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Erratum

The following changes have been made to the on-line copy of the journal: Alexander and North (page 21-30) was retracted from the journal. Braund et al (page 93-105) was updated (see individual article page for details)

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COVER: Photograph of Fluorescence in a Flying Squirrel from Clark Co., SW Arkansas. *From:* Vertebrate Natural History Notes from Arkansas, 2019. R. Tumlison, *et al.*, pp39-45 (Manuscript 3348)

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The Reproductive Season of the Highland Stoneroller, *Campostoma spadiceum*, Evidenced by Museum Specimens

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Running Title: Reproductive Season of *Campostoma spadiceum* in Arkansas

Abstract

The Highland Stoneroller (*Campostoma spadiceum*) was described as a distinct species in 2010. Since then, the only study specific to this species is a survey of distribution, and nothing is known about reproduction. We examined 134 lots including 315 specimens of *C. spadiceum* housed in the Henderson State University collection of fishes to evaluate the timing of reproductive events. We dissected individuals to reveal sex and reproductive status. Females as small as 49 mm total length were yolking eggs, and follicles were in development by October. Ripe (mature) eggs were present in specimens collected from January through May, but were most common in March through May. Several females had oviposited in early March, and most specimens that appeared to have spawned had done so likely in April. Nuptial tubercles appeared on males as early as January and February, but most adult males were fully tuberculated from March through May.

Introduction

The Highland Stoneroller (*Campostoma spadiceum*) was redescribed as a distinct species (Cashner *et al*. 2010), soon after cladistic analysis of mitochondrial DNA data (cytochrome *b* gene) revealed that the population in the Ouachita Mountains region of Arkansas and Oklahoma should be considered a distinct species (Blum *et al*. 2008). Specimens of all sizes and both sexes present obvious red to red-orange coloration in median fins and usually in paired fins. The coloration lasts year-round and is most intense during the summer, but the coloration is not found in contiguous populations of any other species of *Campostoma* (Cashner *et al*. 2010).

Since the description, few other studies have dealt with the biology of this species. Some parasites have been documented from Highland Stonerollers, such as Acanthocephalans (McAllister *et al*. 2016), “black grubs” (McAllister *et al*. 2013), “white grubs” (McAllister *et al*. 2014), and leeches (Richardson *et al*. 2013; 2014). Hodges and Magoulick (2011) followed movement of *C. spadiceum* from riffles to pools during periods of stream drying, and Schanke (2013) studied genetics of subpopulations produced by isolating barriers such as stream drying, waterfalls, and culverts. The only other study specific to this species is a survey of distribution (Tumlison and Robison 2018).

Information concerning reproduction in *C. spadiceum* consists of a few anecdotal observations. Nuptial colors of males peaked in March or April (Cashner *et al*. 2010), and tuberculated males were observed from March through late April (Tumlison *et al*. 2017). Mature specimens of both sexes were observed in Clark Co. on 3 March (deemed to be early), and a tuberculated male was collected on 15 November, thought to be long after the breeding season (Tumlison *et al*. 2017). In Oklahoma, gravid *C. spadiceum* were collected on 13 February (McAllister and Robison 2016). Little is known about sizes of individuals in which reproduction begins, or the peak or duration of oviposition. We sought to provide information about the peak and duration of the reproductive season, and to assess the possibility of multiple spawning in this newly described stoneroller.

Methods

We examined 134 lots including 315 specimens of *C. spadiceum*, housed in the Henderson State University collection of fishes, to evaluate the timing of reproductive events. Individuals had been fixed in 10% formalin prior to washing and transfer to 45% isopropanol for storage. Total length (TL) was measured (mm) to determine at what size individuals became reproductive. We dissected individuals by cutting them open from vent to pectoral fins, then snipping at those points along the left side to create a fold to expose the abdominal cavity. We examined specimens under a dissecting microscope to reveal sex and reproductive status.

We adapted criteria in Núñez and Duponchelle (2008) and Timms (2017) to classify developmental status of ovaries in preserved museum specimens. Ovarian development was treated as having 5 stages (Fig. 1). Stage 1 ovaries are very thin, small, and contain no oocytes, representing the period between reproductive seasons (Timms 2017). We did not include those ovaries in our analysis. Stage 2 ovaries are small but have small, white follicles in which vitellogenesis is not apparent. For our purposes, we included ovaries containing only undeveloped white follicles and those that were white but had started deposition of lipids in Stage 2. Stage 3 ovaries are larger and contain yolking eggs, making them yellow to orange, but some previtellogenic white eggs also are present. Stage 4 ovaries are large and are dominated by ripe eggs that are deep orange to reddish, but eggs from earlier stages also usually are present. Stage 5 ovaries represent individuals that have spawned, so the ovary is a large hollow flat structure, and a few ova may remain. Recent spawners often have a distended, hollow abdomen likely caused by deposition of the eggs leaving space before food added to the intestine refills the vacated space in the abdominal cavity.

Results and Discussion

Left ovaries usually were larger than right ovaries, particularly noticeable in maturing specimens, which was consistent with observations for another stoneroller, *C. oligolepis* (Timms 2017). Females as small as 49 mm TL were yolking eggs, and follicles were in development by October. Ripe eggs (Stage 4 ovary) were present in specimens collected from January to May, but were most common in March and April. Females that had oviposited were found in early March, but most specimens that appeared to have spawned had done so likely by May (Fig. 2). Stage 3 ovaries were still present into June, which may indicate multiple spawning. Núñez and Duponchelle (2008) noted that multiple spawners have Stage 3 and 4 ovaries containing a mixture of developmental stages of ova. We noted this condition in almost all of our specimens.

Stage 2 ovaries usually had begun deposition of lipids by January or February, causing the eggs to begin to enlarge, but they remained white. Beginnings of development were seen as early as 31 October, and 2 specimens collected on 21 November also were developing eggs. One specimen of only 49 mm TL was undergoing vitelloegenesis, and had yellow eggs

Figure 1. Stages of development of ovaries in *C. spadiceum*. Top view shows a Stage 3 ovary with smaller white follicles (left) that have lipid deposition but have not started vitellogenesis (yolking), and right side shows larger yellow-orange ova into which yolk is being added. Middle image is a Stage 4 ovary, full of reddish ripe eggs (with other stages of egg development present) as seen just prior to spawning. Bottom image is a Stage 5 ovary, wide and empty of mature eggs which indicates spawning has occurred.

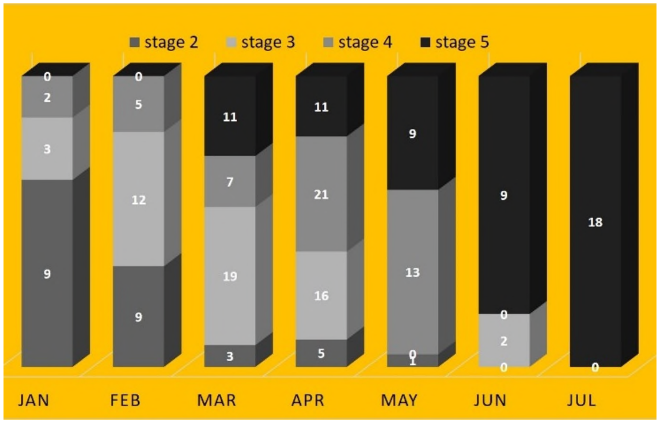

Figure 2. Bar graph of frequencies of ovarian stages in *Campostoma spadiceum* from January to July. Sample sizes per stage are represented in white on each bar, and height of bars represents data standardized to 100%.

developing on 16 February. The average size (TL) of females with Stage 2 ovaries declined over the reproductive season (Table 1), and the smallest specimen with Stage 2 ovaries in January was about the same size as the largest one in April. This was likely because older, larger females were able to spawn earlier in the season. Younger, smaller females might have been able to spawn later in the season, after gaining more resources to develop eggs.

Stage 3 ovaries were bright and distinctly colored. Yellowish eggs were seen as early as 14 January, but because they were not the dominant egg type, those ovaries were classified as Stage 2. However, other specimens collected in January and February had ovaries full of such eggs, so were classified as Stage 3. These would be expected to mature to Stage 4 and spawn in March or April, and we did find Stage 5 ovaries (spawned) beginning in those 2 months. However, most of the Stage 3 ovaries appeared in March and April.

The average TL of females with Stage 3 ovaries appeared to increase over the spawning season (Table 1). This seems to be in contrast with observations for Stage 2 females. However, we hypothesize that early spawners might have been able to spawn again within the season. If this was true, females that are larger in the latter part of the spawning season may be individuals delivering a second clutch (a group of eggs laid at the same time for fertilization). This hypothesis is supported by the fact that many of the individuals classified with Stage 3 ovaries in March and April were of greater TL, often over 100 mm. Two Stage 3 individuals collected at a late date of 4 June were 115 and 120 mm TL. Further, Stage 3 ovaries very often contained white eggs consistent with Stage 2 ovaries, and Núñez and Duponchelle (2008) noted that Stage 3 and 4 ovaries containing a mixture of developmental stages of ova indicate multiple spawners. With the exception of the 2 individuals collected on 4 June, Stage 3 ovaries were not seen after 23 April.

Stage 4 ovaries were dominated by fully yolked ova, considered to be ripe and ready to be fertilized. We found such ovaries as early as 17 January and through 24 May. This stage was most common in April and May, indicating the peak of spawning for this species (Fig. 2). January and February specimens with this stage of ovary tended to be small, but the average size was consistent from March to May (Table 1). The range in TL of specimens with Stage 4 ovaries was greater in April and May. This is consistent again with the notion of multiple spawners, as some larger specimens probably laid a second clutch of eggs while some smaller individuals might have just come into full breeding condition. Most of these ovaries also contained ova in both of the earlier stages of development, suggested by Núñez and Duponchelle (2008) to represent multiple spawners. Such ovaries with eggs in multiple stages of development will release ripe eggs for oviposition while immature eggs are held within the ovary until they are matured, at which point they are released in a second spawn.

Ripe eggs were found in 6 small specimens (55-60 mm TL) collected 17 January, 30 March, 2 April, 24 April, and 1 May. We suggest that the 3.5 month difference in these similarly-sized small but sexually maturing individuals represents the time when they were

Table 1. Sizes of female *Campostoma spadiceum* (mm, TL) at each ovarian stage per month of the spawning season in southern Arkansas.

	Jan	Feb	Mar	Apr	May
Stage 2					
N	9	9	3	5	0
Mean ± SE	71.6 ± 3.4	65.9 ± 6.2	55.0 ± 3.6	55.6 ± 2.0	
Range	60-87	55-113	50-62	49-61	
Stage 3					
N	3	12	19	16	0
Mean ± SE	71.0 ± 8.0	74.8 ± 5.4	78.7 ± 5.0	80.5 ± 4.6	
Range	63-87	49-106	55-125	55-128	
Stage 4					
N	2	5	7	21	13
Mean ± SE	59.5 ± 0.5	73.8 ± 4.3	73.0 ± 4.2	73.0 ± 2.8	70.0 ± 3.8
Range	59-60	64-89	60-93	55-98	59-111

hatched. Individuals too small to become sexually mature last season could mature earlier in the next season, and those hatched later in a previous year also matured later. This effect would extend the length of the spawning season for this stoneroller.

Stage 5 ovaries, representing the post-spawning condition, appeared by 2 March (Fig. 2). Stage 5 ovaries in which the body cavity was expanded but intestines had not refilled the vacated space were found on 2 March, 2 April, 4 May, 15 June, and 30 July. These observations indicate spawning events from March through July, but most specimens had completed spawning by late May.

