Garnet formulation, $\upsilon = 2$ produces the Bruggeman formulation, and $\upsilon = 3$ gives the coherent potential approximation.

Results and Discussion

In the following subsections, the results of the calculations are organized by the optical properties of the components such that major and inclusion components with similar optical properties are viewed together. To this end, soot and dust are categorized as absorbing components while water and sulfate are categorized as non-absorbing. Refractive indices are computed for each two-component mixture using the volume fraction, f, of the inclusion equal to 0.0001, 0.01, 0.1. The formulations explored are all expected to be the same for f < 0.1. It is only for f > 0.1 that the formulations are expected to differ (Sihvola 1999). The point at which this difference occurs is important to note when choosing a model to use.

The computed effective refractive indices are reported here in terms of the real term which indicates stronger light scattering as it increases and the imaginary term which indicates more light absorption by the mixture as it increases. The terms of the refractive indices are split when recorded to facilitate the observation of trends within the formulations.

Non-absorbing materials with non-absorbing inclusions

There are two combinations of materials that are included in this category: water with sulfate inclusions, and sulfate with water inclusions. Both materials have very small imaginary refractive index terms at 550 nm which indicate that they do not efficiently absorb light at this wavelength. Table 1 includes the imaginary term for the refractive index, n_{eff},for these mixtures, and Table 2 includes the real term.

The results of these formulations are not unexpected. In the case of water with sulfate inclusions, all of the formulations produce the same imaginary term for n_{eff}.

For sulfate with water inclusions, the CPA produces a slightly larger imaginary term at f =0.1. That is also the only instance that an EMA formulation produced an imaginary term larger than that of the linear average.

Table 1: Imaginary term of n_{eff} computed for the linear average (Lin); Maxwell-Garnet (MG); Bruggeman (B); and coherent potential (CPA) formulations for listed volume fractions, f.

	Water with Sulfate Inclusions			
f =	**Lin**	**MG**	**B**	**CPA**
0.0001	1.97E-09	1.96E-09	1.96E-09	1.96E-09
0.01	2.94E-09	1.96E-09	1.96E-09	1.96E-09
0.1	1.18E-08	1.05E-08	1.05E-08	1.05E-08

	Sulfate with Water Inclusions			
f =	**Lin**	**MG**	**B**	**CPA**
0.0001	1.00E-07	1.00E-07	1.00E-07	1.00E-07
0.01	9.90E-08	9.88E-08	9.88E-08	9.88E-08
0.1	9.02E-08	8.94E-08	8.94E-08	9.06E-08

Table 2: Real term of n_{eff} computed for the linear average (Lin); Maxwell-Garnet (MG); Bruggeman (B); and coherent potential (CP) formulations for listed volume fractions, f.

	Water with Sulfate Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	1.33	1.33	1.33	1.33
0.01	1.33	1.33	1.33	1.33
0.1	1.35	1.35	1.35	1.35

	Sulfate with Water Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	1.47	1.47	1.47	1.47
0.01	1.47	1.47	1.47	1.47
0.1	1.46	1.46	1.46	1.46

The real terms of n_{eff} are all the same as the linear average. In both cases, you can begin to see the models differing only at f = 0.1 indicating that below this volume fraction, the interactions between inclusions is negligible.

Absorbing materials with absorbing inclusions

This category contains two combinations: soot with dust inclusions and dust with soot inclusions. The imaginary and real terms of the computed n_{eff} are given in Tables 3 and 4 respectively.

In these combinations it is notable that even at the low inclusion volume fractions, the imaginary term of the refractive index changes significantly in the dust mixture with soot inclusions indicating the large influence a small amount of a strong absorber can make on the overall material even when the major component is also somewhat absorbing. In the case of soot with dust inclusions, the linear average is only slightly larger than the EMA formulations. Where dust is the major component, this difference is larger. All EMA formulations produce identical imaginary terms at the volume fractions explored.

Table 3: Imaginary term of computed n_{eff}. Abbreviations are the same as in previous tables.

	Soot with Dust Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	4.40E-01	4.40E-01	4.40E-01	4.40E-01
0.01	4.36E-01	4.35E-01	4.35E-01	4.35E-01
0.1	3.97E-01	3.95E-01	3.95E-01	3.95E-01

	Dust with Soot Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	5.54E-03	5.54E-03	5.54E-03	5.54E-03
0.01	9.85E-03	9.63E-03	9.63E-03	9.63E-03
0.1	4.90E-02	4.70E-02	4.70E-02	4.70E-02

Table 4: Real term of computed n_{eff}. Abbreviations are the same as in previous tables.

	Soot with Dust Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	1.75	1.75	1.75	1.75
0.01	1.75	1.75	1.75	1.75
0.1	1.73	1.73	1.73	1.73

	Dust with Soot Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	1.53	1.53	1.53	1.53
0.01	1.53	1.53	1.53	1.53
0.1	1.55	1.55	1.55	1.55

As seen in Table 4, the real term of the computed n_{eff} is identical for all formulations and the linear average at these volume fractions.

Non-absorbing materials with absorbing inclusions

This category of particles includes either water or sulfate as the major component and soot or dust as the inclusion. The imaginary and real terms of the computed n_{eff} are given in Tables 5 and 6 respectively. The values included here agree very well with data published previously (Erlick 2006). The few differences seem to be a result of the precision carried through the calculations and are differences of ±1 in the last reported digit only. The values that differ from the published data are identified with an asterisk in Table 5.

Table 5: Imaginary term of computed n_{eff}. Abbreviations are the same as in previous tables. Values that differ from those reported previously are marked with an asterisk (Erlick 2006). It should be noted that the differences are insignificant and only occur in the last reported digit.

	Water with Dust Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	5.52E-07	5.18E-07*	5.18E-07*	5.18E-07*
0.01	5.50E-05	5.17E-05	5.17E-05	5.17E-05
0.1	5.50E-04	5.19E-04	5.21E-04	5.23E-04
	Water with Soot Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	4.40E-05	3.76E-05	3.76E-05	3.76E-05
0.01	4.40E-03	3.77E-03	3.78E-03*	3.78E-03
0.1	4.40E-02	3.80E-02*	3.87E-02	3.90E-02*
	Sulfate with Dust Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	6.50E-07	6.42E-07	6.42E-07	6.42E-07
0.01	5.51E-05	5.43E-05	5.43E-05	5.43E-05
0.1	5.50E-04	5.43E-04	5.43E-04	5.43E-04
	Sulfate with Soot Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	4.41E-05	4.08E-05	4.08E-05	4.08E-05
0.01	4.40E-03	4.07E-03	4.08E-03	4.08E-03
0.1	4.40E-02	4.10E-02	4.12E-02	4.12E-02

As expected, the addition of an absorbing inclusion causes a much larger increase in the imaginary term of n_{eff} as compared to that of the major component without inclusions. Similar to the previous mixtures studied, the linear average again provides the upper bound for the imaginary term when compared to the formulations at low volume fractions.

Also, the three EMA formulations are very similar for all volume fractions tested, although the mixtures with water as the major component seem to already be beginning to produce differing estimations for the imaginary term at the $f = 0.1$ volume fraction. This may indicate that the models will not be equivalent at some moderately small volume fractions just over $f > 0.1$ and that care should be taken when choosing a formulation to use for particles modelled as a non-absorbing matrix with absorbing inclusions.

The real terms of the n_{eff} computed for non-absorbing materials with absorbing inclusions are provided in Table 6. In the case of a non-absorbing component with absorbing inclusions, the linear average again produced nearly the same value as all of the EMA formulations at all of the volume fractions tested.

Table 6: Real term of computed n_{eff}. Abbreviations are the same as in previous tables.

	Water with Dust Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	1.333	1.333	1.333	1.333
0.01	1.335	1.335	1.335	1.335
0.1	1.353	1.352	1.352	1.352
	Water with Soot Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	1.333	1.333	1.333	1.333
0.01	1.337	1.337	1.337	1.337
0.1	1.375	1.376	1.376	1.375
	Sulfate with Dust Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	1.470	1.470	1.470	1.470
0.01	1.471	1.471	1.471	1.471
0.1	1.476	1.476	1.476	1.476
	Sulfate with Soot Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	1.470	1.470	1.470	1.470
0.01	1.473	1.473	1.473	1.473
0.1	1.498	1.500	1.500	1.500

Absorbing materials with non-absorbing inclusions

The final category of mixtures considered is that of an absorbing material with non-absorbing inclusions. The real and imaginary terms of n_{eff} are provided in Tables 7 and 8. The main component of these particles is either soot or dust, and the inclusion is taken to be water or sulfate.

As can be seen in Table 7, the imaginary term of the soot with water inclusions mixture decreases in this instance, and all formulations result in the same value as the linear average. It is only a very slight amount larger than the value from the other formulations.

It is also striking that the effect of either sulfate or water inclusions is virtually identical on the computed imaginary term in both the dust and soot cases.

Table 7: Imaginary term of computed n_{eff}. Abbreviations are the same as in previous tables.

	Soot with Water Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	4.40E-01	4.40E-01	4.40E-01	4.40E-01
0.01	4.36E-01	4.35E-01	4.35E-01	4.35E-01
0.1	3.96E-01	3.95E-01	3.94E-01	3.94E-01
	Soot with Sulfate Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	4.40E-01	4.40E-01	4.40E-01	4.40E-01
0.01	4.36E-01	4.35E-01	4.35E-01	4.35E-01
0.1	3.96E-01	3.94E-01	3.94E-01	3.94E-01
	Dust with Water Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	5.50E-03	5.50E-03	5.50E-03	5.50E-03
0.01	5.45E-03	5.44E-03	5.44E-03	5.44E-03
0.1	4.95E-03	4.94E-03	4.93E-03	4.93E-03
	Dust with Sulfate Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	5.50E-03	5.50E-03	5.50E-03	5.50E-03
0.01	5.44E-03	5.44E-03	5.44E-03	5.44E-03
0.1	4.95E-03	4.94E-03	4.94E-03	4.94E-03

As is the pattern for the other combinations of materials, the real term of n_{eff} seems to be predicted identically regardless of the EMA used. These are also identical to the linear average.

Conclusions

The effective refractive indices (n_{eff}) of various two-component materials have been calculated using various EMA formulations as well as a simple linear average. Effective refractive indices were computed at volume fractions (f) of inclusions in the matrix material equal to 0.0001, 0.01, and 0.1 which is within the region that the EMAs are thought to be valid. The main difference between the formulations is the amount of interaction included between the inclusions themselves. At low volume fractions the formulations should produce identical results (Sihvola 1999). Comparison of results computed through these models generally support that conclusion.

The values of the real term computed for any of the two-component combinations show that no formulation produces a difference from the linear average. This is true at any volume fraction tested.

Table 8: Real term of computed n_{eff}. Abbreviations are the same as in previous tables.

	Soot with Water Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	1.750	1.750	1.750	1.750
0.01	1.746	1.746	1.746	1.746
0.1	1.708	1.707	1.707	1.707
	Soot with Sulfate Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	1.750	1.750	1.750	1.750
0.01	1.747	1.747	1.747	1.747
0.1	1.722	1.721	1.722	1.722
	Dust with Water Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	1.530	1.530	1.530	1.530
0.01	1.528	1.528	1.528	1.528
0.1	1.510	1.510	1.510	1.510
	Dust with Sulfate Inclusions			
f =	**Lin**	**MG**	**B**	**CP**
0.0001	1.530	1.530	1.530	1.530
0.01	1.529	1.529	1.529	1.529
0.1	1.518	1.524	1.524	1.524

In general, the data supports the understanding that the linear average serves as an upper bound on the imaginary term of the refractive index (Sihvola 1999), although in some cases this term is the same magnitude as the ones computed with the EMA formulations.

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Organochloride Pesticides Present in Animal Fur, Soil, and Streambed in an Agricultural Region of Southeastern Arkansas

M.E. Grilliot[1], J.L. Hunt[2]*, and C.G. Sims[2]

[1]*Auburn University—Montgomery, Biology Department, College of Arts and Sciences, P.O. Box 244023, Montgomery, AL 36124*
[2]*University of Arkansas at Monticello, School of Mathematical and Natural Sciences, 397 University Drive, Monticello, AR 71656*

*Correspondence: huntj@uamont.edu

Running Title: Organochloride Pesticide Residues in Southeastern Arkansas

Abstract

Animals in agricultural settings may be subject to bioaccumulation of toxins. For the last several years, we collected hair samples from bats and rodents in an agricultural area near Bayou Bartholomew in Drew County, Arkansas. Samples were submitted to the Center of Environmental Sciences and Engineering at the University of Connecticut for wide-screen toxin analysis. Several of these samples contained measurable amounts of organochloride pesticides or their metabolites, including some that have been banned for decades, such as dichlorodiphenyltrichloroethane (DDT) and chlordane. In addition, we collected several samples of soil from within an agricultural field, from adjacent edge habitat, from alongside the bank of the Bayou, and from the bed of the Bayou itself. Although none of these samples tested positive for DDT or chlordane, all of the samples except one contained measurable amounts of metabolites from these pesticides. This study raises questions about environmental persistence of DDT/DDE and other organochlorides. There may be risks to wildlife populations that warrant further investigations into effects of long-term exposure to these toxins.

Introduction

Toxic heavy metals and agricultural chemicals are being found in soil and water and pose serious human health and wildlife risks (USEPA 1997). Bats have been demonstrated to be prone to high levels of toxins in some agricultural regions (Geluso *et al.* 1976). This study is part of a longer-term study that will focus on recent evidence that suggests a connection between toxins in bats and susceptibility to WNS (Kannan *et al.* 2010). However, our investigation has led us to consider the possibility that agricultural toxins may be spread through entire landscapes. Herein we report the results of our research so far.

Materials and Methods

Our original focus was on a colony of Rafinesque's big-eared bats (*Corynorhinus rafinesquii*). This bat roosts in abandoned buildings, caves, hollow trees, and under bridges across the southeastern United States (Jones 1977; Trousdale and Beckett 2004). We were made aware of a maternity colony of over 100 individuals roosting in the Taylor House, an abandoned antebellum building located at the edge of an agricultural field adjacent to Bayou Bartholomew in Drew County, Arkansas. We noticed that the agricultural field was routinely being crop-dusted, and grew concerned about the potential impacts to local wildlife.

During the summer of 2014, we began monitoring this colony as part of a project to determine possible effects of agricultural chemicals on the bats. We collected 10 Rafinesque's big-eared bats and took blood and hair samples from each. In addition, we collected a hispid cotton rat (*Sigmodon hispidus*) and obtained blood and hair samples. All animals were released unharmed at the site of capture. Samples were sent to the Center for Environmental Sciences and Engineering (CESE) at the University of Connecticut for wide-screen toxin analysis.

Our preliminary results led us to begin testing other mammals in the vicinity. We found traces of agricultural chemicals in these animals, and decided to again broaden our survey, but because we detected chemicals only in hair samples, we no longer collected blood. In 2015, we collected 7 white-footed deermice (*Peromyscus leucopus*) by Sherman trap. Hair samples were collected and submitted for wide-screen toxin analysis at CESE. The animals were released at the point of capture. Results of the analyses again led us to broaden the scope of our investigations.

In 2016, we captured two specimens of marsh oryzomys (*Oryzomys palustris*) and one hispid cotton rat by Sherman trap and collected hair samples from each. All rodents were released unharmed at the point of capture. Additionally, we collected a hair sample from a coyote (*Canis latrans*) pup killed on the highway nearby, and collected a blue jay (*Cyanocitta cristata*) feather from the ground at the study site. We also collected water from Bayou Bartholomew, soil from the edge of the Bayou, soil from a point away from the bank but still within the flood plain of the Bayou, and from points within and near an agricultural field adjacent to the Bayou and the Taylor House. All samples were sent to CESE for wide-screen toxin analysis.

Results and Discussion

In 2014, 2 of 10 hair samples from Rafinesque's big-eared bats and the hair sample from the hispid cotton rat contained measurable traces of 4,4-DDT, a pesticide which has been banned since the 1970s, or 4,4-DDE, a metabolite of DDT (Table 1). In 2015, 1 of the 7 hair samples taken from white-footed deermice tested positive for 4,4-DDD, another metabolite of DDT. Another of the 7 contained measurable traces of trans-nonachlor, a component of the pesticide chlordane, which was banned in 1988. In 2016, all 5 environmental samples and 4 of the 5 samples taken from wildlife contained measurable traces of 4,4-DDE. Three of the environmental sites also contained 4,4-DDD. Various samples also contained the banned pesticide heptachlor or its product heptachlor epoxide, cis-nonachlor, a product of chlordane, and the banned pesticides hexachlorobenzene and aldrin.

DDT was banned from general use in the United States in 1972 (USEPA 1990). It is considered to be a persistent pesticide, with a half-life of approximately 3 years, and a 95% disappearance period of 10 years (Brown 1978). Levels that we detected in the environment are not inconsistent with these data, and are comparable to many previous studies (Edwards 1973).

Table 1. Concentration of pesticide residues detected in various samples collected around the Taylor House, Drew County, Arkansas. All concentrations are in parts per billion.

Year	Sample No.	Species and Samples	4,4 DDE	4,4 DDT	4,4 DDD	Trans-nonachlor	Heprachlor epoxide	Trans (gamma)-chlordane	1,2,4,5 tetrachlorobenzene	Hexachlororbenzene	Heptachlor	Aldrin	Cis-nonachlor
2014	3A	*Corynorhinus rafinesquii*	14545.0										
	5A	*Corynorhinus rafinesquii*		3929.0									
	15A	*Sigmodon hispidus*	5323.0										
2015	18	*Peromyscus leucopus*			629.0								
	20	*Peromyscus leucopus*				647.0							
2016	117	*Oryzomys palustris*	87.0				43.5	87.0					
	118	*Sigmodon hispidus*	100.0				60.0		40.0				
	120	*Cyanocitta cristata*	12.8							5.1			
	121	*Canis latrans*	10.7						1.8				
	1	Bayou water	5.7		0.9						15.5	8.3	5.7
	2	Edge of bayou	4.6										
	3	Bayou floodplain	7.5		1.9		4.8						
	4	Trapline	2.5		0.7						21.2		
	5	Cornfield	9.0				0.2		0.7				

However, levels of DDT and its metabolites that we observed in mammals are greater than some that were reported in studies that occurred before the substances were banned (e.g., Korschgen 1970). Very few recent studies have examined residual organochlorines on an ecosystem level, although at least one (Femmer *et al.* 2004) found detectable amounts of some of the same chemicals as in this study in tissues of fish collected in Arkansas and surrounding states. Our sample sizes are small (due to the expense of analysis), but we believe they suggest the utility of further study, especially given the propensity of these pesticides to biomagnification and possible risk to health of wildlife and humans (Snedeker 2001).

Acknowledgments

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New Applications of Radio Frequency Identification Stations for Monitoring Fish Passage through Headwater Road Crossings and Natural Reaches

I.R. MacLeod[1,2]* and C.J. Gagen[1]

[1]*Arkansas Tech University, Department of Biological Sciences, 1701 N Boulder Ave, Russellville, AR 72801*
[2] *Author's current address: California Department of Conservation, Division of Mine Reclamation, 801 K St., MS 09-06, Sacramento, CA 95814*

*Correspondence- ian.r.macleod@gmail.com

Running Title: RFID Stations for Monitoring Fish Passage

Abstract

Within the Ouachita National Forest, roads and streams intersect each other thousands of times. Many of these road crossings alter stream hydrology and potentially limit longitudinal fish movement. To investigate the potential impacts of these road crossings on fish passage, we monitored movements of 3 native fish species (n = 2,171) individually tagged with radio frequency identification (RFID) tags in 2012 and 2013. We installed solar-powered RFID stations in 2 streams with road crossings and 2 reference streams without road crossings. Each of the 4 monitoring stations included a pair of antennas bracketing a road crossing (or similarly-sized natural reach) to continuously detect upstream or downstream passage. To monitor natural reference streams, we avoided full-duplex RFID technology, which would have required rigid in-stream structures. Alternatively, we utilized new applications of RFID technology such as direct in-stream installation of half-duplex wire antennas and figure-eight crossover antenna designs. These techniques appear promising, but technical difficulties limited the consistency of fish passage detection and consequently limited the strength of ecological conclusions. Even so, we report evidence that fish passed at significantly higher rates across reference reaches than reaches with road crossings. Furthermore, Creek Chub (*Semotilus atromaculatus*) passed reference reaches at significantly higher rates than Highland Stonerollers (*Campostoma spadiceum*), which passed at higher rates than Longear Sunfish (*Lepomis megalotis*). Stream intermittency appeared to exacerbate reduced passage rates associated with the road crossings.

Introduction

Stream fragmentation can disrupt life history strategies of even non-migratory fishes, which rely upon dispersal to maintain population connectivity (Fagan *et al.* 2002; Labonne and Gaudin 2006; Cook *et al.* 2007). This issue threatens the biodiversity and persistence of fish communities in the central and southeastern United States (Sheldon 1988; Bessert and Ortí 2008; Kashiwagi and Miranda 2009; Perkin and Gido 2011). In North America, road crossings in headwater streams commonly obstruct fish passage and fragment fish habitat (Gibson *et al.* 2005; Hendrickson *et al.* 2008; Park *et al.* 2008; Price *et al.* 2010; Peterson *et al.* 2016).

Road crossings in the Ouachita National Forest reduce fish passage by altering stream hydrology in 2 principal ways: 1) reducing water depth to levels too shallow for efficient swimming (Blank *et al.* 2005) and 2) increasing water velocity so that it exceeds fish swimming abilities (Belford and Gould 1989; Warren and Pardew 1998; Burford *et al.* 2009; Norman *et al.* 2009; Bourne et al. 2011). Road crossings often act as semipermeable barriers (Bouska and Paukert 2009) and passability varies with fluctuations in discharge (Connolly *et al.* 2008), road crossing hydraulics, and fish species and size (Norman *et al.* 2009). Road crossing designs that more severely constrict natural stream flow appear more detrimental to fish passage (Warren and Pardew 1998) and multiple barriers along a drainage network may lead to cumulative effects (Helfrich *et al.* 1999).

Researchers assess potential impacts of aquatic fragmentation via several methods including hydraulic modeling software (Bourne *et al.* 2011), analysis of population genetic structuring (Wofford *et al.* 2005; Bessert and Ortí 2008; Schanke *et al.* 2017), direct observation of individuals (Cahoon *et al.* 2007), and recapture of marked specimens (Belford and Gould 1989; Morita and Yamamoto 2002). Researchers also use radio frequency identification (RFID) tags, also known as passive integrated transponder (PIT) tags, in conjunction with RFID detection stations to study fish movement in streams (Bond *et al.* 2007; Horton *et al.*

2007; Connolly *et al.* 2008), and monitor passage through road crossings (Blank *et al.* 2005), fishways (Castro-Santos *et al.* 1996; Thiem *et al.* 2011), and hydroelectric dams (Axel *et al.* 2005).

Traditional RFID systems for fish detection were based on full-duplex (FDX) transmission technologies, which are sensitive to small antenna movements and therefore require installation of rigid, watertight housings on structures such as dams, fishways, or weirs (Bond *et al.* 2007). Alternatively, systems based on newer half-duplex (HDX) equipment do not require rigid antennas and are considerably less expensive than FDX systems (Burnett *et al.* 2013; Roghair *et al.* 2014).

The ability of an RFID system to detect a tagged fish passing its antenna array is called fish detection efficiency and has 2 components: 1) path efficiency- the proportion of tags that physically pass through the antenna array, rather than around it and 2) antenna efficiency- the proportion of tags that are detected of those tags passing through the antenna (Zydlewski *et al.* 2006). Whereas HDX systems benefit from less stringent antenna design requirements, they have lower antenna efficiency than comparably-sized FDX designs. Small tags, such as the compact 12-mm tags used for small stream fish, have a smaller read range (the maximum distance at which a tag can be detected) and result in lower antenna efficiency than larger tags (Zydlewski *et al.* 2006; Burnett *et al.* 2013).

The Ouachita National Forest, located primarily in Arkansas and managed by the USDA Forest Service, has a high density of flow-constricting road crossings and a high diversity of warmwater fishes. However, researchers have published few studies assessing the effects of road crossings on fish movement in this region (apart from: Warren and Pardew 1998 Standage and Gagen 2007; Schanke *et al.* 2017). Our initial ecological question focused on the potential impact of road crossings and hydrologic regimes (i.e., water level fluctuations, including intermittency) on individual fish movement in headwater streams.

We chose to monitor fish with RFID detection stations because they increase reencounter probabilities over other techniques (Hewitt et al. 2010; Roghair *et al.* 2014). Although many researchers have installed RFID systems on the upstream and downstream edges of existing instream structures (for example: Blank *et al.* 2005; Burford *et al.* 2009; Roghair *et al.* 2014), we sought to monitor fish movement through not only road crossings, but also unaltered natural reaches (reference streams). Therefore, to minimize environmental alteration and hydraulic disruption to the natural reference reaches in headwater streams, we installed HDX systems with light-weight wire antennas directly in the stream without rigid supporting structures. However, the methodology of HDX, RFID technology to monitor small-bodied native fishes in unaltered natural reaches with small RFID tags was largely untested. Hence, to address our ecological questions, we explored new applications of RFID detection stations in remote, natural stream reaches devoid of man-made structures capable of supporting antennas. Our study thus shifted focus towards the development of new RFID applications while also showcasing the potential for ecological observations.

Materials and Methods

Field-site description

We examined fish movements in 4 similarly-sized, low-order, warmwater streams in the Ouachita National Forest within the Ouachita Mountains, an ecoregion of approximately 4.8 million hectares in Arkansas and Oklahoma (Table 1; Fig. 1). We selected 2 *culverted* streams (each intersected by multiple engineered road crossings within the study reach) and paired each with a nearby *reference* stream without road crossings. To represent the diverse hydrologic regimes of the region, these 4 streams consisted of 2 *intermittent* streams in the northeastern portion of the Ouachita Mountains and 2 *perennial* streams towards the south. We established 2-kilometer study zones (1 km upstream and downstream) centered on the study reach in each stream.

The 2 intermittent streams drained into the Fourche La Fave River in the Arkansas River watershed. Bear Creek had 3 total road crossings within the 2-km study zone (including another vented ford and a slab ford). Alternatively, Crystal Prong was located in the Flatside Wilderness Area and had no road crossings. We studied a vented ford (also known as a pipe culvert) road crossing on Bear Creek (Fig. 2). However, prior to this study, the culvert pipes had filled in with gravel and cobble so that they conveyed only trickles of water and presumably precluded fish passage. This type of flow restriction is common to this road crossing design in the Ouachita National Forest and affects stream hydrology much like a slab ford or low head dam. These intermittent

Table 1. The locations of the 4 study reaches.

Stream	Latitude	Longitude
Bear Creek	34.788809°	-93.169854°
Crystal Prong	34.861274°	-92.934850°
Long Creek	34.399824°	-93.934639°
Little Missouri River	34.430157°	-93.944220°

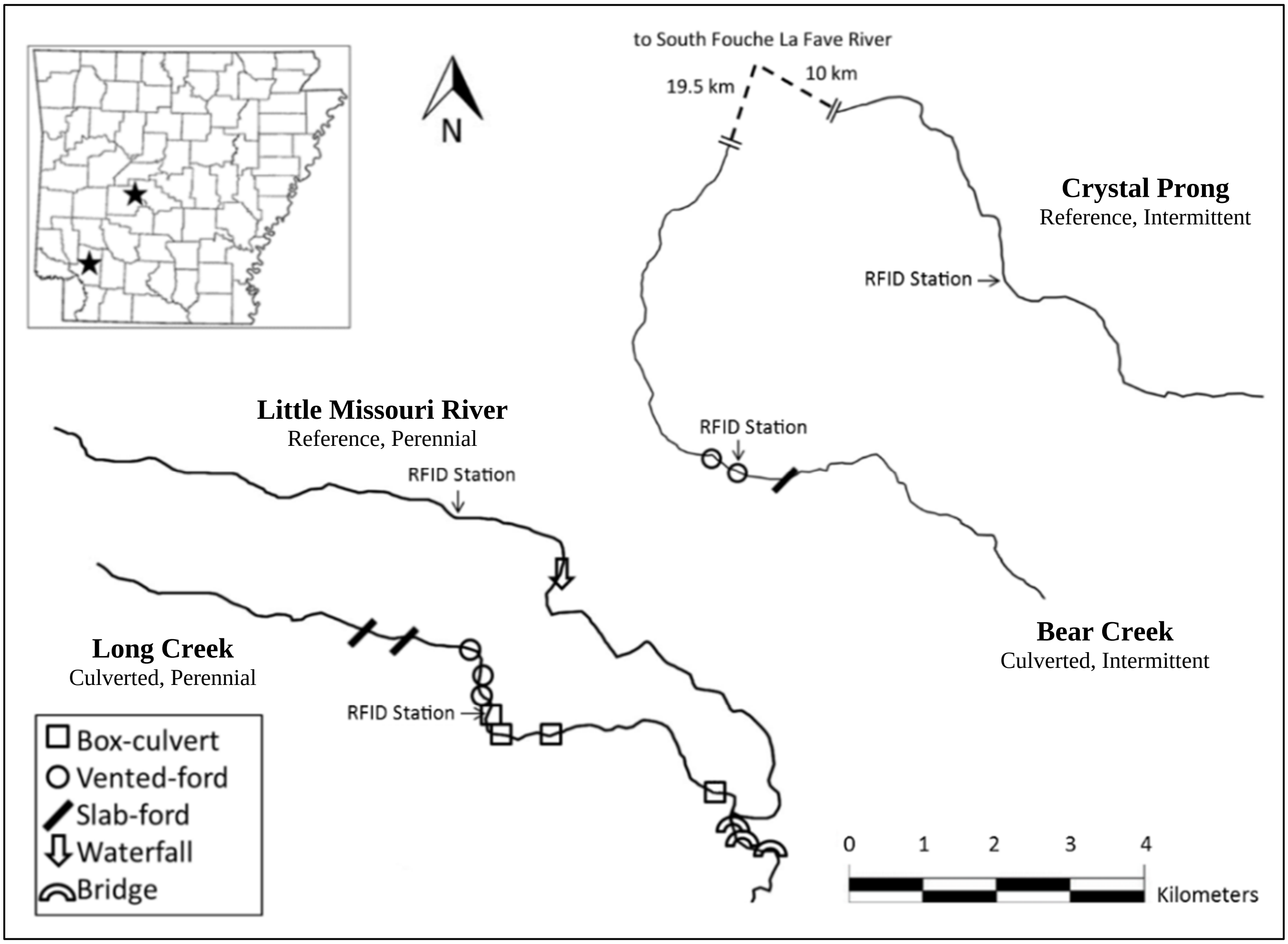

Figure 1. Map of the 4 study streams located in the Ouachita National Forest, Arkansas. Two intermittent streams, Bear Creek (culverted) and Crystal Prong (reference), were located in the central part of the state. To the southwest were 2 perennial streams, Long Creek (culverted) and Little Missouri River (reference). Bear Creek and Crystal Prong flow downstream to the South Fouche La Fave River (adapted from Schanke 2013).

streams show reduced surface flow during the summer such that wetted portions become isolated pools.

The 2 perennial streams drained to the Ouachita River watershed. Long Creek had 6 total road crossings, including vented fords and box culverts, within the 2-km study zone. The adjacent Little Missouri River had no road crossings within the study zone (though some existed in the headwaters and farther downstream). On Long Creek, we focused on a box culvert (constructed in 2008; Fig. 2) to contrast with the older style vented ford on the other culverted stream, Bear Creek. This box culvert consisted of five 2.4-m-wide boxes and spanned 6.2 m of longitudinal stream distance. The bottoms of the boxes were constructed below stream grade, resulting in a natural gravel and cobble substrate bottom (covering the concrete) that contributed to roughness and slowed water velocity. Neither of the 2 road crossings we studied had measurable outlet drops that would impede fish movement via outlet drop height or outlet pool depth barriers.

Fish collection and marking

We encountered and tagged 9 fish species (additional details in MacLeod 2013)—however we restricted analysis and reporting to 3 species that we commonly encountered in all 4 of the streams to enable comparisons across streams. Target species, in order of captured abundance, included: *Semotilus atromaculatus* (Mitchill) (Creek Chub), *Campostoma spadiceum* (Girard) (Highland Stoneroller), and *Lepomis megalotis* (Rafinesque) (Longear Sunfish; Table 2).

Between May 2012 and February 2013, we tagged 2,171 fish in the 2-km study-zones of the 4 streams. Beginning downstream, we proceeded upstream in 50-m

Figure 2. Water moves from right to left in both photographs of the 2 culverted streams. At the clogged **vented ford** on intermittent Bear Creek, water pooled upstream and flowed over the concrete slab. The turbulent water in the left foreground indicates the 2 culvert outlets. At the **box culvert** on perennial Long Creek, water flowed easily through the road crossing, even during summer low-flow conditions.

sections and collected fish via single pass, backpack electrofishing (Smith-Root LR 20- Smith-Root, Vancouver, Washington), in continuous sweeps without block-nets. We repeated this procedure on subsequent visits to meet nominal tagging quotas (approximately 500 fish per stream). Fish were more abundant in the perennial streams and we met quotas in 2 complete sweeps, whereas the intermittent streams required additional sweeps (average 3.9 sweeps/section for Bear Creek and 4.3 sweeps/section for Crystal Prong).

We held fish in screen-bottom buckets or mesh baskets for processing and released them immediately after tagging. We injected fish larger than 85 mm total length with 12.0 mm x 2.2 mm half-duplex RFID tags (Oregon RFID, Portland, Oregon). We inserted the syringe-style implanter (MK7 Implanter- Biomark, Boise, Idaho) subcutaneously between the dorsal fin and the lateral line and deposited the tags at least 5 mm distant from the incision to reduce chances of tag loss. We recorded RFID tag numbers with a handheld RFID reader (APR 350- Agrident, Barsinghausen, Germany).

Table 2. The number of fish tagged and their distribution relative to the RFID station within each stream.

Species	Downstream	Upstream	Total
Bear Creek- vented ford, intermittent			
Creek Chub	303	251	554
Highland Stoneroller	25	17	42
Longear Sunfish	15	11	26
Total	**343**	**279**	**622**
Crystal Prong- reference, intermittent			
Creek Chub	137	150	287
Highland Stoneroller	81	120	201
Longear Sunfish	52	20	72
Total	**270**	**290**	**560**
Long Creek- box culvert, perennial			
Creek Chub	8	40	48
Highland Stoneroller	82	85	167
Longear Sunfish	93	158	251
Total	**183**	**283**	**466**
Little Missouri River- reference, perennial			
Creek Chub	104	178	282
Highland Stoneroller	102	31	133
Longear Sunfish	64	44	108
Total	**270**	**253**	**523**

Fish detection

We began installing the autonomous RFID detection stations in January 2012. The stations recorded the timing and direction of fish passage across the study reaches (Fig. 3). In the culverted streams, the middle of the RFID station spanned the target road crossings; whereas, in reference streams, we positioned the middle of the station across a riffle (devoid of human structures). Each station included an RFID reader (Multi-antenna, Half-duplex Reader- Oregon RFID) and 2 in-stream antennas (1 upstream and 1 downstream). To accommodate the large road crossings and locate suitable locations for antenna installation, we placed antennas ~60 m apart. We installed antennas in pools, runs, and riffles.

The station's RFID reader recorded the RFID tag number of passing fish coupled with a timestamp. When a fish was detected by both antennas, the timing of detection events indicated the direction of upstream or downstream movement. We powered the RFID reader with a 12-volt, 205-watt photovoltaic solar panel that charged four, 6-volt batteries (216 amp-hour, heavy-

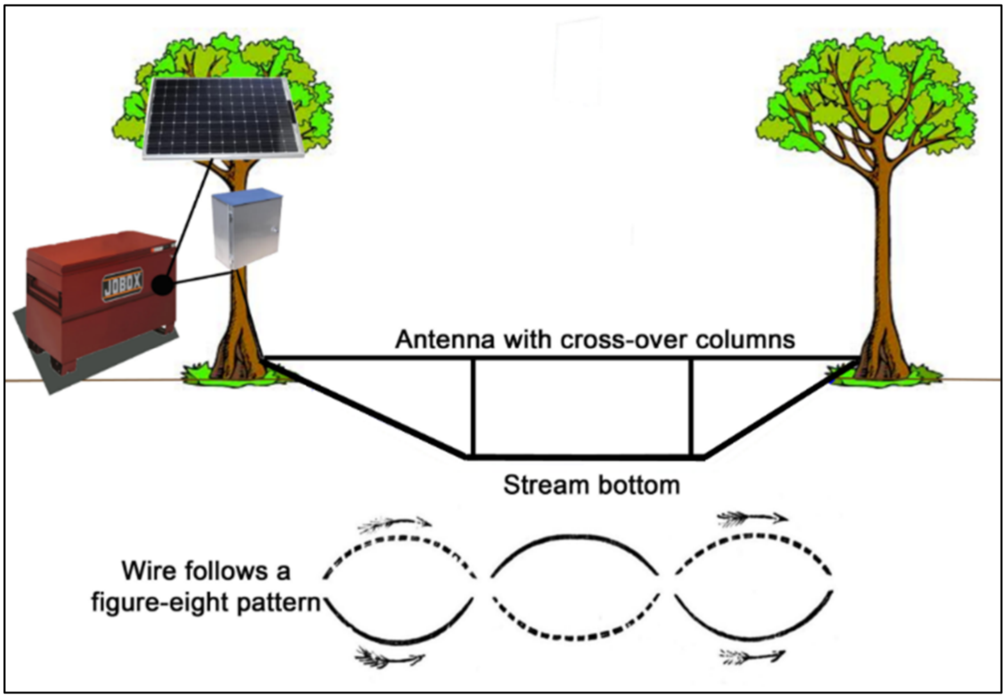

Figure 3. Each RFID station operated 2 wire antennas on either side of the road crossing (on culverted streams) or riffle (on reference streams). This figure shows a cross-section of 1 antenna spanning a stream (2nd antenna not shown). We designed antennas with cross-over columns (in a figure-eight pattern) to improve antenna efficiency. We installed the RFID reader, batteries, and solar charge controller in a locked in a steel box, utilized a solar panel to charge the station, and mounted an antenna tuner on nearby trees for each antenna.

duty, deep-cycle; Interstate Batteries, Dallas, TX) via a solar charge controller (ProStar 30M- MorningStar, Newton, PA). The batteries supplied the RFID reader with 12 volts and ~1 amp of direct current electricity (see MacLeod 2013 for additional technical details).

We built pass-through antennas that encompassed as much of the stream cross-section as possible (maximizing path efficiency) and oriented the antenna plane perpendicular to stream flow and tag direction (maximizing antenna efficiency; for more discussion of pass-through versus pass-over antennas, see Armstrong *et al.* 1996 and Zydlewski *et al.* 2006). The antenna widths ranged between 5 and 10 m and heights ranged from 0.2 m in riffles to 0.6 m in deeper pools. We constructed antennas from common stranded household electrical wire, specifically 10 or 12 American Wire Gauge (AWG) and supported the antennas with rope. We mounted antenna tuners (Oregon RFID) to nearby trees so that we could adjust the antenna inductance and ensure antennas effectively transmitted and received radio communication with the RFID tags (see MacLeod 2013 for additional installation details). The RFID stations began operating on all 4 streams by February 2012 and the 8 antennas operated for an average of 372 days (SE 10).

We continually adjusted (weekly or biweekly) the antennas to maximize antenna efficiency. We assessed antenna efficiency during site visits by manipulating a test tag perpendicularly through the antenna plane at ~1 m/s. Antennas demonstrating "good performance" detected all tags: (1) within 5 cm (upstream or downstream) of the antenna wire plane and (2) within 10 cm above or to the side of the antenna loop. This study required large antennas at large distances from the readers, which exceeded dimensions reported in other RFID studies (e.g., Bond *et al.* 2007; Aymes and Rives 2009) and reduced antenna efficiency. Additionally, high stream flows periodically damaged the antennas and technical challenges (including electrical noise and equipment failure) periodically contributed to failure or decreased performance of one or both antennas leading to gaps in the monitoring data, sometimes for several months (more details in MacLeod 2013; Table 3).

In August 2012, we developed a crossover antenna design that dramatically improved antenna efficiency and read range relative to our initial efforts with single-loop designs (Fig. 3). By dividing the antenna loop into smaller cells in a figure-eight pattern, we reversed the electrical polarity within the cells thereby minimizing electrical noise and improving antenna efficiency (Warren Leach, Oregon RFID, *personal communication*). We formed the antenna cells by

Table 3. The percentage of time that each RFID station operated at different performance levels.

	RFID Station Performance		
Stream	Excellent or Good	Fair	Poor or Off
Bear Creek	48%	25%	27%
Crystal Prong	28%	19%	53%
Long Creek	57%	15%	28%
Little Missouri River	28%	25%	48%
Mean	41%	21%	38%

crossing the top and bottom strand of wire in opposing directions through a column made of either 1/2” (1.2 cm) plastic irrigation hose or PVC pipe. We optimized antenna designs on a site-by-site basis and constructed crossover columns every 1.5–3 m, with more crossovers needed on taller antennas.

While electrofishing, we recorded the locations of recaptured individuals that had been previously tagged, which we termed “incidental recaptures". By spring 2013 we had already met our tagging quotas, but we performed additional electrofishing to expand the spatial scale of detected fish (some of which were distant from the fixed RFID stations) and assess the efficacy of the detection stations. Specifically, in March and April 2013 we detected “intentional recaptures” by operating 2 electrofishing units simultaneously in a single pass through the 2-km study zone of each stream.

Hydrology at crossings

To characterize the hydrologic conditions associated with fish passage, we measured water levels on the culverted streams from July 2012-April 2013 and estimated the highest water level occurring during fish passage events (more detail in MacLeod 2013). We installed continuous water level recorders (Vented WL-16– Global Water Instrumentation, Dallas, TX) on the 2 culverted streams. We did not install water level recorders on the 2 reference streams, but we installed staff gauges on all 4 streams and estimated water level fluctuations on the reference streams based on the respective water level recorders on the culverted streams (the pairs of intermittent and perennial streams responded similarly to precipitation events). To estimate water levels on the study streams for May and June 2012, prior to installation of the water level recorders, we consulted USGS stream gauge data for nearby streams (more details in MacLeod 2013).

We assessed the road crossings for barrier effects caused by high velocity and low water depth and searched for the presence of favorable passage conditions at a wide range of water levels. We used an electronic flow meter (Flo-Mate, Model 2000– Marsh-McBirney, Loveland, CO) and wading rod to measure depth and velocity along transects at the inlet and outlet of the box culvert on perennial Long Creek and along multiple transects across the trapezoidal vented ford on intermittent Bear Creek. Minimum swimming depth varies with species and length (Schaefer 2001; Rodríguez *et al.* 2006) but Blank *et al.* (2005) determined 3 cm to be the minimum swimming depth permitting passage for several species of trout. Hence, we identified areas with sufficient water depth for swimming based on the presence (or absence) of a water column equal to or greater than 3 cm. Additionally, we measured water velocity at 3 cm above the substrate to represent the lowest velocity path for fish movement, following Belford and Gould (1989) and Rajput (2003).

Data analyses

We concluded that a fish had passed a study reach when we could confirm its location at least once on both the upstream and downstream side of the RFID station. We used all available data sources, including the RFID detection stations and locations of tagged fish, “incidental recaptures", and “intentional recaptures" to detect passage. Some fish passed across the RFID station more than once, resulting in multiple detected passages. The number of fish tagged varied across streams and species, so we generated a normalized *passage rate*—dividing the number of *detected passages* by the number of *tagged fish* that did not pass.

We used chi-squared analyses (χ^2) and log-linear modeling to elucidate relationships between the passage rate and the following design variables: 3 species and 4 streams, which represented different crossing types (culverted versus reference) and hydrologic regimes (intermittent versus perennial).

We studied 2 crossing types and 2 hydrologic regimes; however, because we were limited to 4 RFID stations, we were not able to replicate the design. Additionally, variability among the 4 streams (e.g., the operating time of the RFID antennas was not uniform, the streams supported different fish communities, etc.) created additional complications for direct comparisons. Thus, the experiment was a comparative mensurative experiment (*sensu* Hurlbert 1984), and although we were able to investigate RFID technology, we did not design the study to make strong inferences regarding fish movements or ecology.

Results

RFID detection efficiency

The RFID stations recorded more than 260,000 detection events (i.e., instances when the reader logged an RFID tag). This resulted in detection of 290 fish (of 2171 tagged individuals; average detection rate per stream 13%; SE 2.3%; Table 4). In March and April 2013, we detected 47 tagged individuals (of 1,128 fish captured) through the intentional recapture electrofishing. Within the 4 streams, 1–12% of these captured fish carried tags (mean, 8%; SE, 2%).

The RFID dissection stations were effective—data from the RFID detection stations, in conjunction with the original tagging location, identified 94% of all observed passages; whereas the electrofishing recapture data only identified 6% of observed passages. However, the RFID stations, often crippled by poor performance, had limitations. For example, the stations detected 24% of observed passing fish on only 1 antenna (rather than both). These fish passed undetected through or around one of the two antennas and we were only able to infer that the fish had passed a study reach by also consulting the electrofishing location data (both original tagging and recapture locations).

Movement and passage

We detected 118 fish passing the study reaches 246 times (Fig. 4). In all streams, fish passed at similar rates both upstream and downstream. The RFID stations did not always operate both antennas continuously, but fish passages scaled to RFID station operating time (which varied among streams) followed a similar pattern as data presented here.

When we pooled the 3 species, passage rates appeared dependent on "stream" (four study streams; $\chi^2 = 166$, df = 3, $P < 0.01$). We also observed this pattern of significant stream-effects when we analyzed the 2 most abundant species, Creek Chub and Highland Stoneroller, separately ($\chi^2 = 230$, df = 3, $P < 0.01$ and $\chi^2 = 9.62$, df = 3, $P < 0.05$, respectively). However, this analysis was inappropriate for Longear Sunfish because of the low frequency of detected passages.

Table 4. The percentage of fish (by species) that were detected by the RFID station on each the 4 streams.