Nuptial tubercles appearing on the head and body of males correlates with sexual development. We observed that the first bilateral pair of tubercles developed just in front of the eyes, followed by other pairs behind the eyes and continuing on the head. As the head became fully tuberculated, the structures began to develop centered along the anterior dorsal scales. A specimen collected on 21 November (65 mm TL) was just developing the first pair of tubercles. Larger specimens (80-135 mm TL) had full head tuberculation by mid-January, while smaller individuals (54 mm TL) had developed only 1-2 pairs at the same time. From February through May, males > 90 mm TL typically were fully tuberculated on the head and dorsal scales, whereas the smaller individuals tended to lack tubercles on dorsal scales and some of those typical for the head. Tubercles were found forming on the heads of smaller males (52-60 mm TL) on 17 January, 21 February, 8 March, 2 April, 10 April, 20 April, 24 May, and 5 June. Similar to reproductive development seen in females, we suggest that the > 4 month difference in these small but sexually maturing individuals represents the time when they were hatched. Individuals produced earlier in a previous year could sexually mature earlier in their first reproductive season, and those hatched later also matured later in a following season.

We speculate that the small females with ripe eggs should be able to deliver them into a spawning nest created by the large, dominant males. However, it is unknown whether the smaller males that show characteristics of sexual maturity are able to spawn. It is unlikely that they could compete with larger males for spawning sites. It would be interesting for further research to determine whether smaller males act as “sneaker” males to fertilize eggs deposited into the nests of large males (Lennon and Phillip 1960; Taborsky 1994; Stoltz and Neff 2006).

Although the length of the spawning season for *C. spadiceum* may be extended by multiple spawns, variation in seasonal temperatures also may affect the length of the breeding season. In New York, *C. anomalum* begins nest-building or territorial behavior when water temperatures reach 13-16°C (Miller 1964), Robison and Buchanan (1988) commented that spawning in Arkansas begins in March and April when water temperatures exceed 14.6°C (58°F), and Etnier and Starnes (1993) noted that peak spawning in Tennessee occurs at 12-14°C. Waters warmed earlier in a given year result in earlier onset of reproduction (but do not shift or truncate the season), therefore yearly variation in the arrival of spring temperatures can extend the period of reproduction (South and Ensign 2013). Because our specimens were collected over a period of about 25 years, the length of reproductive season could include such variation.

We examined average monthly air temperature for January, February, and March, and compared it with normal temperatures (available from 2007-2018; www.usclimatedata.com/climate/arkadelphia/arkansas/united-states/usar0016). Assuming a strong correlation between air and water temperatures, we attempted to infer whether yearly and monthly temperature variation might induce variations in the timing of reproductive development. Of the 12 years of data, 3 years had warmer than average beginnings (for all 3 months), 4 had cooler than average beginnings, and 5 were mixed. Because most of our specimens were collected in the 1990-2005 period, we could not match specimen data with the available climatological data. However, available climate data reveal that earlier development is likely during some years, and delayed in others. The reproductive season reported herein represents a composite of seasons beginning earlier or later due to yearly variations in seasonal temperatures.

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Occurrence of the Sinus Nematode *Skrjabingylus* sp. (Nematoda: Metastrongyloidea) Inferred from Sinus Lesions in Arkansas Mustelidae and Mephitidae, with Review of Relevant Literature

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Running Title: Sinus Nematodes in Arkansas Mustelidae and Mephitidae

Abstract

Nasal nematodes of the genus *Skrjabingylus* occur in the mammalian families Mustelidae and Mephitidae, and in North America occur from Canada to Costa Rica. Ingestion of infected snails, frogs, snakes, or mice can infect mammalian hosts. Infection often causes pathology to bones in the sinus region, which may lead to discoloration, enlargement, and fenestrations of the bone. Examination of museum specimens for evidence of infection has been used to detect prior infection, but prevalence and intensity cannot be interpreted without actually recovering the parasite. We examined Mustelids and Mephitids in collections of mammals housed at Arkansas State University (ASU), Henderson State University (HSU), and the University of Arkansas at Little Rock (UALR) to evaluate the possible occurrence of nasal nematodes in Arkansas mammals. Evidence of infection was found in skulls of the Striped Skunk (*Mephitis mephitis*), Spotted Skunk (*Spilogale putorius*), Mink (*Neovison vison*), Long-tailed Weasel (*Mustela frenata*), and North American River Otter (*Lontra canadensis*) from Arkansas. We report for the first time evidence of the presence and distribution of *Skrjabingylus* sp. infecting mammals in Arkansas.

Introduction

The trematode *Troglotrema acutum* and species of the nematode *Skrjabingylus* can cause cranial lesions in skulls of certain mammals (Heddergott *et al*. 2015a), but *T. acutum* occurs only in Europe so does not affect New World mammals. In North and Central America, only nasal nematodes of the genus *Skrjabingylus* have been identified from several geographic locations and in several members of the mammalian families Mustelidae and Mephitidae. Records are known from as far north as the Northwest Territories of Canada (Dougherty and Hall 1955) to as far south as Costa Rica (Carreno *et al.* 2005). The infective third stage larvae of *Skrjabingylus* may be ingested when a host species consumes an intermediate host (such as snails) or consumes paratenic hosts such as frogs, snakes, or mice (Lankester and Anderson 1971; Hansson 1967; Gamble and Riewe 1982; Jennings *et al*. 1982; Weber and Mermod 1985).

Skrjabinglyus nasicola is the most widely distributed species of the genus (Santi *et al*. 2006), and is common and cosmopolitan. The definitive hosts of *S. nasicola* are members of the genus *Mustela* (then including mink, which is now in the genus *Neovison*) (Hawkins *et al.* 2010). Other species of *Skrjabingylus* appear also to have host specificity; *S. petrowi* of the genus *Martes* (Heddergott *et al.* 2015b), *S. chitwoodorum* of the skunks *Mephitis* and *Spilogale* (Hill 1939, Hobmaier 1941; Goble 1942), and *S. lutrae* of river otters (Lankester and Crichton 1972).

In mink, up to 63 worms were counted in an individual from Minnesota (Kinsey and Longley 1963), but the average infection was 4.1 worms per cavity in Ontario (Santi *et al*. 2006). Significant infection often causes bone pathologies of the sinus region, which may present as discoloration, swelling or enlargement, and fenestrations of bone in the region of the sinuses (Santi *et al*. 2006). Lesions, known as local rarefying osteomyelitis, result from a reaction to the worms in the sinuses, which eventually can result in damage to bone (Kierdorf *et al*. 2006). Significance of the infection, suggested by hypotheses of a relatively smaller braincase caused by swelling of the frontal sinsues in older, infected animals, was supported for striped skunks (*Mephitis mephitis*) (Maldonado and Kirkland 1986), older male mink (Bowman and Tamlin 2007), and also occurred with a bias toward males in river otters (Scherr and Bowman 2009).

Methods

Because no data exist about occurrence of

Skrjabingylus in Arkansas, we sought to determine which Arkansas members of the Mustelidae and Mephitidae might show evidence of infection. We examined skulls housed in collections of mammals at Arkansas State University (ASU), Henderson State University (HSU), and the University of Arkansas at Little Rock (UALR). We examined skulls of the Striped Skunk (*Mephitis mephitis*), Spotted Skunk (*Spilogale putorius*), Mink (*Neovison vison*), Long-tailed Weasel (*Mustela frenata*), and North American River Otter (*Lontra canadensis*), all of the members of the Mustelidae and Mephitidae in Arkansas with the exception of the American Badger (*Taxidea taxus*). We collected data regarding the county of origin of each specimen to examine distribution within Arkansas.

Results and Discussion

The apparent oldest skull from Arkansas exhibiting damage from infection with *Skrjabingylus* was a new subspecies of ermine named by Brown (1908) after collection from the Conard Fissure in Newton County. Originally named *Putorius cicognanii angustidens*, and now known as *Mustela erminea angustidens*, one specimen shown in plate XVII of Brown (1908) had a circular lesion on the left side just behind the postorbital process of the skull. The lesion is consistent with infection by *Skrjabingylus*.

In the modern species we examined, we detected lesions in all species. Skulls of smaller species appeared to be more likely to demonstrate lesions, and to have larger openings, likely due to the thinner bones constituting the frontal region. Bones of the North American River Otter are more substantive, and were deemed less likely to show lesions (Scherr and Bowman 2009).

Examination of museum specimens for bone pathologies in the sinuses often has been used to provide evidence of occurrence, prevalence, and intensity of infection. Stegeman (1939) found bone lesions in New York *Mephitis* and suggested infection by *Skrjabingylus*, and Tiner (1946) used occurrence of lesions to infer the presence of this nematode in Texas skunks. Reliance on observation of damage in museum specimens to determine prevalence and intensity of infection have added confusion, however, due to problems in inferring levels of parasite burden without actually recovering the parasite (Dougherty and Hall 1955).

Presence or absence of osteological damage may not be a good index of the incidence of these parasites. Goble and Cook (1942) examined the sinuses of 10 minks and 10 weasels and found infections in 4 of each species, but no external enlargement or abnormality was observed. On the other hand, they found no worms on opening the sinuses of a Bonaparte weasel that had an enlarged frontal region. Levine *et al*. (1962) also reported that these parasites were present in the brain cases of skunks that did not show the characteristic lesions. Thus, damage in the sinuses may indicate that infection has occurred, but not that it is current, and absence of damage does not mean no infection is present.

Considerable variation exists with respect to reported patterns of occurrence and manifestation of infection. Several studies report evidence that both prevalence and intensity of infection tend to be higher in older host animals (Dougherty and Hall 1955; Gamble and Riewe 1982; Fuller and Kuehn 1984), but other studies find this in juveniles (Santi *et al*. 2006). Both prevalence and intensity of infection were observed to be higher in male ermine (Dubay *et al*. 2014), and male otters were found to suffer greater damage to their skulls due to these parasites (Scherr and Bowman 2009). Skulls of male Least Weasels (*Mustela nivalis*) were more severely damaged in England (King 1977), as were skulls of male Long-tailed Weasels (*Mustela frenata*) in Manitoba (Gamble and Riewe 1982). In contrast, Debrot and Mermod (1980) reported no sex- or age-related differences in mustelids from Switzerland. Santi *et al*. 2006 summarized studies in which an infection bias to one sinus was believed to have caused asymmetrical damage to the sinus region, whereas other studies found no difference in damage between the sinuses (Hansson 1967; Lewis 1967).

Therefore, we present data only to demonstrate the inferred presence of the nematode in mustelids and mephitids from Arkansas, the distribution of such occurrences, and relevant literature to elucidate how our observations fit with other North American information.

Spotted Skunk (*Spilogale putorius*) - Hill's (1939) description of a new species of sinus nematode, *Skrjabingylus chitwoodorum*, was based partly on specimens collected from *Spilogale interrupta* collected in Oklahoma. In California, *S. chitwoodorum* was reported to occur frequently in spotted skunks taken from the Davis area (Mead 1963). Lesions assumed to have been cause by an infestation by *S. chitwoodorum* were found in skulls of 3 *Spilogale* from Texas, wherein damage varied from bulging and osteitis to holes in the frontal sinuses (Tiner 1946).

Spotted Skunks are not well represented in collections in Arkansas. We examined 5 specimens, originating from Franklin, Izard, Pulaski, and Sebastian Counties. Those from Franklin, Pulaski and Sebastian Counties, showed evidence of infection (Fig. 1). The Pulaski Co. specimen had a small hole in the right sinus, the Sebastian Co. specimen had a large hole on the left sinus, and the Franklin Co. specimen had 2 holes in the right sinus (see Fig. 1).

Figure 1. Skulls of the Spotted Skunk (*Spilogale putorius*) from Arkansas, showing significant sinus lesions creating large holes due to infestation by *Skrjabingylus* sp. Top image shows two large holes in the right sinus, and bottom image is a large hole in the left sinus of a different specimen.

Striped Skunk (*Mephitis mephitis*) – Stegeman (1939) noted skull lesions attributable to *Skrjabingylus* parasitism in skunks from New York, and Hill (1939) described *Skrjabingylus chitwoodorum*, based on specimens collected from skunks (*Mephitis* and *Spilogale*) in Oklahoma. Goble (1942) reported *S. chitwoodorum* from *Mephitis* near Schenectady, NY, Tiner (1946) reported lesions attributed to this parasite in 3 *Mephitis* skulls from Texas, Levine *et al*. (1962) reported them in Illinois, and Bailey (1971) recorded them in Ohio. Dyer (1969) listed records of *S. chitwoodorum* in *Mephitis* from Maryland, Illinois, California, Kansas, and Quebec. Carreno *et al*. (2005) described *Skrjabingylus santaceciliae* based on specimens from a hooded skunk, *Mephitis macroura* from Costa Rica.

We examined 94 skulls of Striped Skunks, of which 5 (5.3%) showed lesions. Manifestations of infection included swollen discolored sinuses, a swollen bubbly appearance with tiny perforations, numerous small perforations, and a remodeled forehead with a high rise ridge (Fig. 2). Specimens originated from 26 counties, and 5 counties were represented by lesioned skulls (Fig. 5).

Figure 2. Skulls of the Striped Skunk (*Mephitis mephitis*) from Arkansas, showing significant sinus lesions due to infestation by *Skrjabingylus* sp. Top, the bubbly appearance resulting from swelling and tiny perforation; middle, numerous small perforations; bottom; swelling with considerable bone restructuring.

American Mink (*Neovison vison*) – The American Mink is a common mustelid of North America. Heavily infected specimens often exhibit inflated, discolored, and perforated sinuses (Sealander 1943, Kinsey and Longley 1963), though infections may occur with no visible manifestations (Goble and Cook 1942). In Minnesota, up to 63 worms were counted in an individual mink (Kinsey and Longley 1963).

Our mink sample originated from 15 counties, and specimens with damage caused by *Skrjabingylus* came from 8 counties (Fig. 6). Of 94 specimens we examined, 51 (54.3%) showed evidence of infection (Fig. 3). Prevalence of infection by *S. nasicola* in mink appears to be generally high throughout North America. Sealander (1943) found *Skrjabingylus* to be the most common parasite of mink in Michigan, occurring in 94% of specimens. Goble and Cook (1942) found 40% infection in New York, Dorney and Lauerman (1969) found 85% infection in Wisconsin, and Kinsey and Longley 1963) reported 75% infection in Minnesota. Santi *et al*. (2006) reported that 80.5% of mink were infected in Ontario, whereas Schulte- Hostedde and Elsasser (2011) reported infection in 43.6% of male mink in Ontario.

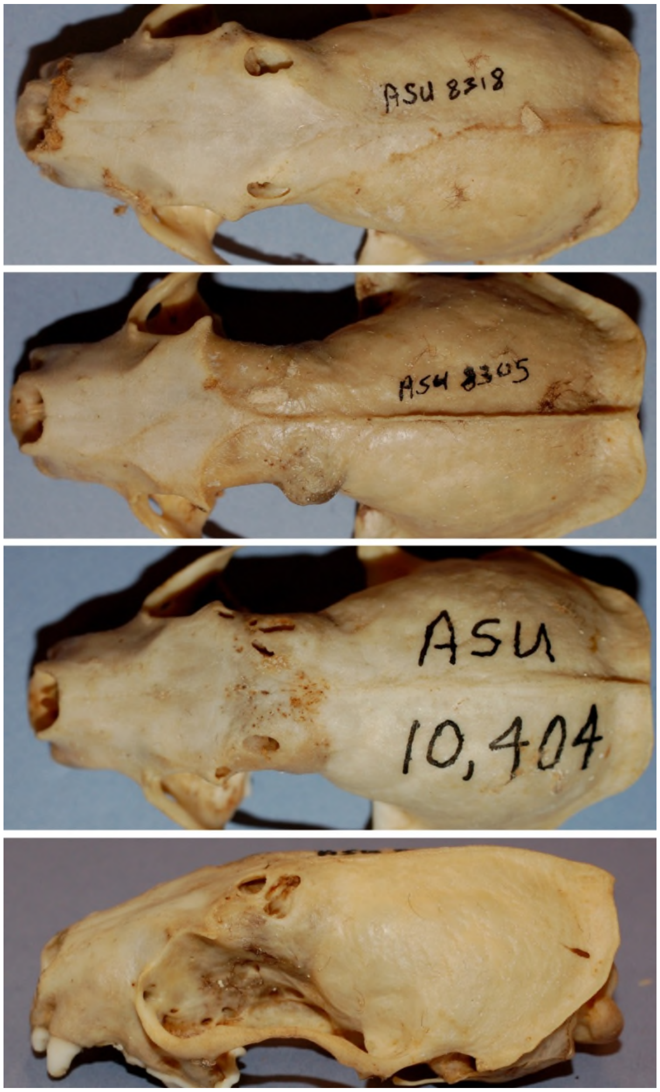

Figure 3. Skulls of American Mink (*Neovison vison*) from Arkansas, showing significant sinus lesions due to infestation by *Skrjabingylus* sp. Top, bilateral presentation of large holes: second from top, bulge in left sinus; third, bubbly appearance with bilateral large holes; and bottom, left sinus with 2 large holes.

The higher numbers of infected specimens allowed us to examine occurrence in more detail than for other species examined. Infections were represented bilaterally (in both sinuses) in 78.4% of the specimens. Santi *et al*. (2006) reported that a higher proportion of mink (58.2%) from Ontario were infected in both sinus cavities concurrently (double sinus infection), but frequency or intensity of infection did not differ significantly between left and right sinus cavities.

We ranked nature of infection for each side of each specimen according to the degree of damage, classifying based on the highest level of damage. Evidence of infection ranged from discoloration only (14.7% of sinuses), to a bubbly appearance with tiny holes (18.9%), small holes (< 2 mm; 33.7%), medium holes (2-4 mm; 15.8%), and large holes (> 4 mm, or 2 or more medium-sized holes; 16.8%). Sinuses with holes could also have a bubbly texture, and be discolored (Fig. 3). Our infected specimens originated from 8 counties (Fig. 6).

High frequencies of infection may have a yet unknown effect on behavior. Bowman and Tamlin (2007) found that infection with *Skrjabingylus* in older male mink caused down-warping of the braincase, resulting in reduced braincase volume caused by swelling of the frontal sinuses.

Long-tailed Weasel (*Mustela frenata*) – Goble and Cook (1942) found *Skrjabingylu*s infections in 4 weasels, though they had caused no external enlargement or abnormalities in the skull. Clapp (1952) reported *S. nasicola* in Long-tailed Weasels from Oregon, where up to 14 individuals were taken from 1 specimen.

Long-tailed weasels are not collected often in Arkansas, and our sample consisted of only 2 specimens, from Bradley and Craighead Counties. The weasel collected in Craighead Co. had a small hole resulting from infection of the right sinus.

North American River Otter (*Lontra canadensis*) – Lankester and Crichton (1972) described *Skrjabingylus lutrae* from river otters in Ontario. We examined 188 skulls of river otters from Arkansas, of which 16 (8.5%) showed lesions. Because skulls of otters are more robust than those of other hosts, it is less likely that they will show lesions. Most of our affected specimens presented discoloration, swelling, and small openings, but 1 specimen had a relatively large hole (4 mm) on the left sinus (Fig. 4). Our sample originated from 31 counties, of which 10 produced specimens infected by *Skrjabingylus* (Fig. 7).

Otters may harbor many *S. lutrae* and have major skull lesions without showing obvious clinical disease (Addison *et al*. 1988), but other infected otters have nasal discharge and neurologic signs (Petrini 1992). However, Scherr and Bowman (2009) found that skulls of male otters lesioned by infection also had reduced braincase volume, and to a lesser extent, female otters were similarly affected. This might affect behavior and survival of otters.

Conclusions

Through sinus lesions attributed to infection by the nasal nematode *Skrjabingylus*, we document the inferred occurrence of this parasite in the Mustelidae and Mephitidae of Arkansas. If host specificity is absolute, we have *S. nasicola* in Long-tailed Weasels and American Mink, *S. chitwoodorum* in Striped and Spotted Skunks, and *S. lutrae* in North American River Otters. We believe these parasites occur throughout the distribution of their hosts in Arkansas.

Figure 4. Skulls of North American River Otter (*Lontra canadensis*) from Arkansas, showing significant sinus lesions due to infestation by *Skrjabingylus* sp

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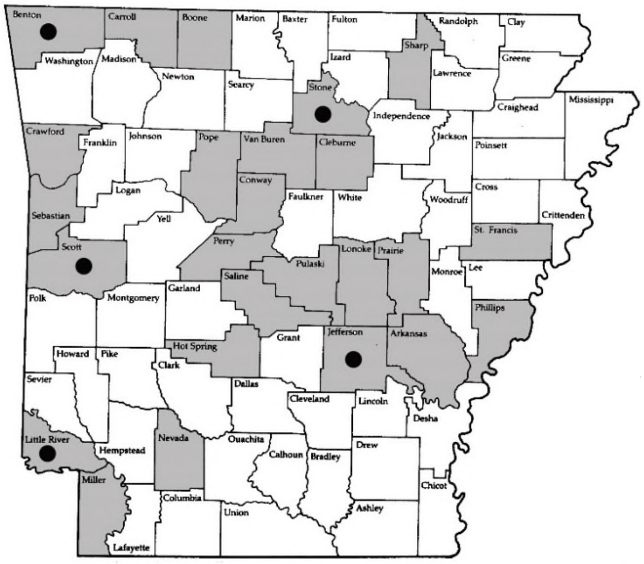

Figure 5. Distribution of Striped Skunks (*Mephitis mephitis*) in the sample (gray-shaded counties) and occurrence of lesions caused by *Skrjabingylus* sp. (dots). Counties with infected Striped Skunks included Benton, Jefferson, Little River, Scott, and Stone.

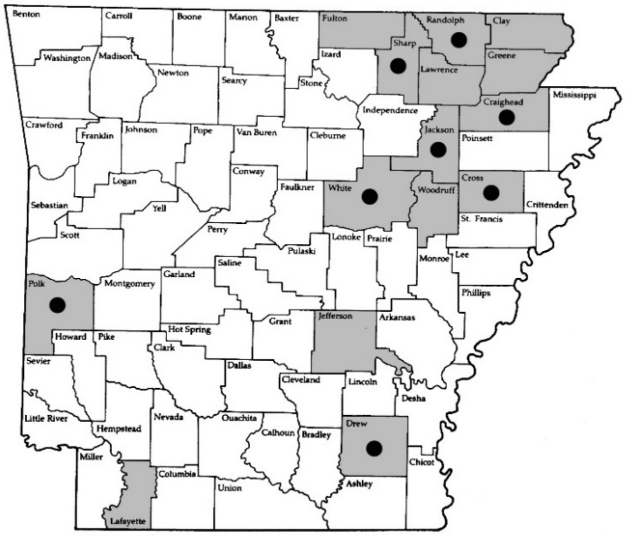

Figure 6. Distribution of Mink (*Neovison vison*) in the sample (gray-shaded counties) and occurrence of lesions caused by *Skrjabingylus* sp. (dots). Counties with infected Mink included Cross, Craighead, Drew, Jackson, Polk, Randolph, Sharp, and White.

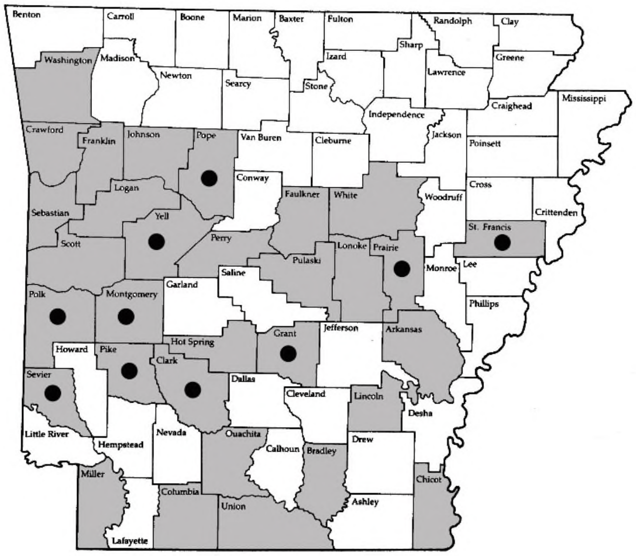

Figure 7. Distribution of River Otters (*Lontra canadensis*) in the sample (gray-shaded counties) and occurrence of lesions caused by *Skrjabingylus* sp. (dots). Counties with infected otters included Clark, Grant, Montgomery, Pike, Polk, Pope, Prairie, Sevier, St. Francis, and Yell.