	Species			
Stream	Creek Chub	Highland Stoneroller	Longear Sunfish	**Mean**
Bear Creek	12%	5%	4%	**11%**
Crystal Prong	10%	18%	4%	**12%**
Long Creek	8%	11%	10%	**10%**
Little Missouri River	24%	16%	14%	**20%**
Mean	**14%**	**14%**	**9%**	**13%**

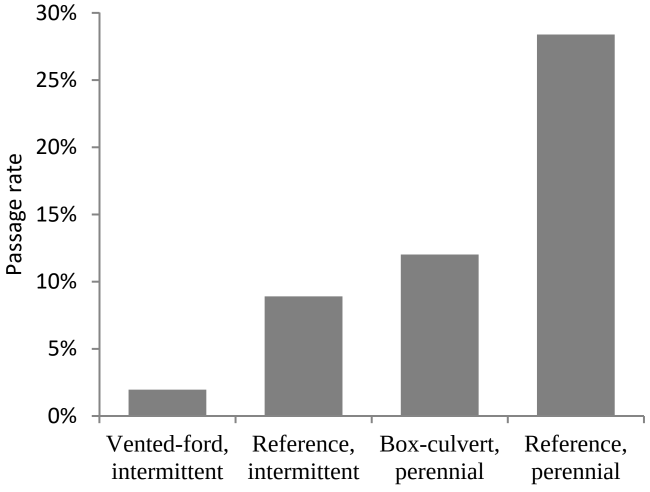

Figure 4. Fish passage rate (proportion of detected passages to RFID-tagged fish [that did not pass] within each stream).

When we grouped the streams into pairs (based on hydrologic regime) and pooled the 3 species, passage rate was significantly greater in the reference reaches than the road crossings for both pairs of streams (perennial: $\chi^2 = 31.7$, df = 1, $P < 0.01$ and intermittent: $\chi^2 = 20.3$, df =1, $P < 0.01$). Likewise, when we grouped the streams into pairs (based on crossing type) and pooled the 3 species, passage rates in perennial streams were greater than in intermittent streams (culverted streams: $\chi^2 = 34.4$, df = 1, $P < 0.01$ and reference streams: $\chi^2 = 56.4$, df = 1, $P < 0.01$).

Log-linear modeling indicated significant three-way interaction among the design variables, stream and species, with respect to passage rate as a response variable ($G^2 = 1080$, df = 17, $P < 0.01$). Each of the associated two-way interactions were also significant. Passage rates differed among the 3 species for both road crossings ($\chi^2 = 25.5$, df = 2, $P < 0.01$) and reference reaches ($\chi^2 = 38.3$, df = 2, $P < 0.01$). Furthermore, the pairwise comparisons indicated a significant trend across species with respect to passing the reference reaches; with Creek Chub passing at higher rates, followed by Highland Stoneroller, and finally Longear Sunfish (each $\chi^2 > 10$, df = 1, and $P < 0.01$).

For Highland Stonerollers and Longear Sunfish, the lengths of fish passing versus not passing were similar, but passing Creek Chub were significantly longer than those not passing (126 mm versus 109 mm, respectively; ANOVA, $F = 42.5$, df = 1, $P < 0.01$). Four individuals (2 Creek Chub and 2 Highland Stonerollers) moved as far as 950 m from their original capture locations to pass a study reach, but the average movement was approximately 262 m (SE, 22.3 m); thus, we considered the 2-km study zone sufficiently large to avoid biased assessment of fish movements.

Hydrology and passage

Water levels, as measured by the water level recorders from July 2012–April 2013, fluctuated between 0.07 and 1.35 m (mean, 0.47 m; SE, 0.002 m) near the vented ford on intermittent Bear Creek and ranged from 0.08 to 1.01 m (mean, 0.18 m; SE, 0.001 m) near the box culvert on perennial Long Creek. At Long Creek, water depth always exceeded 3 cm and we never observed swim zone velocities >0.5 m/s within the culvert.

Alternatively, the vented ford on intermittent Bear Creek presented hydraulic challenges for fish passage. The culvert pipes were clogged—thus, fish passage was restricted to movement over the top of the large concrete slab or to adjacent portions of the floodplain. At low water levels (less than 0.24 m on the staff gauge), water did not pass over the road crossing. As water levels rose above 0.45 m, water flowed over the concrete roadway and down the steep concrete slope on the downstream side of the road prism, but never exceeded 3 cm depth and velocities ranged from 2.3–2.8 m/s. At higher flows (water levels ≥0.69 m), the downstream slope supported a potential swim-zone with depths ≥3 cm. However, during these conditions, water velocities of 2.5–4.5 m/s exceeded typical swimming speeds of warmwater fishes (Leavy and Bonner 2009).

We categorized water levels on the culverted streams as either "low" and "high" to assess passage rates based upon water levels. For the vented ford on intermittent Bear Creek, water levels <0.7 m did not produce the 3-cm potential swim zone. Therefore, we assigned passages when water levels were <0.7 m to the low category and >0.7 m to the high category. Because the perennial streams supported a swim zone at all measured conditions, we did not identify a clear hydraulic cutoff for water levels and arbitrarily categorized water levels as low or high relative to 0.6 m, which mimicked the categories assigned to the intermittent streams and distinguished baseflow levels from less frequent high water events.

Fish passed study reaches in the 3 streams with passable "swim zones" predominantly when water levels were low (corresponding with baseflow hydrologic conditions) and only passed study reaches 7–17% of the time during high water levels (Table 5). Alternatively, 25% of fish passages for the vented ford on intermittent Bear Creek were at high water levels. This high-water passage rate was significantly greater than the rates for the other 3 streams (when the 3 dominant species were pooled for sufficient sample size in a log-linear analysis with flow regime (i.e., perennial vs. intermittent) and crossing presence (i.e., road crossing vs. reference reach) as design variables; $G^2 = 4.62$, df = 1, $P < 0.05$). We hypothesize that fish opportunistically utilized higher water levels to pass the hydraulically-challenging vented ford on Bear Creek.

We utilized all available location data (i.e., RFID station data *and* electrofishing data) to determine if fish passed the study reach because the RFID stations alone sometimes failed to detect fish passage. However, despite our best intentions, these data had often recorded a fish's location prior to the actual moment of passage, sometimes even months before the fish was detected on the far side of the study reach; therefore, we could not always ascertain the precise moment for a particular passage. Consequently, we conservatively analyzed the relationship between water level and passage based on the highest water level occurring between the 2 detection events. We acknowledge that this presents a potential bias, whereby it appears that fish passed during high water levels.

Discussion

RFID detection stations

The RFID stations improved the probability of re-encountering tagged fish over traditional methods such as electrofishing mark and recapture (similar to benefits observed by Roghair *et al.* 2014). Additionally, except when damaged, the RFID stations detected passage during high flow conditions when electrofishing was unsafe or less efficient and they enabled us to link fish passage to flow fluctuations in natural stream cross-sections. Alternatively, electrofishing sampling allowed us to detect fish in more locations, ameliorating the inherent limitation of a fixed-location RFID station.

Our approach could not accurately assess detection (or missed detection) rates for the RFID stations as others have reported for more controlled settings such as fishways (Axel *et al.* 2005; Aymes and Rives 2009). The RFID stations (without help from the electrofishing detection data) missed 24% of the total observed passages. Thus, we acknowledge that the reported counts of fish passages were biased low. We designed the RFID stations to provide uniform and efficient fish

Table 5. The number of fish passages when water levels were "low" and "high". The vented ford on Bear Creek did not sustain a hydraulically-favorable "swim zone" at low water levels; whereas, both perennial streams supported a hydraulically-favorable swim zone at all water levels measured.

	Number of passages		
Species	Low water levels	High water levels	Proportion of high water passages
Bear Creek- vented ford, intermittent			
Creek Chub	9	2	0.18
Highland Stoneroller	0	0	N/A
Longear Sunfish	0	1	1.00
Total	**9**	**3**	Mean 0.25
Crystal Prong- reference, intermittent			
Creek Chub	17	5	0.23
Highland Stoneroller	21	1	0.05
Longear Sunfish	3	0	0.00
Total	**41**	**6**	Mean 0.13
Long Creek- box culvert, perennial			
Creek Chub	4	2	0.33
Highland Stoneroller	21	3	0.13
Longear Sunfish	19	4	0.17
Total	**44**	**9**	Mean 0.17
Little Missouri River- reference, perennial			
Creek Chub	98	9	0.08
Highland Stoneroller	23	0	0.00
Longear Sunfish	4	0	0.00
Total	**125**	**9**	Mean 0.07

detection, but unavoidable intra-station and inter-station variations limited the validity of such comparisons (also noted by Aymes and Rives 2009). Station downtime led to missed detections, which contributed to fewer observed passages. These design limitations probably contributed to the observed interactions among species and streams (with respect to the frequency of detected passage). Future studies seeking more rigorous analysis could address this limitation by installing additional RFID stations to achieve greater replication.

We explored the technological limits of the RFID detection equipment by building large antennas located substantial distances from the reader without rigid, in-stream structures. Antenna efficiency, already limited by the read range of 12-mm HDX, RFID tags, declined when we added a second antenna to the multiplex reader and as each antenna increased in size and distance from the reader. To mitigate the limited antenna efficiency associated with the simple pass-through loop designs, we developed a figure-eight crossover design, which produced multi-fold improvements in antenna efficiency and largely eliminated tag detection gaps within the antenna plane. However, the crossover design, with its vertical columns, was more prone to damage during high flow events and was more complex to build and repair. Fortunately, an experienced a two-person team could rebuild and tune this type of antenna in less than 3 hours using inexpensive and widely available materials.

We initially designed larger antennas that encompassed more of the floodplain above bankfull to maximize antenna cross-section (and hence *path efficiency*) and detect fish during higher stormflow events. We later reduced antenna size to achieve consistently higher *antenna efficiency* during average flow conditions. These small antennas were also less vulnerable to high flow damage. An antenna installed in a pool increased detection probability because fish often resided in the pool for extended periods. However, to accommodate the pool depth, these antennas were large and had reduced antenna efficiency. Runs and riffles permitted squat antennas with excellent antenna efficiency, but the high stream velocity and associated debris damaged antennas.

Norman *et al.* (2009) called for longer-term studies (months to years rather than weeks) to evaluate the impact of hydrologic variability (i.e., fluctuations in stream discharge) on fish passage at semi-permeable road crossings. We sought to measure the water level at the moment of fish passage to investigate passability at various hydrologic conditions. However, our conclusions were limited by the long spans of time between detection events, which were exacerbated by equipment downtime. Future improvements to RFID technology will likely yield smaller tags, improved antenna read range and efficiency, more resilient antenna designs, and more stable electrical operating systems. Pass-over antenna designs, such as those designed by Connolly *et al.* (2008), may someday offer the same level of detection performance as more vulnerable pass-through designs. By placing multiple antennas on each stream (i.e., more than just 1 upstream and 1 downstream of the study reach e.g., Connolly *et al.* 2008) researchers can improve the spatial accuracy of fish movement studies and minimize missed detections. We believe RFID detection stations can help evaluate and prioritize the removal of the worst passage barriers in large stream networks as called for by Kemp and O'Hanley (2010).

Fish detection and passage

The community of fishes within the 4 streams varied in both assemblage and abundance—as evidenced by the variable tagging rates of the 3 species across streams (Table 2)—and we detected fish at different rates across species and streams (Table 4). To enable comparisons, we attempted to control for differences by focusing on only three common species and normalizing detection and passage rates to tagging rates. However, we acknowledge that ecological and experimental variability impacted our study.

Alternatively, the higher detection rates may not be experimental variability but may indicate higher movement rates (hence greater likelihood that the RFID station would detect these individuals). Specifically, species that were more likely to move (e.g., Creek Chub and Highland Stoneroller) or streams that may have allowed more movement (e.g., Little Missouri River) resulted in higher detection rates.

Even when accounting for differences in numbers of fish tagged and RFID station operating time, Creek Chub and Highland Stoneroller passed reference reaches more often than road crossings. This conclusion is consistent with results of other studies of fish passing road crossings in warmwater streams (Benton *et al.* 2008; Bouska and Paukert 2009). Data for Longear Sunfish across treatment type was inconclusive due to low numbers of observed passages.

Passage was higher on the perennial streams, likely because the hydrologic discontinuities of the intermittent streams, most pronounced during the dry summer months (see Girondo 2011 for related details), cumulatively reduced movement. In other words, stream dryness converted the intermittent streams into a series of isolated pools that reduced long-distance fish movement and passage across the study reaches.

Passage rates were lowest at the vented ford on intermittent Bear Creek where the clogged culvert pipes prevented water and fish passage *through* the road crossing. Furthermore, the stream's summer intermittency appeared to exacerbate passage problems by causing discontinuous surface flow that prevented water and fish from passing *over* the road crossing—a common condition among road crossings in this ecoregion. Consistent with these observations, Schanke *et al.* (2017) concluded that culverts and stream intermittency in this and other nearby streams contributed to reduced gene flow among Longear Sunfish and Highland Stoneroller subpopulations. When water did flow over the crossing at moderate water levels, the steep downstream slope of the structure produced a sheet of water with high velocity and insufficient depth for most fish species to pass (especially upstream). Thus, we conclude that fish opportunistically crossed this barrier when water rose near or above bankfull and flowed over and around the road crossing, creating low-velocity swim zones. Helfrich *et al.* (1999) and Norman *et al.* (2009) have also observed fish opportunistically crossing otherwise impermeable barriers during high flows. Our results should be interpreted cautiously due to the inconsistent performance of the RFID detection stations and small sample sizes, but we document a trend of reduced movement that may concern conservationists and resource managers.

At the full range of water levels observed, the box culvert on perennial Long Creek sustained hydraulic conditions (i.e., adequate swimming depth and low velocities) that appeared favorable for fish passage. Indeed, fish passed this box culvert at higher rates than they passed the vented ford on intermittent Bear Creek. While the confounding factor of hydrologic regime (intermittent versus perennial) and lack of replication preclude strong inference, this observation is corroborated by an independent analysis of gene flow patterns (Schanke *et al.* 2017) and supports previous observations that box culverts facilitate more passage than other types of road crossings that restrict flow (Warren and Pardew 1998; Standage and Gagen 2007; Norman *et al.* 2009).

The RFID stations on reference streams detected higher passage rates of Highland Stonerollers than Longear Sunfish, which is consistent with their respective swimming abilities (inferred through swimming velocity) as reported by Leavy and Bonner (2009) and with gene flow studies in these headwater streams (Schanke *et al.* 2017). Leavy and Bonner (2009) reported that the Creek Chub's swimming ability in the laboratory was poor among Cyprinidae; however, our results support the possibility that Creek Chub have a greater tendency to move in natural settings. On average, Creek Chub that did not pass our study reaches were likely two-year-old fish; whereas those that did pass were likely three-year-old fish (based on Gunning and Lewis's [1956] study of length at age in Illinois). Because even the two-year-old Creek Chub were likely sexually mature (Schemske 1974) and thus motivated to move frequently, we attribute the higher passage rates of the larger Creek Chub to their more powerful swimming capabilities.

Fish traveling on the culverted streams encountered not only the road crossing with the RFID station, but additional road crossings, which may have compounded the challenges of long-distance movements. Conclusions about long-distance movement and passage at a particular crossing must be considered in the context of the overall stream system because the benefit of any given "fish-friendly" road crossing is likely diminished by other barriers along the stream continuum (Helfrich *et al.* 1999; Zydlewski *et al.* 2006; Cote *et al.* 2009; Kashiwagi and Miranda 2009; Ryles 2012).

Despite ongoing research (Park *et al.* 2008; Bouska and Paukert 2009; Schanke *et al.* 2017), there are still many unknowns regarding the ecological effects of stream fragmentation and the degree of road crossing permeability necessary to maintain genetic diversity and viability of non-migratory fish populations over longer time scales. This presents a challenge to resource managers who wish to maintain access to remote areas while preserving natural fish movements. Flow-constricting road crossings are ubiquitous in many headwater streams, where they were commonly designed to intentionally restrict and intensify cross-sectional stream flow to ensure that strong flows would clear debris and substrate from the road surface. Thus, additional effort is needed to identify and improve aging road crossing designs, such as vented fords, which restrict flow, alter stream hydraulics, and impair fish passage (also see Warren and Pardew 1998; Bouska and Paukert 2009).

In this study, we applied RFID technology in a novel way to investigate road crossings as barriers to individual fish movements. Concurrently, Schanke *et al.* (2017) reported patterns of DNA microsatellite variability in 2 fish species in the same stream systems and documented evidence of longer-term, population-level, impacts of road crossings. A combination of research methods, such as genetic analyses at the population level, hydraulic evaluation of road crossings, and observation of individual fish movements may help answer the question—how much passage is enough?

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Prevalence of *Cytauxzoon felis* (Protista: Apicomplexa) in Feral Cats in Russellville Arkansas

C.H. Jacobs

Department of Biological Sciences, Arkansas Tech University, Russellville, AR 72801

Correspondence: cjacobs@atu.edu

Running title: Prevalence of *Cytauxzoon felis* in Feral Cats

Abstract

Cytauxzoon felis (*C. felis*) is a protozoan hemoparasite of domestic and wild felids. Transmitted by ixodid ticks, the sylvatic reservoir for this organism in North America is the bobcat (*Lynx rufus*) in which the infection is apparently self-limiting. In domestic cats (*Felis catus*), *C. felis* causes a highly fatal disease with a distribution that covers much of the central, southcentral and southeastern U.S. and parallels that of the primary vector, the lone star tick (*Amblyomma americanum*). Interestingly, there appears to be an increased survival rate in domestic cats in the geographic area of the Ozark Plateau. In this study, convenience blood samples from apparently healthy feral cats were microscopically evaluated for the presence of *C. felis* merozoites. Positive samples were submitted for PCR confirmation by a commercial laboratory. Results indicated a prevalence of 13% (4/32) in this population. Understanding the prevalence of *C. felis* infection in feral cats is central to evaluating their potential role as a reservoir for the disease and may also further our understanding about the variable pathogenicity of this organism.

Introduction

Cytauxzoonosis is a hemoparasitic infection of felids that is characterized by a rapid course of disease with a high morbidity and mortality in the domestic cat (*Felis catus*). First reported in North America in Missouri (Wagner 1976) this emerging disease has since been identified in wild and domestic felids from the Midwest to the mid-Atlantic states in the U.S. (Birkenheuer *et al.* 2006a; Haber *et al.* 2007; Shock *et al.* 2011; Tarigo *et al.* 2013; Zieman *et al.* 2017). Cytauxzoonosis is caused by an apicomplexan hematozoan parasite, *Cytauxzoon felis* (*C. felis*), that is transmitted by ixodid (hard shell) ticks. Although *C. felis* is thought to infect felids only, a closely related genus *Babesia* causes tick-borne disease in a wide variety of vertebrate hosts, including man. Thus *C. felis* belongs to a family of hemoparasites that has agricultural and zoonotic importance worldwide (Alvarado-Rybak *et al.* 2016; Wang *et al.* 2017). In the United States the principal vector of *C. felis* appears to be the lone star tick (*Amblyomma americanum*) (Reichard *et al.* 2009). The American dog tick (*Dermacentor variabilis*) is also known to carry the protozoan, but its ability to transmit the disease may be limited (Reichard *et al.* 2009). Both vectors have extensive ranges that overlap with the current distribution of *C. felis*. Both tick vectors are found in Arkansas (McAllister *et al.* 2016).

In North America, the bobcat (*Lynx rufus*) is the primary sylvatic reservoir for this parasite. The reported prevalence of infection in bobcats ranges from 7-70% (Birkenheur *et al.* 2008; Shock *et al.* 2011; Zieman *et al.* 2017) and the natural infection in these animals is apparently self-limiting and asymptomatic. In the domestic cat, on the other hand, the course of disease is rapid and highly fatal. Clinical signs of cytauxzoonosis are non-specific and typically include acute onset of fever, inappetance, lethargy or weakness, palor, icterus, and/or respiratory distress. Death occurs as a result of multiple organ failure caused by widespread vascular occlusion. With treatment, a 60% survival has been reported (Cohn *et al.* 2011). Thus the domestic cat has historically been considered to be an aberrant or dead-end host.

The life cycle of *C. felis* is complex involving sexual reproduction within the tick vector, as well as asexual replication (schizogony, or merogany) in the felid host (Fig. 1). The red blood cell inclusions, called merozoites, are the basis of the cytological screening test for the disease. Domestic cats that manage to survive the acute schizogenous phase of the disease do not completely clear the organism from their system. Instead they become chronic carriers and a potential reservoir for the parasite. In these animals, a low level parasitemia may persist for years following natural infection (Brown *et al.* 2008).

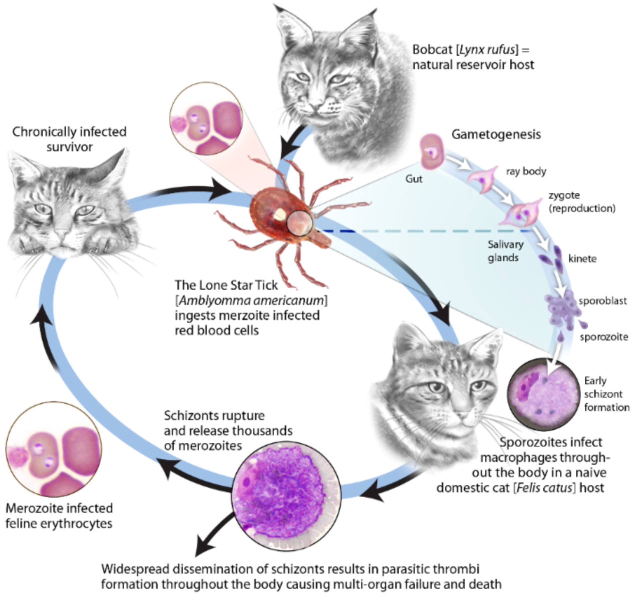

Figure 1: Life cycle of *Cytauxzoon felis*. The acute schizogenous phase is typically fatal in the domestic cat. Hosts that survive develop a chronic erythroparasitemia with merozoite-infected red blood cells that are the basis of cytologic screening. CCAL image from: Tarigo JL, *et al.* (2013).

Interestingly, the pathogenicity of *C. felis* in domestic cats appears to vary between enzootic regions. In the 1990s reports began to emerge of pet cats that survived infection with *C. felis* (Walker *et al.* 1995; Meinkoth *et al.* 2000). These early reports of subclinical disease were from cats that originated in the Ozark Plateau of Arkansas, Missouri, and Oklahoma. A 2007 study of asymptomatic free-roaming cats enrolled in trap-neuter-release (TNR) programs on the east coast and in Tennessee found a low prevalence of subclinical infection, 0.3% (n=961) (Haber *et al.* 2007). More recently, a study in apparently healthy domestic cats in and around the Ozark Plateau of Oklahoma, Arkansas, and Missouri identified an overall disease prevalence of 6.9% (n=902). Within this region, the prevalence of infection ranged from 3.4% in Oklahoma to 12.9% in southern Missouri and 15.5% in northwest Arkansas (Rizzi *et al.* 2015).

The difference in the prevalence of inapparent carriers between the eastern region of the U.S. (0.3%) and the Ozark Plateau (3.4-15.5%) may suggest the existence of a less pathogenic strain of *C. felis* in the Ozark region. To test this hypothesis, molecular studies have explored the genetic variability and revisited the taxonomy of these piroplasms (Brown *et al.* 2010; Shock *et al.* 2012; Schreeg *et al.* 2016; Pollard *et al.* 2017). Other factors may influence the pathogenicity of *C. felis* in domestic cats such as differences in the innate immunity of the host or the dose of the infectious inoculum. (Tarigo *et al.* 2013). It is possible that a difference in the biology of the tick vector affects the pathogenicity of the parasite. It is also possible that the increasing availability of more sensitive diagnostic tests are simply increasing the frequency of detection of asymptomatic carriers. In a recent review article on cytauxzoonosis in domestic cats, it was suggested that non-fatal, clinically inapparent infections should no longer be considered rare; especially in enzootic regions. (Wang *et al*. 2017). Still, the epidemiology of cytauxzoonosis is complex and gaps in our understanding remain.

The purpose of this study was to measure the prevalence of *C. felis* in apparently healthy feral cats in Russellville Arkansas. Russellville is located in the Arkansas River Valley, adjacent to the Ozark Plateau. Venous blood samples were microscopically evaluated for *C. felis* merozoites and positive samples were submitted for PCR confirmation by a commercial veterinary diagnostic laboratory. Measuring the prevalence of this infection in feral animals may improve our understanding of the epidemiology of this disease and the role of feral cats as a disease reservoir.

Materials and Methods

From April through August 2013, convenience blood samples were obtained from 33 feral cats that were live-trapped as part of a wildlife science graduate study of the feral population in Russellville, Arkansas (Norman 2014). Consistent with institutional requirements at the time, all trapping and handling procedures were performed in accordance with the guidelines of the American Society of Mammalogists (Sikes *et al.* 2011). Briefly, a general exam was performed by the graduate student while the animal was under sedation with dexmeditomidine HCl (Dexdomitor®, Zoetis U.S.A.) The animal's sex, approximate age based on dentition, weight, temperature and body condition score (BCS) were recorded. Additional health information collected included a visual inspection for ectoparasites, external signs of illness such as naso-ocular discharge, or evidence of diarrhea, and evidence of pregnancy or lactation. Approximately 0.5ml of venous blood was collected in lithium heparin, or ethylenediaminetetraacetic acid (EDTA) micro-vacutainers (Becton Dickenson Co., Franklin Lakes, N.J.). The sedation was reversed with atipamezole HCl (Antisedan®, Zoetis U.S.A) and the animal was released following full recovery. The blood samples were chilled and transported in a cold pack back to the lab where they were used for whole blood analysis for feline leukemia (FeLV) and feline immunodeficiency virus (FIV) as part of the primary research project.

Within 24 hours blood smears were made from the residual blood for cytological screening for *C. felis*. The slides were prepared in triplicate for each sample and air dried prior to staining. Two of the slides were stained with a Romanowsky stain (DipQuik, Jorgenson labs) and the third unstained blood smear was stored for future reference. Stained specimens were examined under oil immersion (1000x) for the presence of *C. felis* merozoites in erythrocytes. Fifty to 75 high power fields (hpf) were examined, focusing on the monolayer region of the smear. The feather edge was also screened for schizonts. Although *C. felis* merozoites may present in several forms, only the signet and 'safety pin' forms were counted. The sample was considered positive if 5 or more affected red blood cells were identified per 50 hpf. This represents an estimated parasitemia of ≥ 0.05%; assuming an average of 200 rbc/hpf in the monolayer region of the blood smear.

Samples from the animals that were positive on cytology were submitted for confirmation testing by a commercial veterinary diagnostic laboratory (Idexx laboratories, Westbrook, ME) where unstained blood smears were used for DNA extraction and polymerase chain reaction (PCR) analysis (*C. felis* RealPCR™, Idexx labs). One cytologically negative specimen was also submitted for PCR testing as a negative control.

The overall disease prevalence was calculated as the proportion of the specimens that were positive on both cytology and PCR. Binomial confidence intervals were calculated at the 95% confidence level.

The stated purpose of this study was to identify *C. felis* infections in apparently healthy feral cats. Due to the non-specific signs associated with this disease and the difficulty in assessing these signs in feral animals, the presence of fever (>102.5°F) was used as an exclusion criterion for identifying clinically affected animals.

Results

Stained blood smears from 33 feral cats were examined microscopically for the presence of *C. felis* merozoites. Six of the specimens tested positive on cytology. Unstained slides were available for 5 of these samples and they were subsequently submitted for confirmation testing by PCR at a commercial veterinary diagnostic laboratory. Four of the cytologically positive specimens also tested positive on PCR (Table 1). This indicates an overall prevalence of 13% (CL 95%, CI 3-32)

Three of the animals that tested positive for *C. felis* were afebrile at the time of sample collection. The initial temperature for one animal was not recorded; however, the general exam for that animal was normal. Thus all 4 animals were considered to be inapparent carriers of *C. felis*. All of the infected animals were adults (> 6 months). Three were males and one was a female.

As mentioned earlier, one cytologically negative specimen was also submitted for PCR confirmation as a negative control (not shown in Table 1). This sample tested negative on PCR as well.

Discussion

This study measured the prevalence of *C. felis* in apparently healthy feral cats living in an exurban environment in the Arkansas River Valley. The study area is located adjacent to the Ozark Plateau where cytauxzoonosis is enzootic and the sylvatic reservoir (bobcat) and principal vector (lone star tick) are common. The prevalence of subclinical *C. felis* infection in this population of feral cats was 13% (4/32; CL 95%, 3 - 32). These findings are similar to levels previously reported in free roaming and pet cats from the Ozark Highlands of eastern OK (16.9%, 9.7-27.2), northwest AR (15.3%, 10.3-21.7), and southern Missouri (12.9%, 6.1-24.0) (Rizzi *et al.* 2015).

The prevalence of cytauxzoonosis has been shown to vary within enzootic regions. For example, in central Oklahoma the reported prevalence (3.4%) was lower than that found in eastern Oklahoma (16.9%) (Rizzi *et al.* 2015; Nagamori *et al.* 2016). A recent study utilized an ecological niche model to predict the distribution of *C. felis* in domestic cats in Oklahoma, Missouri, and

Table 1. Results of cytology and PCR diagnostic testing for *Cytauxzoon felis* in 33 feral cats. Overall prevalence (specimens positive on both tests) was 13% (4/32). Note: 5 of 6 samples that were positive on cytology were available for PCR confirmation.

C. felis	Cytology	PCR*	Cytology + PCR
Positive	6	4	4
Negative	27	1	28
Total	33	5	32
Prevalence (95% CL)	18% (7 - 35)		13% (3 - 32)

Arkansas (Mueller *et al.* 2013). The model used confirmed cytauxzoonosis case records, vector and sylvatic reservoir distribution data, and other environmental factors to generate maps that predict high risk areas for *C. felis*. Notwithstanding the author's caution that limited data was available from Arkansas, the model predicted a lower prevalence of disease in the River Valley in Arkansas versus the surrounding highlands of the Ozarks to the north and the Ouachita mountains to the south. The results of the current study would suggest that *C. felis* is more evenly distributed in western Arkansas.

Although health assessment in feral animals is a challenging prospect, a general exam was performed on the sedated animals in this study. None of the animals that tested positive for *C. felis* exhibited fever or other external signs of disease consistent with feline cytauxzoonosis at the time of blood draw. Since inapparent carriers can remain parasitemic for a prolonged period of time, they present an increased risk of infection for naïve free roaming cats and a reservoir of disease in this exurban setting.

In addition to the small sample size in the current study, there are inherent limitations for each of the diagnostic tests used (cytology, PCR) that should be considered when interpreting these results. Cytological screening for merozoites in asymptomatic animals is challenging in part because of the low levels of parasitemia that are commonly encountered. The erythrocytic merozoites of *C. felis* are small (1-2 µm) and are found in several different forms (signet, 'safety pin', punctate). The later can be difficult to distinguish from Howell Jolly bodies, stain precipitate and even drying artifacts (Fig. 2). Further, some of the convenience blood samples used in this study were originally collected in heparinized tubes. Heparin is not an ideal anticoagulant for examining the morphology of erythrocytes as it can cause cellular distortion and variable stain uptake. Thus cytological screening, although rapid and inexpensive, has limitations. PCR analysis for parasitic DNA is a more sensitive and specific test when compared to cytology. (Birkenheuer *et al*. 2006b). Ideally, all samples (positive and negative on cytology) would be confirmed by PCR. These tests are commercially available but expensive to run and as with any test, false positives and false negatives are possible. In this study, one sample was identified as positive on cytology but tested negative on PCR. Because PCR is a more specific test than cytology, the sample was recorded as negative for *C. felis* (Table 1).

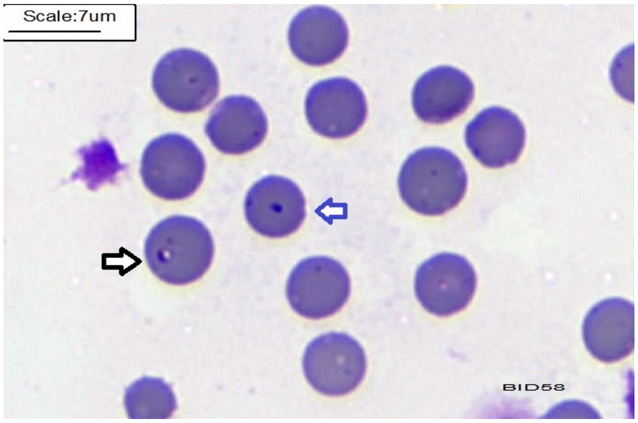

Figure 2. *Cytauxzoon felis* merozoite (black arrow) on a peripheral blood smear. The blue arrow indicates a Howell Jolly body. Blood is stained with a Romanowski differential stain (DipQuik). Oil (1000x).

Conclusion

The current study measured a 13% prevalence of *Cytauxzoon felis* infections in apparently healthy feral cats living in Russellville, Arkansas. A comparable level of infection was reported previously in asymptomatic cats in the adjacent Ozark Highlands region (Rizzi *et al* 2015). Thus, the results of the current study suggest that the distribution of *C. felis* extends into the Arkansas River Valley at a similar level. The relatively high prevalence of *C. felis* reported here also supports the hypothesis that feral cats serve as a reservoir of infection for free roaming, naïve cats in exurban environments where the sylvatic reservoir (bobcat) is less common. Further study of the epidemiology of cytauxzoonosis is needed to assess whether the prevalence of asymptomatic carriers reflects a change in host-parasite interaction.

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Optimum Magnetometer Transect Spacing to Locate Legacy Oil and Gas Wells: Preliminary Results

J.A. Patton, M.G. Davis, K.J. Gowing, and H.B. Vickers

Department of Physical Sciences, Arkansas Tech University, Russellville, AR 72801

Correspondence: jpatton@atu.edu

Running Title: Magnetometer Transect Spacing to Locate Legacy Oil and Gas Wells

Abstract

The purpose of this project was to examine the optimum transect spacing to locate legacy oil and gas wells using an Overhauser magnetometer. Widely known to be a potential environmental hazard, legacy oil and gas wells may act as a conduit for methane and/or deeper subsurface fluids (naturally occurring brines, injected waste fluids, or injected CO_2) to the surface or shallow subsurface. Many plugged wells have all surface equipment removed leaving no visible trace at the surface and thus making the environmental assessment of these wells difficult. Using a magnetometer along a set of predefined transects, magnetic anomalies from the metal casing can be detected. In order to assess large numbers of wells, understanding the typical anomaly size is critical to maximize the transect spacing and therefore minimize magnetometer field work time. Here we briefly review the wide range of transect spacings reported in the literature and show the results of five wells with an initial survey grid at two meter spacing. Although there is significant variation in the anomaly size (X, Y, and Z), transect spacing of 20 m was sufficient to identify all buried wells using the method described herein. The anomalies associated with four of the wells ranged from approximately 1000-4000 nanoteslas (nT), while one well anomaly exhibited more than 10,000 nT above background.

Introduction

Legacy wells (defined as any oil or gas well that is at the end of its production life cycle regardless of its current plugged status) resulting from historic oil and gas production have the potential to cause environmental harm through two primary mechanisms: contamination of surface and/or groundwater and release of methane to the atmosphere (Boothroyd *et al.* 2016; Chilingar and Endres 2005). This situation arises when either the well was never properly plugged or when the state-mandated plugging system fails due to poor construction or old age and allows the migration of deeper fluids and/or gases to the shallow subsurface.

When modern wells are plugged, cement is usually pumped into the well to isolate the production zone (perforated interval) and the shallow fresh water zone. In older wells, standard plugging procedures may not have existed, so the methods utilized varied greatly. As an example, in Pennsylvania, until the year 1955, an oil or gas well was required to be permanently plugged with a “well seasoned, round wooden plug” (Dilmore *et al.* 2015). Recent studies from other states show that failure rates in older wells range from 1.9% to 75% (Davies *et al.* 2014) with older wells being particularly susceptible to plugging failures, even when plugging procedures were followed properly.

As an example of the possible scope of the problem in the Arkoma Basin of Arkansas (an area essentially equivalent to the Arkansas River Valley geographic region), there are approximately 5230 wells that were drilled before 1967 (defined here as older than 50 years)(AOGC 2018). If wells in this area have the same magnitude of failure rates as other areas, then a conservative estimate of 262 wells (5% failure rate of 5230 wells) in the Arkoma Basin may currently be releasing contaminants to the atmosphere or groundwater.

Most of these wells no longer have any surface expression – the pipe is cut off below the land surface and is buried during the plugging procedure. Before these wells can be assessed for risk, their location must be accurately known. Many older wells throughout the country do not have exact locations associated with them and were only required to submit location information equivalent to an area of approximately 16 hectares (40 acres). To determine if older wells are a risk to the environment, their exact location must be known before any environmental assessment can be performed.

The use of magnetics in finding oil and gas wells has a rich history (Frischknecht *et al.* 1983; Aller 1984;

Table 1. General information for wells in this study. Note that the latitude and longitude listed here are approximate locations only. Data obtained from AOGC (2018).

Well Name and Number	Permit	Location Section, Township, Range			Latitude	Longitude	Year Drilled	Year Plugged
Ozark Highlands Unit 11-20 1-23	44836	23	11N	20W	35.59442	-93.08168	2011	2012
Ozark Highlands Unit 11-21 1-23	44835	23	11N	21W	35.58397	-93.18634	2012	2013
Silex Federal 1-4	34225	4	10N	21W	35.54996	-93.22791	1990	1990
Pilot Mountain 1	25547	18	11N	21W	35.60672	-93.27351	1979	1979
Pilot Mountain ES 13114 1	23519	6	11N	21W	35.63615	-93.26085	1977	1977

Frischknecht *et al.* 1985; Hammack and Veloski 2016; Hammack *et al.* 2016). In general, to perform a ground based magnetic survey, a series of transects is created at a predefined spacing. The magnetometer is carried along the transects, while the earth's magnetic field is constantly measured. Any large metallic objects – including buried objects - will create a magnetic anomaly that is detected by the sensor. The size of the anomaly produced by the object is proportional to the mass, geometry, orientation, and distance of the buried object to the magnetometer (Aller 1984). In addition, both Frischknecht *et al.* (1983) and Jordan and Hare (2002) noted that corrosion of well casings may affect the size of the anomaly, but neither estimated the influence of degradation on the anomalous signal.

Although this technique has been used for both aerial and ground surveys, justification for the transect spacing chosen is commonly absent from most reports (e.g. Xia 2002; Hammack and Veloski 2016; Hammack *et al.* 2016) with transect spacing for ground surveys ranging between 2 m (Hammack and Veloski 2016), 3 m (Martinek 1988), 10 m (Hammack and Veloski 2007), 15.2 m (Frischknecht *et al.* 1983), 30.5 m (Xia 2002) or even increasing as distance from the suspected well location increases (Frischknecht *et al.* 1983; Jachens *et al.* 1986). Jordan and Hare (2002) stated "between-line station spacing is based on the survey goals and size of anomalies expected" (p 7-10) and later noted that transect spacing should be no more than 6-9 m, while Martinek (1988) stated that while 6 m may be sufficient to detect an anomaly, 3 m spacing was needed to ensure the anomaly was a buried well casing. The wide variation in transect spacing reported in these studies makes it difficult to determine what the maximum spacing can be at any one location where a well is suspected to be located. This study investigated the maximum transect width for ground-based magnetometer surveys and presents data from five natural gas wells in the Arkoma Basin of Arkansas.

Methods

Five wells of varying age were chosen to collect field magnetometer data (see Table 1 for general description of each well). For each well location, a grid was set-up with north-south oriented transects with a 2 m nominal spacing between each transect. Although the exact location of each well was not known, the grid was centered over the estimated location derived from well records obtained from the Arkansas Oil and Gas Commission (AOGC 2018). The two most recently drilled wells (permit 44836 and 44835) were located on large, open, accessible well pads. The remaining wells were all located on well pads with significant vegetative overgrowth, making locating the transect spacing less regular.

The magnetic data were collected using a GEM System GSM-19W Overhauser Magnetometer with integrated GPS. The data were collected by setting the magnetometer to take readings every two seconds while the operator walked the transects. Diurnal corrections were not made to the field data due to the expected relative magnitude difference between typical diurnal variations and well casing anomalies (Hammack and Veloski 2016) and the short duration of field data collection (<30 minutes at each site).

After field collection of the data was complete, the data were downloaded for further analysis. The data were imported into ArcGIS 10.2 (ESRI) and Microsoft Excel for mapping and data analysis purposes, respectively. In ArcGIS, the data were gridded using the IDW interpolation technique with a 1 m grid size. No reduction to the pole transformation or other transformations were performed because the intent of this study was to maximize transect spacing.

To differentiate between probable well casings and non-target metallic debris, we used two criteria: the presence of a monopole signature and anomaly amplitude threshold value of 50 nT above background.

Several authors (Hammack and Veloski 2016; Jordan and Hare 2002) have noted that well casing anomalies typically exhibit a monopole form, due to the orientation of the well casing relative to the earth's magnetic field (Breiner 1973). In addition, Breiner (1973) noted that most small metallic objects have magnetic anomalies well below 50 nT, while other authors have shown that most well casings have anomalies that are more than 1000 nT (Jordan and Hare 2002). The 50 nT anomaly contour was plotted over the monopole signature to be used as a basis for estimating the maximum transect size needed to detect the well casing.

Results

Significant variation was found in the amplitude of the magnetic anomaly from the five wells surveyed (Table 2). Anomalies ranged from 1021 nT to 10,343 nT. Magnetic surveying at one well location (Permit 25547) was impeded by the presence of dense vegetation and a small pond, therefore results from that location are incomplete. Figure 1 show the results of the interpolation of the field data and identification of the 50 nT anomaly boundary. Figure 2 shows a north-south oriented profile through the highest magnetic reading at each well location. Although wider spacing may be able to capture most wells, the smallest anomaly found suggests that a 20 m spacing would identify the 50 nT threshold for each of these wells and is therefore appropriate for further testing and refinement.

Table 2. Magnetic values measured at each well location. Note that the survey for Permit 25547 was not completed. Background values were collected at each site during field work.

Permit	Max Value	Min Value	Back-ground	Anomaly Amplitude
44836	53066	50418	50652	2414
44835	51859	50612	50651	1208
34225	51719	50685	50698	1021
25547	54907	50473	50561	(4346)*
23519	60835	50296	50492	10343

Discussion

Several interesting features should be noted regarding these findings. First, the amplitude of the anomaly of Permit 23519 was unexpected. Amplitudes commonly range from 2000 – 5000+ nT (Jordan and Hare 2002). Permit 23519 had an anomaly amplitude of more than 10,000 nT over background, which was at least four times greater than the other wells in the study with similar construction. Interestingly, Permit 23519 is also the oldest well in our survey, having been drilled and plugged in 1977 (Table 2), and presumably may be somewhat degraded compared to the newer wells we examined. Although both Frischknecht *et al.* (1983) and Jordan and Hare (2002) noted that corrosion of well casings may affect the size of the anomaly, it is assumed well corrosion would decrease the amplitude, not increase the anomaly size as observed here.

The minor amplitude variation found in the others wells, ranging between 1021 and 2414 nT, may simply be related to transect location. Further analysis of points near the well location show that the gradient of the magnetic field was sufficient to cause a change of more than 1000 nT over a range of about one meter. The wells with lower values (e.g. Permit 34225) may not have had a transect directly over the top of the well, while the higher value wells may have. In an attempt to further explain the variation between all wells, a search of the plugging and completion data for each well was performed and is shown in Table 3. No noticeable correlation exists between pipe size or pipe length and the total anomaly size, similar to what was found in Frischknecht *et al.* (1983). What is not known about each well is the total burial depth. Only one well (Permit 34225) had information about how far below the surface the pipe was cut before burial. Plugging records throughout the area commonly state the burial depth to between 1 and 2 m. This depth may affect the total magnetic anomaly size in that a well with a shallow casing would produce a greater anomaly. Corrosion, transect location, burial depth, and pipe construction details may all have contributed to variations in anomaly amplitude.

Table 3. Type and amount of pipe left in each well location.

Permit	Conductor Width (cm)	Conductor Length (m)	Surface Casing Width (cm)	Surface Casing Length (m)
44836	40.64	12.5	24.45	319.1
44835	40.64	12.5	24.45	366.7
34225	-	-	21.91	155.8
25547	29.85	9.1	21.91	91.4
23519	29.85	12.8	21.91	196.3

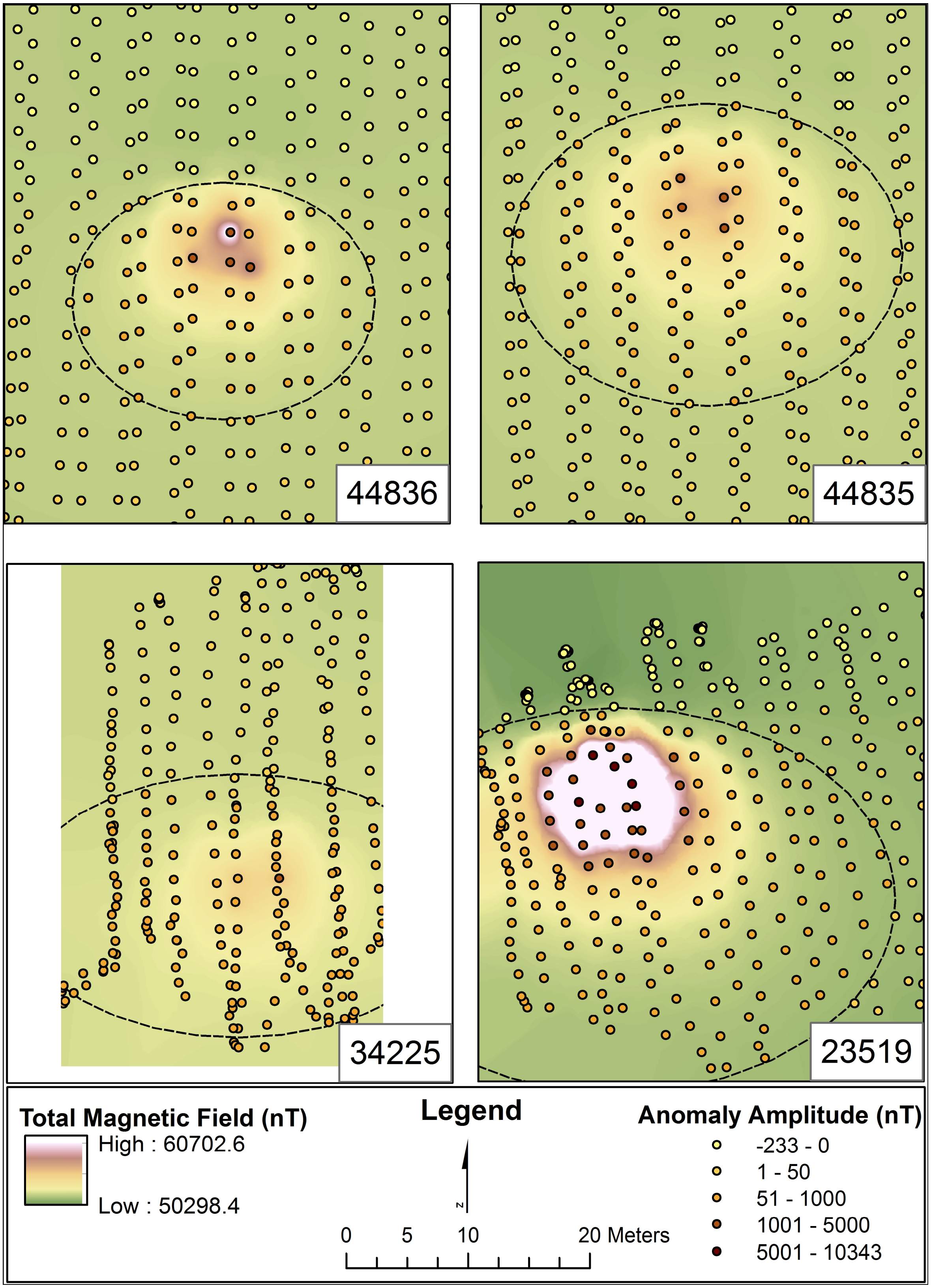

Figure 1. Magnetic Data from All Well Locations Along Nominal 2 m Spacing. Permit number is shown in corner. Dashed line shows 50 nT anomaly boundary. Wells 44836 and 44835 were located in areas with well-defined pads, whereas wells 34225 and 23519 were located in areas of heavy vegetation.