Moore's Law and Space Exploration: New Insights and Next Steps

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Running title: Moore's Law and Space Exploration

Abstract

Understanding how technology changes over time is important for industry, science, and government policy. Empirical examination of the capability of technologies across various domains reveals that they often progress at an exponential rate. In addition, mathematical models of technological development have proven successful in deepening our understanding. One area that has not been shown to demonstrate exponential trends, until recently, has been space travel.

This paper will present plots illustrating trends in the mean lifespan of satellites whose lifespans ended in a given year. Our study identifies both Wright's law and Moore's law regressions. For the Moore's law regression, we found a doubling time of approximately 15 years. For Wright's law we can see an approximate doubling of lifespan with every doubling of accumulated launches. We conclude by presenting a conundrum generated by the use of Moore's law that is the subject of ongoing research.

Introduction

It has been observed that the rates of increase of technological capability in a variety of domains often follow exponential trends. For such domains there is a fairly predictable time constant at which the capability of the technology doubles although the time constants themselves vary quite a bit across domains (Magee *et al*. 2014). These trends are exponential and often described as conforming to "Moore's law," which originally described how the number of components that can be built into an integrated circuit doubles approximately every 18 months (Moore 1965).

But what causes these exponential patterns? Some noteworthy research done in this area suggests that this exponential progress is due to innovators applying lessons and principles from one domain to another domain (Basnet and Magee 2016; Arthur and Polak 2006; Axtell *et al.* 2013). The newly generated ideas will then be available for use in another domain and so on. The complexity of the technological system itself as well as functional requirements of the system influence how quickly the technology can be improved and leads to differing rates of progress (McNerny *et al*. 2011; Basnet and Magee 2016; Basnet and Magee 2017).

Another important description of technological progress was discovered by the engineer Theodore Paul Wright. This principle, known as "Wright's law," describes how as the volume produced of a manufactured good increases, the per-unit cost of the good falls at a predictable rate (Wright 1936). While Wright's law has important implications for operations management and business strategy it has also proven useful for technology foresight. An influential report indicates an equivalence between Wright's law and Moore's law when volume produced increases exponentially over time (Sahal 1979). A study in 2013 further compared Moore's law and Wright's law (Nagy *et al*. 2013).

While such patterns have been observed for fields as diverse as genome sequencing, LEDs, and 3D printing, they have not been observed for space travel. In fact, it is widely held that progress in space travel "has stalled" (Hicks 2015). A primary focus of our research program has been to determine if we are in a "space winter" or if there are in fact exponential trends to be found (Berleant *et al*. 2017).

The question of how to measure progress is not an easy one to answer. In fact, the wrong choice of metric may obscure the fact that space travel is improving (Roberts 2011). Cost may show an improvement trend, but collecting and analyzing the required data has proven non-trivial. As an alternative approach, evidence has been found suggesting an exponential trend with regards to spacecraft lifespan (Berleant *et al*. 2019). A key question (Nagy *et al.* 2013) has been whether this trend best fits a Moore's law-like pattern (improvement with respect to time) or a Wright's law-like pattern (improvement with respect to accumulated production volume).

One reason given for the apparent lackluster progress of space technology is the lack of commercialization. Matt Ridley in his book *The Rational Optimist* and others make the case that financial incentives play an important role in the development of any technology. From British capital markets during the industrial revolution to venture capitalists on Sandhill Road in Silicon Valley, history gives us good reason to believe that the expectation of profit is a strong driver of technological progress. Satellite technology represents the most commercialized aspect of space technology today. For this reason, we hypothesized that a data analysis of satellite technology may provide indications of exponential trending.

Analysis of Satellite Data

Figure 1 shows the mean lifespan of all satellites whose lifespans ended in a given year. A more detailed discussion of the data appears in Berleant *et al*. (2019), while here we emphasize those aspects most salient to (1) the focus of the present article, and (2) those elements of Figure 1 that represent an advance on the analogous figure in Berleant *et al*. (2019). The Moore's law regression is provided in equation (1) and the Wright's law regression is provided in equation (8). The top curve, Annual Count, shows the number of satellites whose lifespans ended (not launched) in the year given on the x-axis.

Both the Wright's law and Moore's law regressions show a general upward trajectory. Wright's law displays some irregular variations when plotted with respect to time, which is to be expected since Wright's law defines volume produced as the independent variable and not the x-axis variable, passage of time. If the x-axis showed volume instead, the regression curve would be free of such variations (but the Moore regression would then have them). For the Moore's law regression we have a doubling time of approximately 15 years. For Wright's law we can see an approximate doubling of lifespan with every doubling of accumulated launches.

Some of the earliest years were discarded from both regressions due to their inclusion leading to a poor fit to the regression curves. While this may seem contrary to the point of doing a regression it is useful for maintaining the ability of the model to predict, when early data is outlying or seemingly anomalous, and the primary interest is in extrapolating to the future. In this case, early launches were not representative of satellite technology as a whole and later data is more relevant than earlier data for the purpose of making predictions.

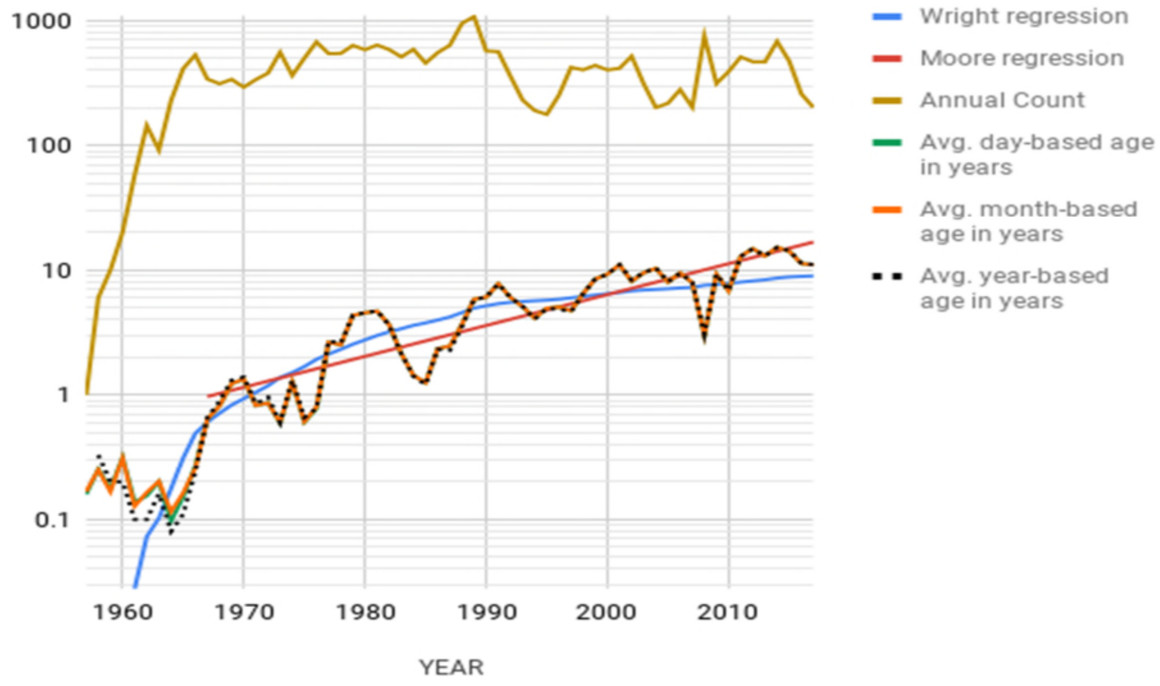


Figure 1. Annual count (top curve) and average age of satellites ending their lifespans each year.

An important question for measuring spacecraft lifespans is the correct unit of time to use. Lifespans were measured in days, months, and years (and then normalized so they could be directly compared) to examine how much using years and months distorted the graphs compared to more precise measurements in days. From Figure 1 it appears that years is not as good as months or days which are nearly identical. This occurs because measuring lifespan using years consists of subtracting the launch year from the end year. For example, suppose that a satellite was launched in December of 2016 and stopped functioning in March of 2017. Using years to measure this satellite's lifespan would give us a value of one year when in fact it had a lifespan of only three months.

End year was chosen rather than launch year because recently launched satellites would often still be in orbit, with only the shortest lived of them therefore contributing lifespan data for recent years, skewing the results and preventing a meaningful analysis.

Figure 2 illustrates an example of this phenomenon with lifespan data for spacecraft sent on deep space missions. When measured with respect to launch year we see that average lifespan increases until approximately 2000 and then decreases afterward, as significant numbers of craft launched in post-2000 years are still operational. This is because only short-lived spacecraft from this period are measured because these shorter-lived are the ones whose lifespans are available, leading to the noticeable decline in average lifespan due to the biased data, beginning in approximately 2000 and continuing to the present.

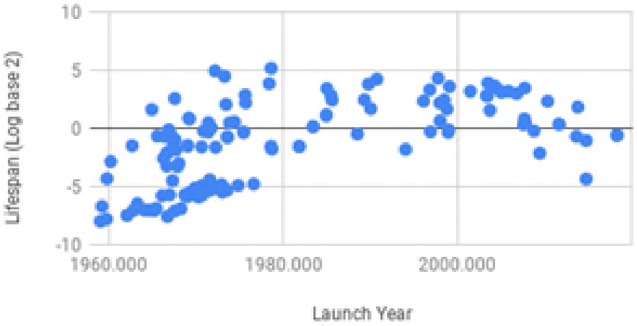


Figure 2. Lifespan vs. launch year for deep space craft.

The Moore's law conundrums

When comparing the RMS error of the Moore and Wright regressions it initially appears that Wright's law has a slightly better fit (Berleant *et al*. 2019). However, Wright's law may be more useful for another reason as well which isn't so obvious. While choosing the end year rather than start year made the analysis more feasible by removing the bias problem mentioned earlier, it also introduced another problem. If the observed doubling in satellite lifespan continues to hold then we must eventually reach a point where lifespan is increasing faster than the passage of time. This would require satellites dying in later years to be launched before satellites dying in earlier years, a seeming contradiction. Eventually we would reach a year for which satellite lifespans ending in that year would be predicted to be longer than the entire history of satellite technology. Since this scenario clearly makes no sense it remains an open problem of how it should be handled. Some progress is explained next.

If we still wish to associate lifespans with end year, when will Moore's law lose its predictive power? For this analysis let Moore's law be defined as:

$$y = a * 2^{\frac{(x-1957)}{b}} \qquad (1)$$

where *a* and *b* are function parameters and *x* represents time and is used to model the current end year. Parameters *a* and *b* are set to 0.549 and 12.17 respectively since this minimizes RMS error (Berleant *et al*. 2019). The input value *x* represents end year and the value *y* is expected lifespan. The first year against which lifespans can be measured is 1957 since that is the year the first satellite was launched, this value is subtracted from *x* and only positive values are considered. For this reason, the historical time span *y* of satellite technology at year *x* is:

$$y = x - 1957 \qquad (2)$$

In order to determine when Moore's law breaks down, we need to determine when the rate that lifespan increases with respect to time equals (immediately following which it will exceed) the rate that time increases with respect to time. In order to do this, we can solve (1) and (2) simultaneously, take the first order derivative and determine the year the two expressions are equal to one another.

$$0.549 * 2^{\frac{(x-1957)}{12.17}} = x - 1957 \qquad (3)$$

Moving both expressions to one side:

$$0.549 * 2^{\frac{(x-1957)}{12.17}} - x + 1957 = 0. \qquad (4)$$

Taking the derivative of the expression:

$$\frac{d}{dx}0.549 * 2^{\frac{(x-1957)}{12.17}} - \frac{d}{dx}x + \frac{d}{dx}1957 = 0 \qquad (5)$$

$$0.549\left[\frac{1}{12.17}*\ln(2)*2^{\frac{(x-1957)}{12.17}}\right]-1 = 0. \qquad (6)$$

If we simplify and solve for x we obtain:

$$x = 12.17*log_2\left(\frac{12.17}{0.549*\ln(2)}\right) + 1957 = 2017.84. \qquad (7)$$

Thus 2017 was the year that lifespans of satellites dying in a given year are predicted to begin increasing faster than the passage of time, a conundrum. What about the point where satellite lifespan is predicted to be greater than the length of the history of satellite technology? If we graph both equations (1) and (2), we can visually observe the points at which they intersect and thus the year that this predicted event might occur. Doing this shows that this point is reached in the year 2046 when average satellite lifespan is predicted to be approximately 89 years, and thus launched prior to 1957, when Sputnik became the first artificial satellite (Figure 3).

So, returning to the earlier point on which law is better for predicting future satellite lifespans based on year of death, Wright's law seems superior simply because (1) Moore's law based on lifespan as a function of end year began failing in principle in 2017 and will reach an even greater level of impossibility in 2046; and (2) launch year cannot work for recent years for which longer-lived craft are still operational.

Discussion

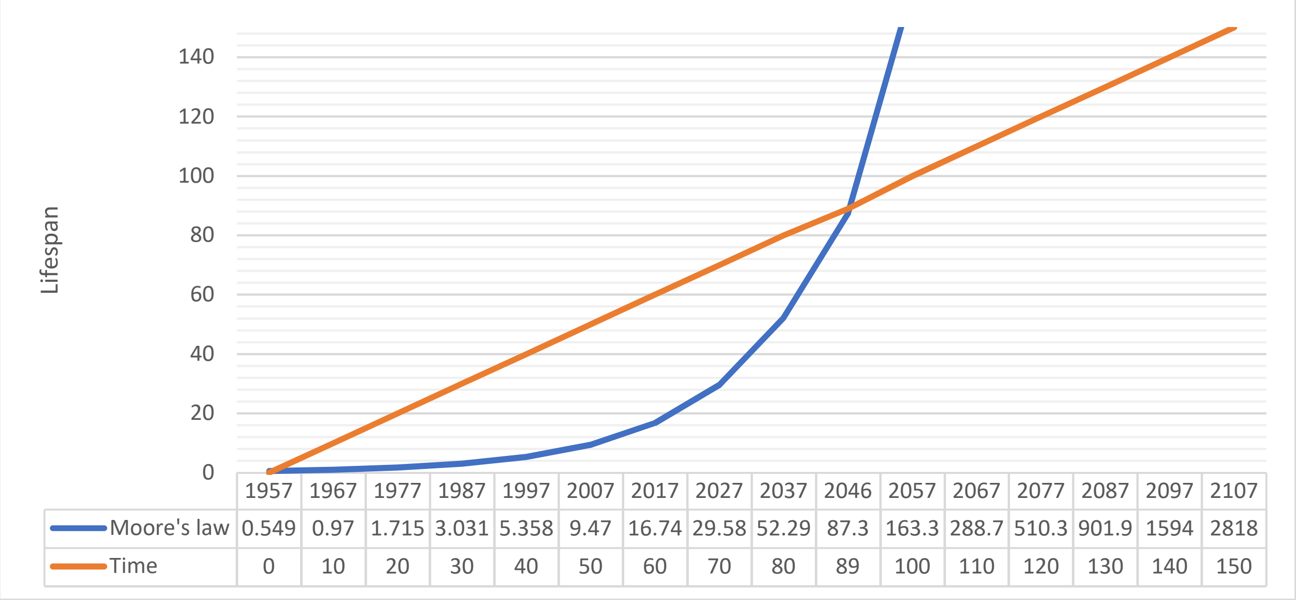
The Moore's law regression was described earlier in equation (1). The simple regression equation for Wright's law is as follows:

$$y = 0.0002446*ordinality^{1.04} \qquad (8)$$

Where y is the average lifespan for satellites ending in that year and *ordinality* is determined by the number of satellites ending in that year and previous years. Our preliminary research suggests that the conundrum associated with Moore's law that was described previously may also apply to the Wright's law regression, although at a much later year, in which case a Wright's law model would not form a principled alternative to a Moore's law model in the case of lifespan as a function of end year. However, this remains to be fully investigated.

Conclusions

It may appear that satellite technology has been progressing in an approximately exponential way, perhaps a little less vigorously than a Moore's law



	1957	1967	1977	1987	1997	2007	2017	2027	2037	2046	2057	2067	2077	2087	2097	2107
Moore's law	0.549	0.97	1.715	3.031	5.358	9.47	16.74	29.58	52.29	87.3	163.3	288.7	510.3	901.9	1594	2818
Time	0	10	20	30	40	50	60	70	80	89	100	110	120	130	140	150

Figure 3. Satellite lifespan vs. passage of time, showing a Moore's law crossover

model, but a little more vigorously than a Wright's law model (Figure 1). However, we can confidently predict based on the mathematical deduction presented earlier that the data in coming years must soon break decisively from the Moore's law trend line of Figure 1 and show that lifespan will soon not fit an exponential function of satellite year of death. Importantly however, we have certainly not ruled out the possibility of an exponential trend for some characteristic other than lifespan as a function of year of satellite death.

We plan to empirically verify the analysis we have introduced here against future satellites. Future research is needed to circumvent this mathematical problem and accurately identifying the degree to which space travel is an accelerating technology.

Finally, we close by pointing out that key results presented here should also apply to lifespans of other engineered artifacts besides satellites

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Energy Content of Seeds of Texas Doveweed (*Croton texensis*) from the Diet of Mourning Doves *(Zenaida macroura*) from Southeastern New Mexico

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Running title: Energy Content of Seeds of Texas Doveweed from Mourning Dove Diet

Abstract

We analyzed the energy content of seeds of Texas doveweed (*Croton texensis*) obtained from the crops of mourning doves (*Zenaida macroura*) collected from plains-mesa sand-scrub habitat in Eddy and Lea counties, New Mexico. Seeds were removed from crops and dried for 48 hours at 60°C to remove moisture and to standardize masses. Seeds were then analyzed for gross caloric value (i.e., energy content) in an oxygen bomb calorimeter. Energy content of seeds of Texas doveweed was greater than that of many seeds previously reported from the diet of mourning doves.

Introduction

Knowledge of the energy content of food items is critical to understanding why an animal might choose one food item over another, and is necessary for conservation and management of game species. While feeding habits of mourning doves (*Zanaida macroura*) are well studied (Mirarchi and Baskett 1994), daily energy requirements have not been determined. Only a single study (Schmid 1965) has measured the energy content of food of free-living mourning doves, although another (Shuman *et al*. 1988) measured some known and potential food items of mourning doves in conjunction with determination of how well captive mourning doves metabolized various food items. No study of energy content of food of mourning doves has been conducted with birds from sand-scrub habitat of New Mexico.

A study of feeding habits of mourning doves in southeastern New Mexico determined that seeds of Texas doveweed (*Croton texensis*) made up the largest portion (32%) of the total mass of crop contents, and were present in 55% of the crops of mourning doves (Hunt 1999). Texas doveweed is also reported to be an important food item of other birds, such as northern bobwhites (*Colinus virginianus*—Hunt and Best 2001*a*) and scaled quail (*Callipepla squamata*—Hunt and Best 2001*b*). We used an oxygen bomb calorimeter to determine the energy content of seeds of Texas doveweed.

Methods and Materials

Mourning doves were collected at the Waste Isolation Pilot Plant site in southeastern New Mexico in conjunction with long-term studies of lead poisoning of game birds (Best *et al*. 1992*a*; 1992*b*) and studies of feeding habits of game birds in southeastern New Mexico (Hunt 1999; Hunt and Best 2001*a*; Hunt and Best 2001*b*). Most of the study area is in eastern Eddy County, but it also extends into western Lea County. All mourning doves were collected in uncultivated, shinnery oak-honey mesquite (*Quercus havardii-Prosopis glandulosa*) habitat, part of the plains-mesa sand-scrub vegetation type (Dick-Peddie 1993). Several studies of the feeding ecology of mourning doves have been conducted in this area (Davis 1974; Best and Smartt 1986; Hunt 1999). The study area is heavily grazed by cattle, and several man-made stock tanks are located on the site.

In late summer and autumn 1988, 150 mourning doves were collected by shooting as encountered. Collected birds were placed on ice within 10 minutes of shooting to minimize effects of post-mortem digestion (Dillery 1965; Farner 1960; Sedinger 1986); no effect of digestion on crop contents was observed. Crops were removed, placed into plastic vials, and frozen. Contents of crops were later thawed, separated by type of food, and placed into envelopes for drying. Food items were dried for 48 hours at 60°C to standardize masses. Food items were identified by comparison with samples of plants collected at the study site, and by using identification manuals (Davis 1993; Martin and Barkley 1961).

Samples of seeds of *Croton texensis* were analyzed for gross caloric value (i.e., energy content) in an oxygen bomb calorimeter (Model 1341, Parr Instrument Company, Moline, Illinois). Samples of seeds from 9 individual mourning doves with crops that contained enough seeds for analysis were selected; each sample weighed approximately 1 g. Seeds were combusted in the oxygen bomb; after combustion, the bomb was washed and bomb washings were titrated with sodium carbonate to allow adjustment of results for nitrate content.

Results

The 9 samples analyzed contained an average of 5.2 kcal/g (range, 4.4-6.2; standard deviation, 0.5—Table 1). This figure is greater than that for most previously tested food items in the diet of mourning doves.

Table 1. Gross caloric value (energy content) of seeds of Texas doveweed (*Croton texensis*) from the crops of mourning doves (*Zenaida macroura*) collected from Eddy and Lea counties, New Mexico.

Sample No.	Energy in kcal/g
MD003-88	4.6
MD004-88	5.0
MD005-88	5.0
MD006-88	5.2
MD007-88	5.3
MD009-88	5.3
MD088-88	5.5
MD122-88	4.4
MD138-88	6.2

Discussion

Mourning doves are known to be selective in food choices (Browning 1959; Davison and Sullivan 1963), although criteria for their selection are imperfectly understood. Among suggested criteria are taste (Davison and Sullivan 1963), color (Goforth and Baskett 1971), nutrient content (Hayslette and Mirarchi 2001) and energy content (Schmid 1965; Shuman *et al.* 1988). Although no study has demonstrated that mourning doves preferentially select Texas doveweed over other food items, Davison and Sullivan (1963) categorized Texas doveweed as a "choice" food plant for mourning doves, meaning it was readily eaten when encountered. Our study demonstrates that Texas croton has an energy content comparable to or greater than food items from previous studies. For example, in a study of 9 food items collected from crops of mourning doves in North Dakota, Schmid (1965) found only 2 that had a greater energy content—seeds of flax (*Linum usitatissimum*, 6.3 kcal/g) and field mustard (*Sinapis arvensis*, 5.98 kcal/g). Other seeds tested from North Dakota had less energy content; examples include wild plants such as green foxtail (*Setaria viridis*, 4.4 kcal/g) and cultivated crops such as corn (*Zea mays*, 4.06 kcal/g) and wheat (*Triticum aestivum*, 3.96 kcal/g). Likewise, Shuman *et al.* (1988) tested 8 varieties of seeds that were considered to be potential food items for mourning doves in Kansas, and found only 2 that had greater energy content—thistle (*Cirsium*, 6.2 kcal/g) and Maximilian sunflower (*Helianthus maximiliani*, 5.6 kcal/g). Other seeds analyzed in Kansas had less energy content; examples include proso millet (*Panicum millaceum*, 4.5 kcal/g) and timothy (*Phleum pratense*, 4.7 kcal/g).

Doveweed and other crotons are associated with disturbance, particularly with areas grazed by cattle (Fessler 1960). Much of southeastern New Mexico is heavily grazed by cattle (Hunt 2004), so that Texas doveweed grows in abundance. Availability of seeds of Texas doveweed, coupled with the relatively great energy content, helps explain its prevalence in the diet of mourning doves (Hunt 1999).