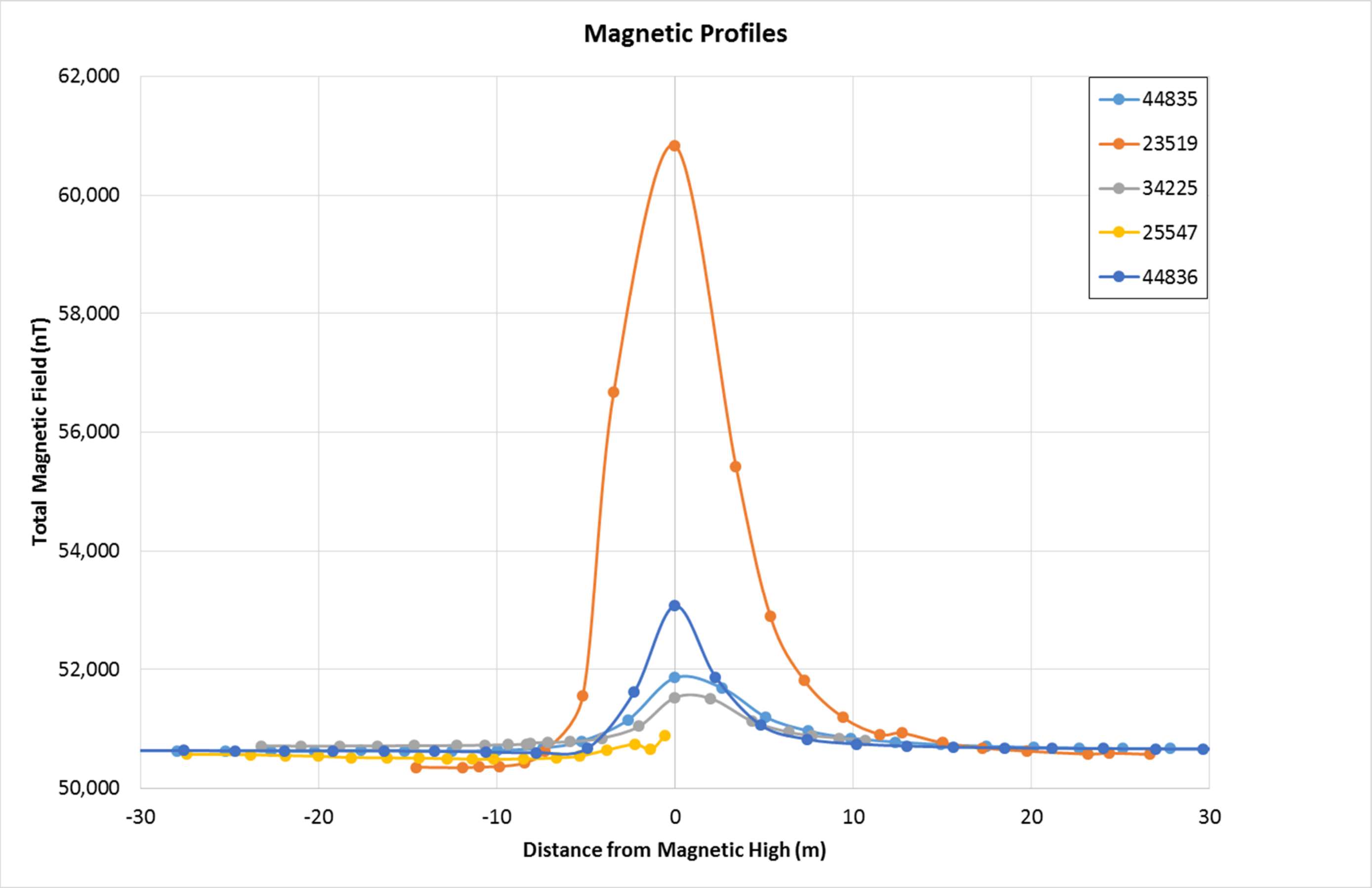

Figure 2. Magnetic Profiles of Well Locations. Note that each profile is centered around highest reading. Negative distance values are north of the high, positive distance values are south of the high.

A 20 m grid spacing was sufficient to identify all well locations using the methods described herein and as shown by the size of the 50 nT contour on Figure 1. When considering that most wells without detailed locations have general location data down to the Quarter Quarter Section of the Public Land Survey System, a 20 m spacing would allow surveying an area of this size in only a few hours.

Conclusion

As finding the exact location of these wells becomes more important in the future so that an environmental assessment can be performed, the maximum transect size that will find all wells, including the smallest signatures, will be critical. A transect spacing of 20 m was sufficient to accurately identify all of the well locations in this study, but more research is needed into variations in anomaly size so that future studies do not inadvertently miss wells with small anomaly signatures.

Acknowledgements

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A Preliminary Checklist of the Stoneflies (Arthropoda: Insecta: Plecoptera) of Arkansas

H.W. Robison[1] and C.T. McAllister[2*]

[1]9717 Wild Mountain Drive, Sherwood, AR 72120
[2]Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

[*]Correspondence: cmcallister@se.edu

Running Title: Stoneflies of Arkansas

Abstract

Seventy-seven species of stoneflies representing 8 families and 24 genera are herein reported from Arkansas. The most diverse families of state stoneflies were Perlidae (26 species), Capniidae (14 species), and Perlodidae (12 species) while Pteronarcyidae had only a single representative species. Additional taxa will surely be added to the list with future taxonomic studies incorporating molecular analyses.

Introduction

Stoneflies (Plecoptera) are a diverse group of aquatic insects which occur worldwide including both the Northern and Southern hemispheres (Steward and Stark 2008). They are valuable components of stream food webs and serve as biological indicators of water quality (Hynes 1972; Resh and Unzicker 1975; Harper and Stewart 1984). Stoneflies are also important prey for a variety of game and non-game fishes in Arkansas (Robison and Buchanan 1988) and are well known to fly fishermen. Worldwide, there are approximately 3,500 species of stoneflies placed within 16 families (Fochetti and Tierno de Figueroa 2008; Stark *et al.* 2009; Stark 2017; DeWalt *et al.* 2018). About 715 species in 9 families are known for North America (DeWalt *et al.* 2018) while approximately 294 species are currently recognized from, or adjacent to, the southeastern region of the United States (Morse *et al.* 2017).

Previously, Poulton and Stewart (1991) documented 88 stonefly species in 8 families and 24 genera from the Interior Highlands (Ozark and Ouachita Mountain) region of Arkansas, Kansas, Illinois, Missouri, and Oklahoma); however, no specific list of stoneflies has been published for Arkansas.

The major objective of this study is to provide an updated checklist of the stoneflies currently known from Arkansas so that state biologists, naturalists, resource managers, and anglers have such a list available to them. While there has been no comprehensive study of the stoneflies of the state, a larger study of the Arkansas stonefly fauna is currently in progress with stonefly specialists R. Edward DeWalt (Illinois Natural History Survey [INHS]) and Barry C. Poulton (United States Geological Survey), in collaboration with HWR and CTM which will deal with current taxonomy, distribution, and status of those species in the state.

Historical Review

Although there are lists of stoneflies available for some contiguous states to Arkansas such as Oklahoma (Stark and Stewart 1973), Louisiana (Stewart *et al.* 1976), Mississippi (Stark 1979), Texas (Szczytko and Stewart 1977), and Kansas (Stewart and Huggins 1977), there is no current official checklist of the stoneflies of Arkansas. Earlier Illie's (1966) catalog attributed 33 stonefly species to the state while Stewart and Stark (1988) later reported 60 stonefly species from Arkansas. Further records of Ozark and Ouachita mountain species in Arkansas have been provided in taxonomic papers (Ricker and Ross 1968; Ross and Ricker 1971; Stark and Stewart 1973; Stark and Ray 1983; Ernst *et al.* 1986; Grubbs *et al.* 2013, 2014), faunal surveys (Robison and Harp 1971; McGary and Harp 1972; Cather and Harp 1975; Guntharp and Harp 1982; Huggins and Harp 1983; Cochran and Harp 1990; Chordas *et al.* 1996; Robison 2003; Harp and Robison 2006; Sheldon and Warren 2009), and from ecological studies of Arkansas streams such as the Illinois (Brown and Ricker 1982) and the Little Missouri rivers (Feminella and Stewart 1986). To date, the most authoritative accounts of Arkansas stoneflies is that of Poulton (1989) and Poulton and Stewart (1991) which covered the stoneflies of the Interior Highlands and provided a great deal of data on stoneflies of the state.

Basic Life History

Stoneflies are a small group of hemimetabolous insects distributed primarily in the mountainous regions of North America. The nymph is similar in form to the adult and differs mainly in the incompletely developed condition of the wings and genitalia. Larvae typically live in cold, clear, well-oxygenated creeks, streams, rivers and lakes. They generally resemble wingless adults, but often have external gills, which may be present on almost any part of the body. Larvae are typically herbivorous and feed on submerged leaves and benthic algae; however, there are species which function as predators of other aquatic arthropods. Adults are terrestrial and survive for only a few weeks after transforming from nymphs and usually emerge only during certain times of the year. Adult stoneflies have a rather generalized body anatomy, a relatively soft body, simple mouthparts with chewing mandibles, long, multiple-segmented antennae, large compound eyes, and 2 or 3 ocelli (Merritt *et al.* 2008). Legs are large and end in 2 claws. Long, paired cerci, project from the end of the abdomens of both adults and nymphs. Adults tend to be herbivorous if they feed at all and because they are not strong fliers, adults tend to remain close to the stream or lake where they hatched.

Adults mate on vegetation, stones, bridges, and other physical situations. Females may lay up to 1,000 eggs. Hatching generally occurs in 2 to 3 weeks; however, some species undergo diapause. After hatching, the nymphal stage may last from 1 to 4 years, depending on species, and undergo anywhere from 12 to 36 molts before emerging and becoming an adult. Nymphs leave the water and attach to a fixed surface and molt one last time becoming a fully terrestrial adult.

Methods

Previous collection records for Arkansas stoneflies as well as continuing light trap collections were used to develop this checklist of Arkansas stonefly species. Stoneflies were obtained from several sources including >600 collections made between the mid-1980s and 2017 by Barry C. Poulton (BCP), R. Edward DeWalt (RED), and George L. Harp (GLH) and additional collections made by former students of HWR (see **acknowledgments**). Between 1975 and 2017, >500 ultraviolet (UV) light trap collections were made by HWR from the Arkansas River Valley and Ozark and Ouachita Mountains, as well as collections from the Gulf Coastal and Mississippi Alluvial plains. In addition, aquatic stonefly collections were also made by hand collecting along seeps, springs, and streams, as well as sweeping riparian vegetation for adults with an insect net in various areas of the state. Also available were previous collection records of Arkansas stoneflies made by BCP between 1983 and 1988 (Poulton 1989; Poulton and Stewart 1991), a list of state species provided by RED from his research on North American stoneflies, and an online Plecoptera species file database (DeWalt *et al.* 2018). Unpublished records and unidentified material were obtained from the insect collections of the INHS, a thorough literature search was made of macroinvertebrate studies in Arkansas, and faunistic investigations in the state specifically targeting stoneflies (see ***Historical Review***).

Identifications of stoneflies were made initially by BCP, the late K.W. Stewart (University of North Texas), and by RED. Dr. DeWalt is continuing his systematic investigations of stoneflies using DNA analysis of specimens collected in Arkansas in conjunction with HWR and CTM. Pinned and larval specimens in the INHS collection were examined by the authors. All stonefly voucher specimens collected for this project are to be deposited in the INHS collection. Use of common names (**Appendix**) for each stonefly follows Stark et al. (2012).

Results and Discussion

Our study found a total of 77 stonefly species within 24 genera and 8 families to inhabit Arkansas (APPENDIX). The 3 most speciose families were the Perlidae (26 species), Capniidae (14 species), and Perlodidae (12 species). The remaining 5 families included Taeniopterygidae (8 species), Leuctridae (7 species), Chloroperlidae (5 species), Nemouridae (4 species), and Pteronarcyidae (1 species). The largest represented genera are *Allocapnia* with 12 species, *Isoperla* with 8 species, *Neoperla* with 7 species, and *Taeniopteryx, Acroneuria,* and *Perlesta,* with 6 species each.

Within the last decade, the most recently described stonefly species in Arkansas are *Perlesta ephelida* Grubbs and DeWalt (Grubbs and DeWalt 2012) and *Prostoia ozarkensis* Baumann and Grubbs (Grubbs *et al.* 2014). In addition, there are several undescribed species of stoneflies currently being studied and these descriptions will appear in the near future, thus they were not included in our checklist. These additional undescribed stonefly species will increase the number of species known for the state. Collecting by light traps and winter stonefly collecting also continues across the state by the authors and others which will reveal more about stonefly distributions in Arkansas.

Of the 77 species of stoneflies documented from Arkansas, 8 are endemic to the state, most known only from their type locality (Robison and Allen 1995). These state endemics include *Allocapnia oribata* from Searcy County, *A. ozarkana* from Madison County, *A. warreni* from Washington County, *Alloperla caddo* from Garland and Perry counties, *Al. ouachita* from Hot Spring and Montgomery counties, *Isoperla szczytkoi* from Logan County, *Leuctra paleo* from Columbia and Dallas counties, and *Zealeuctra wachita* from Polk and Scott counties.

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APPENDIX. List of Arkansas Plecoptera (Stoneflies).*

NEMOURIDAE – FORESTFLIES (4 SPECIES)

Amphinemura delosa (Ricker, 1952) - Eastern Forestfly
Amphinemura nigritta (Provancher, 1876) - Little Black Forestfly
Prostoia ozarkensis Grubbs & Baumann, 2014 - Ozark Forestfly
Shipsa rotunda (Claassen, 1923) - Intrepid Forestfly

CAPNIIDAE – SNOWFLIES (14 SPECIES)

Allocapnia granulata (Claassen, 1924) - Common Snowfly
Allocapnia jeanae Ross, 1964 - Osage Snowfly
Allocapnia malverna Ross, 1964 - Gulf Snowfly
Allocapnia mohri Ross & Ricker, 1964 - Ouachita Snowfly
Allocapnia mystica Frison, 1929 - Moraine Snowfly
Allocapnia oribata Poulton & Stewart, 1987 - Bowed Snowfly
Allocapnia ozarkana Ross, 1964 - Ozark Snowfly
Allocapnia peltoides Ross & Ricker, 1964 - Shield Snowfly
Allocapnia rickeri Frison, 1929 - Midwest Snowfly
Allocapnia sandersoni Ricker, 1952 - Notched Snowfly
Allocapnia vivipara (Claassen, 1924) - Shortwing Snowfly
Allocapnia warreni Ross & Yamamoto, 1966 - Arkansas Snowfly
Nemocapnia carolina Banks, 1938 - Southern Snowfly
Paracapnia angulata Hanson, 1942 - Angulate Snowfly

LEUCTRIDAE – NEEDLEFLIES (7 SPECIES)

Leuctra paleo Poulton & Stewart, 1991 - Arkansas Needlefly
Leuctra tenuis (Pictet, 1841) - Narrow-lobed Needlefly
Zealeuctra cherokee Stark & Stewart, 1973 - Cherokee Needlefly
Zealeuctra claasseni (Frison, 1929) - Common Needlefly
Zealeuctra narfi Ricker & Ross, 1969 - Northern Needlefly
Zealeuctra wachita Ricker & Ross, 1969 - Ouachita Needlefly
Zealeuctra warreni Ricker & Ross, 1969 - Early Needlefly

TAENIOPTERYGIDAE – WILLOWFLIES (8 SPECIES)

Strophopteryx arkansae Ricker & Ross, 1975 - Arkansas Willowfly
Strophopteryx fasciata (Burmeister, 1839) - Mottled Willowfly
Taeniopteryx burksi Ricker & Ross, 1968 - Eastern Willowfly
Taeniopteryx lita Frison, 1942 - Small Willowfly
Taeniopteryx lonicera Ricker & Ross, 1968 - Honeysuckle Willowfly
Taeniopteryx maura (Pictet, 1841) - Spinyleg Willowfly
Taeniopteryx metequi Ricker & Ross, 1968 - Shortwing Willowfly
Taeniopteryx parvula Banks, 1918 - Hooked Willowfly

CHLOROPERLIDAE – SALLFLIES (5 SPECIES)

Alloperla caddo Poulton & Stewart, 1987 - Caddo Sallfly
Alloperla caudata Frison, 1934 - Ozark Sallfly
Alloperla hamata Surdick, 1981 - Barbed Sallfly
Alloperla ouachita Stark & Stewart, 1983 - Ouachita Sallfly
Haploperla brevis (Banks, 1895) - Least Sallfly

PERLIDAE - SUMMER STONEFLIES (26 SPECIES)

Acroneuria evoluta Klapálek, 1909 - Constricted Stone
Acroneuria filicis Frison, 1942 - Illinois Stone
Acroneuria frisoni Stark & Brown, 1991 - Central Stone
Acroneuria internata (Walker, 1852) - Lobed Stone
Acroneuria ozarkensis Poulton & Stewart, 1991 - Ozark Stone
Acroneuria perplexa Frison, 1937 - Enigmatic Stone
Agnetina capitata (Pictet, 1841) - Northern Stone
Agnetina flavescens (Walsh, 1862) - Midwestern Stone
Attaneuria ruralis (Hagen, 1861) - Giant Stone
Neoperla carlsoni Stark & Baumann, 1978 - Spiny Stone
Neoperla catharae Stark & Baumann, 1978 - Slippery Stone
Neoperla choctaw Stark & Baumann, 1978 - Choctaw Stone
Neoperla falayah Stark & Lentz, 1988 - Curved Stone
Neoperla harpi Ernst & Stewart, 1986 - Arkansas Stone
Neoperla osage Stark & Lentz, 1988 - Osage Stone
Neoperla robisoni Poulton & Stewart, 1986 - Slender Stone
Paragnetina kansensis (Banks, 1902) - Smoky Stone
Paragnetina media (Walker, 1852) - Embossed Stone
Perlesta baumanni Stark, 1989 - Darkwing Stone
Perlesta browni Stark, 1989 - Toothed Stone
Perlesta cinctipes (Banks, 1905) - Plains Stone
Perlesta decipiens (Walsh, 1862) - Widespread Stone
Perlesta ephelida Grubbs & DeWalt, 2012 - Speckled Stone
Perlesta fusca Poulton & Stewart, 1991 - Tinted Stone
Perlinella drymo (Newman, 1839) - Striped Stone
Perlinella ephyre (Newman, 1839) - Vernal Stone

PERLODIDAE - STRIPETAILS AND SPRINGFLIES (12 SPECIES)

Clioperla clio (Newman, 1839) - Clio Stripetail
Helopicus nalatus (Frison, 1942) - Ozark Springfly
Hydroperla crosbyi (Needham & Claassen, 1925) - Early Springfly
Hydroperla fugitans (Needham & Claassen, 1925) - Austin Springly
Isoperla burksi Frison, 1942 - Banded Stripetail

Isoperla davisi James, 1974 - Alabama Stripetail
Isoperla dicala Frison, 1942 - Sable Stripetail
Isoperla irregularis (Klapálek, 1923) - Texas Stripetail
Isoperla namata Frison, 1942 - Ozark Stripetail
Isoperla ouachita Stark & Stewart, 1973 - Ouachita Stripetail
Isoperla signata (Banks, 1902) - Transverse Stripetail
Isoperla szczytkoi Poulton & Stewart, 1987 - Magazine Stripetail

PTERONARCYIDAE – SALMONFLIES (1 SPECIES)
Pteronarcys pictetii Hagen, 1873 - Midwestern Salmonfly

***Current number of taxa found is 77.**

Tree-Ring Dating of the Ficklin-Imboden Log Structures, Powhatan Historic State Park, Arkansas

K.R. McAdoo[1], and D.W. Stahle[2]

[1,2]*Department of Geosciences, University of Arkansas, Fayetteville*

[1]Correspondence: krmcadoo@uark.edu

Running Title: Tree-Ring Dating of the Ficklin-Imboden Log Structures

Abstract

Powhatan Historic State Park in Powhatan, Arkansas preserves and interprets five historical structures from 19th century Arkansas, including the Ficklin-Imboden Log House. This structure, which is actually two separate log buildings with uncertain construction dates and functions, is believed to be the earliest surviving structure at Powhatan Historic State Park and is on the National Register of Historic Places. Powhatan Historic State Park contracted with the University of Arkansas Tree-Ring Laboratory to develop a more accurate dating and interpretation of the log structures. Dendrochronology (tree-ring dating) was used to determine the true felling dates of bald cypress (*Taxodium distichum*) logs in both buildings. Core specimens were extracted from 22 wall logs and 16 were dated with dendrochronology. The measured ring width data were used to develop a chronology for the buildings that was then correlated against other absolutely dated tree-ring chronologies from the region.

The strong correlation among the ring width time series from both structures provided evidence that the trees from which the logs were cut grew contemporaneously in the same area. The derived mean ring width chronology is highly correlated with tree-ring reconstructions of the Palmer Drought Severity Index (PDSI) across Arkansas and the central US from 1726 to 1846 and with other regional tree-ring chronologies, particularly one created from living bald cypress trees at Allred Lake in southeast Missouri. All possible correlation analyses between the continuous Ficklin-Imboden chronology and the Allred Lake chronology, advancing one year at a time over the past 808 years (1185–1992), indicate that the highest correlation ($r = 0.45$) is observed precisely where the log specimens were dated based on microscopic analysis and skeleton plot crossdating. The derived cutting dates extended from 1843 to 1846, with logs in both structures cut as late as 1846. Because the structures are in situ and the wall logs have not been extensively repaired or replaced, the cutting dates indicate that the structures were likely erected simultaneously during or soon after the growing season of 1846. This date is slightly earlier than the current interpretation by Powhatan Historic State Park, but still consistent with the documentary evidence that Andrew Imboden and his wife used the building as a home by 1851.

Introduction

After the Louisiana Purchase in 1803, many non-French European settlers immigrated into modern-day northeast Arkansas. Some pushed farther west into the interior Ozark Plateau and beyond, but many chose to settle in what is now Lawrence County, Arkansas. A business man named John Ficklin arrived in present-day Powhatan in 1837 and established a ferry on the Black River in 1839 (Land Deed Book F 1845-1849, Court Deed Book C 1834-1844). In 1838, Ficklin's nephew, John Lindsey, moved to the area and is credited with designing the town plan for Powhatan in 1849 (Historic Structures Report, 1991). In these early years of western expansion, river travel was the most efficient way of transporting goods and people, and the Black River was no exception. The ferry landing at Powhatan helped make the town a regional center of trade and commerce. Multiple structures were erected in early Powhatan, including schools, churches, businesses, a courthouse, and the Ficklin-Imboden log house (Figure 1).

The Ficklin-Imboden log house is one of the earliest examples of a residential dwelling in Lawrence County, and is believed to be the oldest surviving structure in the town of Powhatan. The original construction date is thought to be ca. 1850. The property that the house and attached log room are located on once belonged to Andrew Imboden, and it is the opinion of park interpreters that the building was used as a first home with his wife, Lusinda Ficklin, niece of John Ficklin, by 1851 (Deed of Sale 1850). Original documents from this time, including census records and land deeds, provide some information on the construction, ownership, and

Figure 1. The Ficklin-Imboden log house is depicted in this historic photograph of the log structure (left) and the Lawrence County Courthouse (right) taken in ca. 1940 (photo courtesy of K. Watts). Both structures have been stabilized and restored as part of Powhatan Historic State Park

original purpose of the two log structures, but the actual date of construction is not known. This study uses dendrochronology to determine felling dates for the hewn wall logs in the Ficklin-Imboden house. These felling dates, obtained from the unhewn 'waney' edge of the logs, combined with original town records, deeds, census information, and historical maps, will help clarify when the structure was built, who owned the property at the time of construction, and how the Ficklin-Imboden house fits into the early history of Powhatan. This more detailed chronological information will also contribute to the interpretive mission of Powhatan Historic State Park.

Materials and methods

Tree-ring core specimens were collected non-destructively from 10 wall logs in the southernmost structure of the Ficklin-Imboden House and 12 wall logs from the northernmost structure, under contract with Powhatan Historic State Park. All 22 specimens were identified as bald cypress (*Taxodium distichum*). All wall logs were hewn on the interior and exterior face, but the top and bottom of most logs as they rest in the wall preserve the original bark surface of the tree (i.e., half-hewn logs; Stahle 1979).

The 12 mm diameter cores were collected with an electric drill and specialized coring bit. The sampling procedure began with a careful inspection to determine which logs would likely yield long ring series potentially suitable for tree-ring dating. Where possible the cores were taken from areas of the logs with evidence of outer bark surface. After thorough visual inspection of the structures and the archive records, we found no evidence that the structures were extensively remodeled or moved from their original construction sites. Therefore, any dates derived from the wall logs should provide insight into the original construction of the structures and not some subsequent remodeling or log replacement episode.

Each specimen was mounted and polished according to standard techniques (Stokes and Smiley 1968). The Douglass method of crossdating (Douglass 1941, Stokes and Smiley 1968) using skeleton plots was used to exactly date the specimens. The specimens were measured to 0.001 mm on a stage micrometer. The computer program COFECHA was used to check the accuracy of the tree-ring dating and measurement (Holmes 1983; Grissino-Mayer 2001) and to conduct sequential correlation analyses of the derived Ficklin-Imboden chronology against the 808-year long bald cypress chronology based on living trees from the Allred Lake site in southeastern Missouri (Stahle 2018). The computer program ARSTAN (Cook 1987, Cook and Peters 1997, Cook *et al.* 2007a) was used to detrend and standardize each ring width series and then compute the mean ring-width index chronology for the Ficklin-Imboden log structures, i.e., the raw ring width measurements were power transformed and then detrended with an age-based spine, the mean index chronology was computed with a biweight robust mean of the standardized indices from each core, and the variance of the computed chronology was detrended by fitting a 100-year spine to the absolute values of the chronology (see Stahle *et al.* 2016 for further details and citations; Cook 1987; Cook and Peters 1997). The bald cypress tree-ring chronology developed from Allred Lake, Missouri (Stahle 2018) was used with COFECHA for all possible correlation analyses over the past 808 years to check the crossdating identified with the skeleton plot method (e.g., Grissino-Mayer 2001). The tree-ring reconstructions of the summer Palmer Drought Severity Index (PDSI) available in the North American Drought Atlas (NADA; Cook *et al.* 2007) were also used to confirm the dating of the Ficklin-Imboden log structures and to map the spatial pattern of correlation between the Ficklin-Imboden chronology and the exactly dated moisture reconstructions during the late 18th and early 19th centuries (e.g., Stahle *et al.* 2016).

Results

Cores were obtained from 22 wall logs in the two structures (Table 1). However, only 16 samples could be conclusively dated, including 8 from the south structure and 8 from the north structure. The 6 specimens that were not dated were low quality due to pest damage and contained few annual rings.

One cutting date at 1843 and ten cutting dates at 1845 and 1846 were identified. Some of the other 5 logs may also have been cut in 1845 or 1846 (Table 1), but the outer rings were too compressed to determine the exact outer date with certainty. We concluded that both structures were built with logs cut in 1845 and 1846.

Most cutting dates in 1846 had complete terminal rings (Table 1). This indicated the logs were mostly cut after the growing season of 1846 but before the growing season of 1847 (the radial growth of bald cypress in the southeastern United States typically occurs from March to July; Stahle *et al.* 2012). The single felling date at 1843 suggested that cutting began at least 3 years before the structures were erected. The wall logs therefore appear to have been cut from 1843–1846 and stockpiled prior to the actual erection of the log buildings. Because cutting dates in 1846 were identified in both the north and south structures, it appears that the two structures were erected simultaneously, most likely in late 1846 or soon thereafter. All dated wall logs from both structures were measured, detrended, and standardized and are illustrated in Figure 2.

The Ficklin-Imboden chronology is only 121 years long (1726–1846) and some of the log samples do not span this entire period (Table 1). Nonetheless, the historic chronology is significantly correlated with other tree-ring chronologies from the region, and with the tree-ring reconstructions of the summer PDSI that have been produced in the NADA (Cook *et al.*, 2007b). The spatial pattern of correlation with the reconstructed PDSI is illustrated in Figure 3 which includes strong positive correlation over Arkansas and adjacent states.

Table 1. Tree-ring dating results from the Ficklin-Imboden Log House, Powhatan, Arkansas, are arranged chronologically by the date of the outermost ring. Core specimens were all extracted from bald cypress *(Taxodium distichum)* wall logs. Column Headers: ID = specimen identification number; PROVENIENCE = location of timber specimen ("SW" = south wall of building; "L8" = wall logs were counted consecutively from the lowest log); BARK = true outermost ring is or is not present on the core (OS = outer surface [likely bark ring]; B = bark); INNER DATE = innermost dated ring on the specimen; OUTER DATE = outermost dated ring on the specimen; TR = terminal ring (C = complete [cut during dormant season]; I = incomplete [cut during the growing season, if a true cutting date]); CUT DATE = outer date represents the true cutting date of the tree (yes or no).

ID	PROVENIENCE	BARK	INNER DATE	OUTER DATE	TR	CUT DATE
Southern Structure						
PSP04A	SW L8	OS	1745	1837	I	No
PSP01A	WW L8	—	1735	1844	C	No
PSP03A	WW L10	OS	1758	1845	C	**Yes**
PSP08A	WW L5	OS	1742	1845	C	**Yes**
PSP09A	WW L6	B	1771	1845	C	**Yes**
PSP02A	WW L9	OS	1780	1846	C	**Yes**
PSP05A	EW L10	OS	1740	1846	C	**Yes**
PSP07A	WW L2	OS	1791	1846	C	**Yes**
Northern Structure						
PSP13A	EW L1	—	1726	1841	C	No
PSP14A	EW L4	OS	1725	1842	C	No
PSP20A	WW L2	—	1739	1844	I	No
PSP12A	SW L3	B	1740	1843	C	**Yes**
PSP18A	NW L5	OS	1730	1845	C	**Yes**
PSP17A	NW L4	OS	1733	1846	C	**Yes**
PSP22A	WW L5	B	1757	1846	I	**Yes**
PSP15A	EW L5	OS	1754	1846	C	**Yes**

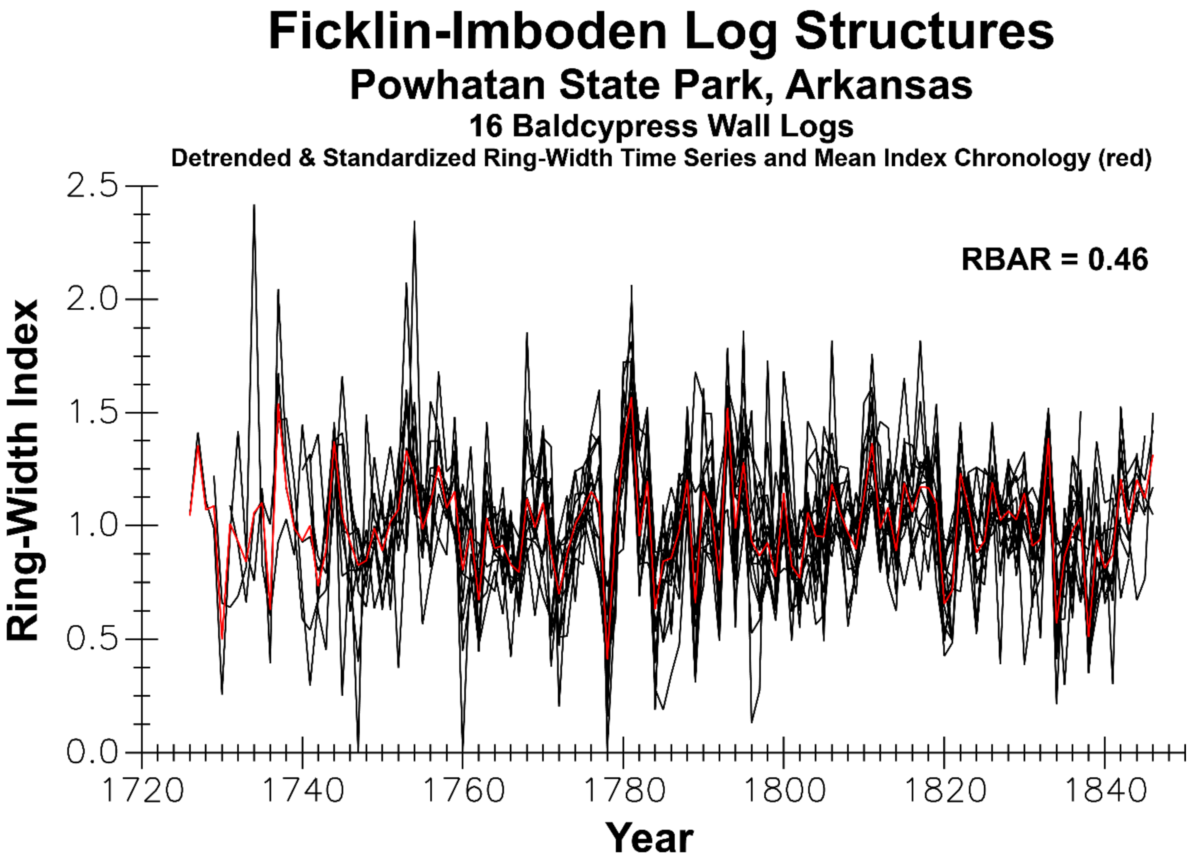

Figure 2. The detrended and standardized ring-width indices from the 16 dated and measured wall logs from the Ficklin-Imboden log structures are plotted (black time series) with the mean (red time series). Note the strong time series matching among the various cores, especially during 1762, 1778, 1789, 1792, 1809, 1810, 1820, and 1834, which were years of inferred dryness over the central U.S. The average correlation among the 16 cores is RBAR = 0.46.

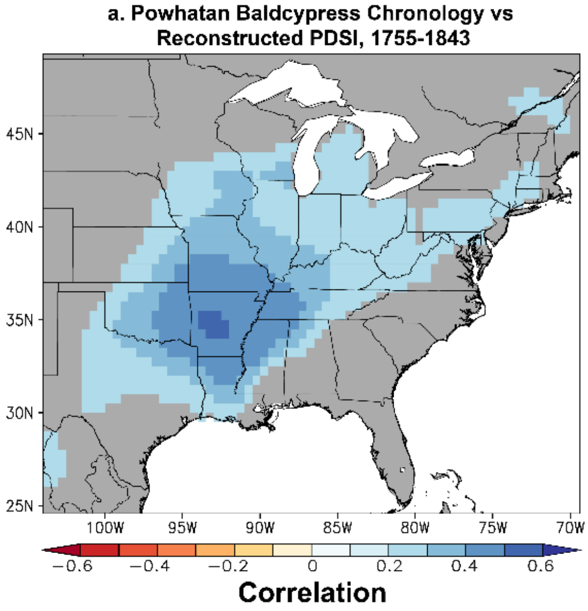

Figure 3. The Ficklin-Imboden chronology is correlated with the gridded tree-ring reconstructions of summer PDSI available in the NADA (Cook et *al.* 2007). Note the strong correlation over Arkansas and adjacent states.

For a time series comparison, tree-ring reconstructed PDSI was extracted from the NADA and averaged for the central Arkansas region (within 34–35°N and 91.5–93.5°W; e.g., Stahle *et al.* 2016). The Ficklin-Imboden chronology is plotted with summer PDSI from 1726–1846 in Figure 4a, and from 1755–1843 in Figure 4b when the historic chronology is replicated with at least 10 logs per year. There is generally good agreement between these series despite the fact that the Ficklin-Imboden chronology is only 121 years long.

The mean index chronology for the Ficklin-Imboden structures correlates significantly with tree-ring reconstructed summer PDSI for central Arkansas, especially from 1755–1843 when the historic chronology is best replicated ($n \geq 10$ for each year). This time series agreement is plotted in Figure 4ab. The correlation between the regional PDSI and the Ficklin-Imboden time series is $r = 0.50$ ($p < 0.001$; 1755–1843).

For a statistical test of the dating, the best replicated continuous 89 years of the Ficklin-Imboden historic

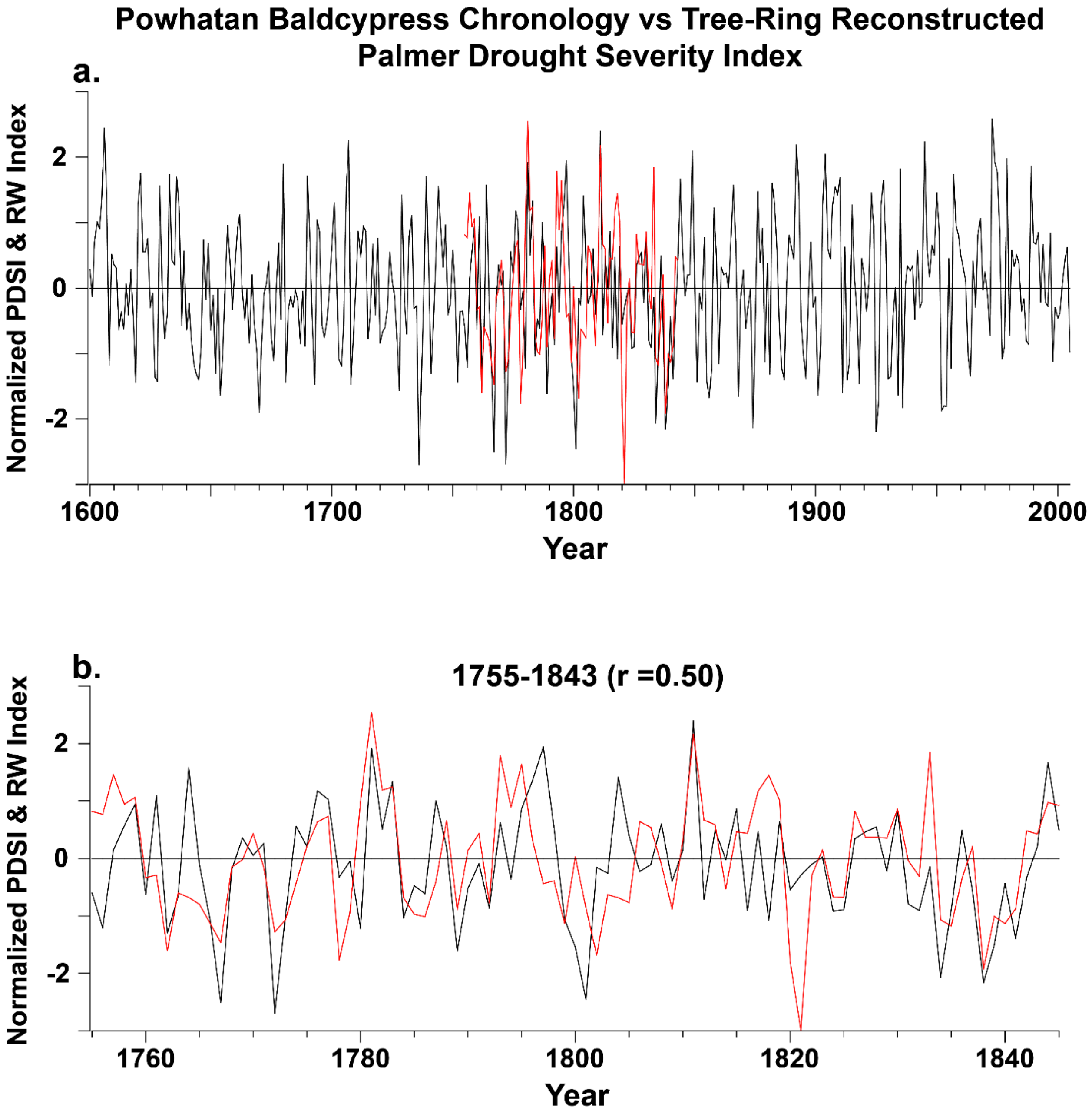

Figure 4. (a) The Ficklin-Imboden chronology (red) is compared with the tree-ring reconstructed summer PDSI for central Arkansas (black) from 1726–1846. (b) The best replicated portion of the Ficklin-Imboden chronology (red) is plotted with the PDSI (black) for 1755–1843 and the two series are correlated at r = 0.50.

chronology (1755–1843) was compared with all possible continuous 89-year intervals from 1185 to 1992 against the 808-year long bald cypress chronology from Allred Lake in southeast Missouri (Stahle 2018) using the computer program COFECHA. The highest single correlation computed when comparing the Ficklin-Imboden chronology with the Allred Lake series was r = 0.45 at the exact chronological position (1755–1843) determined visually using the skeleton plot method.

Discussion

The 11 cutting dates determined from the wall logs of the Ficklin-Imboden log structures ranged from 1843 to 1846. The single cutting date from 1843 indicates that cutting began at least three years prior to construction, and logs were likely stockpiled prior to the actual construction of the buildings. Logs cut in 1846 were identified in both structures, indicating that they were likely built simultaneously, contrary to the current interpretation which asserts that the northernmost building was a significantly later addition.

Little is known about the early history of Block 4, Lot 6, where the Ficklin-Imboden House was constructed, prior to 1847. Andrew Balfour received the land from Governor Thomas Drew in 1847 (Deed of Sale 1847) and Balfour sold the land to John Ficklin's nephew John Lindsey in 1848 (Deed of Sale 1848). Though Ficklin died in 1846 while returning from the Mexican-American War (Tipton 2001), he had been involved in the settlement of Powhatan, as evidenced by his establishment of Ficklin Ferry. One possibility is that the Ficklin-Imboden House was under construction at his direction while he was away to facilitate the development of the town. After his death, the land was put up for sale by the governor and sold to Balfour. Lindsey later bought the land and continued his uncle's work of establishing Powhatan, Arkansas. Additional archival and archaeological research might help clarify the early history of the Ficklin-Imboden house and the town of Powhatan.

Conclusions

Tree-ring dates on 16 timbers in the Ficklin-Imboden house indicate that the trees used as wall logs in both the north and south structures were felled primarily in 1845 and 1846. These results largely confirm the available historical information and indicate that the Ficklin-Imboden house is the oldest still standing structure at Powhatan Historic State Park. The current interpretation indicates that the larger southern room was the main living quarters and the smaller north structure was a later kitchen addition. However, the tree-ring dates indicate that these two structures were likely built simultaneously using logs stockpiled for perhaps 2 to 4 years. Archival and archaeological research might help clarify the functions of each log structure and the earliest history of the town of Powhatan. The new tree-ring dates do support the interpretation that the Ficklin-Imboden log buildings were occupied by Andrew Imboden and his wife by 1851.

Acknowledgements

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Gene Flow and Genetic Structure of Two of Arkansas's Rarest Darter Species (Teleostei: Percidae), the Arkansas Darter, *Etheostoma cragini*, and the Least Darter, *E. microperca*

J.S. Baker[1*], B.K. Wagner[2], and R.M. Wood[3]

[1] *Wood Environment and Infrastructure Solutions, Inc. 15933 Clayton Road, Suite 215 Ballwin, Missouri 63011*
[2] *Arkansas Game and Fish Commission, 2 Natural Resources Drive, Little Rock 72205*
[3] *Department of Biology, Saint Louis University, 3507 Laclede Street, Saint Louis, Missouri 63103*

*Correspondence: justin.baker@woodplc.com

Running Title: Genetic Structure of Arkansas's Rarest Darter Species

Abstract

Distinguishing the effects of naturally caused historical fragmentation from those of contemporary landscape modification is critically important to understanding the consequences of human influences on patterns of gene flow and population dynamics. Nonetheless, relatively few recent studies focusing on this issue have dealt with species that showed evidence of historical fragmentation. In the current study, we disentangled the effects of fragmentation operating over separate timescales on two darter species, *Etheostoma cragini* and *E. microperca*, from the Ozark Highlands. Formerly more wide-spread within this region in Arkansas, these species now occur only in highly isolated habitats (*i.e.*, spring-runs). We separated fragmentation effects at distinct spatial and temporal scales by using several molecular loci (i.e., mtDNA/nuclear DNA/nuclear microsatellite DNA), as well as a variety of analytical approaches. Sequence divergence among Ozark and northern populations of *E. microperca* indicate long-standing isolation resulting from vicariant events. Both species were further isolated in unique 'island' habitats, sometimes at fine spatial scales, as shown by sequence divergence among Ozark Highland populations of *E. cragini*. Microsatellite data also revealed additional subdivision among Arkansas populations with *E. cragini* divided into three distinct populations and *E. microperca* into two. Overall, migration rates were similar among contemporary and historical time periods although patterns of asymmetric migration were inverted for *E. cragini*. Estimates of contemporary effective population size (N_e) were substantially lower for both species than past population sizes. Overall, historical processes involving natural fragmentation have had long-lasting effects on these species, potentially making them more susceptible to current anthropogenic impacts.