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The Essentiality of the Vinyl Proton in Anticonvulsant Enaminones

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Running title: The Essentiality of the Vinyl Proton in Anticonvulsant Enaminones

Abstract

Enaminones are chemical compounds consisting of an amino group linked through a C=C bond to a keto group with a vinyl proton. Enaminones analogs have been extensively studied in anticonvulsant therapy. They appear to act through two mechanisms, (a) inhibition of the sodium channel or (b) via a γ-aminobutyric acid-ergic (GABAergic) pathway. A small library of 2,5-dimethyl enaminone derivatives were synthesized and evaluated in a series of acute preclinical seizure models to answer several questions: 1) is the vinyl proton essential for anticonvulsant activity, 2) is the vinyl proton required for activity, and 3) can we substitute with a methyl group? Most of the methyl-substituted enaminones showed protection against maximal electroshock seizure (MES), the pentylenetetrazol seizure (scPTZ), [illegible]Hz psychomotor seizure, and neurotoxicity (Tox). Three compounds (5i, 5l, 5p as listed in Table 1) emerged with activity at 100 and 300mg/kg and no toxicity in MES studies.

Introduction

Epilepsy affects more than 3 million individuals in the United States and 65 million people worldwide. Epilepsy is the second most prevalent neurological disorder worldwide (Johnson 2019). Despite optimal use of available antiepileptic drugs, many patients with epilepsy fail to experience seizure control and some do so only at the expense of significant toxic side effects (White 1999). Hence, the search for [illegible]epileptic agents with more selectivity and lower toxicity continues to be an area of investigation in medicinal chemistry. In a continuing study of potential enaminone anticonvulsants (Alexander *et al.* 2013; Ananthalakshmi *et al.* 20[illegible]; Anderson *et al.* 2006; Edafiogho *et al.* 2006; Edafiogho *et al.* 2003; Edafiogho *et al.* 1992; Edafiogho *et al.* 2007; Eddington *et al.* 2003; Eddington *et al.* 2002; Eddington *et al.* 2000; Foster *et al.* 1999; Gibson *et al.* 2009; Jackson *et al.* 2012; Jackson *et al.* 2009; Laws *et al.* 199[illegible]; Mulzac and Scott 1993; Scott *et al.* 1995; Wilson *et al.* 200[illegible]), we postulated early in our studies (Eddington *et al.* 200[illegible]) that the vinyl proton was essential for activity.

Enaminones are a conjugated system having the general formula of [illegible]-CH=CH-(CH[illegible]C=O (Scott *et al.* 1995) As anticonvulsants they appear to act through two mechanisms, (a) inhibition of the sodium channel or (b) via a GABAergic pathway (Alexander *et al.* 2013; Amaye *et al.* 20[illegible]; Wang *et al.* 2011). Based on the extensive Quantitative structure–activity relationship (QSAR) study, we postulated that the vinyl proton was essential for activity. For this study, we will methylate the vinyl proton and discuss its activity.

Materials and Methods

Chemistry

Melting points (mp) of the target compounds were determined on a Thomas Hoover melting point apparatus and were uncorrected. All reactions were monitored by thin layer chromatography (TLC) on a 5 x 10 cm Whatman K6F glass plates using a solvent system of ethyl acetate (EtOAc): hexane (3:1). Column chromatography (gravity) was performed on silica gel as a method of purification using (200-400 mesh) silica gel and EtOAc: methanol (9:1) or EtOAc: hexane (3:1) as elution mixtures. The ^{1}H proton nuclear resonance and ^{13}C nuclear resonance spectra were determined on a Bruker 1 Ultra Shield-400 MHz NMR spectrometer. The samples were dissolved in deuterated dimethylsulfoxide (DMSO-d_6) containing 0.03% tetramethylsilane (TMS) as an internal reference. The infrared (IR) spectra for the target compounds were obtained on a Perkin Elmer 100 FT-IR spectrophotometer. IR peaks are designated as weak (w), medium (m), broad (br), strong (s) and sharp (s). Commercially available starting materials cyclohexane-1,3-dione, 5,5-dimethylcyclohexane-1,3-dione and the para-substituted anilines were obtained from Sigma-Aldrich Chemical Company, 2-methyl-1,3-

cyclohexanedione was obtained from Acros Organics and used without further purification. 5-Methylcyclohexan-1,3dione, 2,5-dimethyl-1,3-cyclohexanedione, and 2,5,5-trimethyl-1,3-cyclohexanedione were prepared by literature methods (Born *et al.* 1953; Scarborough 1961; Silbermann and Henshall 1957). The proton nuclear magnetic resonance (1H NMR spectra and purity can be seen in North 2011.

2,5-Dimethyl-1,3-cyclohexanedione (3). M.P.: 170-172°C (lit.). yield 54%. **^{1}H NMR:**δ (DMSO-d_6) 0.95 (3H, d), 1.55 (3H, s), 1.8-2.55 (5H, m).

2,5,5-Trimethyl-1,3-cylohexanedione (4). M.P.: 209-211°C (lit.). yield 47%, **^{1}H NMR:**δ (DMSO-d_6) 1.0 (3H, m, br), 1.55 (3H, s), 2.15 (5H, m, br).

3-(4-chlorophenylamino)-2-methylcyclohex-2-enone (5a). M.P.: 195-197°C. Yield 37.9%, **I.R.:** ν_{max} (cm^{-1}) 3260 (w) NH, 2950 (CH stretch; w), 1645 (w) C=[illegible] 1567 (C=C) and 1454 (aromatic); 1198 (s, sh) CN; [illegible]d 1084 (m,sh) Cl-aryl. **^{1}H NMR:**δ (DMSO-d_6) 1.5 [illegible]H, s, CH_3), 1.8 (2H, sextet), 2.2 (2H, t), 6.5-7.0 (4H[illegible]d), 8.2 (1H, s, NH) **^{13}C NMR:**δ (DMSO-d_6) 7.[illegible], 20.9[illegible] 110.08, 115.65, 128.93 129.06, 148.10.

3-(4-bromophenylamino)-2-methy[illegible]clohex[illegible]one (5b). MP.: 167°C. Yield 16.9%[illegible]**R.:** *v*m[illegible] (c[illegible]) = *v*NH 3270 (w); *v*sp^2 2934 (CH[illegible]etch; w[illegible]C=O [illegible]601 (w); *v*C=C 1581 and 1480 (a[illegible]atic); *v*C-N [illegible] (s, sh), and *v*Br-aryl 1070 (m, sh[illegible]**H NMR**[illegible] (DM[illegible]) 1.6 (3H, s, CH_3), 1.8 (2H[illegible]ntet), [illegible]H, t), 2.4[illegible]h), 7.0-7.5 (4H, dd, aro[illegible]tic ring), [illegible].28 [illegible], s, NH) **^{13}C NMR:**δ (DMSO-[illegible] 9.88, [illegible]12, 27.83, [illegible]94, 39.3[illegible] 40.61, 108.60, [illegible].18, 12[illegible]7, 1[illegible]1.93, 139.[illegible]3, 157[illegible], 195.22.

3-(4-iod[illegible]henyla[illegible]-2-m[illegible]yl[illegible]clohex[illegible]enone (5c). [illegible]**P.:** 181[illegible]2°C[illegible]Yield 1[illegible]%. **I.R**[illegible]*v*max (cm^{-1}) = [illegible]H 3269 [illegible] *v*sp^2 [illegible] (CH st[illegible]h; w); *v*C=O [illegible]01 (w); *v*C=C[illegible] and 1482 (ar[illegible]atic); *v*C-N 1136 (s, sh); and *v*I-aryl [illegible]70 (m, sh[illegible] **NMR:**δ (DMSO-[illegible]) 1.6 (3H, s, CH_3), [illegible] (2H, [illegible]ntet), 2.2 (2H, t), 2.5 (2[illegible] 6.9-7.7 (4H, dd, arom[illegible] ring), 8.25 (1H, s, NH) **^{13}C** [illegible]**R:** δ (DMSO-*d*[illegible].92, 22.13, 27.86, 36.96, 39.36, [illegible]57, 40.62, [illegible]03, 108.72, 126.35, 137.81, 140.40, 15[illegible]4, 195[illegible]

3-(4-trifluromet[illegible]yl)phenylamino)-2-methylcyclohex-2-enone (5d). M.P.: 175°C. Yield 25.1%. **I.R.** *v*max (cm^{-1}) = *v*NH 3182 (w); *v*sp^2 2942 (CH stretch; w); *v*C=O 1523 (w); *v*C=C 1590 and 1450 (aromatic); and *v*C-N 1197 (s, sh). **^{1}H NMR:**δ (DMSO-d_6) 1.6 (3H, s, CH_3), 1.8 (2H, quint[illegible] 2.2 (2H, t), 2.49 (2H), 7.1-7.7 (4H, dd, aromatic ri[illegible]), [illegible]50 (1H, s, NH) **^{13}C NMR:** δ (DMSO-d_6) 10.[illegible] 22.07, [illegible].38, 37.09, 111.48, 122.31, 126.33, 144[illegible], 156.72, 19[illegible].

3-(4-trifluromethoxy)[illegible]enylamino)-2-methylcyclohex-2-e[illegible]e (5e). M.P.: 194-196 °C. Y[illegible] 33.3% **I.R.:** *v*ma[illegible]m^{-1}) = [illegible]269 (w); *v*sp^2 29[illegible] (CH stretch; w[illegible]C=O 16[illegible] (w); *v*[illegible]C 1550 and 1[illegible]7 (aromatic); *v*[illegible]ryl 1252 [illegible]sh); and [illegible]-N 1148 [illegible]sh). **^{1}H NMR:**[illegible]DMSO-[illegible]) 1.7 [illegible]H, s, [illegible]), 1.8[illegible] (2H, quintet [illegible]clohex[illegible] ring), [illegible] (4H, d[illegible] aromatic ring)[illegible]30 (1H[illegible]NH) [illegible]C NM[illegible] δ (D[illegible]O-d_6) 9.78, 22[illegible], 27.79, 3[illegible]08.58, [illegible]19.32, [illegible]1.87, 121.97, [illegible].64, 13[illegible]7, 144[illegible] 14[illegible]9, 158[illegible], 195.23.

3-(4-n[illegible]rop[illegible]ylamino)-2-me[illegible]lcyclohex-2-enone (5f)[illegible]**M.P.:** 20[illegible]1°C. Yiel[illegible]9.7% **I.R.:** *v*max (cm^{-1}) [illegible] 3270 (w); [illegible]p^2 293[illegible]CH stretch; w); *v*C=O 1601 [illegible]w); *v*C=C[illegible]581 and 1[illegible] (aromatic); *v*NO2-aryl 1482 [illegible] sh); an[illegible]C-N 11[illegible] (s, sh). **^{1}H NMR:**δ (DMSO-d_6) [illegible](3H, [illegible]CH_3)[illegible] (2H, quintet, CH_2), 2.2 (2H, t, CH_2)[illegible]-8.0 ([illegible] dd, aromatic ring), 10.2 (1H, s, NH) [illegible]**C NMR:** δ [illegible]MSO-d_6) 7.67, 20.99, 110.08, 112.83, 12[illegible]82, 13[illegible]1, 156.13.

3-(p-[illegible]ylamino)-2-methylcyclohex-2-enone (5g). M[illegible] 134-135 °C. Yield 26.9% **I.R.:** *v*max (cm^{-1}) = [illegible]H 3179 (m); *v*sp^2 CH stretch 2942 (m, br); *v*C=O s-trans 1700 (m, sh); *v*C=C 1527 (s); *v*CH_3 1373 (m, sh) **^{1}H NMR:**δ (DMSO-d_6) 1.7 (3H, s, CH_3), 1.8-2.4 (5H, m, cyclohexene ring), 2.3 (3H, d, CH_3), 6.9-7.2 (4H, dd, aromatic ring), 8.1 (1H, s, NH) **^{13}C NMR:** δ (DMSO-d_6) 9.43, 20.90, 22.13, 27.59, 36.85, 106.49, 125.37, 129.69, 134.11, 137.59, 159.25, 194.56.