Introduction

Habitat fragmentation operating both over historical time scales and over more recent timescales results in species with highly fragmented distributions, significantly compromising the maintenance of genetic diversity and population viability (Keyghobadi *et al.* 2005; Zellmer and Knowles 2009). Distinguishing between these time scales is important to conservation efforts because knowledge of historical population structure is essential to assessing the impact of current anthropogenic effects. Several recent studies comparing past and current patterns of gene flow among populations have revealed that recent human activities have substantially altered connectivity among populations, resulting in increased bottlenecks and high levels of inbreeding (Reed *et al.* 2011; Apodaca *et al.* 2012; Blakney *et al.* 2014); others suggest the high levels of structure observed among populations reflect long-standing limited dispersal of the species rather than recent habitat fragmentation (Chiucchi and Gibbs, 2010). These two causes of fragmentation may also act synergistically, such that the historically fragmented populations of a species become reduced in number or each experience declines in membership due to anthropogenic effects. Populations that are both highly fragmented and exhibit reduced population sizes have high rates of local extinction and therefore higher probability of global extinction (Templeton *et al.* 1990). Recent fragmentation may also substantially influence metapopulation dynamics, which may play a critical role in contributing to adaptive differences observed among populations even at small spatial scales (Zellmer and Knowles 2009).

An ideal setting for studying the consequences of natural fragmentation occurs in the Ozark Highlands region extending from southern Missouri to northern Arkansas, USA. This region is well known for having historical climatic fluctuations and complex geology and topography, including distinctive karst topography (Templeton *et al.* 1990). It is hypothesized that many aquatic species entered this region during a cooler and wetter Pleistocene and later became isolated in fragmented habitats as the region dried during arid conditions of the Late Wisconsin (Cross *et al.* 1986). The detrimental effects of recent habitat loss and fragmentation may be amplified for these species because populations often display disjunct distributions and are associated with habitats displaying unique features. For example, species closely associated with groundwater-fed springs in this region exhibit patchy distributions and high endemism due to the sporadic location of habitat islands (Robison and Buchanan 1988; Pflieger 1997). The stable environment provided by spring ecosystems (Hubbs 1995) may have facilitated the persistence of these fragmented populations over time, despite potentially facing reduced genetic diversity and small population sizes (Fluker *et al.* 2010). These habitats are, however, easy targets for anthropogenic impacts which have resulted in further population fragmentation and increased imperilment of spring species (*see*, *e.g.*, Fluker *et al.* 2009).

Although the genetic consequences of habitat fragmentation are well known (Keyghobadi 2007), relatively few studies have disentangled the effects of both past and recent events when assessing the impact on species having naturally fragmented distributions (although *see* Apodaca *et al.* 2012). In this study, we apply a variety of analytical approaches including genetic assignment and coalescent methods to several molecular loci (mtDNA/nuclear DNA/nuclear microsatellite DNA) having different mutation rates and levels of variability to detect genetic structure at distinct spatial and temporal scales. Using this comprehensive approach, we assess the genetic diversity and population structure of two of Arkansas's rarest darter species, the Arkansas Darter, *Etheostoma cragini* Gilbert, and the Least Darter, *Etheostoma microperca* Jordan and Gilbert. Both of these species have highly fragmented distributions in the Ozark Highland region of northwestern Arkansas, with their presence critically dependent on availability of spring-runs filled with abundant aquatic vegetation, and their existence under significant and immediate threats from rapid urban and suburban development (Wagner *et al.* 2011,2012). We evaluate genetic diversity and historical isolation of disjunct populations in Arkansas compared to other populations with sequences from the mitochondrial cytochrome *b* gene and nuclear S7 intron. Analyses of more rapidly-evolving nuclear microsatellite data allow us to independently examine current genetic diversity, and to identify fine-scale subdivision among Ozark populations. Using microsatellites, we estimate contemporary and historical migration rates among populations and effective population sizes to separate the influence of fragmentation processes operating over different time scales. Similarly, we evaluate the significance of any potential recent or historical reduction in population size using several methods to detect population bottlenecks. Our results allow us to make comparisons with spring-endemic taxa elsewhere and to make inferences about metapopulation dynamics of species confronted with reduced habitat connectivity as in the Ozark Highlands.

Materials and Methods

Distribution and sampling

All historic localities for *E. cragini* and *E. microperca* in Arkansas and additional nearby sites were sampled during 2009-2011 (*see* Wagner *et al.* 2011, 2012 for more details). For *E. cragini*, caudal fin clips were taken from a total of 117 individuals from 13 locations, representing the entire range of the species in Arkansas (*see* Fig. 1a). Fin clips from two additional locations were obtained from Missouri, in Shoal Creek and the headwaters of Spring River. For *E. microperca*, caudal fin clips or in some cases whole specimens were taken of 235 individuals from 29 locations. Samples were obtained from three major areas in Arkansas: Little Osage Creek/Osage Creek; Flint Creek; and Clear Creek (Fig. 2a). We obtained comparative material from 22 individuals from northern populations in Illinois (1), Indiana (3), Ohio (5), Michigan (11), Ontario (1), and Wisconsin (1). For both species, total genomic DNA was isolated from each individual using DNeasy Tissue Extraction Kits (Qiagen, Valencia, CA, USA) following manufacturer's instructions.

DNA sequencing and microsatellite genotyping

The complete mitochondrial cytochrome *b* (cyt *b*) gene (1140 base pairs) was amplified using primers located in flanking tRNA^{GLU} and tRNA^{THR} genes (Schmidt and Gold, 1993) for a subset of individuals from populations selected for each species (*E. cragini*, n = 32; *E. microperca*, n = 65). PCR was performed in 25 µl reactions containing 10-50 ng DNA, 0.8 mM dNTP, 0.4 µM each primer, 1.5 mM $MgCl_2$, and 1 Unit Taq polymerase (Promega, Madison, WI). Cycling conditions were 4 min at 94° C followed by 40 cycles at 94° C (1 min), 48° C (1 min), 72° C (2 min), with a final extension at 72° C for 7 min. The first intron of the nuclear S7 ribosomal protein intron 1 was amplified with universal primers (Chow and Hazama, 1998) for the same individuals amplified for cyt *b*; the thermal profile for cyt *b* was modified to include an annealing temperature of 59° C for S7 amplifications. Sequencing reactions were performed by htSEQ High-Throughput Genomics Unit (University of Washington, USA). The first 57 and last 78 nucleotides of the cyt *b* gene were excluded prior to analyses due to poor quality, resulting in the final cyt *b* fragment length of 1005 nucleotides and a S7 fragment length of 540 nucleotides (GenBank accession numbers KC445320-KC445462).

Nine microsatellite loci used previously in studies of darters were identified as having potential variability in *E. cragini*: EosC117, EosC6, EosC112, EosC208, EosD108, EosD11, EosD107, EosC2 (Switzer *et al.* 2008) and Esc26b (Gabel *et al.* 2008). Seven microsatellite loci were chosen for *E. microperca* based on ease of amplification and allele calling: EosC3, EosC6, EosD108, EosC208, EosC2, EosC124 (Switzer *et al.*, 2008) and Esc26b (Gabel *et al.* 2008). PCR for microsatellite loci was performed in 10 µl reactions containing ~10 ng DNA, 0.8 mM dNTP, 0.2 µM each primer, 1.5 mM $MgCl_2$, and 1 Unit Taq polymerase (Promega, Madison, WI) under the thermal cycling conditions of Switzer *et al.* (2008). Labeled PCR products were loaded into ≈ 20 µl reactions with 20 µl SLS and 0.25 µl 400 size STD (Beckman Coulter, Saint Louis, MO, USA) and genotyped using a Beckman CEQ8000 Genetic Analysis System (Department of Biology, Saint Louis University). Alleles within the designated range for each locus were called by eye.

Genetic data analysis

All analyses were conducted independently for each species. Genetic diversity estimates from cyt *b* were computed using ARLEQUINv3.11 (Excoffier *et al.* 2005), including the mean number of pairwise differences (π, nucleotide diversity, Nei, 1987) and the probability that two randomly chosen haplotypes are different (*h*, equivalent to gene diversity, Nei 1987). We performed an Analysis of Molecular Variance (AMOVA) in ARLEQUINv3.11 to test for hierarchical partitioning of genetic structure among populations (*see* supplementary data at the Journal website). Significance was assessed using 1000 permutations for all calculations. Haplotype networks were constructed using the median-joining method in NETWORKv4.610 (Bandelt *et al.*, 1999). Haplotype reconstruction for sequences of the S7 intron with ambiguous sites was conducted using PHASEv2.1 (Stephens *et al.* 2001; Stephens and Donnelly 2003). Missing data and invariable sites were excluded when constructing median-joining networks.

Microsatellite loci were examined for evidence of null alleles and scoring errors using MICROCHECKERv2.2 (van Oosterhout *et al.* 2004). To test for conformity to Hardy-Weinberg equilibrium (HWE), exact tests were performed for all loci using GENEPOPv4.010 (Guo and Thompson 1992) with significance assessed using Markov chains with 1000 dememorizations and 100 batches with 1,000 iterations per batch. Standard genetic diversity was measured in GENALEXv6.2 (Peakall and Smouse 2006) as the total number of alleles (N_A), mean number of alleles per locus (A_M), Shannon's Information Index (I), observed heterozygosity (H_O), unbiased heterozygosity (H_E), and number of private alleles unique to a population (PA). Allelic richness (AR) and private allelic richness (PAR) independent of sample size were also calculated using HP-RAREv1.0 (Kalinowski, 2005). Using the microsatellite DNA data, the Bayesian clustering method in STRUCTUREv2.3.3 (Pritchard *et al.* 2000) was implemented to determine the appropriate number of genetic clusters without any *a priori* group assignment. A model allowing admixture of genotypes and correlated allele frequencies between populations was used to assess the best value of K, the number of discrete populations (Falush *et al.* 2003). Twenty replicate runs consisted of a burn-in of 100,000 generations followed by an additional 500,000 iterations for each K ranging 1 to 10. The best value of K was chosen by determining ΔK, whereby the rate of change in the log likelihood values between successive K values was assessed (Evanno *et al.* 2005). The final results for chosen K values were visualized with DISTRUCT (Rosenberg 2004).

Three methods were used to detect genetic signatures of changes in population size due to bottlenecks using the microsatellite DNA data. First, the M-ratio test (Garza and Williamson 2001) was used to detect bottlenecks that occurred over relatively long periods of time (>100 generations). The program M P Val was used to estimate M-ratios of the number of alleles to the range in allele size and compare them to population specific critical M values (M_c) estimated using the program Critical M (Garza and Williamson 2001). The M-ratio was estimated using the two-phase model (TPM) with 90% single-step mutations, mean size of non-stepwise mutations=3.5, and pre-bottleneck θ value of 4, as suggested by Garza and Williamson (2001). A total of 10,000 simulation replicates were run for the calculation of M_c (Garza and Williamson 2001). Second, the Wilcoxon's sign rank test was used to detect bottlenecks occurring over approximately the last <$4N_e$ generations. Third, the mode-shift test was used to detect population declines that may have occurred within the last few dozen generations (Cornuet and Luikart 1996; Luikart *et al.* 1998). Both of these tests were performed using BOTTLENECKv1.2.02 (Piry *et al.* 1999). A total of 10,000 replicates were run under a two-phase model (TPM) with 95% single step mutations and 5% multi-step mutations, variance for mutation size was 12, as suggested by Piry *et al.* (1999). For all analyses, populations were defined based on the results obtained with STRUCTURE.

The Bayesian coalescent approach of the program Migrate-*n* v3.2.11 (Beerli 2009) was used to estimate the historical effective population size ($\theta = 4N_e\mu$, where μ = mutation rate) and past (roughly $4N_e$ generations) migration rates between populations ($M = m/\mu$, where m = migration rate) from the microsatellite datasets (Beerli 2009). The Bayesian inference method was used with uniform priors (range = 0 - 100, delta = 10) and slice sampling with one long chain and a sample increment of 1000 for 50,000 recorded steps, with 500,000 discarded as initial burn-in. Five replicates were run using a static heating scheme (1, 1.5, 3.0, 10000) with a swapping interval of 1, for a total of 250,000,000 visited parameter values. Final priors and starting values for θ and M were chosen based on results of multiple trial runs. Final runs were performed in parallel on the bioserv cluster at the University of Nebraska-Lincoln.

We used the program BayesAss v3.03 (Wilson and Rannala 2003), which implements a Bayesian MCMC approach and genetic assignment method, to estimate contemporary rates of migration within the last five generations. For each species three independent runs were conducted with 10 million iterations and a 1 million generation burn-in, sampling every 1,000 generations. To compare estimates of historical migration rates from Migrate ($M = m/\mu$) with contemporary gene flow estimates from BayesAss (m) we multiplied all M values by the mutation rate (μ), where $\mu = 5.0 \times 10^{-4}$ (Yue *et al.* 2007). To estimate contemporary effective population sizes (N_e), we performed the sibship assignment method implemented in COLONY V2.0 (Wang 2009a). The sibship assignment method is more accurate than e.g., the heterozygote excess method, the linkage disequilibrium method, and the temporal method at estimating N_e (Wang 2009a).

Results

Etheostoma cragini

Only two unique haplotypes of cyt *b* were recovered from 16 individuals from Arkansas (Fig. 1b). Consequently, mean uncorrected cyt *b* divergence among Arkansas samples was low (0.102%); however, divergence among Arkansas and Missouri populations was relatively high (2.086%). For the nuclear S7 intron, thirteen unique haplotypes were detected from 34 phased sequences. Five S7 haplotypes representing Arkansas populations were separated by 6 mutation steps from the nearest Missouri haplotypes. Genetic diversity (π and h) estimates from cyt *b* for Arkansas populations were low compared to Missouri populations (unpaired t-test, $P = 0.0001$ and $P = 0.5309$, respectively; *see* supplementary data at the Journal website).

For the microsatellite DNA data there was no evidence of scoring errors or allelic dropout detected by MICRO-CHECKER. Four localities deviated from HWE: Clabber Creek AR, Healing Spring AR, Osage Creek AR, and Shoal Creek MO. Following Bonferroni correction, only one locus (EosD11) for Shoal Creek MO was significant. The number of alleles per locus was generally low, ranging from 1-16 with an average of 2.496 (±0.2) alleles per locus. Microsatellites also indicate low genetic diversity of Arkansas populations (Table 1). Estimates of allelic richness were the highest for Missouri populations (avg. 3.68). Missouri populations also had a high number of private alleles (15 and 17, respectively), indicating isolation and reduced gene flow among populations.

STRUCTURE analysis identified two distinct genetic clusters: (1) Arkansas populations and (2) Missouri populations. Secondary runs containing only Arkansas populations identified an additional four genetic clusters: (1) Clabber Creek/Wilson Spring, (2) Turentine Spring, (3) Chamlin-Wise Spring/Osage Creek, and (4) Lick Creek/Unnamed Spring; with remaining individuals having mixed assignment to either the third or fourth cluster preventing these clusters from being clearly separated (Fig. 1c).

Overall, migration rates averaged across all comparisons were similar during both time periods (0.0225 vs. 0.0297, two-tailed $P = 0.6167$; *see* supplementary data at the Journal website). However, contrasting patterns of asymmetric migration were observed (Fig. 3). Historically, migration rates from Osage Creek to Clabber Creek/Wilson Spring and Turentine Spring were substantially higher (0.0296 and 0.0375, respectively) than rates from these populations to Osage Creek (0.0016 and 0.0026, respectively).

Table 1. Genetic diversity estimates from nuclear microsatellite loci of Arkansas Darter, *Etheostoma cragini*, and Least Darter, *E. microperca*, genetic clusters identified by STRUCTURE

Species/Population	N	N_A	A_M	I	H_O	H_E	PA	AR (PAR)
Etheostoma cragini								
Spring River, MO	24	50	5.556(1.608)	1.042(0.280)	0.489(0.118)	0.493(0.107)	17	3.81(1.40)
Shoal Creek, MO	26	45	5.000(1.658)	1.013(0.296)	0.432(0.134)	0.495(0.126)	15	3.56(1.28)
Osage Creek, AR	52	38	4.222(0.830)	0.854(0.214)	0.311(0.090)	0.446(0.107)	11	2.96(0.69)
Clabber Creek/ Wilson Spring, AR	8	19	2.111(0.423)	0.383(0.131)	0.088(0.037)	0.227(0.074)	3	2.07(0.44)
Turentine Spring,AR	7	14	1.556(0.242)	0.294(0.123)	0.206(0.104)	0.210(0.088)	0	1.56(0.10)
Etheostoma microperca								
OsageCk/FlintCk, AR	54	71	10.143(4.194)	1.221(0.522)	0.395(0.179)	0.430(0.174)	30	6.11(2.61)
Trudell Spring, AR	32	30	4.286(1.507)	0.783(0.336)	0.366(0.144)	0.364(0.146)	4	3.55(0.97)
Northern populations (IL, IN, OH, MI, WI)	63	72	10.286(3.037)	1.299(0.459)	0.397(0.168)	0.475(0.159)	27	6.23(2.04)
Deer Creek, OH	16	31	4.429(1.716)	0.799(0.362)	0.384(0.168)	0.362(0.158)	2	4.03(0.68)
Mallet River, ON/ Doke Lake, OH	16	20	2.857(0.937)	0.594(0.287)	0.265(0.134)	0.314(0.149)	2	2.72(0.35)
Tenmile Creek, OH	12	17	2.429(0.841)	0.458(0.252)	0.190(0.097)	0.246(0.133)	1	2.43(0.22)

N – Number of genotypes examined, N_A – Total number of alleles, A_M – mean number of different alleles, I – Shannon's Information Index, H_O – observed heterozygosity, H_E – Nei's (1987) unbiased heterozygosity, PA – Number of alleles unique to a single population, AR – Allelic Richness with rarefaction, PAR – Private Allelic Richness with rarefaction.

Estimates of contemporary migration, however, exhibit a reversed pattern, with substantially higher rates to Osage Creek (0.0386 and 0.0586) versus from Osage Creek (0.0071 and 0.0104).

Contemporary effective population sizes (N_e = 12 – 31) were consistently an order of magnitude lower than historically (N_e = 208 – 544) (Table 2). Evidence of historical population bottlenecks were observed with *M*-ratios below critical M_c values (M_c = 0.62 – 0.72). More recent population declines were detected by the Wilcoxon's sign rank test for Clabber Creek/Wilson Spring ($P < 0.05$) and by the mode-shift test for Clabber Creek/Wilson Spring and Turentine Spring.

Etheostoma microperca

Two cyt *b* haplotypes were recovered from nine individuals from Arkansas. These haplotypes were separated by more than 62 mutation steps from other populations, representing substantial cyt *b* divergence (7.57%) and indicating long-term isolation from northern populations (Fig. 2b). For the nuclear S7 intron, ten unique haplotypes were detected from 44 phased sequences. Three S7 haplotypes representing Arkansas populations were separated by 9 mutation steps (1.78% uncorrected divergence) from haplotypes of northern populations. Results from cyt *b* indicate significantly lower genetic diversity (π and *h*) of populations from Arkansas relative to northern populations (unpaired t-test, P = 0.0001 and P = 0.0025, respectively; *see* supplementary data at the Journal website).

For the microsatellite DNA data there was no evidence of scoring errors or allelic dropout detected by MICRO-CHECKER. Three localities deviated from HWE: Nichols Lake MI, Spring Creek IL, and Healing Spring AR. Following Bonferroni correction, one locus (EosC6) for Nichols Lake and one locus (EosC2) for Healing Spring were significant. The number of alleles ranged from 1-23 with an average of 3.444 (±0.335) alleles per locus. Analyses of microsatellite data indicate lower genetic diversity (A_M, I, H_E, AR) of Arkansas populations identified by STRUCTURE (Table 1). Comparisons among individual Arkansas localities revealed several having relatively moderate genetic diversity (*see* supplementary data at the Journal website). In particular, Healing Spring had higher genetic diversity with A_M = 8.429, Shannon's I = 1.138, and 11 private alleles; however, after accounting for sample size, this locality had only the fifth highest estimate for allelic richness (2.13). STRUCTURE analysis identified two distinct genetic clusters: (1) Arkansas populations and (2) northern populations. Additional runs containing only Arkansas populations identified an additional two genetic clusters: (1) Trudell

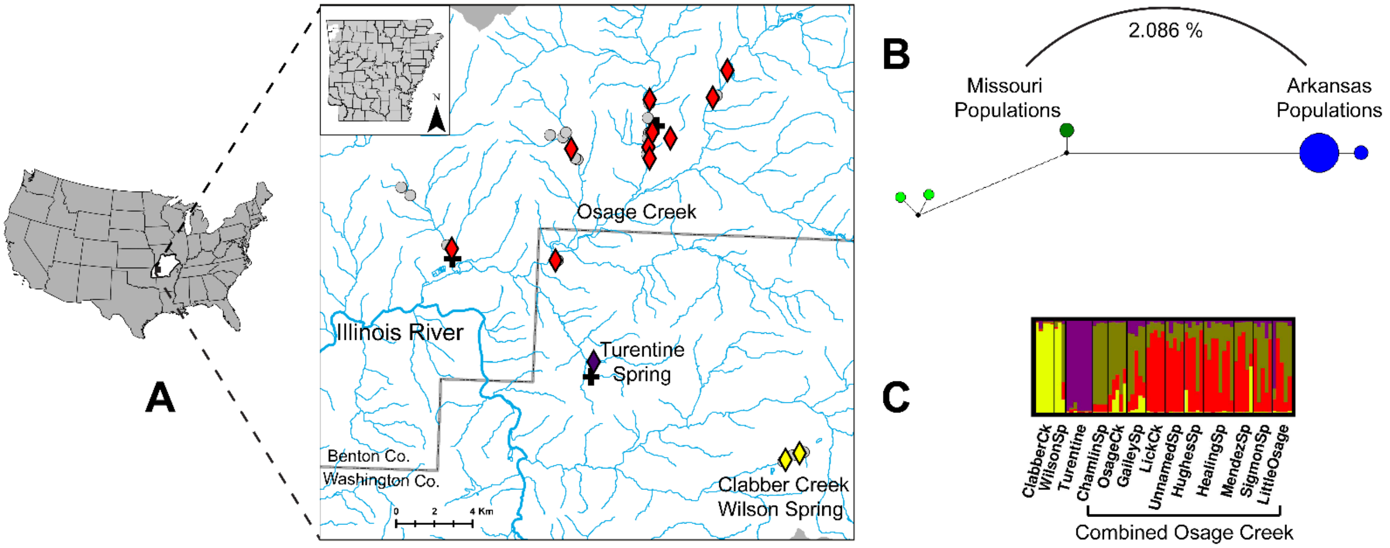

Figure. 1 (A) Map showing collection sites and major river drainages for the Arkansas Darter, *Etheostoma cragini*, in Arkansas, USA with inset map highlighting the Ozark Highlands outlined in white. Symbols designating genetic sites are colored according to populations identified by STRUCTURE analyses (See online version for full color). Extirpated sites are indicated by a cross (*see* Wagner *et al.* 2011 for additional information). (B) Median-joining haplotype network for *E. cragini* based on data from the mitochondrial cytochrome *b* gene. Mean uncorrected sequence divergence is shown among populations. Circle sizes reflect relative frequency of haplotypes, with smallest circles representing extinct or unsampled haplotypes. (C) Results from STRUCTURE analysis of Arkansas localities of *E. cragini*. Shown are individual assignment proportions to four clusters with each vertical bar corresponding to a single individual in the dataset.

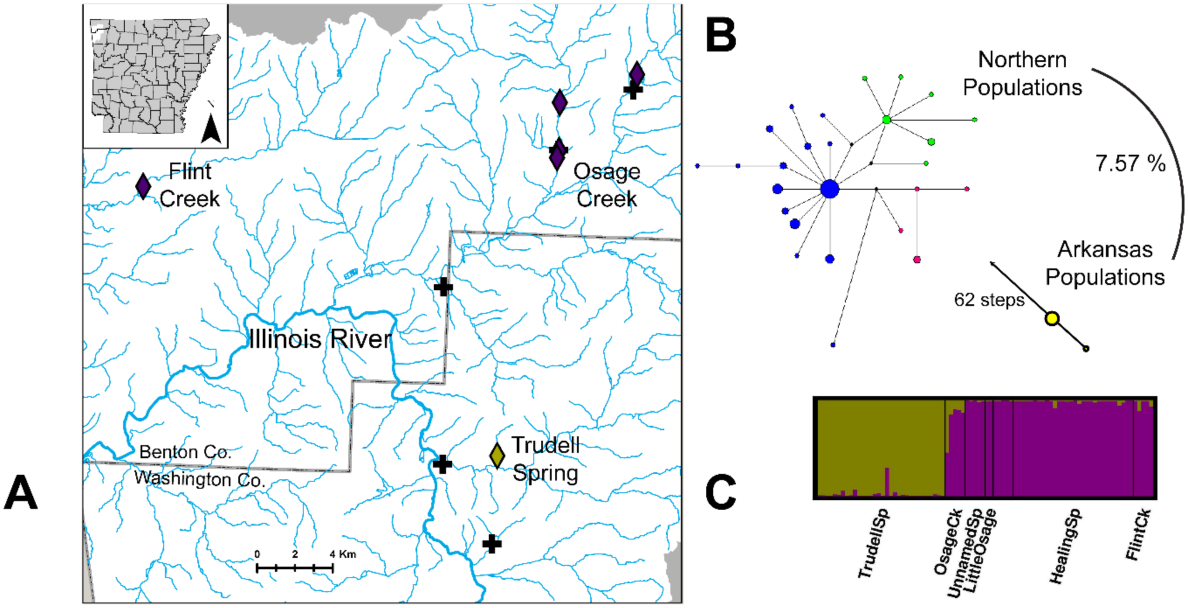

Figure 2. (A) Map showing collection sites and major river drainages for the Least Darter, *Etheostoma microperca*, in Arkansas, USA. Symbols designating genetic sites are colored according to populations identified by STRUCTURE analyses (See online version for full color). Extirpated sites are indicated by a cross (*see* Wagner *et al.* 2012 for additional information). (B) Median-joining haplotype network for *E. microperca* based on data from the mitochondrial cytochrome *b* gene. Mean uncorrected sequence divergence is shown among populations. Circle sizes reflect relative frequency of haplotypes, with smallest circles representing extinct or unsampled haplotypes. (C) Results from STRUCTURE analysis of Arkansas localities of *E. microperca*. Shown are individual assignment proportions to two genetic clusters with each vertical bar corresponding to a single individual in the dataset.

Spring and (2) Osage Creek/Flint Creek (Fig. 2c). Overall, migration rates averaged across all comparisons were similar during both time periods (0.0194 vs. 0.0137, two-tailed $P = 0.7906$), although contrasting migration patterns were observed (Fig. 3; *see* supplementary data at the Journal website). Historical migration rates were asymmetric with a much higher rate ($m = 0.0383$) from Osage Creek/Flint Creek to Trudell Spring versus the opposite direction ($m = 0.0006$). However, contemporary migration rates between populations were more similar (0.0117 and 0.0157).

Contemporary effective population sizes ($N_e = 19 – 35$) were consistently an order of magnitude lower than values historically ($N_e = 660 – 2715$) (Table 2). Evidence of historical population bottlenecks were observed with M-ratios below critical M_c values < 0.7 ($M_c = 0.65 – 0.68$), with more recent bottlenecks detected by the Wilcoxon's sign rank test for Osage Creek.

Table 2. Estimates of contemporary and historical effective population sizes (N_e) of the Arkansas Darter, *Etheostoma cragini*, and Least Darter, *E. microperca*, with 95% confidence intervals shown in parentheses

Species/Population	N_e (MIGRATE)	N_e (COLONY)
Etheostoma cragini		
Osage Creek	544 (0-1360)	31 (19-53)
ClabberCk/WilsonSp	349 (0-1160)	19 (7-∞)
Turentine Spring	208 (0-960)	12 (5-130)
Etheostoma microperca		
OsageCk/FlintCk	2715 (810-4040)	35 (22-59)
Trudell Spring	660 (0-1460)	19 (11-38)

Discussion

In this study we assessed gene flow and population structure of two of Arkansas's rarest darter species. Complete sampling of all known extant localities of *E. cragini* and *E. microperca* in Arkansas, as well as use of several molecular loci (mtDNA/nuclear DNA/nuclear microsatellite DNA) having different mutation rates and levels of variability have allowed a comprehensive analysis of these populations at multiple temporal scales. There are three major results of this study. (1) Historical fragmentation has led to the isolation and genetic distinctness of Arkansas populations, particularly for *E. microperca*. (2) Arkansas populations were isolated in spring habitats at fine spatial scales and exhibited low contemporary and historical migration rates. (3) Both species have experienced significant population declines due to recent fragmentation that may impact overall metapopulation stability. We elaborate on each of these conclusions below and their implications on future conservation strategies.

Historical fragmentation

In other fishes not associated with spring habitats, ancient vicariant events have been suggested to explain congruent phylogeographic patterns revealing a deep split between the Ozark and Eastern Highlands (Near *et al.* 2001; Berendzen *et al.* 2003,2008). More recent geological events (~130,000 YBP) associated with the development of the present Arkansas River contributed to further vicariance, resulting in the isolation of the Ozark and Ouachita Highland regions (Mayden 1985). In addition to being affected by these historical vicariant events, populations of *E. microperca* and *E. cragini* were further fragmented by the availability of favorable spring habitats. This additional level of fragmentation may have resulted in reproductively isolated populations exhibiting substantial differentiation on a small geographic scale.

Prior to the Pleistocene, populations of *E. microperca* from the Ozark Highlands were separated from northern populations, as evident from divergence observed at cyt *b* and S7 loci. Deep divergence between Ozark and northern populations suggests historical vicariance, as opposed to scenarios involving recent dispersal, which predict shallow population divergence with high rates of migration. Comprehensive morphological analyses also confirm the divergence of Ozark populations relative to northern populations, despite relatively high disparity among characters of Ozark populations (Burr 1978). A recent systematic analysis of northern and Ozark populations of *E. microperca*, as well as the disjunct population from the Blue River, Oklahoma revealed additional unrecognized diversity, with evidence of long-term isolation of Illinois River populations representing an ancient lineage (Echelle *et al.* 2015). Levels of sequence divergence among *E. cragini* populations also indicate historical isolation at a fine geographic scale among the Illinois and Neosho River basins of the Arkansas River drainage. In contrast, other fish species exhibit only shallow divergence among Arkansas River samples, which form a separate clade relative to other Ozark drainages (Berendzen et al. 2008). Overall, these results suggest a variety of historical factors, involving vicariance, as well as further fragmentation and isolation in unique spring environments, may have allowed gradual, adaptive, allopatric speciation to occur at small spatial scales. This may be the norm rather than the exception for aquatic organisms associated with spring habitats that are often geographically and genetically isolated (Lucas *et al.* 2009; Timpe *et al*, 2009; Wang 2009b; Fluker *et al.* 2010).

Metapopulation structure and gene flow patterns

Additional subdivision and genetic structure was observed among Arkansas populations of both species with variable, albeit low, levels of historical asymmetric migration among populations (Figs. 1-3). Low levels of gene flow would have permitted the spread of highly advantageous alleles, enabling fragmented Arkansas populations to evolve as a single cohesive unit while simultaneously differentiating at neutral loci (Morjan and Rieseberg 2004). Migration would have also been important in colonizing new habitat islands and will continue to be in the future, especially considering the number of local extinctions observed in past and recent surveys of Arkansas populations (Figs. 1, 2; Harris and Smith 1985; Hargrave and Johnson 2003; Wagner and Kottmyer 2006; Wagner *et al.* 2011,2012). Periodic local extinction and recolonization events may have been common features throughout the evolutionary history of these species, as shown by surveys of *E. cragini* elsewhere in Colorado (Labbe and Fausch 2000), Kansas (Eberle and Stark 2000), and Oklahoma (Blair 1959). Rates of local extinction can be explained, in part, by natural drying of intermittent streams during summer months, although recent anthropogenic impacts including gravel mining, urbanization, and cattle farming have intensified these effects (USFWS 2016). Consequently, recent fragmentation events may be substantially altering metapopulation dynamics by decreasing the number of available habitat islands and altering migration patterns and rates.

Overall comparisons of historical versus contemporary gene flow rates were similar between the focal species; although discordant migration patterns were observed between periods for *E. cragini*, potentially reflecting effects from anthropogenic impacts (Fig. 3). These results could be an artifact of small sample sizes of *E. cragini* from Clabber Creek/Wilson Spring and Turentine Spring rather than from historic and/or contemporary differences. Alternatively, temporal variation in migration patterns could be caused by natural phenomena or may be a successful survival strategy. Distinguishing among these explanations requires a detailed understanding of metapopulation dynamics and landscape changes over time. In the present study, fine-scale genetic structure and asymmetric rates of gene flow among Arkansas populations of both species suggest a hybrid metapopulation model which combines characteristics of the patchy population and source-sink models (*see* Schlosser and Angermeier 1995). In particular, *E. cragini* localities from the Osage Creek drainage have a mosaic of genotypes that display patchy population dynamics caused by rates of dispersal among populations

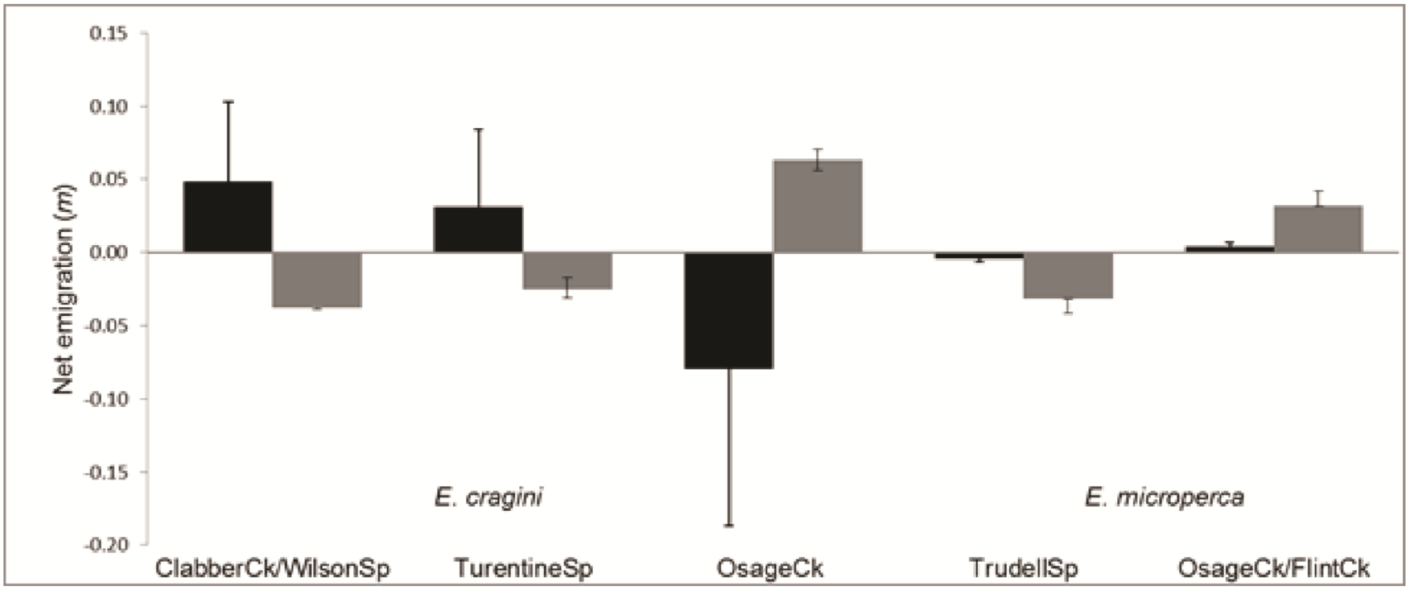

Figure 3. Estimates of net emigration rates (*m*) for Arkansas populations of the Arkansas Darter, *Etheostoma cragini*, and Least Darter, *E. microperca,* calculated by subtracting total immigration rates from total emigration rates for each population. Estimates of contemporary rates are shown in black while historical rates are shown in gray with error bars depicting 95% confidence interval.

that exceed rates of local extinction (Harrison 1991; Schlosser and Angermeier 1995). Historically, this “single” Osage Creek population may have acted as a source for colonizing other favorable habitat islands during high flood events, including Clabber Creek/Wilson Spring and Turentine Spring (Fig. 3). However, comparisons with contemporary migration rates reveal reversed patterns, with *E. cragini* populations shifted from genetic sources to sinks and vice versa (Fig. 3). Moreover, substantial declines in current effective population sizes relative to historical conditions have significantly reduced the effective number of migrants (Table 2).

Metapopulation stability

The combined effects of small population sizes and reduced number of migrants per generation have been associated with the accumulation of deleterious mutations, potentially leading to significant increases in the probability of population extinctions (Couvet 2002). However, other studies have suggested the pattern of migration can be more important than the number of migrants, with conditions under asymmetric migration resulting in reduced fitness (Bouchy *et al.* 2005) and having direct consequences on adaptive evolution (Kawecki and Holt 2002). Contemporary patterns and rates of gene flow among *E. cragini* populations suggest unstable metapopulation dynamics, whereas the current similarity in migration observed among *E. microperca* populations may in theory allow the maintenance of metapopulation viability. Most empirical studies of asymmetric migration indicate dispersal is density dependent, with patterns of higher gene flow from large into small populations (Fraser *et al*, 2004; Manier and Arnold 2005; Jehle *et al.* 2005). Patterns of asymmetric gene flow that reverse over evolutionary time may involve a variety of factors including population dynamics, local adaptation, behavioral and life-history strategies, and environmental stochasticity (Palstra *et al.* 2007). These factors may be involved in the discordant patterns observed among *E. cragini* populations, where directionality of gene flow was not always contingent on population size. Future studies involving resampling of Arkansas populations could determine whether current migration rates and patterns vary over time and their influence on changing metapopulation dynamics.

All Arkansas populations of *E. cragini* and *E. microperca* have experienced relatively severe past bottlenecks as indicated by M-ratio tests. Moreover, recent genetic bottlenecks and population declines were detected for Clabber Creek/Wilson Spring and Turentine Spring for *E. cragini* and Osage Creek for *E. microperca*. Estimates of current effective population sizes for both species were extremely small (N_e = 12 – 35), in addition to being an order of magnitude lower than during historical times (Table 2). Current effective population estimates for *E. cragini* at 12 localities in Colorado were also relatively small, ranging from 20 to 47 (average ± STD = 35 ± 9), though no recent bottlenecks were detected at these localities (Fitzpatrick *et al.* 2014). Corresponding estimates of current and long-term effective population sizes for the Watercress Darter, *Etheostoma nuchale*, a spring-endemic of the south-eastern United States were considerably larger than those for *E. cragini* and *E. microperca*, with the exception of the Osage Creek/Flint Creek historical population of *E. microperca* (Fluker *et al.* 2010). Similarly, estimates of contemporary effective population size were substantially larger for the federally endangered Fountain Darter, *Etheostoma fonticola*, another spring-endemic and close relative of *E. microperca* from the Comal River (N_e = 899) and San Marcos River (N_e = 9,234) in Texas, USA (Olsen *et al.* 2016). Whereas discrepancies may be influenced by different methods employed, particularly for contemporary estimators (i.e., ONeSAMP, LD-N_e and N$_E$ESTIMATOR versus COLONY, *see* Wang 2009a; Luikart *et al.* 2010), they indicate both *E. cragini* and *E. microperca* have experienced significant population declines compared with similar darters dependent on spring habitats. Recent signatures of population bottlenecks, as well as contrasting histories, with *E. nuchale* colonizing a series of springs and diverging from a widespread stream-dwelling relative (Fluker *et al.* 2010) versus *E. cragini* and *E. microperca* that were previously more widespread but affected by vicariance and further fragmentation, may explain the extremely small effective population sizes observed. These results suggest the impacts of natural fragmentation can have long-lasting effects on species, potentially making them more susceptible to contemporary influences from human-imposed changes.

Conservation implications

Conservation and management efforts usually focus on protecting ‘source’ populations that are deemed important for providing migrants for a particular region. However, the results of this study suggest ‘source-sink’ dynamics (Pulliam 1988; Dias 1996) can become reversed over evolutionary timescales. Whether this ‘source-sink inversion’ occurred via demographic changes and adaptive evolution as Dias (1996) originally proposed or through complex interactions involving a variety of mechanisms (*see*, *e.g.*, Palstra *et* *al.* 2007) warrants further attention beyond the scope of this study. However, the lack of permanent 'source' populations identified as having higher net emigration rates suggests conservation efforts should focus on conserving as many habitat islands as possible. Similarly, concentrating efforts on specific localities or populations believed to maintain overall higher genetic diversity may be unreliable, since positive correlations between neutral and adaptive variation might not be particularly high (Hedrick 2001). Moreover, for *E. craigini* and *E. microperca*, uniformly small contemporary effective population sizes defy efforts to prioritize conservation of any single population. The best management strategies for these species would ideally involve protection of all Arkansas populations, as well as protection and enhancement of additional unoccupied habitats that may be important for maintaining connectivity among currently isolated islands. Successful management strategies must address a variety of stressors (e.g. urbanization and development, water depletion, water quality degradation) impacting populations across these species' ranges. Water depletion has already resulted in lowering of aquifers from groundwater withdrawals (Juracek *et al.* 2017) and led to decreases in perennial stream refugia and local extirpations of *E. cragini* localities (USFWS, 2016). Thus, habitat restoration at a landscape-level may be necessary for species inhabiting naturally fragmented landscapes within the Ozark Highland region, independent of whether they rely on disturbance-generated habitats or constantly stable environments (Neuwald and Templeton 2013).

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Update of Distribution of the Chestnut Lamprey (*Ichthyomyzon castaneus*) in Arkansas

J.M. Salinger[1], B.K. Wagner[2], and R.L. Johnson[3*]

[1] *Department of Aquaculture and Fisheries, University of Arkansas at Pine Bluff, Pine Bluff, AR 71601*
[2] *Arkansas Game and Fish Commission, 915 East Sevier Street Benton, AR 72015*
[3] *Department of Biological Sciences, Arkansas State University, State University, AR 72467*

* Correspondence: rlj@astate.edu

Running Title: Update of Distribution of the Chestnut Lamprey in Arkansas

Abstract

Most lamprey species other than sea lampreys have been poorly studied in North America. The chestnut lamprey, *Ichthyomyzon castaneus*, has a distribution within the Mississippi River and Hudson Bay drainage systems, and the Great Lakes. Since the text *Fishes of Arkansas* was published in 1988, few papers have been published to update the statewide distribution of this lamprey. We incorporated our electrofishing sampling results with gray and published literature to describe the distribution of this species in the drainage basins of the State of Arkansas. Reported are records of 250 chestnut lamprey specimens, over a 90-year period, from 47 different waterbodies in the state.

Introduction

Most lamprey species, other than the sea lamprey *Petromyzon marinus* L., have been poorly studied in North America. Rather than being directly studied, lamprey distributions are often identified as part of broader qualitative fish surveys. This information is particularly important, as a majority of lamprey species are imperiled in some fashion (Renaud 1997; Kiabi *et al.* 1999; Mateus *et al.* 2012), and lampreys may also be valuable bioindicators (Lyons *et al.* 1998; Soto-Galera *et al.* 1998; Newall *et al.* 1999).

The chestnut lamprey, *Ichthyomyzon castaneus* Girard, is an obligate blood-feeding parasitic freshwater lamprey (Renaud 2011). Their distribution primarily lies within the Mississippi River drainage system ranging from southern Canada to the Gulf of Mexico (Renaud 1997). The chestnut lamprey is considered to be threatened in portions of its range, but is largely unstudied in these areas (Kansas and Saskatchewan, Mesa and Copeland 2009; Iowa, Flammang and Olson 2010; Nebraska, Steffensen 2015). Since the text *Fishes of Arkansas* was published in 1988 (Robison and Buchanan 1988), few papers have been published updating the distribution of this lamprey in Arkansas. One notable exception was an update on chestnut lamprey collections by Robison *et al.* (2006).

Our goal was to identify the distribution of this species in the context of published historic and gray literature, and provide a summary of the available distribution records for this species. Further, we studied by way of electrofishing, the distribution of the chestnut lamprey in streams where this species was previously documented. We summarize those records by drainage basin within the State of Arkansas.

Methods

We used historical records of gray literature from the Arkansas Game and Fish Commission (B. Wagner),the Arkansas Department of Environmental Quality (ADEQ; J. Wise), and the Arkansas Highway and Transportation Department (AHTD; B. Thesing) in addition to published literature (Robison *et al.* 2006; Tumlison and Robison 2010; Connior *et al.* 2011) and the online database FishNet2. We tabulated those records on the basis of major drainage systems from north to south, including the St. Francis, White, Arkansas, Ouachita, and Red river drainages, as well as the Mississippi River itself. When discrepancies existed between information provided in Robison and Buchanan (1988) and information we had available in archived databases, we chose to not include records from the text. Nonetheless, we refer to discrepancies between archived data and Robison and Buchanan (1988). It must be noted that Robison and Buchanan (1988) do not include number of individuals collected, but rather had a focus on sites of collection. Lastly, we looked at archived records in a historical context (before or after 2000) to try to identify current distribution trends.

In an effort to collect chestnut lampreys, or identify fish hosts parasitized by chestnut lampreys, electrofishing by boat for the present study was conducted from mid-July to mid-November 2015 in 12 streams in Arkansas, USA (29 river km total; Table 1). This included 11 streams previously reporting the presence of this species, as well as 1 adjoining stream (Spring River) suspected of containing this species. Criteria used for the selection of rivers sampled included the absence of the silver lamprey *I. unicuspis* Hubbs and Trautman (documented in the upper White River drainage of northwest Arkansas, confluence of the White River with the Black River in Jackson and Independence counties, and the lower White River; Robison *et al.* 2011), existing historical records of chestnut lampreys, and boat access. Up to ten 250 m reaches on each river were selected for sampling, with at least one km separating sampling sites. Reaches were excluded from sampling consideration if they were inaccessible or too shallow for sampling. In addition to identifying chestnut lampreys, we evaluated their presence by examining potential host species for evidence of lamprey lesions and scars. We assumed based upon the lack of historic silver lamprey records that lamprey parasitism was due to the chestnut lamprey. This assumption may prove incorrect, yet additional specimens of silver lampreys have not been documented since the 2005 collections and re-identification of specimens collected in 1972 and 1998 reported by Robison *et al.* (2011).

Parasitic lampreys have been reported in the federal Norfork National Fish Hatchery by U.S. Fish and Wildlife Service personnel. In an effort to determine the presence of chestnut lampreys and/or parasitism by lampreys, rainbow trout *Oncorhynchus mykiss* Walbaum were examined at the hatchery over a three-month period from the end of January to mid-April 2013.

Results and Discussion

Red River Drainage

The Red River drainage includes the Cossatot/Little Cossatot, Sulphur and Bayou Dorcheat rivers. There were 3 collection dates since 2000 of Chestnut Lampreys (n = 4) within this drainage, and no prior collection dates (Figure 1). One of the sub-watersheds, the Bayou Dorcheat, empties into the Red River below Shreveport, LA, whereas the Little Cossatot and the Sulphur have more proximal connections to the Red River. Additional records are found upstream and downstream of Lake Millwood in Robison and Buchanan (1988; n = 6), yet we were not able to find those records. Therefore, they were not included in the Figure 1 results. Despite intensive electrofishing efforts in the Sulphur River (2.5 river km), we did not

Table 1. Streams electrofished in 2015 and outcomes for the collection of Chestnut Lampreys. Rainbow Trout from Norfork National Fish Hatchery (NFH) were studied for parasitism by lampreys in 2013.

Drainage Basin **Stream**	**River km Sampled**	**Number Collected**	**Parasitized Fishes**
Red River Drainage			
Sulphur R.	2.5	0	0
Ouachita River Drainage			
Little Missouri R.	1.5	0	1
Ouachita R.	2.5	0	1
Arkansas River Drainage			
Cadron Cr.	2.5	0	1
Fourche LaFave R.	2.5	0	0
Petit Jean R.	2.5	0	0
White River Drainage			
Black R.	2.5	3	3
Current R.	2.5	2	1
Little Red R.	2.5	0	0
Norfork NFH	N/A	0	83
Spring R.	2.5	3	3
Strawberry R.	2.5	0	1
St. Francis River Drainage			
St. Francis R.	2.5	0	0

find any evidence of this species directly or indirectly (parasitized hosts) (Table 1).

Ouachita River Drainage

The Ouachita River drainage, including the Caddo, Little Missouri, Ouachita, and Saline rivers, has extensive records of chestnut lamprey collections (Figure 1). There were 37 specimens collected from the Caddo River in the past 45 years, with 10 of those collections occurring since the year 2000. This indicates the ongoing presence of this species. There are 13 records in the Little Missouri River and its tributaries, with only one individual collected since 2000. There were 81 specimens collected from the Ouachita River and streams entering the river. Included in these collections were specimens from DeGray, Hamilton, and Webb lakes. Collections were regular throughout the archival records. Fewer individuals (n = 6) were collected from the Saline River, with most of those collections occurring over 30 years ago. In comparison Robison and Buchanan (1988) identified 5 collections from the Ouachita River, including Lake Ouachita, 4 records from the upper Caddo River, and 1 each from the Little Missouri River, the Antoine River, and the upper Saline River.

Despite our sampling efforts in both the Ouachita and Little Missouri rivers, no chestnut lampreys and only

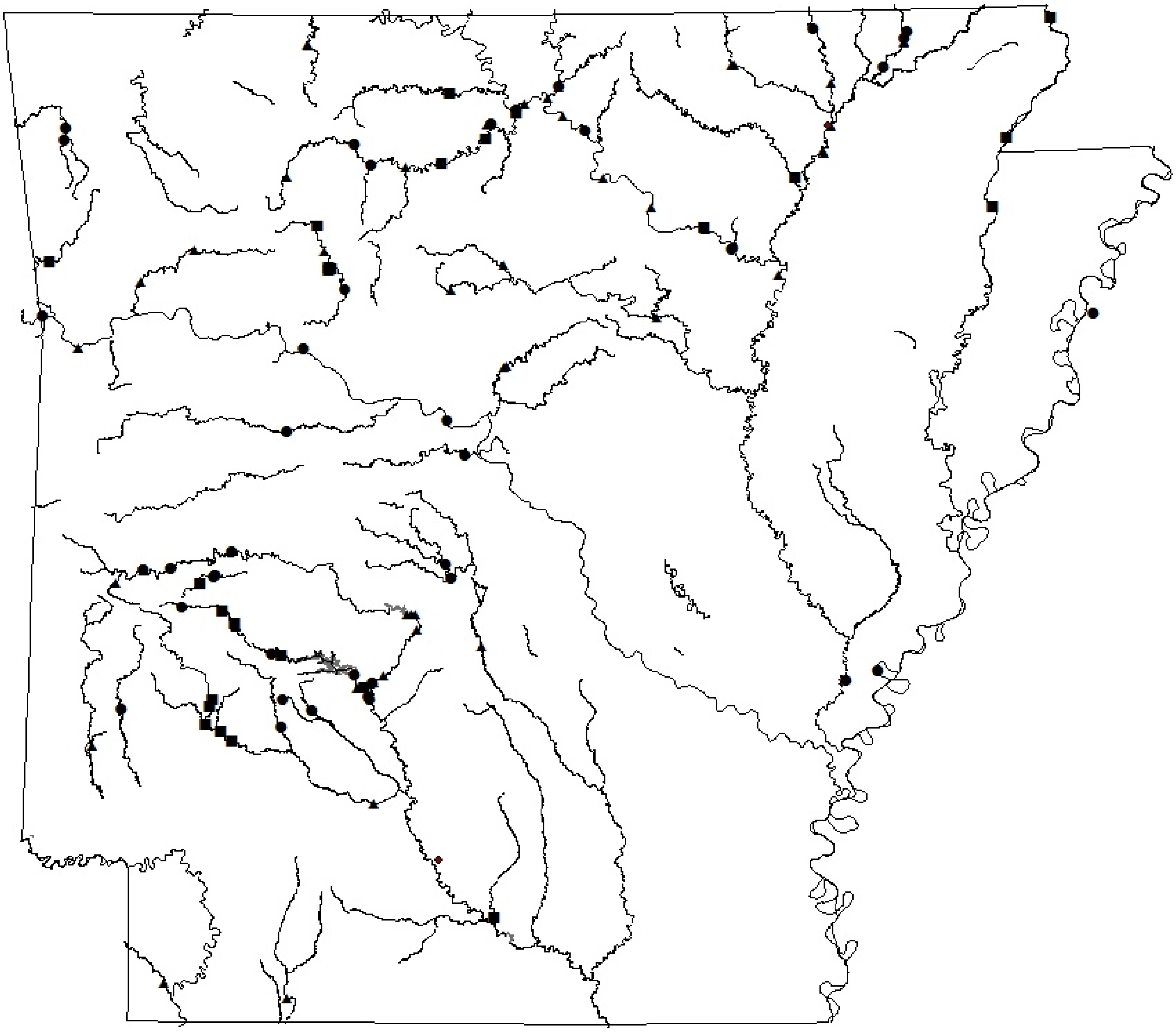

Figure 1. Chestnut lamprey distribution records in Arkansas, 1928-present. Legend: records from 1928-1986 are represented by a circle, records from 1987-2000 by a square, and records from 2000-present by a triangle. Records for which no date could be confirmed are represented by a diamond. See supplemental table (Appendix I) which contains all individual records at the journal website.

1 potential host (a river redhorse *Moxostoma carinatum* Cope) presumptively parasitized by a chestnut lamprey were collected in the Little Missouri River (Table 1).

Arkansas River Drainage

Thirty-four chestnut lampreys have been documented from the Arkansas River drainage, encompassing eight streams (Figure 1). Specimens were first collected from this drainage in 1937. Sub-watersheds of the Arkansas River where chestnut lamprey were observed include the Illinois (n = 3), Mulberry (n = 2) and Petit Jean (n = 2) rivers, in addition to Big Piney (n = 5), Cadron (n = 2), Garrison (n = 1), and Lee (n = 1) creeks. Nonetheless, only 9 individuals have been collected from the Arkansas River and tributaries since 2000. During our 2015 sampling, no individuals were collected from the 3 streams sampled, but 1 potential host (a spotted bass *Micropterus punctulatus* Rafinesque) was collected from Cadron Creek (Table 1). In contrast, Robison and Buchanan (1988) had 5 records from the Arkansas River, 1 from the Illinois River, 1 from Lee Creek, and 1 from the Petit Jean River.

White River Drainage

Chestnut Lampreys in the White River drainage have been identified in several of tributaries, including the Black (n = 4), Buffalo (n = 13), Current (n = 5), Eleven Point (n = 2), Fourche (n = 2), Little Red (n = 10), Spring (n = 2), Strawberry (n = 1), and White (n = 16) rivers (Figure 1). Specimens were first collected from this drainage in 1928, representing the first documented collection of the species in Arkansas. Nine of the specimens collected from the Little Red River were from streams feeding Greers Ferry Lake, with 1 record in a downstream creek; each was collected over the past 2 decades. All of the Spring River collections were from our 2015 survey (Table 1). Creeks directly feeding the White River that have chestnut lamprey records include Crooked Creek and Long Creek (Figure 1). A single record exists for the species from an unnamed pit lake on the White River National Wildlife Refuge in Phillips County. Within this drainage collections of Chestnut Lampreys have been regular and ongoing.

In our 2015 survey, the 8 specimens we collected were from tributaries feeding the lower White River: the Black (n = 3), Current (n = 2), and Spring (n = 3) rivers (Table 1). Further, we collected parasitized fishes from the Black River (n = 3), Current River (n = 1), Spring River (n = 3), Little Red River (n = 1), and Strawberry River (n = 1). These fishes included a spotted bass and 2 common carp *Cyprinus carpio* Linnaeus from the Black River, a single common carp from the Current River, 3 common carp from the Spring River, 1 spotted sucker *Minytrema melanops* Rafinesque, and 1 common carp from the Strawberry River. Two chestnut lampreys were also collected from the federal Norfork National Fish Hatchery in the summer and fall of 2012 by United States Fish and Wildlife Service personnel. Subsequent work by Salinger (2016) in this hatchery found 83 cases of rainbow trout parasitized by lampreys believed to be chestnut lampreys. Our sampling did not include the upper and lower White River in an effort to avoid the capture of silver lampreys or fishes parasitized by silver lampreys.

Robison and Buchanan (1988) reported that specimens were collected from the lower White River (n = 3), the middle section of the White River (n = 8), the South Fork of the Little Red River above Greers Ferry Reservoir (n = 1), the Little Red River below Greers Ferry Reservoir (n = 1), the and 1 record each from the Norfork River, the Buffalo River, the Spring River, the Eleven Point River, and Crooked Creek.

St. Francis River Drainage

Three individuals have been collected from the St. Francis River, with each specimen collected during the years of 1987-1988 (Figure 1). No collection records were identified within the St. Francis River in Robison and Buchanan (1988). Despite sampling of 2.5 river km, no specimens or evidence of lamprey parasitism on other species was identified (Table 1).

Mississippi River

There have been 2 collections of Chestnut Lampreys (n = 3) from the Mississippi River. One collection record comes from an apparently unnamed oxbow lake in southern Phillips County, whereas the other was collected in the main stem of the river in southern Mississippi County. Both records date to the early to mid-1980s, and no other collection records have been documented since. This lack of records is in spite of the fact that the US Army Corps of Engineers has extensively sampled the Mississippi River in Arkansas for the past two decades, and large host fishes are abundant within the river (T. Buchanan, *pers. comm.*).

Conclusions

The distribution of chestnut lampreys occurs throughout much of the state, although few individuals have been collected in the Mississippi River or the Red River or the St. Francis River drainages. Recent electrofishing results with a goal of specifically targeting chestnut lampreys showed a low frequency of individuals with all individuals collected in the White River drainage. Further, few parasitized individuals were collected in other drainages.

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Second record of Gray-headed Junco (*Junco hyemalis caniceps*) in Arkansas

K.G. Smith[1*] and K. Garrett[2]

[1]*Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701*
[2]*1208 N 37th St, Rogers, AR 72756*

*Correspondence: kgsmith@uark.edu

Running title: Gray-headed Junco in Arkansas

Once considered a separate species, the Gray-headed Junco (*Junco hyemalis caniceps*) is now part of the *caniceps* group within the species Dark-eyed Junco (*Junco hyemalis*). It is primarily a bird associated with the Rocky Mountains (Nolan *et al*. 2002), but single birds are occasionally found east of the Rocky Mountains (see below).

The first record of Gray-headed Junco in Arkansas occurred in late February and early March of 1963 at a feeder in Little Rock (Pulaski County) (James 1964). This bird had a pink upper mandible (Figure 1), confirming it was a Gray-headed Junco and not the closely related Red-backed Junco (*J. h. dorsalis*), which has a dark upper mandible and is thought to be relatively sedentary.

The second record for Arkansas occurred on 28 February 2018 when Garrett photographed a Gray-headed Junco at the bird feeders at Hobbs State Park – Conservation Area, east of Rogers (Benton County). The bird was seen and photographed by many (Figure 2) and was last observed at the bird feeders on 14 March.

First documented in western Kansas in late April of 1966 (Rising 1966), there have been several more records for that state (Thompson and Ely 1992). Similarly, most records in Oklahoma have been in the western panhandle with a few records in eastern Oklahoma (Van Els *et al*. 2009). The first Gray-headed Junco in Missouri spent the winter from early December 2000 to April 2001 (Rowe 2002) and there has been a second record during 13-18 February 2011 (Rowe 2012). Louisiana has had 2 specimens collected, one on 3 February 1957 and one on 4 July 1990. There are also sight records of single birds from Louisiana on 19 December 1966, 12 January 1990, and 13-14 October 1990 (S. W. Cardiff, *pers. comm.*) and 2-3 March 2000 (Dittman and Cardiff 2003). There is one record from Tennessee on 24 May 1982 (Anderson 1984).

Further north in the Great Plains, Gray-headed Juncos occasionally occur in states like Nebraska (Svingen and Martin 2005) and there are 3 recent records for single birds in Ontario in May (Holden 2014).

Figure 1. The first Gray-headed Junco found in Arkansas on 22 February 1963 at a feeder in Little Rock. It was last seen on 2 March 1963. Picture from Arkansas Audubon Society archives.

Figure 2. Gray-headed Junco found at Hobbs State Park – Conservation Area on 1 March 2018. It was last observed on 14 March. Photograph by Michael Linz.

Most of these reports would suggest that many birds are strays during spring migration. The report of a flock Gray-headed Junco in Louisiana of at least 8 birds on 1 November 1990 is unprecedented (S. W. Cardiff, *pers. comm.*).

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Observations of Townsend's Solitaires (*Myadestes townsendi*) on Mount Magazine in Logan County, Arkansas

D.R. Simons[1*] and K.G. Smith[2]

[1]*Mount Magazine State Park, 16878 South State Highway 309, Paris, AR 72855*
[2]*Department of Biological Sciences, University of Arkansas, Fayetteville AR 72701*

[*]Correspondence: don.simons@arkansas.gov

Running Title: Townsend's Solitaires on Mount Magazine

Townsend's Solitaires (*Myadestes townsendi*) (Fig. 1) normally range throughout the Rocky Mountains. In winter months they are known to occur eastward into the Midwest, with occasional reports from eastern states. Range maps in most bird field guides do not indicate that they visit Arkansas. However, the range map for Townsend's Solitaire in Dunn and Alderfer (2017) has a small symbol indicating an "extent of irregular or irruptive range in some winters" in northwestern Arkansas. This was based on multiple reports from Mount Magazine in Logan County beginning in 2005 that are summarized in this report.

Figure 1. Townsend's Solitaire *Myadestes townsendi* photographed by Simons on Mount Magazine on 15 January 2008.

Townsend's Solitaires were previously considered to be a rare bird in Arkansas. Prior to 1986, there was only one record of a bird near Springdale (Washington County) during winter of 1963-1964 (James and Neil 1986). Since that time, single birds were discovered in February of 1991, October of 1995, and January 2001 (Arkansas Audubon Society 2015).

Mount Magazine rises out of the Arkansas River Valley over 670 m (2,200 ft) to an elevation of 840 m (2,753 ft) above mean sea level. It is the highest point in Arkansas and the highest point between the Minnesota/ Canada border and the Gulf of Mexico. The mountain supports a variety of plant communities including Eastern redcedar (*Juniperus virginiana*) thickets just above bluff lines along the north and south facing rims. A lodge and cabin complex is located approximately 1.5 km along the south rim with cabin #1 being the western most. The northern rim of the mountaintop includes a scenic overlook drive and hiking. The eastern end of the mountain is split into two legs by Bear Hollow, which has redcedar thickets along its northern rim.

Mount Magazine is presently the only known (in Arkansas) and eastern most breeding site for the Rufous-crowned Sparrow (*Aimophila ruficeps*) (Collins 1999). A small population was discovered on the southern rim, below the lodge and cabin complex in 1972. The population, present throughout the year, has fluctuated over the years (James and Neal 1986).

There is a seldom used term in the birding world called the "Arizona Roadside Rest Area Effect" (Arizona Audubon Society 2011). Someone reports a rare bird at a highway rest area prompting other birders to visit the site. They report additional unexpected species, bringing more attention to the site. This is how Simons and others accidently became aware that solitaires were wintering on Mount Magazine.

A Snow Bunting (*Plectrophenax nivalis*) was discovered on Cameron Bluff Overlook Drive on 17 December 2005. It was reported to the Arkansas Rare Bird Alert system. The next morning the bunting could not be relocated. During the search, a pair of solitaires was discovered feeding in an Eastern redcedar at the first parking area on Cameron Bluff drive. This information was posted on the rare bird alert and many birders drove up Mount Magazine to add solitaires to their lists. The last known date this pair was seen on the mountain was 6 March 2006. This was the Arkansas's 5th record for the species.

A single solitaire was found on 2 April 2007. This bird was foraging in cedars along the southern rim of the mountaintop west of the cabin complex and was seen by a group of experienced observers 4 days later. This bird was silent. It was not relocated afterwards.

Suspecting the occurrence of solitaires on Mount Magazine might be more common, Simons began purposefully searching cedar thickets for them each November and December. Searches began with simple observation on the edge of cedar thickets. After a period of about ten minutes with no solitaires, he played a recording of their song on an iPod.

When present, birds responded almost immediately by flying high and dropping down to perch on an exposed treetop. If a pair was defending a territory, one would perch close to the source of the sound and the other further away. Often the nearest one would start to call with single whistle-like notes. On a few occasions, it (presumably a male) would begin to sing a song described as "a loud, complex, melodious warbling". The song was only uttered when in the presence of a second (probably a female) who seemed to be more passive by staying further away.

On 9 January 2008 a single solitaire was located on Cameron Bluff. It was last seen on 1 March 2008.

Drought affected the cedar berry crop for several years. No solitaires were reported until 23 December 2012 when 3 were found on the rim above Ross Hollow which is between Cameron Bluff and Brown Springs. That winter, solitaires also appeared on the southern rim of the mountain at the hang glider launch, below the lodge, and near the eastern most cabin. Also, there was an unconfirmed report of a solitaire along the Bear Hollow trail on the eastern part of the park. We suspect there could have had more than 3 solitaires in the park that season. The last sighting for any of these birds was 17 April 2013.

Four solitaires spent winter from 14 November 2014 through 22 February 2015 along the rim of Ross Hollow. On one occasion, a solitaire broke out into song from a prominent perch along the western rim when another flew across from the eastern side to harass the singer then returned to the eastern side.

Playback of a solitaire song one afternoon in November 2014 resulted in the audible response of a Northern Saw-whet Owl (*Aegolius acadicus*). The owl was only heard and was not located. Until recent banding efforts (Pruitt and Smith 2016), saw-whets was considered rare in Arkansas.

Two solitaires were discovered below the cabins east of the lodge on 28 December 2017. One was seen feeding on winged sumac (*Rhus copallina*) berries. The last sighting was 27 January 2018.

In summary, since 2005, Simons has been able to confirm the occurrence of solitaires on Mount Magazine 6 out of 13 winters. Each time many birders and bird photographers made special efforts to add this species to their lists on Mount Magazine without looking for them in other areas with similar habitat. This suggests solitaires are probably overlooked in suitable cedar habitat in other parts of Arkansas. During this period, there was only one report of a solitaire in Arkansas not found on Mount Magazine. That bird was at Holla Bend National Wildlife Refuge on 10 January 2010 (Arkansas Audubon Society 2015), about 50 km due east of Mount Magazine.

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House Finch (*Haemorhous mexicanus*) Nesting in December in Arkansas

K.G. Smith

Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701

Correspondence: kgsmith@uark.edu

Running title: House Finch nesting in December

The House Finch (*Haemorhous mexicanus*) is native to the southwestern United States and Mexico, but was introduced to the northeastern United States around 1940 (Elliot and Arbib 1953). Over the course of the next few decades, the House Finch expanded its range southward and westward in eastern United States, arriving in eastern Arkansas in 1971, with evidence of breeding there in 1985 (James and Neal 1986). By 1991, finches were breeding in the northwestern corner of the state (Arkansas Audubon Society 2015). Today it is found throughout the state and is one of the most common birds in urban and suburban areas.

Given that House Finches now occur throughout the United States, there is a great variation in the timing of nesting from early February to April (Badyaev *et al.* 2012). Here I document a nesting attempt by a pair of finches in December of 2017 in central Arkansas.

On 28 November 2017, Anthony Woods put up a wreath at his house in Maumelle (Pulaski County), Arkansas. On or about 12 December, he noticed a pair of House Finches investigating the wreath and when he took down the wreath on 29 December, he discovered a nest with 4 eggs (Figure 1). Wreaths are commonly used as nesting sites in Arkansas (*pers. observ.*)

Pair bonds persist from one breeding season to the next in some House Finch pairs and paired birds do nest earlier than newly paired birds (McGraw and Hill 2004). However, photoperiod is thought to play a role in male breeding activity (Hamner 1966), so finding a nest in December is certainly surprising. There were some warm days early in December in nearby Little Rock, with it reaching 22 °C on 11 December. Temperatures were near normal after that until 23 December when it became much colder.

Two other winter nesting attempts of House Finches have been reported in California: nests with eggs in late November (Smith 1930) and in late December (Howell and Burns 1955). There is also one report of a female starting to build a nest in November in southern Florida (Johnson 2010).

The House Finch joins the Killdeer (*Charadrius vociferous*) as the only unusual December breeders in Arkansas (Smith *et al*. 1999). Koenig and Stahl (2007) suggested that fall breeding might be more common than originally thought. Possibly winter breeding will become more common as well, particularly at southern latitudes, as the climate continues to change.

Figure 1. Typical House Finch nest with 4 eggs discovered on 29 December 2017 in Maumelle, Arkansas. Photograph by Anthony Woods.

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Proportionality of Population Descriptors of Helminth Infections of Smallmouth Bass (*Micropterus dolomieu*) from the Buffalo National River

J.J. Daly Sr.* and K. Wagner

University of Arkansas for Medical Sciences (retired)

*Correspondence: jamesdalysr@yahoo.com
6700 Granada Drive, Little Rock AR 72205

Running title: Proportionality of Helminths Descriptors

Mean abundance (MA), standard deviation (SD), maximum number (Max) and prevalence (Prev) are standard descriptors for defining helminth parasite populations in their hosts with mean intensity and its standard deviation used less often. Daly and coworkers (Daly Sr. *et al*. 2013; Daly Sr. 2014) previously demonstrated that these population descriptors in infections of *Clinostomum* metacercariae in smallmouth bass exhibited a principal of proportionality between the factors where each one effects the value of the other *as a given ratio*. These relationships could be seen where the descriptors are used as the x and y variables in regression analyses in which very significant R and probability values are seen. Also, it was found that ratios of descriptive values of standard deviation/mean abundance, maximum abundance/mean abundance, and maximum abundance/standard deviation produced normal distributions with low standard deviations. The values of these ratios, obtained from a collective of population infections, would produce numbers that were relatively predictive for *Clinostomum* infections in any bass population from Ozark and Ouachita mountain streams (Daly Jr. *et al*. 1999; Daly 2014). Importantly, the ratio of SD/mean of *Clinostomum* populations in 16 smallmouth bass populations were mostly above 1 (with 3 exceptions) indicting a tendency toward non-normal, non-parametric, overdispersed distributions rather than randomly acquired infections. Overdispersion or aggregation is where a few hosts have the largest number of parasites and is commonly found in helminth infections. Daly and Wagner (2016), using data from a study of an acanthocephalan infection in stream *Gammarus* in England (Croft 1971) demonstrated that another helminth beside *Clinostomum* can also show proportionality in their population descriptors.

The study of Kilambi and Becker (1977) on populations of 24 helminth parasites of smallmouth bass from the Buffalo National River in North Central Arkansas offered the opportunity to use their data to determine if the same principal of proportionality and overdispersion also existed in helminths other than *Clinostomum* infections of stream smallmouth bass or an acanthocephalan in *Gammarus*. Those investigators collected bass hosts by electro-shocking from three locales; one upstream (Ponca), one halfway (Hasty), and one close to the terminus of the stream (Rush). Collections were taken in spring, summer, fall and winter. A total of 127 hosts were examined. Analysis was presented for all collections but for our purpose we used the refined data from their table 5 that combined the data from all of the collections. Regression analysis was done with the Excel analytical package (2010). Table 1 shows the data that was used for this study. Table 2 shows the results of the regression analysis for the smallmouth helminth populations. Monogenea, digenea, and nematoda populations all show very high correlation coefficients between the three major population descriptors of mean abundance,

We have found that this agrees with data from previous host-parasites relationships in smallmouth data from Ozark and Ouachita mountain streams. The exception is the tapeworm correlations. The mean abundance – standard deviation shows a strong R but a less than significant probability using p = 0.05 as the baseline. However, the number of populations was only three. Still it may mean that cestodes may not fit the principle of proportionality that is seen with other helminths. The density of infections with stream tapeworm infections are light compared to those from hosts from lakes (Daly Sr. *et al.* 2006). Another special case is with the acanthocephalan *Neochinorhynchus cylindratus* since only one set of descriptor values was available therefore the four seasons and the three locales were used herein as separate populations. The regression relationships for these populations were similar to the other helminths (Table 2). Prevalence for all infections (Table 3) was poorly related to other descriptors. This is usual for helminth infections where prevalence can be significant or not. We have found that density of infection is a primary cause of this (Daly and

Wagner; manuscript *in preparation*). The prevalence of light infections is significantly correlated with mean abundance but heavy infections are not.

The major value of the principle of proportionality is as an estimate of where the population fits in the spectrum of randomness to aggregation. All of the helminth populations from the Buffalo River data tend toward aggregation (Table 4). From our previous data only three of the sixteen individual *Clinostomum* populations from streams tended toward randomness (Daly 2014). Other such random populations from our studies are *Clinostomum* in pond-raised channel catfish (Singleton *et al*. 2018) and tapeworm plerocercoids in lake bass (Daly *et al.* 2006). Catfish in a pond would be moving about and would be randomly exposed to cercaria from snail hosts that would probably be territorial and limited in their dispersal in the pond. As for lake bass it is known that *Micropterus punctulatus* roam the lakes in schools and would randomly ingest infected small fish hosts. Some investigators prefer to use variance as the numerator in the overdispersion ratio Poulin (2007) has pointed out that variance has a power function and violates a rule of not using an exponential value in regression studies. It's not clear what the other ratios represent and to our knowledge they have not been studied to any extent.

In conclusion helminth infections in Buffalo River smallmouth hosts show strong proportionality indicating similar life cycle structures. Also, a tendency toward aggregated populations was similar to most other helminths studied elsewhere and it indicates a general principle of proportionality in helminth ecology.

Table 1. Data from Kilambi and Becker (1977) for population descriptors from monogenean, digenean, cestode, acanthocephalan, and nematode helminths from smallmouth bass (*Micropterus dolomieu*) collected from the Buffalo National River, Arkansas and used for regression and ratio analysis in this study. Mean = mean abundance, SD = standard deviation, and Maximum = maximum abundance.

	Helminth	Prevalence	Mean	SD	Mean
Monogenea	*Alcolpenteron uretereroecetes*	16	<0.0	0.2	2
	Acinocleidus fusifornos	31.5	1.3	2.94	15
	Clavunculus bursatus	15.7	0.4	1.31	9
	Cleidodiscus banghami	23.6	0.8	2.48	15
	Leptocleidus megalonchus	0.8	0.1	0.71	8
	Urocleidus principalis	31.5	15.3	64.4	541
Digenea	*Clinostomum marginatum*	33.1	1.4	5.54	59
	Crepidostomum cornutum	38.6	6.2	28.07	298
	Cryptogonimus chyli	44.9	73.6	335.6	3480
	Leuceruthrus micropteri	44.9	1	1.58	8
	Neascus sp.	78.7	9.2	13.58	83
	Pisciamphistoma reynoldsi	10.2	0.2	0.88	6
	Posthodiplostomum minimum	7.9	3.4	17.45	51
	Rhipidocotyl papillosum	20.5	0.7	2.04	14
	Rhipidocotyl septpapillota	7.9	0.2	0.9	8
	Rhipidocotyl sp.	64.6	26.9	66.56	587
Cestoda	*Bothriocephalus cuspidatus*	3.2	0.1	0.59	6
	Proteocephalus ambloplites adult	15.7	0.4	1.49	15
	Proteocephalus ambloplites larva	14.2	0.4	1.08	7
Acanthocephala	*Neochinorhynchus cylindratus*	79.5	11.6	16.53	88
Nematoda	*Capillaria catenate*	7.1	0.4	3.07	34
	Contracoecum sp.	19.7	2.8	9.5	59
	Philometra sp.	12.6	0.4	1.4	8
	Spinitectus carolina	59.8	4.2	7.2	34
	Nematode cyst	93.7	190.9	336.9	2055
	Rhabdochona cascadilla	2.4	<0.0	0.15	1

Table 2. Regression analysis of helminth populations collected from smallmouth bass (*Micropterus dolomieu*) hosts from the Buffalo River.

	N	X variable	Y variable	R	*p*	Intercept	Slope
Monogenea	5	Mean	SD	0.99	2.4E-05	-0.88	4.3
		Mean	Max.	0.99	7.6E-05	-11.2	36
		SD	Max.	0.99	2.8E-06	-1.99	8.5
Digenea	10	Mean	SD	0.98	3.0E-07	-7	54.4
		Mean	Max.	0.94	4.9E-05	-1902	448.3
		SD	Max	0.98	2.9E-07	-1328	104.4
Cestoda	3	Mean	SD	0.89	0.3	0.36	2.32
		Mean	Max	0.59	0.6	4.3	16.7
		SD	Max.	0.89	0.3	-0.93	19.7
Acanthocephala	1	Mean	SD	0.97	3.7E-07	0.29	0.97
		Mean	Max.	0.97	6.5E-07	-1.93	3.59
		SD	Max.	0.99	1.5E-08	0.91	0.27
Nematoda	5	Mean	SD	0.98	0.004	5	0.17
		Mean	Max.	0.99	7.5E-06	18.4	10.7
		SD	Max.	0.98	0.003	-271.9	61.1

Table 3. Regression analysis of mean, standard deviation (SD), and maximum number (Max.) population descriptors as the X variables and population prevalence (% hosts infected) as the Y variable from helminth populations collected from smallmouth bass (*Micropterus dolomieu*) from the Buffalo River.

	N	X Variable	Y Variable	R	*p*	Intercept	Slope
Monogenea	5	Mean	Prevalence	0.53	0.36	-1.99	0.3
		SD	Prevalence	0.5	0.39	-8.0	1.1
		Max.	Prevalence	0.48	0.41	-65.7	8.9
Digenea	10	Mean	Prevalence	0.36	0.36	0.3	0.35
		SD	Prevalence	0.51	0.51	0.51	0.06
		Max.	Prevalence	0.15	0.15	0.68	67.5
Cestoda	3	Mean	Prevalence	0.99	0.07	-0.72	39.2
		SD	Prevalence	0.94	0.23	-3.9	14.2
		Max.	Prevalence	0.67	0.53	2.37	0.93
Acanthocephala	1	Mean	Prevalence	0.65	0.03	-6.4	0.19
		SD	Prevalence	0.53	0.1	58.5	1.83
		Max.	Prevalence	0.52	0.1	60.2	0.48
Nematoda	6	Mean	Prevalence	0.84	0.07	24	0.37
		SD	Prevalence	0.87	0.053	12.6	2.2
		Max.	Prevalence	0.83	0.08	23.5	0.03

Table 4. The SD/Mean and other ratios of Buffalo River helminth parasite populations of smallmouth bass (*Micropterus dolomieu*).

Host Populations	N	Sd/Mean	Max/Mean	Max/Sd
All helminths	24	3.6±1.7	31.1±21.7	7.8±2.2
Monogenea	5	4.0±1.3	33.6±27.3	7.5±2.4
Digenea	10	3.6±1.3	31.2±15.5	8.3±2.0
Nematoda	5	3.6±2.4	29.3±32	6.7±2.5

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New Records of the American Badger (*Taxidea taxus*) in Arkansas, with an Updated Distribution Map

R. Tumlison[1*] and D.B. Sasse[2]

[1]*Department of Biology, Henderson State University, Arkadelphia, AR 71999*
[2]*Arkansas Game and Fish Commission, 213A Highway 89 South, Mayflower, AR 72106*

*Correspondence: tumlison@hsu.edu

Running Title: Updated distribution of American badgers (*Taxidea taxus*) in Arkansas

Within 30 years of the first published record of American badgers (*Taxidea taxus*) in Arkansas, only 3 specimens had been documented. In 1964, the first specimen was collected in Washington County in the northwestern corner of the state (Sealander and Forsyth 1966). Franklin County later produced a specimen trapped near the Arkansas River (Cartwright and Heidt 1994). A roadkill specimen from Stone County, far eastward of the first specimens, became the third verified record of occurrence (Cartwright and Heidt 1994).

Fur harvest records maintained by the Arkansas Game and Fish Commission (AGFC) have included reports of badgers as far back as 1976. No county-level data are available for the early records, as they were saved only by region, and most reports were from the Ozark Mountains. In 1984, a record 10 badgers was reported to have been taken in Arkansas, 7 from the Ozarks and 3 of unreported origins. Otherwise, 5 badgers were reported taken in 1986 and 1993, and 1-3 were reported during 16 of the other years since 1976. In the 17 years since 2000, only 5 badgers have been reported. The cause of the shifting numbers is not known, but could be related to trapping effort or pelt prices. Although occurrences in fur reports have dwindled since highs in the 1980s and early 1990s, verifiable records based on specimens or photographs have increased.

Tumlison and Bastarache (2007) noted eastward expansion of range in Oklahoma, near the Arkansas border. This observation was soon followed by new records in Sebastian and Crawford Counties, bordering Oklahoma, and providing evidence of a recent range expansion into the state along the Arkansas River (Tumlison and Sasse 2015).

Beginning about 2003, several new verified observations also significantly expanded the known range into northeastern Arkansas, apparently from the bootheel region of Missouri (Tumlison *et al*. 2012, 2017). Further observations revealed 2 locations, both in Crittenden County, with dens supporting offspring (Tumlison *et al*. 2012).

On 13 November 2017, the AGFC posted information about badgers on their facebook page, and requested that viewers post their observations and images of badgers in Arkansas. Though many people responded in some manner, useful information was sparse, and many reported observations likely were actually woodchucks (*Marmota monax*). However, numerous comments came from areas where badgers have been documented already, and help support the idea that badgers have become established in northern counties of Arkansas.

Current distribution of the badger in Arkansas is dynamic and reflects very recent expansion of historic range, and the species is listed as a Species of Greatest Conservation Need in Arkansas (Fowler 2015). Therefore, herein we report new validated records and consolidate all known records onto one map to provide an updated distribution for this species whose biology in Arkansas began unfolding only in the last 2 decades.

New Records of Distribution

Benton County: near Bentonville, 0.4 km (0.25 mi.) N of Pea Ridge exit. Trevin Tripodi, a predator control trapper, captured a male badger by use of a foothold trap and coyote/skunk gland lure on 14 January 2017. He had set the trap because he recognized badger tracks on a man-made berm covered with vegetation. The trapper also noted dense populations of rabbits and rodents at the site, which would serve well as a food base. This is the first verified record for Benton County with locality data.

Boone County: about 5 km N of Harrison, 36.279615°N, 93.097541°W. On 24 July 2017, Wade Grayson photographed a badger in a den located in a field in which he runs cattle. Few records of badgers have been reported from this region of the state in the Ozark Mountains, though other observations are documented from neighboring Marion County (Tumlison *et al.* 2012). This photo-documented individual represents a new county record for Arkansas.

In the evening of 2 December 2017, David Good caught a male badger in a box trap at Hill Top, Arkansas, on Gaither Mountain, 36.15750°N, 93.22556°W. He had set traps to capture a predator attacking his chickens, and unexpectedly caught the badger. The trap was set at the edge between a grassy field and mature mixed woods. He killed and photographed the animal, and upon skinning it observed that it was laden with greasy fat, but no chicken remains were in the stomach at the time. It was 790 mm (31 in.) in total length. This record is about 21 km (13 mi.) SW of the other new Boone County record, and is located near the Carroll and Newton County lines.

Clay County: County Road 528, 18 km ENE of Rector, 36.32157°N, 90.10389°W. Amanda Russom photographed the roadkill individual on 27 July 2017, along the road beside a bean field, about 1.5 km from the St. Francis River. A couple of farmers in the area reportedly claimed to have recently spotted several animals they identified as badgers. This represents a new county record for Arkansas.

Craighead County: Caraway Cemetery, 35.767°N, 90.341°W. Several people on facebook reported this badger, which was trapped after the discovery of an animal digging by tombstones. Jason New provided the verifying photograph. This is the most southeastern occurrence documented in Craighead Co. The area is surrounded by farmland.

Missouri: *Dunklin County*: 0.8 km west of Arbyrd on MO St. Hwy 108, ca. 5.6 km N of Arkmo or about 12.9 km N of Leachville (Mississippi County, AR). A roadkill badger was photographed by Sheila Lambert on 24 July 2017. Though not technically in Arkansas, this record is just north of the Mississippi County line, very close to other records in that county, represents a new locality in Missouri, and connects the likely path of colonization from the Missouri bootheel region into northeastern Arkansas (Tumlison *et al*. 2012).

Previous Records of Distribution

Benton County: no specific locality given, as record was gleaned from a fur buyer's report (Tumlison *et al*. 2012).

Craighead County: County Road 907, 2.0 km N of jct. with Hwy 18, E of Jonesboro, 35.8397228°N, 90.5833338°W; Jonesboro, Johnson Avenue near jct. U.S. Hwy 49 35.8522228°N, 90.6672228°W; Lake City, AR St. Hwy 18, ca. 1.6 km E of the St. Francis River Bridge; Lake City, 0.4 km W jct. of AR St. Hwy 18E and AR St. Hwy 135N, 35.8221258°N, 90.4706348°W; U.S. Hwy 63, N of Trumann, 35.7138478°N, 90.5765088°W (all records from Tumlison *et al*. 2012).

Crawford County: Van Buren, 1.9 km NE of the Arkansas River and 0.6 km S of U.S. I-40, 35.458°N, 94.364°W (Tumlison and Sasse 2015).

Crittenden County: 1.6 km N Ebony (Tumlison *et al*. 2012); near Proctor, 35.081878°N, 90.335088°W (Tumlison *et al*. 2012); Roseboro Island Road, 5.5 km WNW of Marion, 3.2 km N of jct. with U.S. Hwy 64, 35.22627°N, 90.25420°W (Tumlison and Sasse 2015); AR St. Hwy 77, 1.1 km N of Clarkdale, 35.31905°N, 90.23970°W (Tumlison and Sasse 2015); ca. 1.6 km W of previous site, along U.S. I-55, 35.322564°N, 90.259220°W (Tumlison and Sasse 2015); AR St. Hwy 50 and Woollard Road, 35.25642°N, 90.32569°W (Tumlison *et al*. 2017).

Franklin County: 2.5 km S of the Ozark Dam near the Arkansas River (Cartwright and Heidt 1994).

Lawrence County: off US Hwy 412 W of Walnut Ridge, 36.0646198°N, 90.9939258°W (Tumlison *et al*. 2012).

Marion County: Crooked Creek S of Pyatt; AR St. Hwy 14, 8 km S Yellville, 36.152558°N, 92.674058°W (Tumlison *et al*. 2012).

Mississippi County: AR St. Hwy 18 near Manila, 1.6 km W of Big Lake NWR, 35.872112°N, 90.156273°W; AR St. Hwy 119 E of Marie, Sec. 6, T11N, R10E (both records from Tumlison and Sasse 2015).

Newton County: no specific localities given (Sealander and Heidt 1990; Tumlison *et al*. 2012).

Poinsett County: 2.4 km N Shady Grove, 35.6875808°N, 90.5798538°W (Tumlison *et al*. 2012).

Randolph County: no specific locality given (Tumlison *et al*. 2012).

Sebastian County: 100 S 10th Street, Fort Smith, about 1.1 km SE of the Arkansas River (Tumlison and Sasse 2015).

Stone County: AR St. Hwy 5 near Optimus (Cartwright and Heidt 1994).

Washington County: 3.2 km W Fayetteville (Sealander and Forsyth 1966).

Dens

The den occupied by the badger in Boone County was constructed under a tree that had been covered by honeysuckle. The entrance to the den had the typical U-shaped fan of excavated dirt and was situated at the base of a tree (Fig. 1). Dirt around the tree was more elevated than the surrounding grassy field. The immediate area of the den was covered by woody and herbaceous vegetation, and its location at the tree likely protected it from movements of cattle. This den is similar to the only other den previously reported in Arkansas (Tumlison and Sasse 2015), which also was under a tree in elevated ground.

A den believed to be that of the male badger caught in Benton County was located near the capture site in an elevated berm, but not at the base of a tree. The opening was about 30 cm (1 ft.) wide with a fan of excavated dirt below the opening.

Currently, the scattered reports from around the state seem to indicate 3 primary areas in which populations of badgers are becoming more established (Fig. 2). In western Arkansas, the Arkansas River appears to be a corridor down which badgers are moving into the Arkansas River Valley in Crawford and Sebastian Counties, on both sides of the river. In the mountainous regions of the state, the most numerous and recent reports come from the central Ozarks (Marion and Boone Counties), bordering Missouri. However, the largest number of observations covering the widest area flows from southeastern Missouri, mostly between the St. Francis and Mississippi Rivers, in the northeastern section of the Mississippi Alluvial Plain (Foti 1974). This is the only region where reproduction has been observed.

Figure 1. A badger (*Taxidea taxus*) in its den in Boone Co., AR, 24 July 2017. Photo by Wade Grayson.

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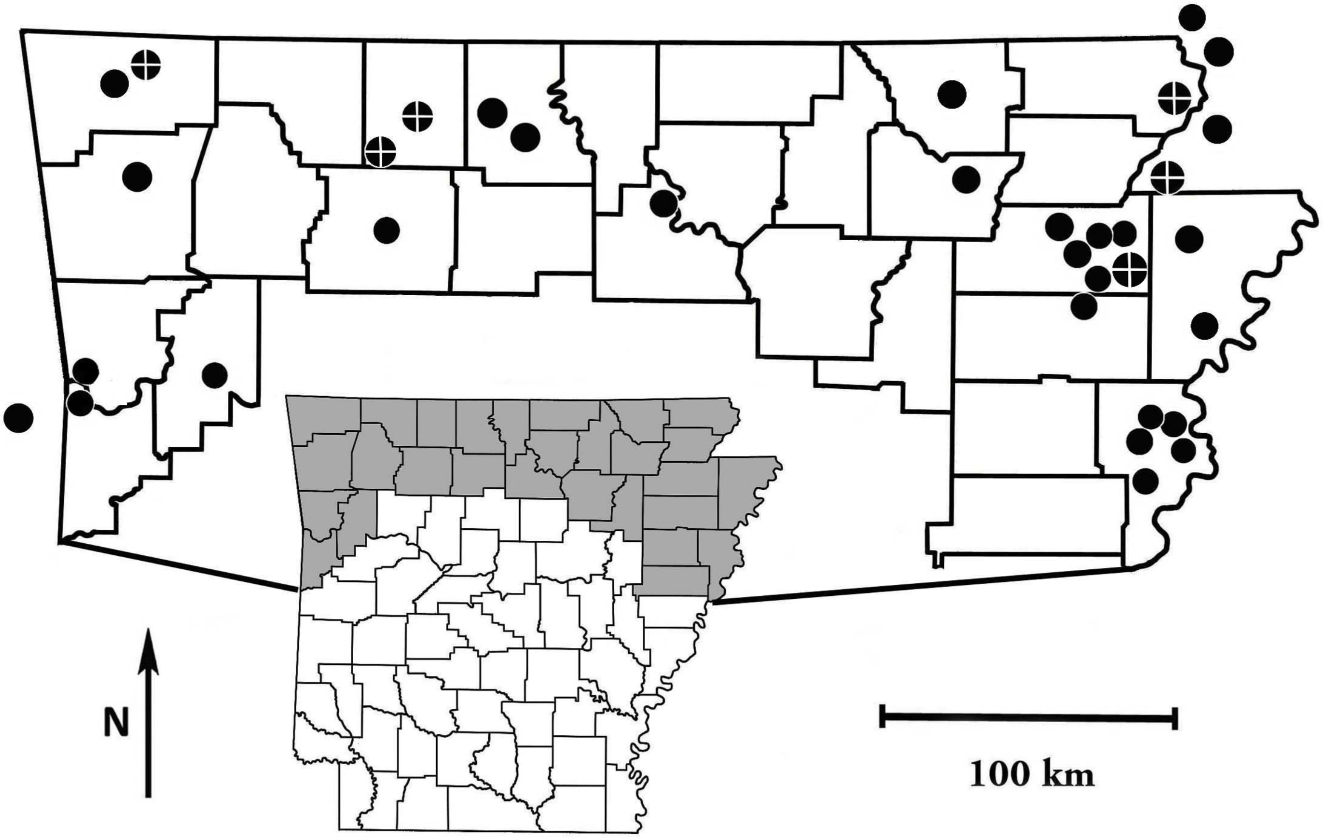

Figure 2. Currently known distribution of American badgers (*Taxidea taxus*) in Arkansas. Historical records are indicated by black dots and new records by crossed black dots. Scale bar is for the enlargement. Nearby record from LeFlore County, OK from Tumlison and Bastarache (2007), and from Dunklin and Stoddard Counties, MO, from Tumlison *et al*. (2012).