3-(4-Chlorophenyl-amino)-2,5-dimethylcyclohex-2-enone (5i). M.P.: 183-185°C. Yield 44.3% **I.R.:** *v*max (cm-1) = *v*NH 3193 (w);*v*sp2 2950 (CH stretch; w); *v*C=O 1704 (m); *v*C=C 1557 and 1491 (aromatic); *v*CN 1162 (s, sh); and *v*Cl-aryl 1083 (m, sh). **^{1}H NMR:**δ (DMSO-d_6) 0.93 (3H, d, CH_3), 1.67 (3H, s, CH_3), 2.02-2.6 (5H, m, cyclohexene ring), 7.0-7.5 (4H, dd, aromatic ring), 8.28 (1H, s, NH) **^{13}C NMR:** δ (DMSO-d_6) 9.72, 21.42, 29.47, 35.74, 45.14, 107.93, 125.99, 128.27, 129.10, 129.19, 139.39, 157.47, 195.11.

3-(4-bromophenylamino)-2,5-dimethylcyclohex-2-enone (5j). M.P.: 189-190°C. Yield 18.2% **I.R.:** *v*max (cm^{-1}) = *v*NH 3446 (w); *v*sp2 2867 (CH stretch; w); *v*C=O 1583 (m); *v*C=C 1519 and 1489 (aromatic); and *v*C-N 1160 (s, sh) *v*Br-aryl 1069 (m, sh). **^{1}H NMR:** δ (DMSO-d_6) 0.93 (3H, d, CH_3), 1.65 (3H, s, CH_3), 1.96-2.34 (5H, m, cyclohexene ring), 7.04-7.50 (4H, dd, aromatic ring), 8.28 (1H, s, NH)

3-(4-iodophenylamino)-2,5-dimethylcyclohex-2-enone (5k). M.P.: 203-204 °C. Yield 14.9% **I.R.:** *v*max (cm^{-1}) = *v*NH 3186 (w); *v*sp^2 3045 (CH stretch; w); *v*C=O 1524 (m); *v*C=C 1489 and 1438 (aromatic); *v*C-N 1166 (s, sh) and *v*I-aryl 1058 (m, sh). **^{1}H NMR:**DMSO-d_6) 1.6 (3H, s, CH_3), 1.8 (2H, quintet), 2.2 (2H, t), 2,49 (2H, t), 7.1-7.7 (4H, dd, aromatic ring), 8.50 (1H, s, NH) **^{13}C NMR:** δ (DMSO-*d_6*) 9.73, 21.83, 29.30, 35.78, 45.25, 88.16, 108.09, 126.15, 137.85, 140.82, 157.28, 195.19.

3-((4-Trifluromethoxy) phenylamino)-2,5-dimethylcyclohex-2-enone (5l). M.P.: 173-175 °C. Yield 23.7% **I.R.:** *v*max (cm^{-1}) = *v*NH 3123 (w); *v*sp^2 3004 (CH stretch; w); *v*C=O 1608 (m); *v*C=C 1534 and 1499 (aromatic); *v*C-N 1102 (s, sh) and *v*F-aryl [illegible] (m,sh). **^{1}H NMR:**δ (DMSO-d_6) 1.6 (3H, s, CH_3), 1.8 (2H, quintet), 2.2 (2H, t), 2.49 (2H, t), 7.1-7.7 (4H, dd, aromatic ring), 8.50 (1H, s, NH) **^{13}C NMR:** δ (DMSO-*d_6*) 9.70, 21.38, 29.49, 35.72, 45.14, [illegible], 108.1[illegible], 119.32, 121.86, 121.99, 122.71, 125.60, [illegible]27.08, 139.70, 144.68, 147.20, 157.34, 195.17.

3-(p-tolylamino)-2,5-dimethylcyclohex-2-enone (5m). M.P.: 225 °C. Yield [illegible]% **I.R.:** *v*max (cm^{-1}) = *v*NH 3158 (w); *v*sp^2 3020 (CH stretch; w); *v*C=O 1612 (m); *v*C=C 1594 and [illegible] (aromatic); *v*CH_3-aryl [illegible]385 (m, sh); and *v*C-N [illegible] (s, sh). **^{1}H NMR:** δ (DMSO-d_6) 0.85 (3H, d, CH_3), [illegible]05 (3H, s, CH_3), 1.[illegible] (3H, s, CH_3), 1.9-2.75 (5H, m, cyclohexene ring), 5.7 (3H, d, CH_3), 7.0-7.3 (4H, dd, aromatic ring), [illegible] (1H, s, NH).

2,5-dimethyl-1,3-(phenylamino)cyclohex-2-enone (5n). M.P. 147-[illegible] °C. Yield [illegible]% **I.R.:** *v*max (cm^{-1}) = *v*NH 3160 (w); *v*sp^2 [illegible] (CH stretch; w); *v*C=O 1590 (w); *v*C=C 152[illegible] and 1490 (aromatic) and *v*C-N 1195 (s, sh). **^{1}H NMR:**δ (DMSO-d_6) 1.[illegible] (3H, d, CH_3), 1.70 (3H, s, CH_3), 2.0-2.8 (5H, m, cyclohexene ring), 7.1-7.8 (4H, dd, aromatic ring), 8.4 (1H, s, NH).

3-(chlorophenylamino)-2,5,5-trimethylcyclohex-2-enone (5o). M.P.: 19[illegible]-197°C. Yield 13.0% **I.R.:** *v*max (cm^{-1}) = *v*NH [illegible] (w); *v*sp^2 3063 (CH stretch; w); *v*C=O 1594 (w); *v*C=C 1566 and 1493 (aromatic); *v*C-N 1153 (m, sh).

2,5,5-trimethyl-1,3-(phenylamino)cyclohex-2-enone (5p). (729 mg, 21.2% yield); **M.P.:** 170-172 °C. Yield 21.2% **I.R.:** *v*max (cm^{-1}) = *v*NH [illegible] (w); *v*sp^2 3063 (CH stretch; w); *v*C=O 1594 (w); *v*C=C [illegible]66 and 1493 (aromatic); and *v*C-N 1166 (s, sh). **^{1}H NMR:** δ (DMSO-d_6): 0.99 (6H, s, gem CH_3), 2.04 (2H, s, CH_2) 2.[illegible] (2H, s, CH_2), 5.3 (3H, s, CH_3), 7.1-7.4 (4H, dd, aromatic ring), 8.89 (1H, s, NH) **^{13}C NMR:** δ (DMSO-*d_6*) 28.[illegible], 32.74, 50.66, 97.[illegible], 123.43, [illegible].78, 129.65, 139.6[illegible], 160.50, 195.84

Pharmacology

The Epilepsy Therapy Screening Program (ETSP) program, a National Institute of Neurological Disorders and Stroke (NINDS) National Institutes of Health (NIH)-funded, preclinical screening program with a mission to facilitate the discovery of new therapeutic agents addressing unmet medical needs in epilepsy provided *in vivo* anticonvulsant screening. Pharmacological testing included maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ), and 6 Hz psychomotor seizure tests, as well as their neurotoxic effects using rotarod tests. An overview of the testing procedures is described (Krall *et al.* 1978; Porter *et al.* [illegible]). Phase I study involved three *in vivo* tests: maximal electroshock seizure test (MES), the pentylenetetrazol seizure test (scPTZ), 6 Hz psychomotor seizure test, and the neurotoxicity test (Tox). Intraperitoneal (ip) administration of the test compounds was carried out as a suspension in 0.5% methylcellulose. Active compounds in the phase I evaluation are subsequently tested either for an ED_{50} quantitation in mice (phase II) or qualitatively in rats (Phase VIA). The Anticonvulsant Screening Program (ASP) classifications for activity are as follows: class 1 = activity at 100 mg/kg or less; class 2= activity > 100 mg/kg, but less than 300 mg/kg; class 3 = no activity at doses up to and including 300 mg/kg. Several of the analogs will be evaluated for oral (po) activity (phase VIA) in the rat to differentiate the result between distinct rodent species. 6 Hz test is a model for therapy-resistant partial epilepsy and tests the ability of a compound to block psychomotor seizures induced by long duration (3s.), low frequency (6 Hz) stimulation (Stables and Kupferberg 1997). 6 Hz testing has identified potential compounds such as levetiracetam which was not active in the MES and/or subcutaneous metrazol (SCM) testing (Stables and Kupferberg 1997). Thus, the ETSP has included 6 Hz test in initial screenings instead of SCM test since 2014. The 6 Hz model was found to be highly sensitive to the antiepileptic drugs that positively modulate $GABA_A$ receptors and thus may be valuable as a tool for the identification of such compounds.

Motor impairment models for toxicity

Rotorod test

Each mouse treated with the test compound and was placed on a rod that rotates at 6 rpm prior to seizure creation. Any mouse that fell off the rod three times in a minute was considered under neurotoxic effect by the compound (Stables and Kupferberg 1997).

Minimal motor impairment assessment

Rats were evaluated for indications of ataxia, which is abnormal, uncoordinated gait. Before seizure creation, each mouse was monitored for circular or zigzag gait, abnormal body posture and spread of the legs, tremors, hyperactivity, lack of exploratory behavior, somnolence, stupor, catalepsy, loss of placing response, and changes in muscle tone. Any animal displaying at least two of these behaviors was considered impaired due to the test compound (Stables and Kupferberg 1997).

Quantification studies

Quantification of effective and toxic doses (ED_{50}/TD_{50}) were performed at the time of peak effect (TPE) of each compound and each model. For determination of the TPE animals were tested at 0.25, 0.5, 1.0, 2.0 and 4.0 h. Groups of four to eight animals were tested at various doses at the TPE until at least two points were established between none to full protection. Using Probit analysis the ED_{50}/TD_{50}, 95% confidence interval, the slope of the regression line, and standard error were calculated.

Animal studies were carried out under the supervision of, and under IACUC approval granted to, Dr. Tracy Chen of [illegible] National Institute of Convulsive Disorders and Stroke.

Results

Chemistry

The unsubstituted series **(5a-h, Scheme 1, Series I)** were formed by reacting 2-methyl-1,3-cyclohexanedione and various anilines under reflux conditions using a Dean-Stark trap. For the mono-methyl series, **(5i-p, Series 2)**, the condensation of ethyl crotonate with tert-butoxy acetoacetate was modified as reported by Friary and co-workers for the synthesis of 4-carbo-tert-butoxy-5-methylcyclohexane-1,3-dione. (Friary *et al.* 1973) The β-diketone tert-butoxy ester underwent hydrolysis and decarboxylation in the presence of 0.05N sulfuric acid to produce the 5-methyl-1,3-cyclohexane-dione in high yields. The 5- methyl-1,3-cyclohexanedione was added to a solution of 4 N aqueous sodium hydroxide and iodomethane and the solution was refluxed for [illegible] h and cooled first to room temperature and then refrigerated overnight to produce 2,5-dimethyl-l,3-cyclohexanedione in good yields. The compound then underwent the same synthesis and work-up procedures to yield the 2,5-dimethyl enaminones (**[illegible]-p, Series [illegible]**).

Preparing the di-methyl series, (**Series 3**), dimedone was added to a solution of [illegible] aqueous sodium hydroxide and iodomethane. The solution was refluxed for [illegible] hours and cooled first to room temperature and then refrigerated overnight to produce 2,5,5-dimethyl-1,3-cyclohexanedione in good yields. The corresponding enaminones were formed using previous methods.