Distribution of *Dracunculus* sp. Infection in River Otters (*Lontra canadensis*) in Arkansas

R. Tumlison*, A. Surf, and H. May

Department of Biology, Henderson State University, Arkadelphia, AR 71999

*Correspondence: tumlison@hsu.edu

Running Title: *Dracunculus* sp. in River Otters of Arkansas

Mature females of the Guinea worm (*Dracunculus* sp.) are large and most commonly observed in the subcutaneous layers beneath the skin of extremities of certain mammals. *Dracunculus insignis* has been reported primarily from raccoon (*Procyon lotor*), but is known also from mink (*Neovison vison*), opossum (*Didelphis virginiana*), muskrat (*Ondatra zibethicus*), domestic dogs, and a variety of mustelids (Crites 1963; Ewing and Hibbs 1966; Crichton and Beverley-Burton 1974; Tumlison *et al*. 1984; Richardson *et al*. 1992). Historically, *Dracunculus insignis* was the only species of *Dracunculus* known in North America, so it was identified by default until Crichton and Beverley-Burton (1973) described *D. lutrae* from the river otter (*Lontra canadensis*) in Canada. Earlier reports of infection by *Dracunculus* sp. in otter occurred under the designation *D. insignis*, but Crichton and Beverley-Burton (1973) argued that *Dracunculus* in river otters throughout North America likely were *D. lutrae,* although definitive diagnosis requires examination of male specimens. In the absence of males, diagnosis was based on host.

Tumlison *et al*. (1984) first reported *Dracunculus* sp. in Arkansas from 3 species of mammals (raccoon, mink, and river otter). Because no males were located, the authors used host identity and personal communication with V. Crichton to suggest *D. lutrae* to be the species found in their river otter samples. More recently, use of DNA barcoding of the mitochondrial cytochrome c oxidase I (*cox1*) gene resulted in identification of both species of *Dracunculus* from river otters (Elsasser *et al*. 2009). Currently, species designation cannot be based on an assumption of host specificity, and identification lacking either males or use of DNA techniques is valid only to the genus level.

Although the fur trade generates a large number of carcasses in many states of the U.S. and provinces of Canada, few studies have attempted to examine the occurrence and geographic distribution of infection by *Dracunculus* sp. in furbearing mammals. Cheatum and Cook (1948) reported them in New York, Toll (1961) in Massachusetts, Crichton and Beverley-Burton (1974) in Ontario, Lauhachinda (1978) in Alabama, Tumlison *et al*. (1984) in Arkansas, and Barding and Lacki (2014) in Kentucky.

We contacted fur trappers and fur buyers in Arkansas through social media and personal communication, to secure carcasses of river otters for examination. We obtained and examined 184 skinned carcasses of river otters harvested during the December-January harvest seasons of 2013-2014, of which 29 (15.8%) were found to be infected by *Dracunculus* sp. In an earlier Arkansas study conducted in 1981-1982, Tumlison *et al*. (1984) found 17 of 105 otters (16.2%) were infected.

Tumlison *et al*. (1984) documented specimens of *Dracunculus* from 12 Arkansas counties (Ashley, Bradley, Conway, Craighead, Greene, Hot Spring, Jackson, Prairie, Pulaski, Randolph, White, and Woodruff). From the 30 Arkansas counties represented in the current sample, we report new records of *Dracunculus* sp. from the counties of Arkansas, Crawford, Franklin, Grant, Miller, Montgomery, Polk, Sebastian, and Sevier (Fig. 1). We found this nematode again in river otters from Bradley, Prairie, Pulaski, and White counties, previously reported by Tumlison *et al.* (1984). No males were found during our survey, but a sample of our specimens has been examined by use of *cox1* sequence analysis (Prosser *et al*. 2013), and all were *D. insignis* (M. J. Yabsley, College of Veterinary Medicine, University of Georgia, *pers. comm.*). It seems, then, that the report of *D. lutrae* in Arkansas (Tumlison *et al*. 1984) more likely represented *D. insignis*.

Besides documenting new county records of this parasite in Arkansas, examination of Fig. 1 reveals presence of the parasites in western portions of the state. It is likely that this parasite occurs in every county of Arkansas in which river otters also occur. The present report extends the known range of occurrence westward in the Arkansas and Ouachita River drainages, and for the first time documents occurrence in the Red River drainage.

The previous Arkansas study (Tumlison *et al*. 1984) reported finding usually 1 to 7 guinea worms per river otter, found mostly in the fascia of the legs. More intense infections including up to 32 immature female worms (50-100 mm length range) were discovered distributed on the head, neck, legs, back, abdomen, inguinal area, axilla, and under the latissimus dorsi of two river otters from Ashley County. A river otter from Conway County harbored 41 mature female dracunculids, located primarily in the legs. In the current study, we detected infection by 41 female *Dracunculus* sp. (140-300 mm length range) in a river otter from White County, and 7 other river otters harbored > 10 dracunculids. Generally, observations of infections were similar between the earlier and current studies.

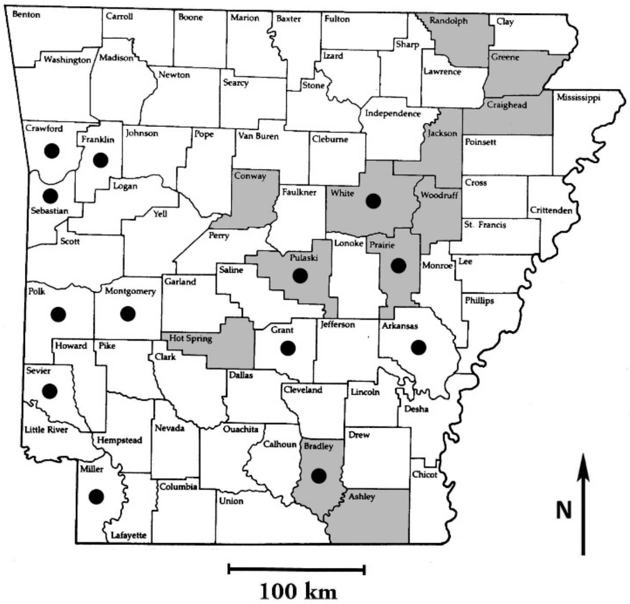

Figure 1. Geographic distribution of *Dracunculus* infections in river otters (*Lontra canadensis*) in Arkansas. Shaded counties represent historic records (Tumlison *et al*. 1984), and black dots represent records obtained during this study.

An interesting new observation during our study was the occurrence of oval cysts up to 30 x 60 mm in width and length, filled with up to 19 individuals of maturing female *Dracunculus* sp. Parasites in these cysts, located in the wrists and ankles of infected otters, ranged in length from 125-300 mm (Tumlison and Surf 2018). Once in the extremities, the parasites create ulcerations through which they deposit larvae into the water when available (Kimber and Kollias 2000).

Other authors have noted such structures to develop at the sites where female *Dracunculus* sp. create a lesion in the extremities of their hosts, allowing them to deposit larvae into the water (Crichton and Beverley-Burton 1977; Langlais 2003; Beyer *et al*. 1999), but none had reported large numbers of parasites within the lesions.

Of 105 otters examined in 1982, 17 (16.2%) contained specimens of *Dracunculus* sp., including 6 of 40 (15.0%) from the Ouachita Mountain physiographic region, 6 of 38 (15.8%) from the Mississippi Alluvial Plain, and 5 of 27 (18.5%) from the West Gulf Coastal Plain (Tumlison *et al*. 1984). In the present study, 12 of 87 (13.8%) otters from the Ouachita Mountains, 13 of 67 (19.4%) from the Mississippi Alluvial Plain, and 5 of 21 (23.8%) from the West Gulf Coastal Plain were infected. Infection rate for females (12 of 80, 15.0%) was only slightly lower than that for males (18 of 98, 18.4%).

Infection rates found in the previous and current studies indicate reasonably consistent infection rates over time. Examination of pelts of harvested river otters, and reports from trappers, reveal efforts by river otters to relieve skin irritation at the site of lesions in their extremities. Hair often has been rubbed off by either scraping or biting at the point of irritation. Based on harvest trends, the river otter population in Arkansas is believed to be stable, so infection by *Dracunculus* sp. does not appear to be a major health issue affecting the population.

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Distribution of the Highland Stoneroller (*Campostoma spadiceum*) in Southern Arkansas

R. Tumlison[1*] and H. W. Robison[2]

[1]*Department of Biology, Henderson State University, Arkadelphia, AR 71999*
[2]*9717 Wild Mountain Drive, Sherwood, AR 72120*

*Correspondence: tumlison@hsu.edu

Running Title: Distribution of *Campostoma spadiceum* in Arkansas

Under the name *Dionda spadicea*, Girard (1856) first described a form of stoneroller based on specimens collected in western Arkansas during an 1853 railroad survey. Variable morphologies of stonerollers created a complex taxonomic history, likely related to their genetic variability. Cladistic analysis of mitochondrial DNA data (cytochrome *b* gene) suggested the population in the Ouachita Mountains region of Arkansas and Oklahoma should be considered a distinct species (Blum *et al*. 2008). Molecular data currently are being examined by Blum and colleagues in further attempts to resolve the phylogeny of this group. Attempting to resolve the confusing nomenclatorial history of this fish from the Ouachitas, Cashner *et al*. (2010) soon redescribed it in morphological terms and renamed it *Campostoma spadiceum*.

Both male and female *C. spadiceum* present obvious red to red-orange coloration in median fins and often also in paired fins, which lasts year-round but is most intense during the summer. Even smaller individuals usually present this coloration, which is not found in contiguous populations of any other species of *Campostoma* (Cashner *et al*. 2010). We find this coloration to be less intense in the more southern part of its range in Arkansas compared to the illustrations in Cashner *et al*. (2010), although the coloration still is present to some degree in young and quite evident in adult females.

The appearance of nuptial tubercles also allows clear species identification for males in breeding condition (Fig. 1). Males of *C. spadiceum* have a single small tubercle on almost all dorso-lateral scales (forming rows of tubercles) whereas males of *C. anomalum* have larger tubercles not present on most scales and not forming rows.

Cashner *et al*. (2010) documented specimens of *C. spadiceum* in tributaries of the Arkansas and Red Rivers, and in the upper reaches of the Caddo, Ouachita, Saline, and Little Missouri drainages, all well above the fall line in the Ouachita Mountains regions of Arkansas and Oklahoma. These upland flowing-stream conditions provide the preferred habitat of clear water over gravel, rubble, and exposed bedrock substrates.

Tributaries of the Red River flowing from the Ouachita Mountains in Arkansas and Oklahoma have *C. spadiceum*, but tributaries of the Red River in western Oklahoma have *C. anomalum* (Cashner *et al*. 2010). Based on habitat preferences of the 2 species, it may be unlikely that both species would occur in sympatry. However, Cashner *et al*. (2010) shows one specimen of *C. spadiceum* in what appears to be Clear Boggy Creek in Oklahoma, though all other specimens shown

Figure 1. Comparison of tuberculation on adult males of *C. spadiceum* (left, with small tubercles in rows) and *C. anomalum* (right, with larger tubercles not in rows) from Arkansas.

from that stream were *C. anomalum*. Sympatry, or at least co-occurrence within the same drainage, seems possible.

Reaches of the same stream that flow through different geological regions may provide appropriate habitats for closely related species. Collections of *Campostoma* sp. in Arkansas (Robison and Buchanan 1988) have been made well downstream of the published collections of *C. spadiceum*, and well below the Fall Line of the Interior Highlands, into the West Gulf Coastal Plain (WGCP) in Arkansas (Foti 1974).

The map of known specimen localities in Arkansas provided by Cashner *et al*. (2010) represented mostly the headwater regions of the sampled streams, and left a large gap in the eastern part of the Ouachita Mountains in Arkansas. The one specimen located farther downstream in the Ouachita River (Clark Co., near Arkadelphia) was based on a photograph of a male specimen taken by RT. Further, *Campostoma* collections from farther downstream below the Fall Line (Robison and Buchanan 1988) have not been evaluated. We examined museum specimens from the Ouachita River drainage to determine whether *Campostoma* sp. in streams flowing southerly from their headwater reaches in the Ouachita Mountains in Arkansas (i.e., streams south of the Fourche Lafave River) were consistent with *C. spadiceum*.

Examination of preserved specimens of stonerollers from the Ouachita Mountains and southward, housed in the Henderson State University collection of fishes, allowed positive identifications based on the nature of nuptial tubercles in male specimens. In all cases, those specimens were *C. spadiceum*. Collection localities of juveniles and females were within the range of the verified males, so identification of these was assumed by location. Fin coloration fades after preservation, so this character was not useful in identification of preserved specimens of females and juveniles. Thus, the possibility of sympatric presence of *C. anomalum* in the lower part of the Ouachita Mountains region cannot be excluded. We used separate map characters to plot known localities based on males and presumed localities based on females and juveniles to produce a detailed map of distribution of *C. spadiceum* in southern Arkansas below the Fourche Lafave River (Fig. 2).

Most locations presented by Cashner *et al*. (2010) represented headwater locations of several stream systems. For example, they documented this stoneroller at 1 location in the upper Saline River drainage (eastern Arkansas), 3 locations in the upper reaches of the Caddo River, 3 locations in the extreme upper Ouachita River drainage, 1 location in the extreme upper Little Missouri River drainage, 2 locations in the upper Cossatot River drainage, and 1 location in the extreme upper portion of the Rolling Fork tributary to Little River.

We are able to document occurrence of *C. spadiceum* in numerous tributaries in the upper Saline River, throughout the Caddo River to its confluence with the Ouachita River, throughout the Ouachita and many of its tributaries down close to the confluence of the Little Missouri River, in the upper reaches of the Little Missouri River drainage, throughout the Cossatot River, and down the Rolling Fork River and Little River to near the mouth of the Cossatot. Historical records (Robison and Buchanan 1988) indicate occurrence of *Campostoma* specimens of unverified identification far down the Saline, throughout the lower Little Missouri drainage, and even farther down the Ouachita drainage well into the WGCP ecoregion (Fig. 2).

Campostoma spadiceum is common in streams throughout the highland Ouachita Mountains ecoregion, and below the Fall Line into the lowland WGCP in those same drainages. A separate portion of the WGCP, called the Southwest Arkansas section (Foti 1974; Fig. 2) borders the Ouachita Mountains. It is distinguished from the majority of the WGCP by Cretaceous origins rather than Eocene (which formed the deposits covering most of the WGCP), and tends to be more elevated than the rest of the WGCP. This area encompasses many of the locality records for *C. spadiceum* S of the Fall Line. Still, verified occurrences of *C. spadiceum* exist into the more lowland regions of the WGCP along the Ouachita River. Historical occurrences even farther down the Ouachita, Red, and Saline systems still require validation of species identification. Hypothetically the lowland habitat might be occupied by *C. anomalum*.

Acknowledgments

We thank the numerous students in Ichthyology classes at Henderson State University who collected many of the specimens used in this study.

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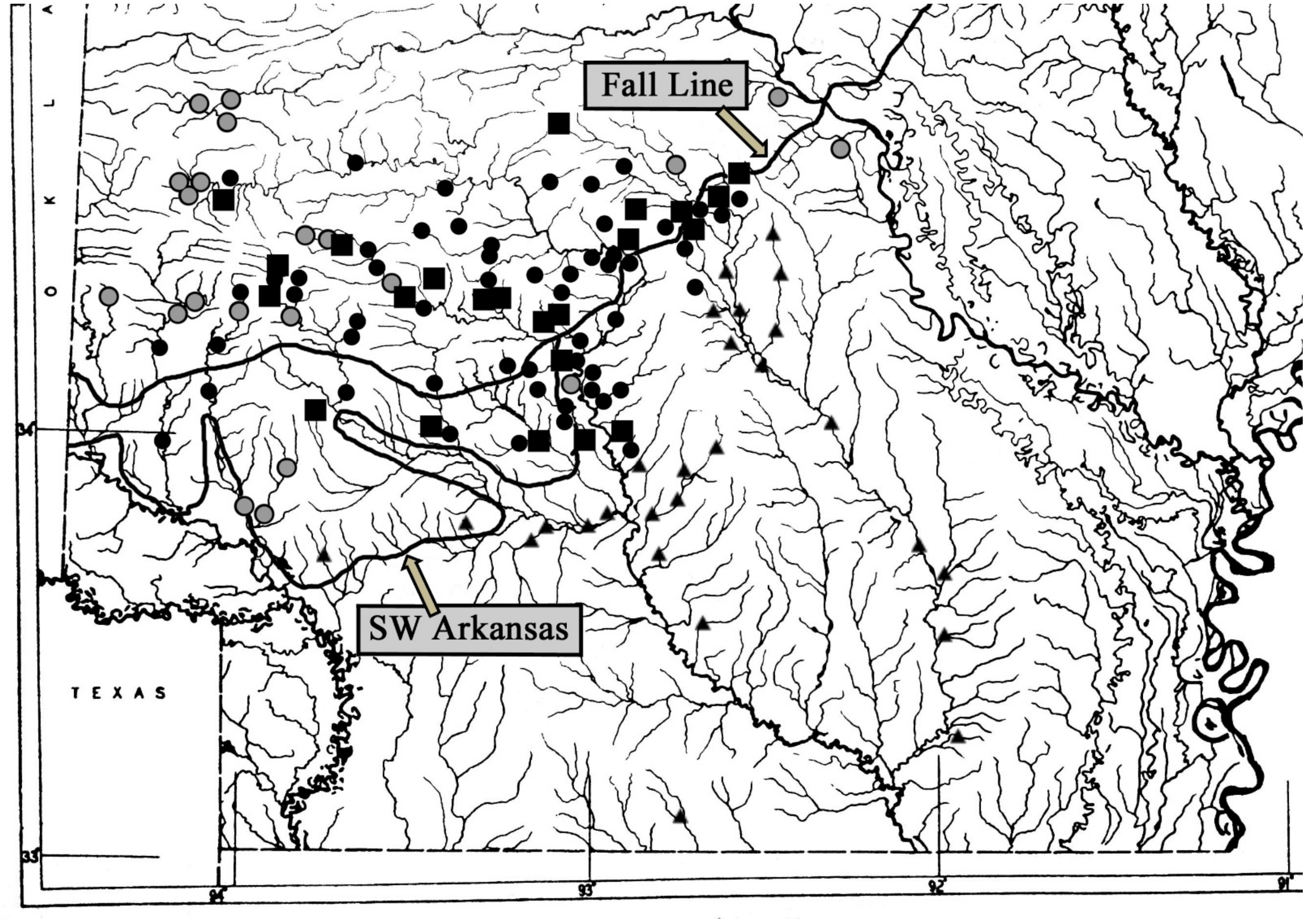

Figure 2. Currently known distribution of the Highland Stoneroller (*Campostoma spadiceum*) in southern Arkansas. Gray-centered circles represent records from the original description (Cashner *et al*. 2010), black squares represent males from the HSU collections positively identified by nuptial tubercles, and black dots represent females and juveniles in the HSU collections. Triangles represent historical records (Robison and Buchanan 1988) found below the Fall Line, which are now of uncertain identification (they hypothetically could be *C. anomalum* based on lowland habitat). The Fall Line is the demarcation separating the Interior Highlands from the southeastern lowlands in Arkansas, and the SW Arkansas section represents a special upland portion of the West Gulf Coastal Plain (Foti 1974).

Incidental Captures of Plains Spotted Skunks (*Spilogale putorius interrupta*) by Arkansas Trappers, 2012-2017

D.B. Sasse*

Arkansas Game and Fish Commission, Mayflower, AR 72106

*Correspondence: blake.sasse@agfc.ar.gov

Running Title: Incidental Captures of Spotted Skunks

The Plains spotted skunk (S*pilogale putorius interrupta*) has historically been found between the Mississippi River and the Rocky Mountains in the central United States (Sasse 2017). Since the 1940s there has been a severe population decline due to causes, possibly including overharvest, that have yet to be conclusively determined which resulted in the filing of a petition to the U.S. Fish and Wildlife Service to list this subspecies as endangered in 2011 (Gompper and Hackett 2005, U.S. Fish and Wildlife Service 2012). Although considered a species of greatest conservation need in Arkansas (Fowler 2015), the harvest of spotted skunks is legal during the regulated trapping season. The species is rarely targeted by trappers and the commercial harvest of spotted skunks has essentially ended (Sasse and Gompper 2006). The purpose of this study was to determine the extent to which the plains spotted skunk is incidentally captured by trappers targeting other species.

Following the conclusion of the 2012-2016 Arkansas trapping seasons, surveys were distributed to everyone who obtained resident and non-resident trapper permits. For the 2012 and 2013 seasons, paper forms with postage-paid return envelopes were mailed to trappers while for the 2014 through 2016 seasons links to an internet-based survey portal were emailed to all permitted trappers with email addresses and paper forms were mailed to those that did not respond to the electronic survey or for whom no email address was available. Both the paper and electronic surveys requested that trappers report the number of nights trapped, the average number of traps set per night, and the county in which any spotted skunks were captured that season.

Post-season survey response rates were 37-46% and 49-65% of respondents reported setting traps each year. Data is reported solely from those that responded to the survey and does not represent an estimate of statewide trapping effort or spotted skunk harvest.

Trappers that reported spotted skunk captures were mailed a follow-up survey requesting additional information including the type of trap, bait, and lure used and were provided with physical descriptions and photographs of spotted skunks and striped skunks (*Mephitis mephitis*) to aid in confirming the species captured. Trappers were requested to submit photographs of these captures when available. Information obtained from six trappers that captured spotted skunks were excluded as they were not relevant to analysis of the impact of recreational trapping seasons; three were attempting to capture nuisance skunks, one was targeting spotted skunks in particular for taxidermy purposes, and two were attempting to capture rabbits and were not actively trapping furbearers.

Trap nights were calculated by multiplying the number of traps set and days trapped for all trappers that responded to this question. If a range of traps or dates was provided, the lesser number was selected for analysis. A few respondents reported trapping more days than were open during recreational seasons, primarily those involved in nuisance beaver and coyote control which can be conducted year-round, and their answers were adjusted to the maximum allowable number of recreational trapping days.

From 2012-2017 a total of 132 trappers that were targeting other species reported capturing spotted skunks. Based upon the follow-up survey, 42 reports of spotted skunk captures were confirmed from trappers that caught a total of 60 spotted skunks. The remaining 90 reports represent trappers that made a mistake while filling out the survey form, reported that they actually caught striped skunks or did not respond to the follow-up survey.

Incidental captures were rare; only 0.35-1.29% of trappers each year caught spotted skunks (Table 1). During the study period confirmed captures came from 32 trappers that caught 1 skunk, 1 trapper caught 2 skunks in 1 season, 5 trappers caught 1 skunk each in 2 seasons, 1 trapper caught 1 skunk in 1 season and 3 in another season, and 1 trapper caught 1 skunk in 1 season and 11 in another season. The unusual capture of 11

Table 1. Trapping effort and incidental capture of spotted skunks in Arkansas, 2012-2017.

Season	Season Length (days)	Permits Issued	Survey Respondents That Trapped This Season	Reported Trap Nights	Confirmed Spotted Skunk Captures	Survey Respondents that Trapped and Had Confirmed Captures (%)
2012	111	4,369	1,086	1,008,270	14	14 (1.29)
2013	112	5,457	1,307	1,057,396	26	14 (1.10)
2014	113	5,590	1,428	887,089	6	5 (0.35)
2015	114	5,044	1,163	593,013	6	6 (0.52)
2016	109	4,526	960	451,687	8	8 (0.83)

individuals in 1 season was by a trapper that had been targeting bobcats with a homemade bobcat lure containing spotted skunk scent glands.

The spotted skunk was found throughout much of the state as recently as the late 1990s and early 2000s (Sasse and Gompper 2006), although recent records outside the Ozarks and Ouachitas are rare except for a spotted skunk that was hit by a car in Hempstead County in the spring of 2016 (Arkansas Game and Fish Commission, unpublished records). The region of capture was determined for 59/60 skunks and they were caught primarily in the Ozark (31) and Ouachita (26) regions with only two from the Gulf Coastal Plain and none from the Delta even though most reported trap nights over these five seasons came from the Delta (31.4%) and Gulf Coastal Plain (27.1%) with less reported effort in the Ozarks (25.1%) and Ouachitas (16.5%)(Figure 1).

The species being targeted in 71% of the traps that captured spotted skunks and for which the target species is known were bobcat (*Lynx rufus*) (20), raccoon (*Procyon lotor*) (18), and a combination of bobcat and raccoon (3). Other target species were fox (*Urocyon cinereoargenteus* or *Vulpes vulpes*) (4), fox and bobcat (4), bobcat and coyote (*Canis latrans*) (2), raccoon and fox (2), bobcat, fox, and coyote (1), bobcat, fox, and raccoon (1), raccoon and opossum (*Didelphis virginiana*) (1), coyote (1), and mink (*Mustela vison*)(1).

Spotted skunks were captured using a broad spectrum of traps including traditional foothold traps (34), dogproof foothold traps (12), body-gripping traps (11), and box traps (2). No spotted skunks were captured with the use of snares. Foothold trap captures were made with size 1.5 (21), 1.65 (1), 1.75 (1), 2 (9), and 3 (2) traps and body-gripping traps were in trap sizes 110 (2), 160 (8), and 220 (1).

Bait use was reported for 23 captures and included fish (10), beaver (*Castor canadensis*) (4), marshmallows (2), dog food (1), cat food (1), mix of dog food and deer (*Odocoileus virginianus*)(1), mix of cat food and sardines (1), marshmallows and grape jelly (1), marshmallow and cherries (1), and a mix of mice and rat carcasses with fried chicken (1).

Scent lure was reported for 42 spotted skunk captures and included homemade bobcat lure with spotted skunk glands (11), fish oil (5), beaver castor (3), fox lure or urine (3), bobcat lure (3), Carman's Canine Call (2), fish oil with anise (1), fish sauce (1), beaver and bobcat gland lure (1), Carman's Coon #1 (1), cat lure and urine (1), coyote urine (1), Dobbin's Back Breaker (1), Lenon's Fox All Call (1), mixture of beaver castor, bobcat urine, Taylor predator bait, and Long Distance Call (1), Caven's Predator Plus (1), Caven's Minnesota brand lure (1), a crayfish-based raccoon lure (1), Carman's Pro Choice (1), a mixture of Voodoo Lure and beaver castor (1), and an unspecified type of homemade lure (1).

Only 24/58 (41%) spotted skunk pelts with known fates were sold by trappers. Fur purchase records are often used to track harvest, but in states where spotted skunk take is legal may significantly underestimate actual harvest since most incidental catches are not sold.

While only a few states allow trapping of plains spotted skunks, the this study suggests that trap types, lures, and baits commonly used to target other species occasionally result in the capture of spotted skunks when present. However, there is no evidence that such incidental take has a negative impact on regional or statewide populations.

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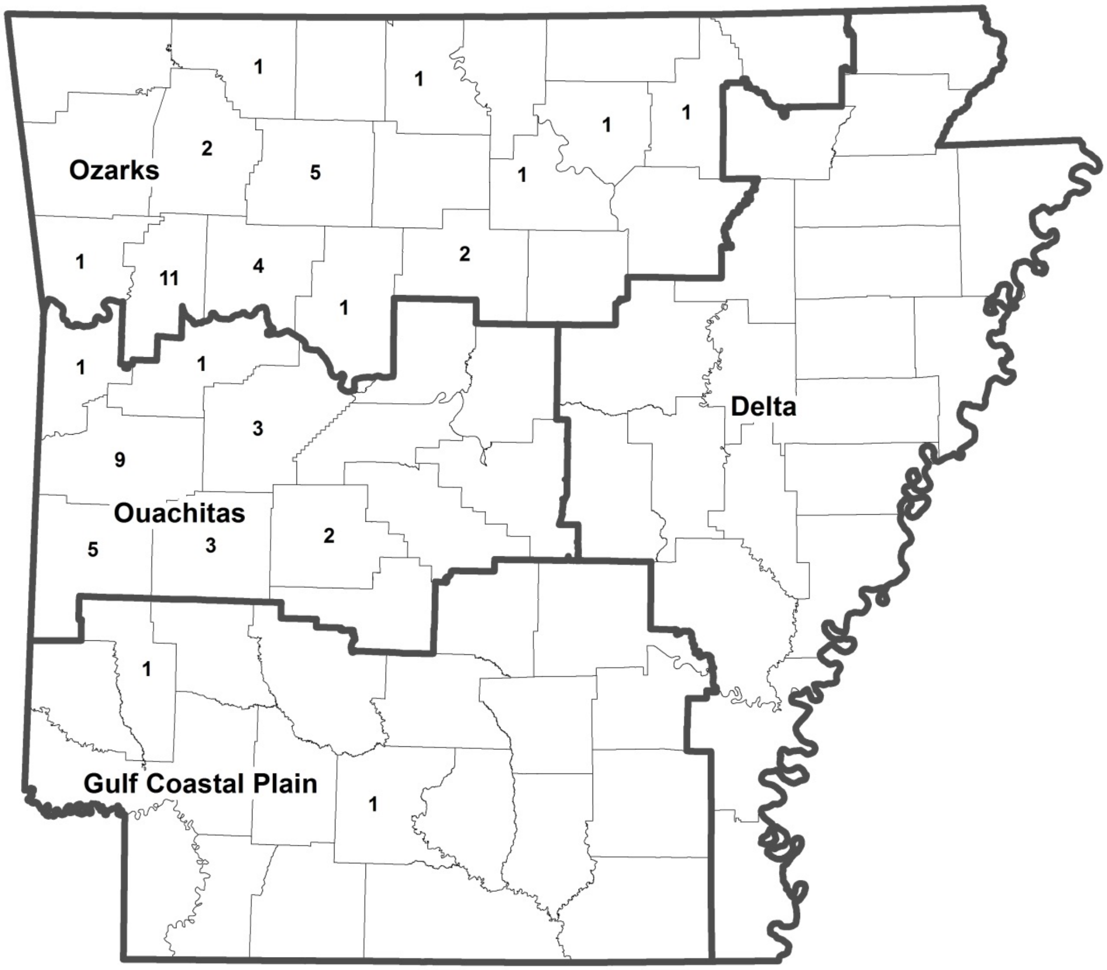

Figure 1. Number of incidental spotted skunk captures by county, 2012-2017.

Primeness in Early Season Arkansas Raccoon Pelts

D.B. Sasse[*]

Arkansas Game and Fish Commission, Mayflower, AR 72106

*Correspondence: blake.sasse@agfc.ar.gov

Running Title: Primeness in Early Season Arkansas Raccoon Pelts

Due to historical overharvesting, the trapping of furbearers is now a highly regulated activity with legal trapping being restricted to certain time periods that are congruent with when fur is in prime condition and most valuable (Hamilton and Cook 1946; Linscomb 1987). Although there are no objective criteria, a commonly accepted definition of prime is “the fur has reached its maximum length, density and finest texture; when the hairs have matured with seemingly no pigment being produced, and as a consequence, the flesh surface of the pelt appears devoid of hair root pigmentation.” (Linde 1963). Reduced hours of daylight during the fall appear to stimulate the start of the priming process (Worthy *et al*. 1987).

Research on when furbearer pelts are in prime condition is surprisingly sparse and as primeness is subjective, can be difficult to perform (Applegate and Predmore Jr 1947; Chabreck and Dupuie 1970; Kellog 1946; Kellog 1947; Linde 1963; Markley 1947). Stains (1979) characterized prime dates of many furbearing species to a great extent based on the opinion of one experienced fur dealer. The only analyses of priming in raccoon (*Procyon lotor*), one of the most commonly trapped furbearers in the United States, were done with pelts from Indiana and Michigan (Stuewer 1942) and Georgia (Hon 1981) and may not be applicable to Arkansas due to latitudinal differences in priming patterns caused by changes in day length.

The purpose of this study was to determine if the current start to the trapping season on the second Saturday in November is either too early or too late based on primeness patterns of Arkansas raccoons.

Raccoons were captured by staff from the Arkansas Game and Fish Commission and cooperating agencies using traditional trapping techniques from November 1-30 of 2014 and 2015. Raccoons were identified to sex and age, although one individual was not assigned an age but is included in later testing involving all raccoons. Two raccoons captured on October 31, 2015 were included in the study and analyzed with the November 1-15 and week 1 records. Raccoons were then skinned and the pelts stretched and dried. Pelts were gathered together and following the conclusion of each season were taken to five experienced Arkansas fur dealers for grading. Pelts were graded as unprime, coming on to prime, or prime (Obbard 1987). When there was not complete agreement among fur dealers as to the grade for a pelt, it was assigned a grade based upon the majority view of the dealers. In order to account for differences in what each fur dealer considered prime, pelts were combined into prime and unprime categories for analysis under both liberal and conservative views of prime. Only pelts graded as prime were considered prime in the conservative system. Under the liberal system, pelts graded as prime and coming on to prime were combined together as prime since dealers indicated that they would generally purchase those pelts for prices similar to that they would give for prime pelts.

Differences in proportions of pelts that were prime were examined using Fishers exact test. Fishers exact test with the Bonferroni correction was used to compare weekly differences in percentages of prime pelts. Logistic regression was used to examine the probability of primeness based on Julian date.

A total of 122 raccoons (75 male:47 female) were captured with the majority (74%) being adults. Effort was not distributed evenly across the state; 58 (48%) of captures were from southern Arkansas (Bradley, Hempstead, Howard, Jefferson, Lafayette, Pike, Sevier counties), 37 (30%) were from central Arkansas (Faulkner, Pulaski, Saline, White counties) and 27 (22%) from northern (Benton, Boone, Cleburne, Craighead, Washington counties) Arkansas.

There were no significant differences in primeness between sexes over the entire month either in juveniles or adults. However, when the first half of November is compared to the last half of November, adults had a higher percentage of prime pelts later in the month under both grading systems and when graded conservatively, adult males and all raccoons were more likely to be prime later in the month. Adults were significantly more likely to be prime under both the conservative ($p= 0.0009$) and liberal ($p= 0.101$) grading systems (Table

1). The only significant difference in primeness within regions was that adult raccoons in the south region were less likely to be prime in the first half of November than later that month (p=0.0031). There was a significant difference between regions (p= 0.0481) however when pairwise comparisons were made none were significant, most likely due to small sample size.

Similarly, weekly analyses found significant differences in percentage of prime pelts among adult males, all adults, and all raccoons when graded conservatively and among all adults and all raccoons when graded liberally (Table 2). However, the only significant weekly pairwise comparisons were for all raccoons between weeks 1 and 4 when graded conservatively (p = 0.0064) and between weeks 1 and 2 (p = 0.0041) and weeks 1 and 4 (p = 0.001) when graded liberally. We calculated a logistic regression between primeness and Julian date using all adults and this also indicated a higher probability of pelts being prime later in the month using both the conservative (X^2=3.38, p<0.001) and liberal (X^2=3.01, p=0.003) grading systems (Figure 1).

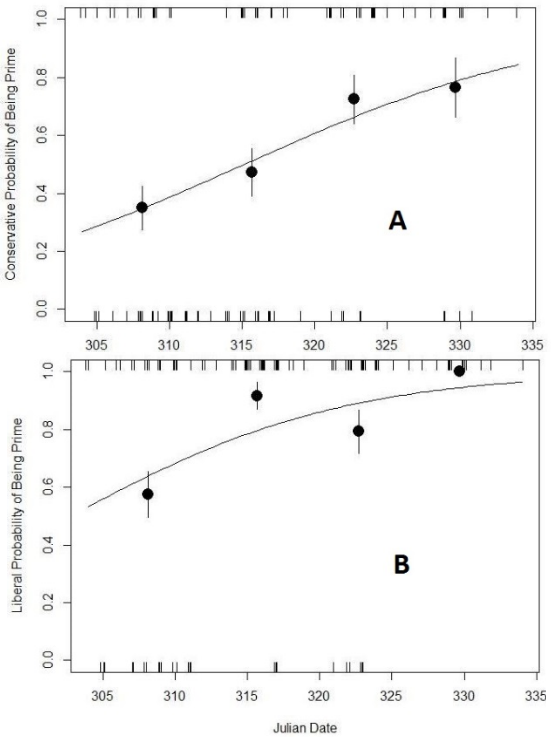

Figure 1. Probability of a raccoon pelt being in prime condition by Julian date under the conservative (A) and liberal (B) grading systems. Plotted values represent mean probability of being prime and error bars represent standard error of a binomial proportion. Lines correspond to the logistic regression of primeness by Julian Date.

Although there are no guidelines as to what percentage of furs must be prime to be acceptable, Hon (1981) suggested that at least 80% of furs should be prime to be considered a good fur market. This study found that under the conservative grading system this percentage of prime furs is not reached until the last half of November or approximately Julian Date 330 or later.

The dealers involved in grading the pelts used in this study indicated that they would generally buy pelts that were “coming on to prime” as if they were prime, which suggests that the liberal grading system may be more representative of the prices received by Arkansas trappers and thus drive trapping effort. When graded liberally the Julian Date by which 80% of furs were prime was approximately 10 days earlier (Julian date 320) than under the conservative grading system.

This study indicates that starting the trapping season in the last half of the month may maximize the percentage of pelts that are in prime condition early in the season, especially in the southern region of the state.

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Table 1. Arkansas raccoon pelt grades by half month, November 2014 and 2015. P value is based upon the Fishers exact test.

	Nov 1-15		Nov 16-30		
	Prime	Unprime	Prime	Unprime	*p*
Conservative Grade					
Juveniles	7	17	2	6	1
Adults (all)	24	27	32	6	0.0004
Males	15	17	23	4	0.0028
Females	9	10	9	2	0.1213
All raccoons	31	45	34	12	0.0004
Liberal Grade					
Juveniles	16	8	4	4	0.4325
Adults (all)	40	11	36	2	0.0366
Males	26	6	26	1	0.1120
Females	14	5	10	1	0.3717
All raccoons	56	20	40	6	0.1106

Table 2. Percentage of Arkansas raccoon pelt in prime condition by week, November 2014 and 2015. P value is based upon the Fishers exact test.

	Week 1		Week 2		Week 3		Week 4		
	Prime	Unprime	Prime	Unprime	Prime	Unprime	Prime	Unprime	*p*
Conservative Grade									
Juveniles	2	8	5	8	1	5	1	2	0.7910
Adults (all)	12	16	12	11	18	4	14	2	0.0034
Males	8	9	7	8	15	2	8	2	0.0214
Females	4	7	5	3	3	2	6	0	0.0816
All raccoons	14	25	17	19	19	9	15	4	0.0053
Liberal Grade									
Juveniles	4	6	11	2	2	4	3	0	0.0290
Adults (all)	19	9	21	2	20	2	16	0	0.0167
Males	12	5	14	1	16	1	10	0	0.0918
Females	7	4	7	1	4	1	6	0	0.3423
All raccoons	23	16	32	4	22	6	19	0	0.0006

Additional County Records of Invertebrates from Arkansas

C.T. McAllister[1,*], H.W. Robison[2], and R. Tumlison[3]

[1]*Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745*
[2]*9717 Wild Mountain Drive, Sherwood, AR 72120*
[3]*Department of Biology, Henderson State University, Arkadelphia, AR 71999*

[*]Correspondence: cmcallister@se.edu

Running Title: Invertebrate County Records of Arkansas

Arkansas is home to a great variety of invertebrate biodiversity. Within the last decade, natural history observations on various invertebrates of the state were reported by Tumlison and Robison (2010) and Tumlison *et al.* (2016). Here, we document additional noteworthy natural history and ecological observations for selected invertebrates from Arkansas.

Field observations and collections were done by the authors and others between 1968 and 2018. Crayfishes were preserved in 70% or 90% v/v isopropanol and are housed at Southern Arkansas University (SAU). Here, we follow the recent updated classification of Crandall and De Grave (2017) for the crayfish family Cambaridae; the number of collected specimens are given in parentheses. Leech identification was made using Klemm (1982) and Williams and Burreson (2005) and voucher specimens in 95% (v/v) DNA grade ethanol are deposited in the Peabody Museum of Natural History (YPM), Yale University, New Haven, Connecticut. Centipede identification was made from field photographs (images available from CTM). Fishes (with invertebrates) were collected with a gill net or backpack electroshocker (DC current) and measured for total length (TL) and a turtle was collected by hand and measured for carapace length (CL). Voucher specimens of land planarians and horsehair worms are deposited in the Henderson State University Collection, Arkadelphia, Arkansas. Latitude and longitude (WGS 84) or township, section, and range are reported when known. Author's initials (CTM, HWR, RT) are also given as collectors.

Twenty-two invertebrate county records in Arkansas are listed below for 8 taxa in an annotated format as follows:

PLATYHELMINTHES: TURBELLARIA: GEOPLANIDAE

***Bipalium kewense* Moseley, 1878 – land or "hammerhead" planarian**. The land planarian, *B. kewense* was first described from a greenhouse at Kew Botanical Gardens near London, England. Although native to tropical SE Asia (Winsor 1983), *B. kewense* has been observed across the southern US (Ducey *et al.* 2007). It has likely been introduced by the international plant trade, as this planarian is frequently found associated with tropical plant pots (Winsor 1983). Indeed, they are commonly observed in the soil of potted plants in greenhouses, and have become established in natural habitats across the coastal southern US (Dundee and Dundee 1963; Ducey *et al.* 2007). This hermaphroditic species is easily identified by its diagnostic spade-like head and bi-colored yellow-brown body. This land planarian was previously documented in Arkansas by Daly and Darlington (1981) from the counties (and cities) of Faulkner (Conway), Ouachita (Camden), and Pulaski (Little Rock) (Fig. 1). These authors also noted that *B. kewense* was found in Little Rock after heavy rains on driveways; otherwise, their specimens were discovered under wet boards, logs, rotting trees, railroad ties, and concrete patio slabs. Other previous reports of *B. kewense* in the state include Tumlison and Robison (2010) who reported specimens from Clark (Arkadelphia) and Columbia (Magnolia) counties, and Tumlison *et al.* (2016) who found specimens in Jefferson (Pine Bluff) and Pope (Russellville) counties. In addition, use of media outlets allowed Daly and Matthews (1982) to locate areas where specimens could be obtained from the Little Rock/North Little Rock area, where they collected 26 specimens, indicating that the species was established at multiple localities.

The following new county records are documented for *B. kewense* as follows: ***Ashley Co.,*** Crossett, spring 1968, RT; ***Chicot Co.,*** Lake Village, 3 Jun. 2014, HWR; ***Dallas Co***., Fordyce (Sec. 34, T10S, R13W), 25 May 2001, HWR; ***Polk Co.,*** Mena, 30 May 2016, C. Holmes. This latter record is not only a new county record for Arkansas, but it also documents the westernmost record of the species in the state. Further west in neighboring Oklahoma, this species has been reported only from a greenhouse in Stillwater, Payne Co., but not from any natural habitats (Wallen 1954). Establishment of this species of land planarian is of concern because it can be detrimental to earthworm populations, on which they feed on by apparently using a tetrodotoxin neurotoxin for paralysis (Dickens *et al.* 2014).

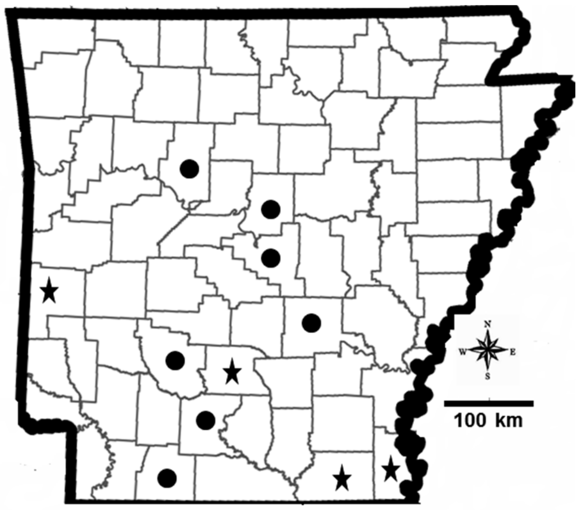

Figure 1. County distribution of *Bipalium kewense* in Arkansas. Solid dots = 7 previous historic records; stars = 4 new county records.

CRUSTACEA: DECAPODA: CAMBARIDAE

Cambarus ludovicianus* Faxon, 1884 – Painted Devil Crayfish**. In his unpublished thesis, Reimer (1963) reported this primary burrower as *C. diogenes ludovicianus* from 4 counties in southern Arkansas. This crayfish is common in Arkansas where it inhabits large burrows in lotic habitats on the Coastal Plain of the southern and southwestern parts of the state (Robison *et al.* 2017). Herein we report 3 new county records as follows: ***Bradley Co., roadside ditch along St. Hwy. 15, 6.4 km NE of Moro Bay (Sec. 11, T16S, R12W), 24 May 1980, HWR (1); ***Clark Co.,*** Saline Bayou at St. Hwy. 7 bridge (34.11737°N, 93.03074°W), 15 Mar. 2007, RT (1); ***Dallas Co.***, in burrow *ca.* 4.0 km N of Fordyce (Sec. 3, T10S, R13W), 19 May 1981, HWR (1).

Procambarus clarkii* (Girard, 1852) – Red Swamp Crayfish**. Reimer (1963) reported this tertiary burrower from 9 counties with lentic and lotic habitats (and burrows) on the Mississippi Alluvial Plain of central and eastern Arkansas in Clay, Chicot, Craighead, Crittenden, Greene, Jackson, Mississippi, Monroe, and Pulaski. This crayfish is commonly raised by commercial crayfish producers in the eastern portion of the state for human consumption and has become a serious introduced agricultural pest (Huner 1977). We herein add 4 additional new counties to its range in Arkansas as follows: ***Lee Co., Bear Creek Lake, *ca.* 6.4 km SE of Marianna (Sec. 9, T1N, R4E), 4 Mar. 1982, HWR (1); ***Phillips Co.***, roadside ditch at Big Creek at side of US 49, *ca.* 6.4 km E of Marvell (Sec. 36, T1S, R2E), 5 Mar. 1982, HWR (1); and ***St. Francis Co.,*** roadside ditch along county (gravel) road in Madison (Sec. 36, R3E, T5N), 4 Mar. 1982, HWR (1); ***Union Co.,*** Felsenthal NWR near Ouachita River at US 82 bridge (33.15098°N, 92.11298°W), 5 Oct. 2008, RT (1 brooding female, photovoucher).

Procambarus viaeviridis* (Faxon, 1914) – Vernal Crayfish.** Reimer (1963) reported the habitat of this crayfish species in Arkansas as "lakes, bar pits, and bayous." It can also be taken from sluggish streams and lentic situations on the Mississippi Alluvial Plain of eastern Arkansas (Robison *et al* 2017). Reimer (1963) reported 5 localities for this burrower in southeastern Arkansas from Lincoln County. An older additional record from the St. Francis River at Greenway, Clay County is known (Faxon 1914). We document 4 new county records for *P. viaeviridis* as follows: ***Ashley Co., 8.0 km SW of Hamburg on US 82, 16 Mar. 1967, J. Cooper (32); ***Bradley Co.***, roadside ditch and culvert, 4.5 km E of Banks, and jct. of St. Hwys. 275 and 4, 18 Apr. 1986, HWR (3); ***Desha Co.***, backwaters of the Arkansas River at Pendleton, 22 Apr. 1981, HWR (2); ***Greene Co.***, roadside ditch *ca.* 3.1 km S of Clay Co. line on St. Hwy. 135, 12 Apr. 1985, HWR (4).

NEMATOMORPHA: GORDIIDA: GORDIIDAE

***Gordius* sp. (“*complex*”) No common name (NCN). –** A single horsehair worm of the *Gordius* sp. (*complex*) was found by CTM in the stomach contents of a 500 mm TL Smallmouth Buffalo (*Ictiobus bubalus*) collected on 9 Feb. 2018 from the Little River, Little River County (33.6129767°N, 93.8217663°W). Cochran *et al.* (1999) provided a summary of fishes known to have eaten horsehair worms. However, this is the first time, to our knowledge, that a nematomorph has been found in the stomach of *I. bubalus*. Robison *et al.* (2012) reported *Gordius* sp. from 8 counties in the state and Little River County represents a new county record.

Two additional county records for *Gordius* sp. are documented as follows: ***Clark Co.,*** Arkadelphia, 10 Sept. 2007, RT (1 photovoucher); ***Union Co.,*** Felsenthal NWR near Ouachita River at US 82 bridge (33.15124°N, 92.11257°W), 6 May 2007, RT (1 photovoucher).

ANNELIDA: HIRUDINIDA: GLOSSOPHONIIDAE

***Placobdella parasitica* (Say, 1824) – NCN.** A single *P. parasitica* (YPM) was removed by CTM from the upper plastron of an adult female (295 mm CL) eastern river cooter (*Pseudemys concinna*) collected on 24 Mar. 2018 from Clear Creek at Savoy, Washington County (36.104939°N, 94.332358°W) (Fig. 2A). As far as we can determine, this represents the third time *P. parasitica* has been reported from this host as Moser (1995) documented *P. parasitica* from an eastern river cooter from Oklahoma, and Moser *et al.* (2006) found it on *P. concinna* in northcentral Arkansas. This turtle leech has been previously reported from Arkansas, Conway, Fulton, Independence, St. Francis, and Van Buren counties (Moser *et al.* 2006). It has also been reported from a variety of chelonian hosts (Moser *et al.* 2006). We here report a new county record for *P. parasitica* in northwestern Arkansas.

PISCICOLIDAE

***Cystobranchus klemmi* (Williams and Burreson, 2005) – NCN.** A single individual (Fig. 2B) of *C. klemmi* (YPM) was taken by CTM from the caudal fin of a tuberculate male (175 mm TL) Central Stoneroller (*Campostoma anomalum*) on 23 Mar. 2018 collected from Flint Creek at Gentry off US 59, Benton County (36.242732°N, 94.487531°W). This represents a new county record in Arkansas for *C. klemmi*. Fourteen other *C. anomalum* from the same site/date were not infested with any leeches. *Cystobranchus klemmi* is primarily found on various stonerollers (Williams and Burreson 2005, Richardson *et al.* 2013) but the host list also includes other cyprinids such as Southern Redbelly Dace (*Chrosomus erythrogaster*), Bigeye Shiner (*Notropis boops*) and Creek Chub (*Semotilus atromaculatus*) (Richardson *et al.* 2013, Thigpen *et al.* 2015). This leech has now been reported from 14 counties of the state and also from sites in Illinois, Missouri, and Oklahoma (Williams and Burreson 2005, Richardson *et al.* 2013, Thigpen *et al.* 2015).

ARTHROPODA: CHILOPODA:
SCOLOPENDROMORPHA: SCOLOPENDRIDAE

Scolopendra heros* Girard, 1853 – Giant Red-Headed Centipede.** This largest North American centipede is brightly colored and has been reported previously from 17 counties in the state (Shelley 2002, McAllister *et al.* 2003, 2006, 2010). Here, we document 2 new county records as follows: ***Polk Co., Shady Community (34.449830°N, 94.120204°W), 21 Aug. 2012; ***Van Buren Co.***, Sugarloaf Mountain, 26 Aug. 2017.

Figure 2A–B. Two leeches from Arkansas vertebrates. A. *Placobdella parasitica* from *Pseudemys concinna*. B. *Cystobranchus klemmi* from *Campostoma anomalum*. Each line on ruler scale = 1 mm.

In summary, we document 22 new county records for various invertebrates of the state. Additional Arkansas county records will be reported in the future as more invertebrates become available from field observations and collections. This should help expand the biological knowledge of this important biota of the state.

Acknowledgments

The Arkansas Game and Fish Commission (AG&F) provided Scientific Collecting Permits to the authors. We thank Eric Brinkman, Jim Cunningham, and Noah Moses (AG&F, Hope) for providing the *I. bubalus*, and Ellie Smith for one of the centipedes. We also thank Dr. Dennis J. Richardson (Quinnipiac University, Hamden, CT) for use of the electroshocker and the McAllister boys, James T. III (University of Arkansas-Fayetteville) and Nikolas H. (Lukfata Elementary, Broken Bow, OK) for assistance with collecting at Clear and Flint creeks.

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ARKANSAS ACADEMY OF SCIENCE

102ndAnnual Meeting April 6-7, 2018
Arkansas State University, Jonesboro

Secretary's Report
MINUTES OF THE 102nd MEETING

ARKANSAS ACADEMY OF SCIENCE SPRING 2018 BUSINESS MEETING MINUTES

April 7, 2018 – 12:00 noon.
Arkansas State University, Jonesboro

The meeting was called to order at 12:09 p.m. by President R. Panneer Selvam.

1. **President's Report**

Panneer welcomed everyone and reported he had a great time at the meeting. He congratulated Andy Sustich and Rajesh Sharma for a job well done. He commented that the AAS had made great progress during the last few years and many people were involved in making that happen. He hoped even more people would take a leadership role in Academy efforts.

2. **Local Arrangements Committee: Rajesh Sharma**

There have been 215 people registered for the meeting including 88 faculty, 126 students, and one sponsor. 150 abstracts are included in the meeting book, including 78 oral presentations and 72 poster presentations. Areas of presentations included Biological Sciences, Chemistry, Physics, Engineering, Computer Science, and Geosciences.

17 universities and 5 government agencies and other institutions were represented at the meeting.

This meeting would not be possible without the efforts of Emily Devereux and the staff of the A-State Research and Technology Transfer Office.

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

Secretary Collis Geren requested help in getting a complete list of all members e-mail addresses.

4. **Treasurer's Report: Mostafa Hemmati**

Mostafa Reported it was a good year for the Academy with a net gain of more than $15K. The full report is available in the Executive Committee Meeting report.

The report was reviewed by the Auditing Committee (Dr. Collis Geren and Dr. Ivan Still, who verified all calculations). All is well, and thanks go to Mostafa for good management (see AAS financial statement in appendix.)

5. **Historian's Report: Kim Smith**

Arkansas State University started as one of 4 agricultural high school, created by an act of the state legislature in 1909. While the campus was being built, the first classes were offered in downtown Jonesboro in 1910. 189 students were taught by 8 faculty, originally emphasizing agriculture, horticulture, and textile manufacture. Curricular expanded into junior college after World War 1, and the name was changed to First District Agricultural and Mechanical College in 1925. A training school was established in the 1920s with K through 12 classes for students who wanted to be teachers. This school lasted into the 1950s and is commemorated by an arch on campus.

In 1931, fire destroyed the main building on campus. It was replaced by Wilson Hall, named for

R. E. Lee Wilson, who built the hall, starting a long association with ASU and the Wilson family. The first 4-year degree was awarded in 1932 and the name was changed in 1933 to Arkansas State College.

During World War II, enrollment dropped to 114, mostly female student, but the end of the war saw a great influx of students on the GI bill in the late 40s, necessitating temporary housing for the new students and their families. Victor Kays resigned in 1943 after 34 years as president.

Carl Reng became president in 1951 and aggressively expanded the campus and lobbied for university standing, which was granted in 1967. Soon after, sports flourished at ASU with strong programs in football, baseball, basketball, and track and field. Reng retired in 1975, after 24 years as president.

In the late 80s, the first doctoral program was established in educational leadership and their sports program moved into Division 1. In the new millennium, the Arkansas Biosciences Institute was established on campus. The growth of the satellite campuses necessitated forming a system in 2006.

Today there are just over 14,000 students enrolled, with an average class size of 29, and a 17 to 1 student to faculty ratio.

The 3 other high schools established in 1909 are now Arkansas Tech University, Southern Arkansas University, and University of Arkansas at Monticello.

6. Journal (JAAS #71) Report: Editor-In-Chief Mostafa Hemmati

During the spring 2017 semester, 39 manuscripts were submitted for consideration for publication in volume 71 of the Journal of the Arkansas Academy of Science (JAAS). Soon after receiving the manuscripts, all manuscripts were sent to reviewers and three Associate Editors. The reviewers sent all manuscripts and their comments back before the end of July 2017.

Reviewers' comments were sent to the authors between July 15, 2017, and July 30, 2017. That process was completed by July 30, 2017. The authors were asked to respond to the reviewers' comments and return their manuscript back to Managing Editor, Dr. Still, by August 31, 2017. That allowed more than a month of time for the authors to respond to the reviewers' comments and pay for the Journal page charges. In the same letter, the authors were asked to mail a check for their page charges as well. August 31, 2017, was also the deadline for receipt of the payment of the page charges; we had to extend the deadline up to September 30 this time.

One manuscript was rejected due to major formatting problems and two manuscripts were rejected by reviewers. Therefore, volume 71 of the Journal will include 36 manuscripts. In the process of manuscript submission, no manuscripts were lost.

Three Associate Editors for Physical Sciences, Dr. Collis Geren, Dr. Frank Hardcastle and Dr. Rajib Choudhury helped considerably with locating possible reviewers for the manuscripts or serving as reviewer for more than one manuscript. I am grateful for all thee Associate Editors' assistance. All activities relating to the handling of the manuscripts were performed electronically, and on the whole this expedited the review process. Managing editor post was performed by Dr. Ivan Still and as usual he did an excellent job. The Journal was completed by December 30, 2017. Printing of the Journal was completed by March 18, 2018. I used used the Russellville Printing Company for printing of the Journal.

Managing Editor Ivan Still

Thirty nine manuscripts were submitted for consideration of publication in volume 71 (2017) of the JAAS. Of these 23 were submitted via email and 15 via the new electronic manuscript submission process on the Journal website. One accepted manuscript was carried over from the previous year, due to late payment of page charges

By the beginning of May, emailed-manuscripts were checked for style, grammar, format, etc, to ensure compliance with the "Instructions to Authors". One paper was rejected at this Editorial stage due issues with formatting that the authors failed to resolve in a timely fashion. With handling manuscripts coming from 2 sources, manuscripts from Scholarworks were not checked for compliance at this stage, but were passed to Dr. Hemmati and Dr. Barron to start the review process.

Abstracts were sent to potential reviewers by mid to late May. Dr. Hemmati handled Physical Science papers and recruited Dr. Collis Geren, Dr. Frank Hardcastle and Dr. Rajib Choudhury to serve as Associate Editors, while Biological Science manuscripts were handled by Dr. Still and Dr. Barron (Ecology/Environmental papers). The majority of manuscripts were sent out electronically for review by the beginning of June.

Authors were informed if their paper was accepted with the need for minor or major revision or whether their paper was rejected in July. Authors were asked to return their revisions to their handling editor, electronically (email or Scholarworks) by August 31, with the page charges being submitted to Dr. Hemmati, Editor-in-Chief. Six manuscripts required major corrections and 2 manuscripts were rejected. Once reviews were returned to handling editors, control of manuscript processing was returned to the Managing Editor.

The total number of manuscripts that will be published this year is 36 (down from the 46 from the 100th meeting), of which 28 were Articles and 8 were in General Note format. Volume 71 is 266 pages long (including cover pages). I have changed the format of the journal, moving the meeting report to the back of the journal, and entering manuscripts in order of processing/payment of page charges. These changes streamlined production editing of the Journal and will expedite processing for future volumes. I have maintained the "Instructions to Authors" in the hardcopy this year, but once I have ensured that the Instructions are fully finalized for electronic submission, the Instructions will be reduced in the hard copy to then link to the Academy's Journal website and the Scholarworks site.

I would like to thank the reviewers and Assistant/Associate Editors for their help in the preparation of volume 71. A special note of thanks to Bennet Grooms and Rachel Urbanek who acted as the initial submission- guinea pigs.

Report on Changes in the Journal Submission system.

This year has marked the complete move to an all-electronic submission process. This will expedite processing and authors can keep track of what is happening with their manuscript.

Over the next few years, I will streamline the reviewer database on Scholarworks and add reviewers that I have used in the past few years. This will grow the database to aid future review of incoming manuscripts. I am doing this as I prepare to retire from this position with the completion of volume 75 in 2021. With that in mind we have put out a call for members who wish to become more involved with the production of the JOURNAL to consider becoming Associate Editors.

7. Webmaster: Rami Alroobi

Dr. Alroobi was not able to attend this year's meeting, but the Committee felt he is doing an excellent job and repeated their feeling that financial resources were available to assist him in doing an even better job.

8. Newsletter: Panneer Selvam

Panneer reported that a single newsletter was produced and distributed in January. The newsletter itself stands as the report. Panneer still has hopes of producing two newsletters annually. There was discussion of how to broaden the distribution of the newsletter to people who are not currently members. The Committee felt that sending the newsletter to appropriate STEM deans statewide and asking them to distribute to their faculties might prove useful.

9. Committee Reports:

Nominations Committee: Mostafa Hemmati

Frank Hardcastle inherited the presidency of the Academy, with Stephen Addison as President-Elect and Paneer Selvam becomes Past President.

Andy Sustich was nominated by the Executive Committee for the 4-year term as Vice President, President Elect, President, and Past President. A call for additional nominations from the floor was met with silence. Andy was elected by acclamation.

Undergraduate Research Awards: Jess Kelly

Mostafa Hemmati reported that due to a sudden severe illness Jess Kelly had not reported the results of the assessment of 13 undergraduate research proposals at the time of the meeting. The Academy will award 3 such grants via e-mail as soon as the results are known. Our best wishes were sent to Jess.

After the meeting, Jess Kelly reported that Andrew Feltmann from UCA, Bipal Simkhada from ATU, and Sarah Martin from UCA were awarded the research grants. Jess also reported he is now cancer free!

AAAS Representative

Abdul Bachri will represent AAS at the national AAAS meeting.

Outreach Committee Report

What follows is the complete report by Ed Wilson on efforts in outreach.

The AAS will enlarge its exposure to the citizens of Arkansas by having articles published in State Newspapers, Magazines and Local Interest

Magazines. These articles will be written on a fifth grade level to increase the exposure to younger people of the State. They will be accurate and complete and be interesting and academic language will be avoided. The articles will contain activities for young and old in so far as possible. The AAS website will host these articles.

An update of activities carried out will be made at the AAS Fall Executive Meeting.

Wilson will make visits to Arkansas Wildlife experts and interview them as well as other groups who are experts (the Audubon Society).

Part of this plan will be to have a “Ask the Experts” column in which citizens will pose questions and be given the best possible answers.

An informal data base of Arkansas Scientists who wish to participate will be collected and the Coordinator will work with them and the newspapers and magazines to produce interesting and information information about the amazing happenings in nature and in science.

Examples of topics might be:
Are there good snakes?
Are starlings helpful or hurtful to the environment and to farming?
What is the biggest bird in Arkansas? the smallest?
What plants/animals are on the brink of extinction or flourishing?
Are spiders good? Bad?

People to contact:

1. Kory Roberts - www.herpsofarkansas.com
2. E.O. Wilson (Harvard) - "Each species, to put the matter succinctly, is a masterpiece. It deserves that rank in the fullest sense: a creation assembled with extreme care by genius."
3. Patrick Ruhl (PhD Perdue; birds) will be joining our dept. faculty this fall at Harding University
4. Nathan Mills – Bird Man of Harding University

11. <u>Business Old and New*:*</u>

Item 1: Upcoming meetings

The 103rd annual meeting in 2019 of the Academy will be hosted by Hendrix College. Todd Tinsley will chair the meeting. The 2020 meeting (104th) is being organized by-Ragupathy Kannan, at UA, Fort Smith.

Item 2: Fellows Proposal

A proposal to establish Fellows and Honorary Fellows in the Arkansas Academy of Science by Kim Smith follows:

Fellows

The Fellows of the Arkansas Academy of Sciences are a group of distinguished scientists, appointed in recognition of their outstanding contributions to the sciences 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 science 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 science profession 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 5 years and have contributed to science in one or more of the following ways: (a) outstanding scientific research, (b) inspired teaching of science, or (c) significant leadership in the Academy. Nominations and seconds for Fellows should be submitted to the current Chair of the Committee on Nominations for Fellows and Honorary Fellows. A nomination consists of 1) a CV of the nominee and 2) a letter outlining the nominee's qualifications and contributions to science in Arkansas and AAS in particular, if appropriate. The Committee will make recommendations on the nominations to the Executive Council, who will vote on the nominations, a simple majority needed for election. Fellows will be announced at the Society’s annual meeting, and recipients will be presented with a certificate. 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, appointed in recognition of their outstanding contributions to the sciences in Arkansas, who are not members of AAS. Any individual who has made a distinguished or substantial contribution in any area of science 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 science 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 within Arkansas. Nominations and seconds for Honorary Fellows should be submitted to the current Chair of the Committee on Nominations for Fellows and Honorary Fellows. A nomination consists of 1) a CV of the nominee and 2) a letter outlining the nominee's qualifications and contributions to science in Arkansas. The Committee will make recommendations on the nominations to the Executive Council, who will vote on the nominations, a simple majority needed for election. Honorary Fellows will be announced at the Society's annual meeting, and recipients will be presented with a certificate. Honorary Fellows are appointed for life. There are no restrictions on the number of Fellows elected each year.

New AAS Committee

Committee on Nominations for Fellows and Honorary Fellows – This committee makes recommendations to the Executive Council based on nominations for Fellow and Honorary Fellow. It consists of 3 Fellows, each appointed to a 3-year term. The Chair will be the person in their 3rd year. The Committee recommends new members to the Executive Committee for approval.

11. <u>Motions and Action Items:</u>

Dr. Mostafa Hemmati requested a discretionary budget of $8,000 for the coming year for items other than for the journal.

Dr. Frank Hardacstle was installed as the President for 2018-2019. He requested that anyone with suggestions for improving the Academy should e-mail him directly at rps@uark.edu. Steve Addison became President-Elect, became Vice President, and Panneer Selvam becomes Past President.

The meeting adjourned at 12:50 pm'

Minutes prepare by Secretary Collis Geren, April 30, 2018.

Treasurer's Report
ARKANSAS ACADEMY OF SCIENCE 2018 FINANCIAL STATEMENT
December 3, 2018

Balance – December 3, 2018	**$150,132.66**
Balance – December 4, 2017	**<u>$142,987.74</u>**
Net Gain	**$7,144.92**

DISTRIBUTION OF FUNDS

Checking Account Dec. 3, 2018 Arvest Bank, Russellville	$4,410.14
PayPal Membership Account: Available funds on Dec. 3, 2018	$1,962.20
PayPal Registration Account – Available Funds on Dec. 3, 2018	$54.60
Certificate of Deposit Dec. 3, 2018 Includes Phoebe and George Harp Endowment Arvest Bank, Russellville	$51,571.04
Certificate of Deposit Dec. 3, 2018 Arvest Bank, Russellville	$51,571.04
Certificate of Deposit Dec. 3, 2018 Arvest Bank, Russellville	$40,000.00

Combined interest from Arvest Bank YTD (December 3, 2018):
$228.89+$105.86+$228.89 = $563.64

TOTAL	**$150,132.66**

INCOME

1. Transfer from Checking to CD (Oct. 15, 2018)	**$11,898.64**

2. GIFTS RECEIVED

a. Contribution, Matthew Moran	$30
	$30.00

3. INTEREST (Interest Earned Year to Date December 3, 2018)

a. Checking Account, Arvest Bank	1290	$0
b. CD1 (Arvest Bank)	1357	$228.89
c. CD2 (Arvest Bank)	1358	$228.89
d. CD3 (Arvest Bank)	1550	$105.86

All interest was added to the CDs	**$563.64**

4. JOURNAL

a. Page Charges	$9,650
b. Subscriptions, University of Arkansas	$250

$9,900

5. MISCELLANEOUS INCOME

$0

6. MEMBERSHIP

a. Associate	$0
b. Individual	$120
c. Individual collected at the meeting	$1,660
d. Institutional	$1,200
e. Life, Kannan, Four $125 Payments through PayPal	$0

$2,980

7. MEETING INCOME

a. PayPal Transfer	$0

TOTAL INCOME **$12,910.00**

EXPENSES

1. STUDENT AWARDS **$1,700.00**

2. AWARDS (Organizations)

a. Junior Science and Humanities Sym.	$400
b. Arkansas State Science Fair	$400
c. Arkansas Junior Academy of Science	$400
d. Arkansas Science Talent Search	$150

$1,350.00

3. UNDERGRADUATE RESEARCH AWARDS

a. Dr. Dr. Gifford, UCA	$898.80
b. Dr. Khan , ATU	$1,000
c. Dr. McDonald, UCA	$982.80

$2,881.60

4. JOURNAL

a. Volume 71 Printing Cost	$2,839.45
b. Journal Mailing Cost	$85.59
c. Journal Return Postage Cost	$3.17

$2,928.21

5. MISCELLANOUS EXPENSES

1. Affiliation to AAAS Dues (Aug. 7, 2018)	$150.00
2. Reimbursed Collis for Plaques	$93.29
3. Reimbursed Mostafa for His Accountant's Post-Card	$20
3. Awards Mailing Cost of Journals to Collis	$10.30

$273.59

6. MEETING EXPENSES

$0

TOTAL EXPENSES **$9,133.40**

ARKANSAS ACADEMY OF SCIENCE
COST OF JOURNAL

VOLUME	COPIES	PAGES	PRINTER CHARGE	TOT. VOL. COST	COST/ COPY	COST/ PAGE
38 (1984)	450	97	$5,562.97	$6,167.72	$13.71	$63.58
39 (1985)	450	150	$7,856.20	$8,463.51	$18.81	$56.42
40 (1986)	450	98	$6,175.20	$6,675.20	$14.23	$68.11
41 (1987)	450	116	$7,122.79	$7,811.25	$17.36	$67.34
42 (1988)	450*	116	$7,210.79	$7,710.15	$17.13	$66.47
43 (1989)	450*	119	$8,057.24	$8,557.24	$19.02	$71.91
44 (1990)	450*	136	$9,298.64	$9,798.64	$21.77	$72.05
45 (1991)	450*	136	$9,397.07	$9,929.32	$22.06	$73.01
46 (1992)	450*	116	$9,478.56	$10,000.56	$22.22	$86.21
47 (1993)	400	160	$12,161.26	$12,861.26	$32.15	$80.38
48 (1994)	450	270	$17,562.46	$18,262.46	$40.58	$67.63
49 (1995)	390	199	$14,725.40	$15,425.40	$39.55	$77.51
50 (1996)	345	158	$11,950.00	$12,640.75	$36.64	$80.00
51 (1997)	350	214	$14,308.01	$15,008.01	$42.88	$70.13
52 (1998)	350	144	$12,490.59	$13,190.59	$37.69	$91.60
53 (1999)	350	160	$13,686.39	$14,386.39	$41.10	$89.91
54 (2000)	350	160	$14,149.07	$14,849.07	$42.43	$92.81
55 (2001)	360	195	$16,677.22	$17,498.22	$48.61	$89.73
56 (2002)	350	257	$18,201.93	$19,001.93	$54.29	$73.94
57 (2003)	230	229	$14,415.12	$15,715.12	$68.33	$68.62
58 (2004)	210	144	$7,875.76	$9,175.76	$43.99	$63.72
59 (2005)	215	226	$16,239.04	$17,835.84	$82.96	$78.92
60 (2006)	220	204	$11,348.06	$12,934.30	$58.79	$63.40
61 (2007)	195	150	$8,196.84	$9,914.69	$50.84	$66.10
62 (2008)	220	166	$2,865.00	$2,967.49	$13.49	$17.88
63 (2009)	213	206	$3,144.08	$3,144.08	$14.76	$15.26
64 (2010)	232	158	$2,713.54	$2,764.30	$11.91	$17.50
65 (2011)	200	194	$2915.12	$2,963.03	$14.82	$15.27
66 (2012)	200	216	$3,087.91	$3,180.29	$15.90	$14.72
67 (2013)	200	238	$3,311.42	$3,396.32	$16.98	$14.27
68 (2014)	180	192	$2,812.75	$2,944.08	$16.36	$15.33
69 (2015)	180	170	$2,622.87	$2,622.87	$14.57	$15.43
70 (2016)	180	307	$3,179.53	$3,320.76	$18.45	$10.82
71 (2017)	180	262	$2,839.45	$2,839.45	$15.77	$10.83

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

- On Volume 42 the Academy received 560 copies, but the printer did not charge us for the extra 110 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 43 the Academy received 523 copies, but the printer did not charge us for the extra 73 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 44 the Academy received 535 copies, but the printer did not charge us for the extra 85 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 45 the Academy received 594 copies, but the printer did not charge us for the extra 144 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 46 the cost was greater than usual due to the high cost of a second reprinting of 54 copies by a different printer.

APPENDIX A

AWARD WINNERS FROM THE 102nd ANNIVERSARY AKANSAS ACADEMY OF SCIENCE (awardees are underlined)

UNDERGRADUATE ORAL PRESENTATION AWARDS: Biology

1st Place (Tie)

Culture-independent analysis of Hot Springs National Park thermophiles by Taylor Stone; Richard C. Murray; Matthew D. Moran. Hendrix College

Effects of recreational boat noise on avoidance and feeding behaviors in an important freshwater stream fish by Claire Turkal; Trystin F. Anderson; Maureen R. McClung. Hendrix College

UNDERGRADUATE POSTER PRESENTATION AWARDS: Biology

1st Place

Genetic engineering of the Mad locus using CRISPR/Cas-9 and Phi-C31 recombination by Jeremy Brown. Southern Arkansas University

GRADUATE ORAL PRESENTATION AWARDS: Biology

1st Place

Distribution Records of the Chestnut Lamprey in Arkansas by Jeremiah Salinger; Ron Johnson. University of Arkansas at Pine Bluff

GRADUATE POSTER PRESENTATION AWARDS: Biology

1st Place

Molecular Mechanisms Underlying the Higher Biomass and Abiotic Stress Tolerance Phenotype of Arabidopsis MIOX Overexpressers by Nirman Nepal; Jessica P. Yactayo Chang; Lucia M. Acosta-Gamboa; Karina Medina-Jimenez; Mario A. Arteaga-Vasquez; Argelia Lorence. Arkansas State University

2nd Place

Investigation of the function of a putative cysteine synthase homolog in Mycobacterium smegmatis using unmarked gene deletion mutation by Saroj Mahato; Ravi D. Barabote. University of Arkansas

UNDERGRADUATE ORAL PRESENTATION AWARDS: Ecology

1st Place

Valuation of ecosystem services of the Arctic National Wildlife Refuge by Margaret Young; Adam C. Turner; Matthew D. Moran; Maureen R. McClung. Hendrix College

2nd Place

Aerobic Pushups: Cutaneous Ventilation in Overwintering Smooth Softshell Turtles, Apalone mutica by Caleb O'Neal; Michael V. Plummer. Harding University

UNDERGRADUATE ORAL PRESENTATION AWARDS: Chemistry & Geosciences

1st Place (Tie)

Development of a Novel Method for Purification of Recombinant Proteins by Amberly Vaughan; Musaab Al-Ameer; T.K.S. Kumar. University of Arkansas

Quantification of Brominated Vegetable Oil in Beverages by LC-MS by Katie Farmer. Harding University

UNDERGRADUATE POSTER PRESENTATION AWARDS: Chemistry & Geosciences

1st Place

Novel Peptoids as Antimicrobial Agents by Eliza Hanson; Francis Umesiri. John Brown University

2nd Place

Extraction and Quantitation of Heterocyclic Aromatic Amines from Cooked Bacon using Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry by Emily Joy Seminara; Lora J. Rogers; Susan Kadlubar; Howard Hendrickson. Hendrix College

GRADUATE ORAL PRESENTATION AWARDS: Chemistry, Geosciences & Engineering

1st Place (Tie)

Phenomics Approaches to Elucidate the Contribution of the Four Ascorbate Pathways to Abiotic Stress Tolerance in Arabidopsis by Lucia Acosta-Gamboa; Nirman Nepal; Zachary C. Campbell; Shannon Cunningham; Karina Medina-Jimenez; Argelia Lorence. Arkansas State University

Variable Frequency Drives, Providing Energy Efficiency and Enhanced Crop Production in Agriculture While Improving Water Management Processes by Robert Straitt; Rajesh Sharma; Paul Mixon; Steve Green; Andrzej Rucinski; Nadya Reingand; Christophe Paoli; Dwight Ellis. Arkansas State University

GRADUATE POSTER PRESENTATION AWARDS: Chemistry

1st Place

Identification of a novel glucan crosslink to feruloylated arabinoxylan in rice bran fiber by Kan Takahashi; Brett Savary. Arkansas State University

UNDERGRADUATE ORAL PRESENTATION AWARDS: Engineering, Math, Physics & Computer Science

1st Place

An Interacting Model between Dark Energy and Dark Matter by Yosuke Kitakaze; Jesse Griffiths. Arkansas Tech University

UNDERGRADUATE POSTER PRESENTATION AWARDS: Engineering & Physics

1st Place

Stratospheric Ballooning RGB System for LiDAR Measurements by Patrick Tribbett; Ross Carroll. Arkansas State University

2nd Place

Rutherford Backscattering Spectromety by Paul Niyonkuru. University of Central Arkansas

GRADUATE POSTER PRESENTATION AWARDS: Engineering

1st Place

Anomalous electrostatic nature of charged particles: An approach towards stable equilibrium in inverted systems by Tamal Sarkar. Arkansas State University

APPENDIX B
RESOLUTIONS

Arkansas Academy of Science
102nd Annual Meeting, 2018 Resolutions

Be it resolved that we, the membership of the Arkansas Academy of Science (AAS) offer our sincere appreciation to Arkansas State University for hosting the 102nd annual meeting of the Academy. We thank the local arrangements committee: Rajesh Sharma (Chair), Andrew Sustich, Emily Devereux, Ross Carroll, Kwangkook (David) Jeong, Zahid Hossian, John Hershberger, Brook Fluker, and Tanya McKay who supported the program and meeting arrangements listed in the AAS proceedings.

We sincerely thank Arkansas State University for providing its facilities and service during the meeting and Sodexho for the catering service.

We especially thank our keynote speaker, Dr. Travis Marsico, for his informative talk.

The Academy recognizes the important role of our session chairs: Brian Wagner (Arkansas Game & Fish Commission), Stan Trauth (A-State), John Hershberger (A-State), Dennis Province (HU), Matthew Young (ATU), Ronald Johnson (A-State), Henry North (HU), Ragupathy Kannon (UAFS), Hamed Shojaei (ATU), Maureen McClung (HC), Antoinette Odendall (SAU), Zahid Hossian (A-State), and Matt Moran (HC).

Even greater appreciation and sincere gratitude is extended to our dedicated judges for the student presentations including David Bowles (National Park Service), Ryan Stork (HU), Mikolaj Sulkowski (SAU), Mary Stewart (UAM), Steve Cooper (HC), Brook Fluker (A-State), Susanne Wache (SACC), Douglas Barron (ATU), Cynthia Jacobs (ATU), Frank Hardcastle (ATU), Ross Carroll (A-State), Andres Caro (HU), John Hershberger (A-State), and Jessica Young (ATU).

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.

The Academy recognizes its leadership and offers its thanks to this year’s set of executive officers including Panneer Selvam (President), Franklin Hardcastle (President Elect), Ed Wilson (Past

President), Stephen Addison (Vice President), Mostafa Hemmati (Treasurer and Journal Editor-in-Chief), Ivan Still (Journal Managing Editor), Panneer Selvam (Newsletter Editor), Rami Alroobi (Webmaster), Kimberly Smith (Historian), and Collis Geren (Secretary).

Respectfully submitted on this 7th day of April, 2018. Resolutions Committee: Panneer Selvam (President), Stephen Addison (Vice President), and Mostafa Hemmati (Treasurer).

2018 ARKANSAS ACADEMY OF SCIENCE MEMBERSHIP

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Gary	Tucker	FTN Associates
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Xin	Yang	
Jessica	Young	Arkansas Tech University
Matthew	Young	Arkansas Tech University

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Steve	Cooper	Harding University
Andy	Sustich	Arkansas State University-Jonesboro
Edmond	Wilson	Harding University

KEYNOTE ADDRESS

Natural History Collections and their Role in Arkansas Science and Education

By Dr. Travis Marsico
Department of Biological Sciences, Arkansas State University

Dr. Marsico’s research focuses on biogeography, biodiversity conservation, natural history and species invasions. He currently researches risk associated with hitchhiking plant propagules at U.S. shipping ports, invasion of herbivorous insect pests, plant diversity patterns in fragmented landscapes of the Upper Mississippi River Alluvial Plain, and plant diversity patterns along elevation gradients in the Neotropics. His work emphasizes making and utilizing natural history collections in research. He also studies biology education and improvements to university-level education, utilizing specimen-based projects in coursework. Marsico has been a faculty member at Arkansas State University for eight years, where he teaches Curation of Collections, Dendrology, Global Change Biology, Mechanisms of Speciation, Natural History Collections Research Design, and Plant Systematics. At A-State, Marsico curates the herbarium and manages the Laboratory Sciences Greenhouse

For the plenary session on Friday evening, Dr. Marsico will discuss the important role that natural history collections have played in shaping scientific knowledge in Arkansas. In his talk, he will also address current biodiversity research being conducted by students and faculty members associated with the newly formed Arkansas Center for Biodiversity Collections (ACBC) at Arkansas State University. He will highlight expanded opportunities for educating students in basic and applied biodiversity science through the ACBC. Finally, Marsico will share his vision for how research utilizing natural history collections is as relevant as it has ever been on a planet with natural ecosystems threatened by anthropogenic pressures.

SECTION PROGRAMS

ORAL PRESENTATIONS

(Presenter is underlined; * - Undergraduate ** - Graduate)

ORAL SESSIONS: FRIDAY 1:00-5:30

AQUATIC BIOLOGY, WHITE RIVER ROOM

1:00
EFFECTS OF RECREATIONAL BOAT NOISE ON AVOIDANCE AND FEEDING BEHAVIORS IN AN IMPORTANT FRESHWATER STREAM FISH
Claire Turkal*, Hendrix College
Co-Authors: Trystin F. Anderson, Maureen R. McClung

1:15
CULTURE-INDEPENDENT ANALYSIS OF HOT SPRINGS NATIONAL PARK THERMOPHILES
Taylor Stone*, Hendrix College
Co-Author: Matthew Moran

1:30
STOCKING ASSESSMENT AND LONG-TERM IMPACTS OF NON-NATIVE WALLEYE ON THE NATIVE POPULATION IN THE ELEVEN POINT RIVER, ARKANSAS
Dustin Thomas**, Arkansas State University
Co-Authors: Brook Fluker, Brett Timmons

1:45
DISTRIBUTION RECORDS OF THE CHESTNUT LAMPREY IN ARKANSAS
Jeremiah Salinger**, University of Arkansas at Pine Bluff
Co-Author: Ron Johnson

2:00
AQUATIC INVERTEBRATE COMMUNITY ASSESSMENTS AT OZARK NATIONAL SCENIC RIVERWAYS, MISSOURI, 2005-2014
David Bowles, U.S. National Park Service
Co-Authors: Lloyd W. Morrison, Janice A. Hinsey, J. Tyler Cribbs

2:15
NEW APPLICATIONS OF RADIO FREQUENCY IDENTIFICATION STATIONS FOR MONITORING FISH MOVEMENT THROUGH HEADWATER ROAD CROSSINGS AND NATURAL REACHES
Charles Gagen, Arkansas Tech University

GENERAL BIOLOGY & ECOLOGY, MOCKINGBIRD ROOM

1:00
IMPACT OF LESPEDEZA CUNEATA INVASION ON ARTHROPOD ABUNDANCE IN A TALLGRASS PRAIRIE
Adam Turner*, Hendrix College *Co-Author: Caroline Kirksey*

1:15
PATTERNS OF LESPEDEZA CUNEATA INVASION IN TALLGRASS PRAIRIES AND ARTHROPOD COMMUNITY STRUCTURE
Carolina Kirksey*, Hendrix College
Co-Author: Adam Turner

1:30
UNCONVENTIONAL GAS DEVELOPMENT EFFECTS ON ECOSYSTEM SERVICES IN THE FAYETTEVILLE SHALE OF ARKANSAS
Varenya Nallur, Hendrix College

1:45
AEROBIC PUSHUPS: CUTANEOUS VENTILATION IN OVERWINTERING SMOOTH SOFTSHELL TURTLES, APALONE MUTICA
Caleb O’Neal*, Harding University
Co-Author: Michael V. Plummer

2:00
GERMINAL EPITHELIUM CYTOLOGY DURING SPERMATOGENESIS IN THE ALLIGATOR SNAPPING TURTLE, *MACROCHELYS TEMMINCKII* (REPTILIA: CHELYDRIDAE)
Stan Trauth, Arkansas State University

2:15
SOIL CRUST ALGAL COMMUNITIES OF WARREN PRAIRIE NATURAL AREA
Karen Fawley, University of Arkansas at Monticello
Co-Authors: Rachel Knight, Caleb Lamb, Brent Baker, Marvin Fawley

MEDICINE, MOLECULAR & CELLULAR BIOLOGY, BLACK RIVER ROOM

1:00
THE MECHANISM OF THYMOQUINONE-INDUCED APOPTOSIS IN ORAL CANCER CELLS
Malcolm Anderson* University of Arkansas at Pine Bluff
Co-Author: Selma Dagtas

1:15
TERBINAFINE BIOACTIVATION: DETERMINING PATHWAY TO REACTIVE METABOLITE ACCUMULATION USING COMPUTATIONAL MODELING AND EXPERIMENTAL APPROACHES
Dustyn Barnette**, University of Arkansas for Medical Sciences *Co-Authors: Mary Davis, Lena Dang, Tyler Hughes, S. Joshua Swamidass, Grover P. Miller*

1:30
COUMADIN (WARFARIN) PHARMACOKINETICS CHANGE FOR PEDIATRIC PATIENTS WITH SINGLE

VENTRICLE PHYSIOLOGY DURING INITIATION OF ANTICOAGULANT THERAPY
Dakota Pouncey, University of Arkansas for Medical Sciences

1:45
DAPHNIA MAGNA AS A MODEL ORGANISM FOR SCREENING THE EFFECTS OF DIETARY SUPPLEMENTS
Antoinette Odendaal, Southern Arkansas University

2:00
CYP3A7 METABOLIZES DEXTROMETHORPHAN LESS EFFICIENTLY THAN CYP3A4.
Dhaval Shah, University of Arkansas for Medical Sciences
Co-Author: Grover Miller

2:15
GEOHELMINTH INFECTION, ANEMIA, AND MALNOURISHMENT IN BAWA, CAMEROON AFTER TEN YEARS OF INTERVENTION BY THE BAWA HEALTH INITIATIVE
Dennis Richardson, Quinnipiac University
Co-Author: Sara Karr

CHEMISTRY & GEOSCIENCES
ARKANSAS RIVER ROOM

1:00
A BOND VALENCE / BOND LENGTH CORRELATION FOR TANTALUM-OXYGEN BONDS
Blake Ludwig*, Arkansas Tech University

1:15
QUANTIFICATION OF BROMINATED VEGETABLE OIL IN BEVERAGES BY LCMS
Katie Farmer*, Harding University

1:30
DEVELOPMENT OF A NOVEL METHOD FOR PURIFICATION OF RECOMBINANT PROTEINS
Amberly Vaughan*, University of Arkansas
Co-Authors: Musaab Al-Ameer, T.K.S. Kumar

1:45
TREE RING DATING OF THE FICKLIN-IMBODEN LOG STRUCTURES, POWHATAN STATE PARK, ARKANSAS
Kaylee McAdoo*, University of Arkansas
Co-Author: David Stahle

2:00
PHENOMICS APPROACHES TO ELUCIDATE THE CONTRIBUTION OF THE FOUR ASCORBATE PATHWAYS TO ABIOTIC STRESS TOLERANCE IN ARABIDOPSIS
Lucia Acosta-Gamboa**, Arkansas State University
Co-Authors: Nirmal Nepal, Zachary C. Campbell, Shannon Cunningham, Karina Medina-Jimenez, Argelia Lorence

2:15
CYP2C19 AND 3A4 GENERATE A POTENTIALLY TOXIC, REACTIVE TERBINAFINE METABOLITE AS REVEALED THROUGH MODELING AND EXPERIMENTAL METHODS
Mary Davis**, University of Arkansas for Medical Sciences *Co-Authors: Anirudh Pidugu, Dustyn Barnette, S. Joshua Swamidass, Grover P. Miller*

ENGINEERING
PINE TREE ROOM

1:00
DESIGN AND DEVELOPMENT OF A SELF-DRIVING RC CAR
Andrew Lea*, Arkansas Tech University

1:15
A STEP BY STEP DESIGN PROCEDURE FOR CURRENT CONTROL OF A 5 MWTHREE-PHASE GRID-CONNECTED INVERTER
Hamdi Albunashee**, University of Arkansas
Co-Authors: Manar Alzahlol, Roy McCann

1:30
PROSPECTS OF NANACLAY AS A PAVEMENT CONSTRUCTION MATERIAL
MM Tariq Morshed**, Arkansas State University
Co-Author: Zahid Hossain

1:45
EFFECTS OF ZEOLLITE ON CHEMICAL ELEMENTALS AND GLASS TRANSITION TEMPERATURE OF ASPHALTS
Mohammad Nazmul Hassan**, Arkansas State University
Co-Author: Zahid Hossain

2:00
VARIABLE FREQUENCY DRIVES, PROVIDING ENERGY EFFICIENCY AND ENHANCED CROP PRODUCTION IN AGRICULTURE WHILE IMPROVING WATER MANAGEMENT PROCESSES
Robert Straitt**, Arkansas State University.
Co-Authors: Rajesh Sharma, Paul Mixon, Dr. Steve Green, Dr. Andrzej Rucinski, Dr. Nadya Reingand, Dr. Christophe Paoli, and Mr. Dwight Ellis

GENERAL BIOLOGY & ECOLOGY
BLACK RIVER ROOM

3:00
CURRENT AND FUTURE THREATS TO THE CHIHUAHUAN DESERT BIOREGION: A LANDSCAPE-LEVEL ANALYSIS
Helena Abad*, Hendrix College. *Co-Author: Nathan Taylor*

3:15
VALUATION OF ECOSYSTEM SERVICES OF THE ARCTIC NATIONAL WILDLIFE REFUGE
Margaret Young*, Hendrix College
Co-Author: Matthew Moran

3:30
A PROPOSAL FOR PRACTICAL AND EFFECTIVE BIOLOGICAL CORRIDORS IN NORTHWEST COSTA RICA
Allison Monroe*, Hendrix

3:45
TERRESTRIAL MAMMAL AND BIRD COMMUNITIES IN PROTECTED AND UNPROTECTED LANDS IN COSTA RICA
Benjamin Zamzow* Hendrix College
Co-Author: Lindsay Stallcup

4:00
TERRESTRIAL MAMMAL AND BIRD SURVEY RESULTS IN THE ARENAL – TILARÁN CONSERVATION AREA OF COSTA
Sarah Nieman*, Hendrix College. *Co-Author: Lindsay Stallcup*

4:15
MIGRATORY BIRDS AND NUTRIENT TRANSFER ACROSS CONTINENTS
Sofia Varriano*, Hendrix College
Co-Author: Maureen McClung

MOLECULAR & CELLULAR BIOLOGY
MOCKINGBIRD ROOM

3:00
RELATIVE GENE EXPRESSION STUDY ON *CENTRUROIDES VITTATUS*: INVESTIGATING SODIUM TOXIN GENE ACTIVITY
Chloe Fitzgerald* and Ashlyn Tedder* Arkansas Tech University
Co-Authors: Alyssa Kool, Aimee Bowman, Taylor Bishop, Cody Chivers, Tsunemi Yamashita

3:15
COMPARISON BETWEEN ENVIRONMENTAL BACTERIA FOUND IN THE SOIL AND THE WOLF SPIDER, *RABIDOSA RABIDA* MICROBIOME USING 16S rRNA SEQUENCING ANALYSIS
Brandon Hogland*, Harding University. *Co-Author: Ryan Stork*

3:30
PRODUCTION OF A PRENYLATED STILBENOID IN MUSCADINE GRAPE HAIRY ROOTS EXPRESSING A PRENYLTRANSFERASE GENE FROM PEANUT
Mohammad Fazle Azim**, Arkansas State University

3:45
GENETIC MODIFICATION OF SWITCHGRASS CELL WALL FOR IMPROVED BIOMASS PROCESSABILITY
Neha Verma**, Arkansas State University
Co-Author: Jianfeng Xu

4:00
USING PROTEOMICS TO INVESTIGATE MICROBIAL ACID TOLERANCE RESPONSE
Newton Hilliard, Arkansas Tech University
Co-Authors: Alan J. Tackett, Kirk L. West

4:15
MOLECULAR ADAPTATION OF MYOGLOBIN PROTEINS IN DEEP-DIVING CETACEANS
David McClellan, University of Arkansas - Fort Smith

TERRESTRIAL & WILDLIFE BIOLOGY
WHITE RIVER ROOM

3:00
DISTURBANCE OF WINTERING WATERFOWL BY TRAFFIC NOISE
John Veon*, Hendrix College

3:15
NEW RECORDS OF DISTRIBUTION OF DRACUNCULUS SP. INFECTING RIVER OTTERS (*LONTRA CANADENSIS*) IN ARKANSAS
Heather May, Henderson State University
Co-Author: Renn Tumlison

3:30
WILD GAME HARVEST AND EFFECTS ON DIET-RELATED CO2 EMISSIONS IN THE U.S.
Nathan Taylor*, Hendrix College. *Co-Author: Jamie Johnson*

3:45
INDIANA BAT OCCUPANCY ESTIMATES OF BUFFALO NATIONAL RIVER USING A MULTI-STATE OCCUPANCY MODEL
James Gore**, Arkansas State University

4:00
NEW RECORDS OF THE AMERICAN BADGER (TAXIDEA TAXUS) IN ARKANSAS, WITH AN UPDATED DISTRIBUTION MAP
Renn Tumlison, Henderson State University
Co-Author: Blake Sasse

4:15
INCIDENTAL CAPTURES OF PLAINS SPOTTED SKUNKS (*SPILOGALE PUTORIUS INTERRUPTA*) BY ARKANSAS TRAPPERS, 2012-2017
David Sasse, Arkansas Game & Fish Commission

MATH, PHYSICS & COMPUTER SCIENCE
ARKANSAS RIVER ROOM

3:00
MATHEMATICAL MODELING OF A ROD-BEAM SYSTEM
Zach Rail*, Arkansas State University
Co-Author: Jeongho Ahn

3:15
WAVE PROFILE FOR CURRENT BEARING LIGHTNING RETURN STROKES
Baylee Landers*, Arkansas Tech University
Co-Author: Ali Alzhrani

3:30
AN INTERACTING MODEL BETWEEN DARK ENERGY AND DARK MATTER
Yosuke Kitakaze*, Arkansas Tech University
Co-Author: Jesse Griffiths

3:45
VERIFICATION AND VALIDATION FOR TURBULENT MIXING SIMULATIONS

Tulin Kaman, University of Arkansas

4:00
NUTRIENT CONCENTRATIONS IN BIG CREEK CORRELATE TO CAFO PRESENCE
David Peterson, University of Central Arkansas

4:15
EFFICACY OF SUPPLEMENTAL INSTRUCTION ON ALGEBRA AND CALCULUS-BASED PHYSICS TEACHING-LEARNING PROCESSES
Puskar Chapagain, Southern Arkansas University
Co-Authors: Lavana J. Kindle, Dipak Rimal

ORAL SESSIONS: SATURDAY 8:00-10:15

GENERAL BIOLOGY
MOCKINGBIRD ROOM

8:30
GENE FLOW AND GENETIC STRUCTURE OF TWO OF ARKANSAS'S RAREST DARTER SPECIES (*TELEOSTEI: PERCIDAE*), THE ARKANSAS DARTER, *ETHEOSTOMA CRAGINI*, AND THE LEAST DARTER, *E. MICROPERCA*
Brian Wagner, Arkansas Game & Fish Commission
Co-Authors: Robert Wood, Justin Baker

8:45
DISTRIBUTION OF *CAMPOSTOMA SPADICEUM* IN SOUTHERN ARKANSAS
Renn Tumlison, Henderson State University
Co-Author: Henry Robison

9:00
NOTES ON TARANTULA (*APHONOPELMA HENTZI*) REPRODUCTION IN MISSOURI
David Jamieson, Crowder College
Co-Author: Austin Jones

9:15
DISTAL UROGENITAL ANATOMY IN MALE SOUTHERN COAL SKINKS, *PLESTIODON ANTHRACINUS* PLUVIALIS (REPTILIA: SCINCIDAE)
Stan Trauth, Arkansas State University
Co-Author: Kevin Gribbin

9:30
EARLY SEASONS PRIMENESS IN ARKANSAS RACCOON PELTS
David Sasse, Arkansas Game & Fish Commission

9:45
AVIAN FRUGIVORY IN A FRUITING MULBERRY TREE (MORUS SPP.) IN ARKANSAS
Jack Jackson, University of Arkansas - Fort Smith
Co-Author: Ragupathy Kannan

10:00
VERTEBRATE NATURAL HISTORY NOTES FROM ARKANSAS, 2018
Renn Tumlison, Henderson State University
Co-Authors: Blake Sasse, Henry Robison, Matt Connior, Chris McAllister, Kelly Jobe, Matthew Anderson

10:15
NEW RECORDS OF DISTRIBUTION OF DRACUNCULUS SP. INFECTING RIVER OTTERS (*LONTRA CANADENSIS*) IN ARKANSAS
Heather May, Henderson State University
Co-Authors: Allison Surf, Renn Tumlison

CHEMISTRY & GEOSCIENCES
WHITE RIVER ROOM

8:30
BOND VALENCE - LENGTH RELATIONSHIPS AND ORBITAL EXPONENTS FOR HYDROGEN THROUGH FLUORINE
Franklin Hardcastle, Arkansas Tech University

8:45
INHIBITION OF CYSTINE TRANSPORT LEADS TO HUMAN GLIOMA GROWTH RETARDATION.
Mariusz Gajewski, Arkansas Tech University

9:00
UNDERSTANDING THE COMPLEXATION OF SMALL MOLECULES WITH A MACROMOLECULE
Rajib Choudhury, Arkansas Tech University
Co-Author: Vaidhyanathan Ramamurthy

9:15
OPTIMUM MAGNETOMETER TRANSECT SPACING TO LOCATE LEGACY OIL AND GAS WELLS
Michael Davis, Arkansas Tech University
Co-Authors: Jason Patton, Kenyon Gowing, Hunter Vickers

9:30
COMPARISON OF EFFECTIVE OPTICAL PROPERTIES OF TWO-COMPONENT INTERNALLY MIXED AEROSOLS USING VARIOUS MIXING RULES
Kristin Dooley, University of Central Arkansas
Co-Author: Jessica DeYoung

ENGINEERING & COMPUTER SCIENCE
ARKANSAS RIVER ROOM

8:30
STEPS TOWARD MEASURING WORLD SPACE EXPLORATION ACTIVITY
Richard Segall, Arkansas State University
Co-Authors: Ronithkumareddy Duggirala, Venkat Kodali, Daniel Berleant, Hyacinthe Aboudja

8:45
DEEP LEARNING-BASED FRAMEWORK FOR FMRI AUTISM IMAGE CLASSIFICATION
Xin Yang, Southern Arkansas University
Co-Authors: Ning Zhang, Saman Sarraf

9:00
RAM PUMP, AN ENERGY INDEPENDENT SOLUTION TO

IMPROVE WATER ACCESSIBILITY TO UNDERDEVELOPED INHABITANTS IN THE WORLD – A PERFORMANCE IMPROVEMENT STUDY
Ashokkumar Sharma, University of Arkansas at Little Rock *Co-Authors: Srikanth Pidugu, Shashank Khaire, Swaminadham Midturi*

9:15
APPLICATIONS OF THEORETICAL ADVANCES IN THE OPTICAL ENERGYMOMENTUM DEBATE: INVISIBILITY CLOAKS, TRACTOR BEAMS, AND REVERSED RADIATION PRESSURE
Brandon Kemp, Arkansas State University
Co-Author: Cheyenne Sheppard

9:30
THE ELECTRODYNAMICS OF KINETIC, CANONICAL, AND HIDDEN SYSTEMS UNDER RELATIVISTIC MOTION
Cheyenne Sheppard, Arkansas State University
Co-Author: Brandon Kemp

9:45
INVESTIGATION OF ACOUSTIC-BASED CRACK DETECTION IN PVC PIPES
Muhammad Safeer Khan, Arkansas Tech University

10:00
A STUDY OF M AND MDYN MASSES AT THE DIFFERENT OF THE DYNAMICAL PARAMETERS OF THE SPIRAL HOST GALAXIES
Ismaeel Al-Baidhany, Al-Mustansaryah University *Co-Authors: Sami Salman Chiad, Nadir Fadhil Habubi, Khalid Haneen Abass, Ehssan S. Hassan, Mohamed Odda Dawod, Wasmaa Jabbar*

10:15
OPTICAL PROPERTIES AND DISPERSION PARAMETERS OF PMMA-$MnCl_2$
Sami Chiad, Al-Mustansaryah University
Co-Authors: Nadir Fadhil Habubi, Khalid Haneen Abass, Ismaeel Al-Baidhany, Ehssan S. Hassan, Mohamed Odda Dawod, Wasmaa Jabbar

PARASITOLOGY
BLACK RIVER ROOM

9:30
PARASITES OF THE SPOTTED SUCKER, *MINYTREMA MELANOPS* (CYPRINIFORMES: CATOSTOMIDAE) FROM ARKANSAS AND OKLAHOMA
Chris McAllister, Eastern Oklahoma State College-Idabel
Co-Authors: D.W. Cloutman, A. Choudhury, T. Scholz, S.E. Trauth, T.J. Fayton, and H.W. Robison

9:45
PREVALENCE AS A PREDICTOR OF OTHER HELMINTH POPULATION DESCRIPTORS (MEAN, STANDARD DEVIATION, MAXIMUM NUMBER) IS RELATIVELY INACCURATE AT HIGH PARASITE DENSITIES IN THE HOSTS.
James Daly Sr., University of Arkansas for Medical Sciences (retired)

10:00
ADDITIONAL RECORDS OF ACANTHOCEPHALAN PARASITES FROM ARKANSAS FISHES, WITH NEW RECORDS FROM MISSOURI FISHES
Chris McAllister, Eastern Oklahoma State College-Idabel
Co-Authors: M.A. Barger, and H.W. Robison

10:15
PROPORTIONALITY OF STATISTICAL PARAMETERS FOR HELMINTH PARASITE POPULATIONS IN SMALLMOUTH BASS (*MICROPTERUS DOLOMEIU*) FROM THE BUFFALO NATIONAL RIVER IN ARKANSAS
James Daly Sr**.,** University of Arkansas for Medical Sciences (retired)

POSTER PRESENTATIONS

BIOLOGICAL SCIENCES POSTER PRESENTATIONS

1 **GENERATION AND ANALYSIS OF CHROMOSOMAL GENE DELETION MUTANTS OF TWO PUTATIVE THIOSULFATE SULFURTRANSFERASES IN *MYCOBACTERIUM SMEGMATIS***
Jasleen Saini**, University of Arkansas *(Co-Author: Ravi D. Barabote)*

2 **INVESTIGATION OF THE FUNCTION OF A PUTATIVE CYSTEINE SYNTHASE HOMOLOG IN *MYCOBACTERIUM SMEGMATIS* USING UNMARKED GENE DELETION MUTATION**
Saroj Mahato**, University of Arkansas, *(Co-Author: Ravi D. Barabote*

3 **THE EFFECT OF SLCO TRANSPORTER POLYMORPHISMS ON PROSTAGLANDIN LEVELS IN HEALTHY POSTMENOPAUSAL VOLUNTEERS**
Kelsey Martin**, Harding University
Co-Authors: Nimmy E. Isaac, Landry K. Kamdem

4 **CHARACTERIZATION OF BIOMASS SMOKE PARTICLES VIA SCANNING ELECTRON MICROSCOPY AND ENERGY-DISPERSIVE X-RAY SPECTROSCOPY**
Mason Rostollan**, University of Central Arkansas

5 **MOLECULAR MECHANISMS UNDERLYING THE HIGHER BIOMASS AND ABIOTIC STRESS TOLERANCE PHENOTYPE OF *ARABIDOPSIS* MIOX OVEREXPRESSERS**
Nirman Nepal**, Arkansas State University
Co-Authors: Jessica P. Yactayo Chang, Lucia M. Acosta-Gamboa, Karina Medina-Jimenez, Mario A. Arteaga-Vazquez, Argelia Lorence

6 **EXPOSURE TO CARBON BLACK NANOPARTICLES DURING LARVAL DEVELOPMENT AFFECTS ADULT PHYSIOLOGICAL STRESS TOLERANCE IN WILD TYPE ISOLATES OF *CAENORHABDITIS ELEGANS***
Brenna Walters*, Harding University
Co-Authors: Gavin Traber, Riley Jones, Kasey Boatwright, Jared Cooper, Emme Copeland, Coleman Dennis, Brandon Gates, Shelby Hamilton, Jon Aaron Howell, Caroline Minton, Joe Tolar, Jo Goy

7 **A RETROSPECTIVE MULTISTATE ANALYSIS OF INFLUENZA PANDEMIC DEATHS**
Spencer Long*, University of Central Arkansas
Co-Author: Ashton Purtle

8 **TRANSIENT RECEPTOR POTENTIAL CHANNEL 3 AND ITS EFFECTS ON SYSTEMIC BLOOD PRESSURE REGULATION IN DISEASED VERSUS HEALTHY MESENTERIC ARTERIES**
Ryan Williams, Arkansas Tech University

9 **DETECTION OF TEN ANTINEOPLASTIC DRUGS IN A CLINICAL SETTING BY WIPE TEST AND LC-MS/MS ANALYSIS**
Spencer Sanson**, University of Arkansas for Medical Sciences. *Co-Author: Howard Hendrickson*

10 **A COMPARISON OF JAPANESE AND AMERICAN CHILDREN'S DIETS, AND A LITERATURE REVIEW OF DIET AND DISEASE.**
Malynn McKay, Arkansas Tech University

11 **THE EFFECT OF GREEN TEA EXTRACT ON *DAPHNIA MAGNA***
Antoinette Davis*, Southern Arkansas University

12 **GENETIC ENGINEERING OF THE Mad LOCUS USING CRISPR/Cas-9 and Phi-C31 RECOMBINATION**
Jeremy Brown*, Southern Arkansas University

13 **IDENTIFY MICROBES IN DRINKING WATER USING PCR**
Jeremy Brown*, Southern Arkansas University

14 **HOUSE FINCH (*HAEMORHOUS MEXICANUS*) NESTING IN DECEMBER IN ARKANSAS**
Kimberly Smith, University of Arkansas

15 **OBSERVATIONS OF TOWNSEND'S SOLITAIRES (*MYADESTES TOWNSENDI*) ON MOUNT MAGAZINE IN LOGAN COUNTY, ARKANSAS**
Kimberly Smith, University of Arkansas

16 **SECOND RECORD OF THE GRAY-HEADED JUNCO (*JUNCO HYEMALIS CANICEPS*) IN ARKANSAS**
Kimberly Smith, University of Arkansas

17 **DOES FREQUENTLY VISITING A BLUEBIRD NEST INCREASE PREDATION RISK?**
Amanda Trusty*, Arkansas State University
Co-Author: Virginie Rolland

18 **DOES AXLE GREASE EFFECTIVELY PROTECT BLUEBIRD NESTS FROM PREDATORS?**
Michael Trusty*, Arkansas State University *Co-Author: Virginie Rolland*

19 **DEVELOPING MICROSATELLITE MARKERS FOR GENETIC IDENTIFICATION OF SONGBIRDS**
Edgar Sanchez*, Arkansas Tech University
Co-Author: Douglas Barron

20 **WINTER SURVEYS OF *COTINUS OBOVATUS* (AMERICAN SMOKETREE) IN THE OZARK MOUNTAINS**
Gary Graves, Smithsonian Institution

21 **HIGH-THROUGHPUT PLANT PHENOTYPING AT THE A-STATE PHENOMICS FACILITY**
Zac Campbell, Arkansas State University
Co-Author: Nirmal Nepal

22 **NOVEL NUCLEAR AND PLASTID LOCI AND THEIR UTILITY FOR INFERRING RELATIONSHIPS AMONG SPECIES OF THE GENUS STREPTANTHUS (BRASSICACEAE) FOUND IN ARKANSAS AND ADJOINING STATES.**
Leila Henning, University of Arkansas at Monticello
Co-Authors: Freddie Rivera, Brent Baker, Karen Fawley, Marvin Fawley

23 **THE HUNT FOR BIGLEAF**
Jennifer Bryant*, Arkansas State University
Co-Author: Travis Marsico

24 **ASSESSING MITIGATIVE PROPERTIES OF VEGETATION IN NORTHEAST ARKANSAS AGRICULTURAL DITCHES USING BIOTIC AND ABIOTIC MEASURES**
Emma Martin**, Arkansas State University

25 **A PRELIMINARY CHECKLIST OF THE STONEFLIES (ARTHROPODA: INSECTA: PLECOPTERA) OF ARKANSAS**
Chris McAllister, Eastern Oklahoma State College-Idabel *Co-Author: H.W. Robison*

26 **ADDITIONAL COUNTY RECORDS OF INVERTEBRATES FROM ARKANSAS**
Chris McAllister, Eastern Oklahoma State College-Idabel
Co-Authors: C.T. McAllister, H.W. Robison, and R. Tumlison

27 **BIOLOGICAL AND ECOSYSTEM-LEVEL CHANGES FROM THE ADDITION OF RESERVOIRS TO HEADWATER STREAMS**
Brian Staley, University of Central Arkansas
Co-Author: Maureen McClung

28 **BIODIVERSITY OF HYMENOPTERA ACROSS SKY ISLANDS OF ARKANSAS**
Sierra Hubbard*, Hendrix College
Co-Authors: Reynol Rodruiguez, Allison F. Monroe, Maureen R. McClung, Matthew D. Moran, Oliver I. Kuhns, Michael W. Gates

29 **ENERGETIC COST OF GIRDLING IN THE NOTODONTID CATERPILLAR, *OEDEMASIA LEPTINOIDES***
Brianna Trejo**, University of Central Arkansas
Co-Author: David Dussourd

30 **ORGANOCHLORIDE PESTICIDES PRESENT IN ANIMAL FUR, SOIL, AND STREAMBED IN AN AGRICULTURAL REGION OF SOUTHEASTERN ARKANSAS**
John Hunt, University of Arkansas at Monticello

Co-Author: Christopher Sims

31 **SOUTHEASTERN MYOTIS AND RAFINESQUE'S BIGEARED BATS SWITCH THEIR ROOSTING HABITS SEASONALLY IN ARKANSAS BOTTOMLANDS**
Stacy Scherman**, Arkansas State University
Co-Authors: Tom Risch, Virginie Rolland

32 **SERIAL FOUNDER EFFECTS IN CROTAPHYTUS COLLARIS: THE INFLUENCE OF GENETIC DRIFT ON PHENOTYPIC DIVERSIFICATION**
Andrew Feltmann, University of Central Arkansas
Co-Author: Matthew Gifford

33 **PHYLOGEOGRAPHIC ANALYSES SUGGEST CRYPTIC DIVERSITY WITHIN THE BLUNTNOSE DARTER, *ETHEOSTOMA CHLOROSOMA***
Hilary Canada*, Arkansas State University

CHEMISTRY POSTER PRESENTATIONS

34 **EFFECTS OF NIR FLUOROPHORES IN SELECTIVE BIOLOGICAL SCENARIOS**
Ben Quattlebaum*, Arkansas Tech University
Co-Author: Hope Parker

35 **THE PURIFICATION OF SODIUM CHANNEL TOXIN FROM *CENTRUROIDES VITTATUS***
Gray Orman*, University of Arkansas
Co-Authors: Srinivas Jayanthi, Ravi Kumar Gundampti, Tsunami Yamashita, T.K.S. Kumar

36 **TESTING THE RE-USABILITY OF DEFINED MEDIUM FOR AN EFFECTIVE INCORPORATION OF UNUSED 15N LABEL INTO RECOMBINANT PROTEINS**
Ryan Coleman*, University of Arkansas

37 **APPLICATION OF ROMP CATALYSTS TO HOMO-COUPLING**
Harper Grimsley, University of Arkansas
Co-Author: Stefan M. Kilyanek

38 **ISOLATING A FAT MOBILIZING SUBSTANCE FROM A FASTING INDIVIDUAL**
Sydnye Shuttleworth, Harding University
Co-Author: Dennis Province

39 **IRON REDUCES MITOCHONDRIAL DNA DAMAGE INDUCED BY MITOCHONDRIAL REACTIVE OXYGEN SPECIES IN HEPG2 CELLS**
Conner Breen*, Hendrix College
Co-Author: Andres Caro

40 **NOVEL PEPTOIDS AS ANTIMICROBIAL AGENTS**
Eliza Hanson*, John Brown University
Co-Author: Francis Umesiri

41 **EXTRACTION AND QUANTITATION OF HETEROCYCLIC AROMATIC AMINES FROM COOKED BACON USING SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/ TANDEM MASS SPECTROMETRY**
Emily Joy Seminara*, Hendrix College
Co-Authors: Lora I. Rogers, Susan Kadlubar, Howard Hendrickson

42 **THE ISOLATION AND CHARACTERIZATION OF A RECOMBINANT FIBROBLAST GROWTH FACTOR-1**
Kylie Cleavenger*, University of Arkansas
Co-Authors: Thallapuranam Krishnaswamy Suresh Kamar, Ravi Kumar Gundampati

43 **ANALYZING COMPOSITION OF PLASTER FROM TEL BETH-SHEMESH ARCHAEOLOGICAL SITE**
Peyton Munch*, Harding University

44 **A NEW AND SIMPLE METHOD FOR THE PURIFICATION OF RECOMBINANT PROTEINS FROM INCLUSION BODIES**
Pooja Lukhi*, University of Arkansas
Co-Author: Musaab Al-Ameer

45 **MITOCHONDRIAL CYP2E1 ACTIVATES ANTIOXIDANT AND MITOCHONDRIAL BIOGENESIS SIGNALING IN HEPATOCYTES.**
Trevor Loew*, Hendrix College. *Co-Author: Andres Caro*

46 **PREPARATION OF 3-PHENYL-2-PROPYNOIC ACID USING UNDERGRADUATE ORGANIC CHEMISTRY LAB TECHNIQUES**
Kaleb Reid*, Harding University

47 **MORPHOLOGICAL INVESTIGATION OF MAMMALIAN CANCER CELLS**
Ayesha Siddiqua**, Arkansas State University

48 **CHARACTERIZATION OF GOLDENSEAL PRODUCT QUALITY BY DISSOLUTION STUDIES**
Alexander Rothenberger**, University of Arkansas for Medical Sciences. *Co-Author: Howard Hendrickson*

49 **DEVELOPMENT AND VALIDATION OF AN LC-MS/MS BASED METHOD TO QUANTITATE OCTREOTIDE IN MOUSE PLASMA**
Sarah Phillips, University of Arkansas for Medical Sciences. *Co-Authors: Qiang Fu, John Seng, Martin Hauer – Jensen, Howard P. Hendrickson*

50 **STRUCTURE, COMPOSITION, AND OLIGOSACCHARIDE PROFILES FOR HEMICELLULOSIC POLYSACCHARIDES ISOLATED FROM RICE BRAN FIBER**
Divya Kandanool**, Arkansas State University
Co-Author: Brett Savary

51 **IDENTIFICATION OF A NOVEL GLUCAN CROSSLINK TO FERULOYLATED ARABINOXYLAN IN RICE BRAN FIBER**
Kan Takahashi**, Arkansas State University
Co-Author: Brett Savary

ENGINEERING POSTER PRESENTATIONS

52 **MAINTAINING QUALITY OF ROUGH RICE DRIED IN A FLUIDIZED BED BY ADDING HOLD-UP PERIODS**
Kaushik Luthra**, University of Arkansas
Co-Author: Sammy Sadaka

53 **SCALING RESISTANCE OF INDUSTRY WASTE MODIFIED CONCRETE EXPOSED TO DEICING CHEMICALS**
Kazi Tamzidul Islam**, Arkansas State University
Co-Author: Zahid Hossain

54 **RAYLEIGH SCATTERING IN MULTIPLE NANOPARTICLE SYSTEMS: A STUDY OF THE SCATTERED MAGNETIC FIELDS**
Md Saber Nazim**, Arkansas State University
Co-Author: Zahid Hossain

55 **QUANTIFICATION OF MOISTURE EFFECTS ON ADHESION AND MODULUS PROPERTIES OF PAVING ASPHALTS**
Summon Roy**, Arkansas State University
Co-Author: Zahid Hossain

56 **ANOMALOUS ELECTROSTATIC NATURE OF CHARGED PARTICLES: AN APPROACH TOWARDS STABLE EQUILIBRIUM IN INVERTED SYSTEMS**
Tamal Sarkar**, Arkansas State University

57 **DEVELOPMENT OF A PEDOT:PSS BUFFER FILM FOR AN ORGANIC SOLAR CELL**
Obande Ikwuyum*, Arkansas Tech University
Co-Author: Matthew Young

GEOSCIENCE POSTER PRESENTATIONS

58 **VARIATION IN SOIL MOISTURE LEVELS IN NO-TILL, COVER CROP, AND CONVENTIONAL TILLAGESYSTEMS IN NE ARKANSAS COTTON**
Haylee Campbell*, Arkansas State University
Co-Authors: Kyle Wilson, Amanda Mann, Michele Reba, Tina Gray Teague

59 **QUALITATIVE ANALYSIS OF PM2.5 ORGANIC COMPOUNDS IN CONWAY, ARKANSAS**
Megan Cassingham*, Hendrix College
Co-Author: Dana Henry

60 **QUANTIFICATION OF SOLUBLE IONS IN ATMOSPHERIC PARTICULATE MATTER USING ION CHROMATOGRAPHY**
Ryan Tumminello*, Hendrix College
Co-Author: Courtney Hatch

PHYSICS POSTER PRESENTATIONS

61. **GAMMA-GAMMA ANGULAR CORRELATION**
Sonja Wagner*, University of Central Arkansas
Co-Author: Rahul Mehta

62 **X-RAY FLUORESCENCE**
Timothy Brown* & Jealen Greer*, University of Central Arkansas. *Co-Author: Rahul Mehta*

63 **DEVELOPMENT AND TESTING OF A SCIENTIFIC PAYLOAD AND GROUND-BASED TRACKING SYSTEM FOR HIGH-ALTITUDE BALLOONS.**
Trae Staggers*, Arkansas State University
Co-Author: Jackson Mixon

64 **DEVELOPMENT AND TESTING OF A SCIENTIFIC PAYLOAD AND GROUND-BASED TRACKING SYSTEM FOR HIGH-ALTITUDE BALLOONS.**
Jackson Mixon*, Arkansas State University

65 **GAMMA-GAMMA ANGULAR CORRELATION**
Jake Bass*, University of Central Arkansas
Co-Author: Rahul Mehta

66 **DEVELOPMENT OF AN ACOUSTIC SCANNER**
Nicholas Scoles*, University of Central Arkansas

67 **GENERATION OF GENERALIZED GAUSSIAN BEAMS VIA A SPATIAL LIGHT MODULATOR**
Samantha Dix*, Arkansas Tech University
Co-Author: Jessica Young

68 **EFFECT OF SIMULATED MICROGRAVITY ON RADIATION-INDUCED ENDOTHELIAL DYSFUNCTION MARKERS**
Yassamine Ghazzali*, Southern Arkansas University
Co-Authors: Abdel Bachri, Rupak Pathak, Marjan Boerman, Martin Hauer – Jensen

69 **STRATOSPHERIC BALLOONING RGB SYSTEM FOR LIDAR MEASUREMENTS**
Patrick Tribbett*, Arkansas State University
Co-Author: Ross Carroll

70 **RUTHERFORD BACKSCATTERING SPECTROMETRY**
Paul Niyonkuru*, University of Central Arkansas

71 **ANALYZING THE CHANGEOVER FROM A MACROSCOPIC SYSTEM TO A NANOSYSTEM BY INVESTIGATING THE MOVING BOUNDARY BETWEEN TWO PHASES**
Paul Niyonkuru*, University of Central Arkansas

In Memoriam: Kimberly G. Smith, 1948-2018

Kimberly Gray Smith, Historian and Executive Committee member of the Arkansas Academy of Science, and a doyen among Arkansas educators, passed away suddenly on April 9, 2018, at his home in Fayetteville, Arkansas. Kim was a community ecologist *par excellence*, and to the legions of his friends, students, and colleagues, the loss of his warm-hearted *persona* and his inspiring mentorship leaves a painful void. Few people mastered so many diverse areas of biology and natural history as Kim did. Birds, bugs, bears, he studied them all, with an insatiable curiosity and inspiring intellect honed by over three decades of nurturing a bevy of students toward productive research and teaching careers. A keen historian who loved to lecture and write on history of ecological thought, Kim clearly became a top ecologist by scrutinizing and emulating the careers of past star performers in ecology.

Born July 19, 1948, in Manchester, Connecticut, to Robert H. and Janet (Simon) Smith, Kim was third of five children. His interest in nature was sparked by his grandfather at an early age, but his passions have always been diverse. At Tufts University (B.S., 1971), he dabbled in lacrosse, swimming, and squash. The ornithology class he took with Herman Sweet in 1969 set him on the path toward a lifetime of studying birds. In August of 1969, he was the first intern at Manomet Bird Observatory, Massachusetts. He was especially adept at shorebird identification. Soon thereafter, Kim was leading bird tours on Cape Cod. Later (with RK), he continued to lead bird tours to the American tropics with profits to support the Arkansas Audubon Society Trust.

Kim came to the University of Arkansas in 1972 for his M.S. degree (1975), under Douglas A. James**.** Doug, and his then wife Frances “Fran” James, nurtured Kim as he elevated his life-long love of ecology into a solid academic career. Fran became a professional mentor to him and that bond lasted through his career. He said that he never made a major, professional decision without first discussing it with her. Kim was particularly capable in the developing field of multivariate analysis as applied to ecological systems. His M.S. work on summer vertical distribution of birds along the Ozark slopes led to his first paper, which appeared in *Ecology* (58:810-819, 1977), propelling him down the path to scientific acclaim.

Kim received his Ph.D. (1982) from Utah State University under James A. MacMahon, studying avian resource partitioning along a montane sere**.** Kim also was deeply involved in the initial studies about the ecological effects from Mount St. Helens eruption. After a brief stint as a post-doctoral research ecologist (1980-81) at the Bodega Marine Lab, University of California, Berkeley, he joined the University of Arkansas as an Assistant Professor in 1981, climbing up the ranks until his impending retirement 36 years later in 2018 as Distinguished Professor of Biological Sciences.

In his nearly four decades in Arkansas, Kim was a committed educator and nurtured students at various stages of career preparation and development. He treated his students like family, always including them, whether in professional or personal get-togethers--the more, the merrier! Altogether, Kim mentored 17 Undergraduate Honors, 29 M.S., 21 Ph.D., and 8 post-doctoral students. His research was funded in part by 29 major (>$20,000) grant awards, including 4 from the National Science Foundation. He served as Chair of the Department of Biology at the University of Arkansas, Fayetteville, between 2004 and 2008. When asked to describe Kim’s leadership style, one professor described his tenure as department chair as a “return to Camelot.” Kim was a Charles Bullard Fellow at Harvard University in 2001-2002.

A prolific writer, Kim authored or co-authored nearly 100 refereed journal articles, and remained active in writing till the end, including the three papers in this issue of the Journal. His papers reflect his remarkably eclectic interests. His notable contributions to avian community ecology include providing insights into community structure and energetics of avian assemblages along a montane sere, and effects of drought, prescribed burns, and tornado damage on community structure. His work on emergence of 13-year periodical cicadas in northwest Arkansas and how predator satiation leads to safety in numbers (Ecology, 1993) is a classic, featured in a leading Ecology textbook (*Ecology: Concepts and Applications*, by M. C. Molles, 4th ed.). His interest in avian breeding biology is evident from his publications on Dark-eyed Junco, Hooded Warbler, Jabiru, Resplendent Quetzal, and Wedge-billed Woodcreeper, from areas as far flung as montane Utah (United States) and cloud forests of Costa Rica, to the rainforests of Ecuador and marshes of Belize. His works on migration and habitat occupancy covered Blue Jays, Shorebirds, raptors, Rusty Blackbirds, and Saw-whet Owls. Kim even penned numerous popular articles. He wrote 41 symposium proceeding articles and book chapters.

A versatile ecologist, Kim was as comfortable with plants, arthropods, and mammals, as he was with birds. His interests ranged the gamut from conservation biology and plant-animal relationships, to gypsy moths, austral migration, and vertebrate breeding ecology and habitat selection. His forays into mammalogy ranged from studying small terrestrial mammals, southern flying squirrels, and elk in Arkansas, to documenting the current status of mammals in South Korea. He teamed with molecular biologists and micro-anatomists to publish on genetic variation and ultrastructure of teeth in black bears. He also spear-headed and documented the successful translocation of black bears in Arkansas—the most successful reintroduction of a bear in the world (Journal of Mammalogy, 1994). He followed it up by documenting the subsequent damage caused by the bears and their influence on land-owner attitudes. His work and participation as an *ex-officio* member of the Arkansas Game and Fish Commission (2004-2008) helped pave the way for improved bear management regulations. Ever the mega-data enthusiast, he brought-together disparate disciplines in population ecology, geographic distribution, and habitat. His contributions to the Arkansas Breeding Birds Atlas http://birdatlas.cast.uark.edu/ provide a template for further planning and protection.

Kim displayed a particularly sharp memory for the when, where, and what, concerning key events, places, and players in ecology. Beginning in 2001, he wrote a series of fascinating, quarterly columns titled "100 years ago in the American Ornithologists' Union" in the Auk (Vol. 118 to present) about the history of the AOU. These immensely readable articles chronicled the history of ornithological thought over the century. Kim brought these global, ecological perspectives to his work—and his life—applying those experiences as an Arkansan naturalist. His passion for history of ecology in Arkansas is evident by his thorough and meticulously researched writings on the subject, e.g., Friedrich Gerstäcker's observations in 1838-1842 (Arkansas Historical Quarterly, 2014), and the history of Saw-whet Owls (Journal of the Arkansas Academy of Science, 2016). He authored numerous small notes from his local studies. His classes instilled the same passion for ecological history into his students, who were often given assignments that asked them to contextualize a discovery in the prevailing scientific thought of the time. He occasionally rented a spare bedroom in his home to struggling graduate students. It was common for Kim to "pepper" those students with questions about ecological history on their way to their first cup of coffee in the morning.

Kim was elected Fellow of the American Ornithologists' Union (AOU, now American Ornithological Society) and American Association for Advancement in Science in 1991. For his service as Editor-in-Chief of The Auk, and for his historical columns, he was awarded the Marion Jenkins AOU Service Award in 2005. He also served as Associate Editor for Southwestern Naturalist (1990-1994) and Ecology and Ecological Monographs (1996-1999), and Book Editor for The Condor (1995-2000). At the University of Arkansas, he won a number of awards over the years, and achieved Distinguished Professor status in 2015.

Kim freely allocated his time to educating birders and mentoring novices. He moderated the popular ARBIRD-L, the Birds of Arkansas Discussion List which is used daily by bird enthusiasts to discuss bird sightings. He also facilitated another listserv for the Department of Defense, the Partners-in-Flight, which pertains to military operations across the globe. His expertise was invaluable to this community of birders seeking a scientific or historical perspective to their conservation activities. Kim served in the Arkansas Audubon Society Trust 1983-88, serving as Chair from 1986. Always the gregarious extrovert, he often jumped at the chance to host field data gathering events, and for many years led Christmas Bird Counts, Breeding Bird Surveys, and Shorebird surveys. He had a particular penchant for departmental parties or mixers in professional meetings, where he synergistically gained intellectual stimulation from rubbing shoulders with like-minded colleagues.

Kim was known for his impish sense of humor and benign sarcasm, often doled out with a beaming smile and a sparkle in his eyes. Once, when he walked in on a group of unsuspecting students in lab playing softball with a wad of paper and umbrella, he set his notes down, grabbed the umbrella from a student’s hand, and said, “I call second base.” He was a great husband, father, mentor, confidant, and friend. Four days before his death, he presented a retirement seminar in the Department of Biological Sciences. He entertained a packed room with an often humorous summary of his life and career, “Life in the Fast Lane: My Life as a Community Ecologist.” He ended with some advice to younger colleagues: “Be curious, be creative, challenge yourself to learn new things, learn the history of things that interest you, take students on field trips, take students abroad,” and finally, “have fun doing what you do … I did …”

Kim is survived by his beloved wife of 45 years, Peggy, their daughter Mallory and her husband Sheldon Steinert, and four grandchildren, Erowyn, Simon, Laura, and Kara.

Ragupathy Kannan
Department of Biology, University of Arkansas - Fort Smith, Fort Smith, Arkansas 72913

Jeffrey Short
122 Riverpark Drive, Malvern, Arkansas 72104

Journal Acknowledgements

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Editorial Board

Editor-in-Chief
Dr. Mostafa Hemmati
Professor of Physics and Director of Undergraduate Research
Department of Physical Sciences,
Arkansas Tech University

Contact Information:
Dr. Mostafa Hemmati
P.O. Box 1950
Russellville, AR 72811
(479)968-0340
mhemmati@atu.edu

Managing Editor
Dr. Ivan Still
Professor of Biology,
Department of Biological Sciences,
Arkansas Tech University
Russellville,
AR 72801
istill@atu.edu

Associate Editor: Ecology
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Assistant Professor,
Department of Biological Sciences,
Arkansas Tech University
Russellville,
AR 72801
dbarron@atu.edu

Associate Editor: Physical Sciences
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University of Arkansas at Fayetteville,
AR 72701
cgeren@uark.edu

Associate Editor: Physical Sciences
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Professor of Chemistry,
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Arkansas Tech University,
Russellville,
AR 72801
fhardcastle@atu.edu

Associate Editor: Physical Sciences
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University Professor,
Department of Civil Engineering
University of Arkansas at Fayetteville,
AR 72701
rps@uark.edu

The editorial staff also extends our heartfelt appreciation for the expertise, assistance and valuable time provided by our colleagues who acted 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.

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 Editor-in-Chief, when the revised manuscript is sent 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 errata. All final decisions concerning acceptance or rejection of a manuscript are made by the Managing Editor (Ivan H. Still) and/or the Editor-in-Chief (Mostafa Hemmati).

Please note that all manuscript processing, review and correspondence will be carried out electronically via the *JOURNAL* web site at http://scholarworks.uark.edu/jaas/. Thus, authors are requested to add the e-mail addresses of the editors (istill@atu.edu and mhemmati@atu.edu) 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. The authors will be provided with an electronic copy of their manuscript after the next annual meeting.

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

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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: http://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 the Editor-in-Chief: Dr. Mostafa Hemmati, P.O. Box 1950, Russellville, AR 72811.** 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: http://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 (istill@atu.edu).

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 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 the Editor-in-Chief (mhemmati@atu.edu): Mailing address: Mostafa Hemmati, P.O. Box 1950, Russellville, AR 72811. The Managing Editor will send an email reminder approximately 1 week prior to the final due date.

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

PREPARATION OF THE MANUSCRIPT

A. General considerations

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

1. Use Microsoft Word 2007 or higher for preparation of the document and the file should be saved and uploaded as a Word Document.
2. The text should be single spaced with Top and Bottom margins set at 0.9″ Left and Right margins, 0.6″. Except for the Title section, the manuscript must be submitted in two column format and the distance between columns should be 0.5″. This can be performed in MS Word by clicking on "Layout" on the Toolbar and then "Columns" from the drop-down menu. Then select "two" (columns).
3. Indent paragraphs and subheadings 0.25″.

4. Use 11 point font in Times New Roman for text. Fonts for the rest of the manuscript must be
   - a) Title: 14 point, bold, centered, followed by a single 12 point blank line.
   - b) Authors' names: 12 point, normal, centered. Single line spaced. Separate last author line from authors' address by a single 10 point blank line.
   - c) Authors' addresses: 10 point, italic, centered. Single line spaced. Separate last author line from corresponding author's email by a single 10 point blank line.
   - d) Corresponding authors email: 10 point, normal, left alignment.
   - e) Running title: 10 point, normal, left alignment.
   - f) Main text: 11 point, justified left and right.
   - g) Figure captions: 9 point, normal.
   - h) Table captions: 11 point normal.
   - i) Section headings: 11 point, bold, flush left on a separate line, then insert an 11 point line space. Section headings are not numbered.
   - j) Subheadings: 11 point, bold, italic and flush left on a separate line.

6. Set words in italics that are to be printed in italics (e.g., scientific names).

7. 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.

8. A **feature article** is 2 or more pages in length. Most **feature articles** should include the following sections: Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgments, and Literature Cited.

9. A **general note** is generally shorter, usually 1 to 2 pages and rarely utilizes subheadings. A note should have the title at the top of the first page with the body of the paper following. Abstracts are not used for general notes.

10. A **review article** should contain a short abstract followed by the body of the paper. The article may be divided into sections if appropriate, and a final summary or concluding paragraph should be included.

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

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

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

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

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

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

B. Specific considerations

1. Title section

(see Fig. 1 above for layout).

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

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

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

iv. Insert a single 10 point blank line after the "Running Title" and add a Continuous section break.

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. Please review your title and abstract carefully to make sure they convey your essential points succinctly and clearly.

3. **Introduction**
   An appropriate sized introduction should be included that succinctly sets the background and objectives of the research.

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

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

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

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

ii. **Figures**: A short caption should be written under each figure in 9 point, normal. Figure 2 shows an example for the format of a figure inserted into the manuscript. All figures should be created with applications that are capable of preparing high- resolution PhotoShop compatible files. The figure should be appropriately sized and cropped to fit into either one or two columns. Figures should be inserted as JPEG, TIFF images or PhotoShop compatible files. Arrows, scale bars etc., must be integral to the figure: i.e. not "added over" the figure once place in the word document: "independent arrows, etc., will be lost in manuscript formatting. While the *JOURNAL* is printed in black and white, we encourage the inclusion of color figures and photographs that can be viewed in the online version. Please note that the figures directly imported from PowerPoint frequently show poor color, font and resolution issues. Figures generated in PowerPoint should be converted to a high resolution TIFF or JPEG file (see your software user's manual for details).

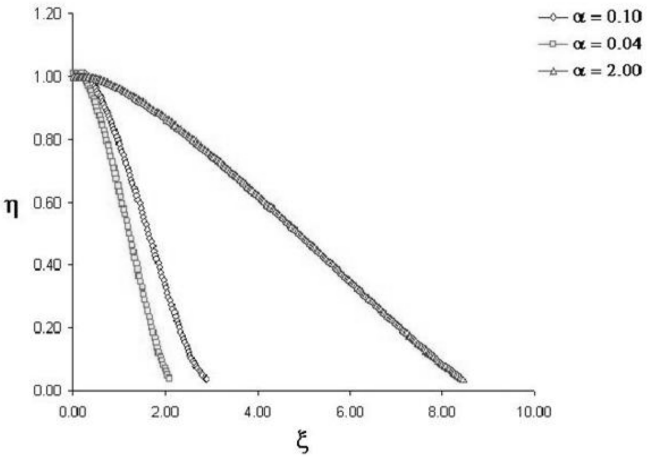

Figure 2. Electric field, η, as a function of position ξ, within the sheath region for three different wave speeds, α.

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 distance measurements are permitted in parentheses.

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

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

7. Biological Specimens

i Common names

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

ii Deposition of materials and sequences in publicly available domains

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

8. Literature Cited

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

ii. Please note how the literature is "cited in text as", i.e. in the introduction, results etc. In general, cite in text by "first author *et al.*" followed by publication date. **DO NOT USE NUMBERS, etc.** Also note that in the Literature Cited section, references should be single line spaced, justified with second and following lines indented 0.25". Column break a reference in Literature Cited that runs into the next column so that the entire reference is together. Insert a "Next Page" Section break at the end of the Literature cited section. If in doubt, see previous issue for format.

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Specific examples:

Standard Journal Article

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

Cited in text as: (Davis 1993)

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

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

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

Cited in text as: (Zheng and Luh 1989)

In press articles

Author(s). Expected publication Year. Article Title. Journal title *in press*.
Cited in text as: (First author *et al*. *in press*)

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

Books, Pamphlets, and Brochures

Box GEP, WG Hunter, and **JS Hunter.** 1978. Statistics for experiments. J Wiley (NY). 653 p.
Cited in text as: (Box *et al*. 1978)

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

Engelberger JF. 1989. Robotics in Service. MIT Press Cambridge (MA). 65 p.
Cited in text as: (Engelberger 1989)

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

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

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

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

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

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

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

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

Dissertations and Theses – General format is given first.

Author. Date of degree. Title [type of publication – dissertation or thesis]. Place of institution: name of institution granting the degree. Total number of pages. Availability statement.

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

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

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

Published Conference Proceedings – General format is given first.

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

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

Scientific and Technical Reports – General format is given first.

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

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

Electronic Journal Articles and Electronic Books should be cited as standard journal articles and books except add an availability statement and date of accession following the page(s):
Available at: www.usfw.gov/ozarkstreams. Accessed 29 Nov 2004.

Online resources

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

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

Cited in text as: (USGS 1979)

Multiple Citations are Cited in text as:

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

8. Submission of Obituaries and *In Memoria*

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

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

Members may receive 1 copy with their regular membership of $30.00, sustaining membership of $35.00, sponsoring membership of $45.00 or life membership of $500.00. Life membership can be paid in four installments of $125. Institutional members and industrial members receive 2 copies with their membership of $100.00. Library subscription rates from 2009 are $50.00. Copies of most back issues are available. The Secretary should be contacted for prices.


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