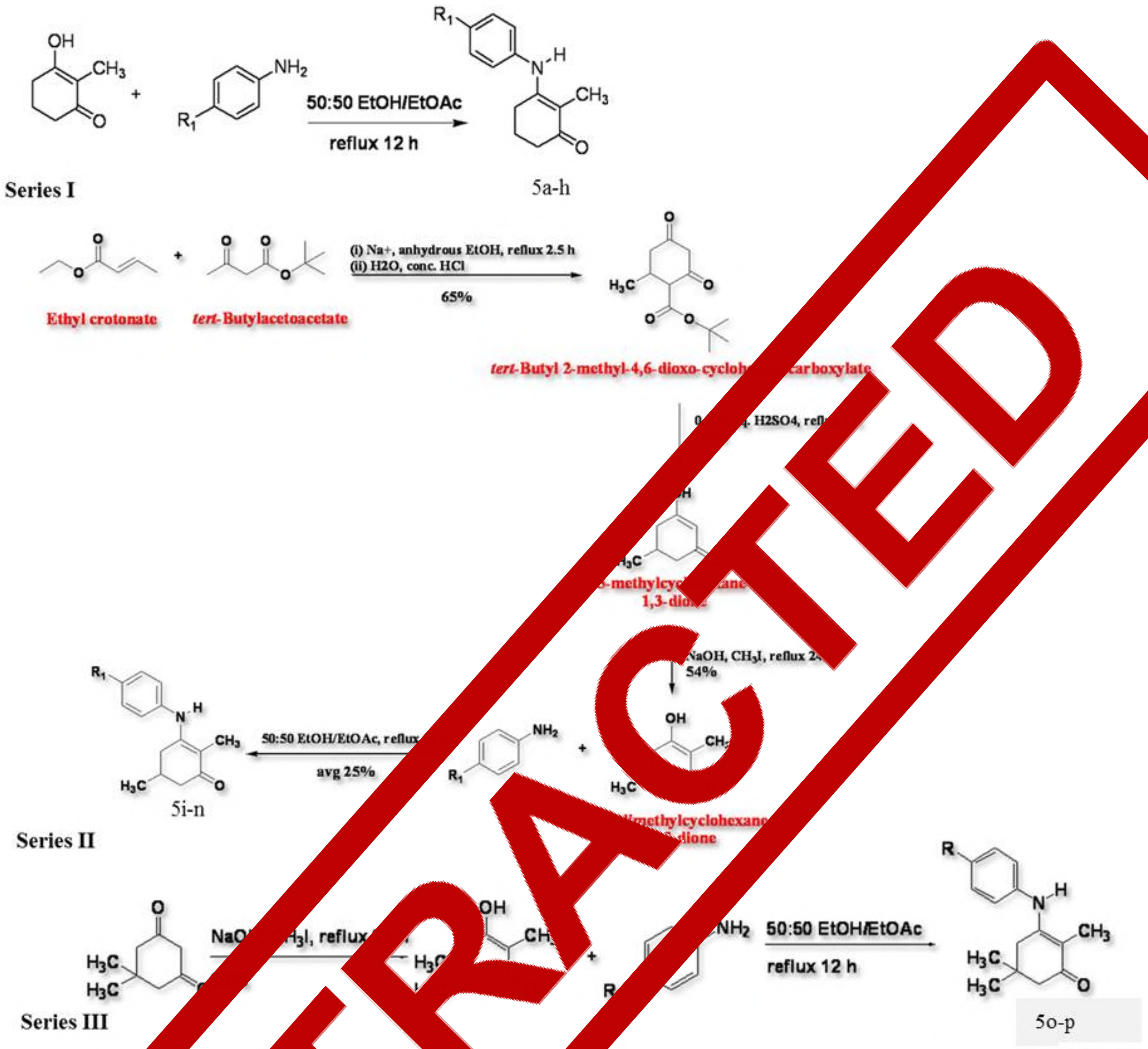
We obtained all of the final compounds in moderate yields (13%-44%), as shown in **Table 1**.

Pharmacology

Anticonvulsant identification, the initial screening of the Epilepsy Therapy Screening Program (ETSP), is where investigational compounds were tested against seizures induced by maximal electroshock (MES), scPTZ, and 6 Hz at two-time points (0.5 and 4h) and three doses (30, 100, and 300 mg/kg) in mice. The MES study in rats is where the investigational compounds were tested at five-time points (0.25, 0.5, 1, 2, 4h) at a dose of 30 mg/kg. Neurotoxic effects were evaluated using the rotorod test. Most of our compounds were active at least at one of the given time points and doses in either or both methods. In mice (i.p.), the exclusively MES active compounds were **5e, 5f, 5i, 5l, 5n**, and **5p**. Compound **5d** was MES active with some scPTZ activity. 5**a** and **5o** were both MES and scPTZ active with notable toxicity. Protection was also noted in **5f, 5l** and **5p** during 6 Hz testing.

Selected compounds **5f, 5l,** and **5p** were chosen for the evaluation of anticonvulsant activity in the 6 Hz test (**Table 2)**. Three compounds were selected randomly as a part of the search of molecules providing anti 6 Hz protection among chemically diversified compounds pursued in the NIH/NINDS.

The unsubstituted anilines **5h**, **5n**, **5p**, provided differing results. **5h** displayed no protection in mouse (ip) studies but displayed some toxicity (1/4) at the 300 mg/kg dose in the rotorod assessment as opposed to the unsubstituted which was highly active and toxic. In **5n** mice (ip) studies, the substance was protective against MES-induced seizures at 300 mg/kg. However, the



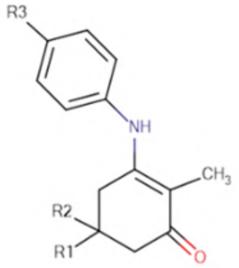
Scheme 1. Synthesis of 2-methyl enaminones.

compound caused toxicity as well as one animal death during toxicity assessment [illegible] protective dose. There was no protection in the scPTZ study. **5p** exhibited MES protection at [illegible] 300 mg/kg in mice (ip). Interestingly, neither scPTZ activity nor toxicity was detected at the [illegible] points employed. Minor MES protection ([illegible]) was observed at 2 hours post dosing while no overt toxicity [illegible] These encouraging results lead to further testing in the 6 Hz and NP assays. **5p** [illegible] active in the 6 Hz test at 50 mg/kg, 1/4 mice (ip) was protected at 0.5 hour and 3/4, 2/4, 2/4 at 0.25, 0.5, and 1.0 hours at 100 mg/kg dose with no toxicity.

Nitroaniline derivative, **5f**, provided MES protection in mice at the 300 mg/kg dose with neither scPTZ nor toxicity found during testing. The level of protection was relatively low, and 6 Hz testing at a dose of 100 mg/kg in mice displayed activity at 1/4, 2/4, and 1/4 at 0.5 to 2 hours post-dose with no toxicity displayed.

MES protection is displayed at 100 and 300 mg/kg in mice (ip) in **5e**. In rats (po), the MES protection is displayed at 30 mg/kg at 4 hours post-dose. There was no toxicity reported up to 4 hours post-dose. In the 6 Hz model, **5l** was active at a dose of 75 mg/kg (1/4 rats were protected) at 0.5, 1.0 and 2.0 hours post dose. No toxicity was presented. **5l** showed MES protection at 100 and 300 mg/kg in mice (ip). When screened in our oral rat and 6 Hz assays, using a dose of 30 mg/kg in rats (po), some MES protection was observed at 4 hours post-treatment with no overt toxicity found during the testing. When evaluated at a dose of 75 mg/kg in the 6 Hz study, the substance exhibited some minor protection without any noticeable toxicity.

Table 1. Chemical Properties of 2-methyl enaminones.



Compound	R_1	R_2	R_3	Yield	MP, °C
5a	Cl	H	H	[illegible]	[illegible]
5b	Br	H	H	[illegible]	167
5c	I	H	H	15.57%	[illegible]
5d	CF_3	H	H	25.10%	[illegible]
5e	OCF_3	H	H	[illegible]	[illegible]
5f	NO_2	H	H	[illegible]	[illegible]
5g	CH_3	H	[illegible]	[illegible]	[illegible]
5h	H	H	H	15.78%	129
5i	Cl	CH_3	H	44.28%	183-185
5j	Br	[illegible]	H	18.21%	[illegible]
5k	I	[illegible]	H	[illegible]	[illegible]
5l	OCF_3	[illegible]	H	[illegible]	[illegible]
5m	CH_3	CH_3	H	[illegible]	[illegible]
5n	H	CH_3	H	[illegible]	147-150
5o	[illegible]	[illegible]	CH_3	13.00%	196-197
5p	[illegible]	CH_3	[illegible]	21.20%	170-172

Yield percentage and melting point values obtained for synthesized analogs **5a-5p**.

Table 2. Anticonvulsant activity [illegible] 6-Hz 44-MA in mice

Compound	Dose (mg/kg)	Time (h)	Activity	Tox
5f	100	0.25	0/4	0/4
		0.5	**1/4**	0/4
		1	**2/4**	0/4
		2	**1/4**	0/4
		4	0/4	0/4
[illegible]	75	0.25	0/4	0/4
		0.5	**1/4**	0/4
		1	**1/4**	0/4
		2	**1/4**	0/4
		4	0/4	0/4
[illegible]	50	0.25	0/4	0/4
		0.5	**1/4**	0/4
		1	0/4	0/4
		2	0/4	0/4
		4	0/4	0/4
[illegible]	100	0.25	**3/4**	0/4
		0.5	**2/4**	0/4
		1	**2/4**	0/4
		2	0/4	0/4
		4	0/4	0/4

[a] Ratios where at least one animal was protected have been highlighted in bold for easier data interpretation. Data indicate the number of mice protected / number of mice tested. Pretreatment times of the test analogs vary from as early as 15 min up to 4 h. This is the Epilepsy Therapy Screening Program (ETSP) protocol to identify compounds with an early onset of action as well as a long duration of action. [b] Rotarod neurologic toxicity test (Tox).

In the initial mouse (ip) studies, **5d** (ADD 427036) showed MES protection at 100 and 300 mg/kg. Some scPTZ protection was seen at a dose of 300 mg/kg while animals displayed myoclonic jerks during testing. In the toxicity assay, none was observed at the doses and time points employed.

5i, a close analog to **5g**, seems to be more potent within the series. In the MES rat oral administration (po) study, 1/4 of the animals show positive MES outcome at doses of 30 mg/kg at 1 hour and 2 hours with no toxicity. The Pilocarpine-induced status test in rats (Test 71), displayed no protection at doses 100, 300, 600 mg/kg at 0.25, 0.5, 1, 2, and 4 hours.

On the basis of data obtained in mice according to the Anticonvulsant Screening Project (ASP) disposition, three compounds (**5i, 5l, 5p**) were selected and examined for their anticonvulsant activity (MES screen) and neurotoxicity after po administration into rats at a dose of 30 mg/kg. The results obtained are presented in **Table 3**.

Compared to the unsubstituted aniline, **5p** seems to be more potent than **5f** in the 6 Hz test, however, in TOX assessment, neither compound displayed any detectable toxicity.

As seen from the data, none of the three compounds exhibit 100% protection at any of the times tested. **5i** exhibited 50% protection at 4 hours and none at other times. The other two exhibited protection at 25% at time intervals 1 and 2 hours. No neurotoxicity was exhibited by the three compounds.

5f, administered intraperitoneally at a dose of 100 mg/kg, revealed marginal activity and protected [illegible] of mice at 1 hour and 25% at 0.5 and 2 hours. A dose of 75 mg/kg of **5l** exhibited 25% of protection at 0.5, 1 and 2 h. Last, **5p** was tested with a dose of 50 and 100 mg/kg with 25% of mice protected at 0.5 hour and in the 100 mg/kg dose 75% at 0.25 and 50% at 0.5 and 1 hour interval[illegible]

During further studies compounds **5[illegible], 5j, 5k, 5m** and **5p** were examined in the in vitro hippocampal slice culture neuroprotection assay; however they did not exhibit neuroprotection against Kainic Acid (KA) and N-methyl-D-aspartate (NMDA). NMDA inhibition is analogous to GABA activity.

Discussion

In our quest to design and synthesize novel enaminones for anticonvulsant studies, our group began to question the essentiality of the vinyl proton. Methyl group substitution in position 2 was assumed to prevent hydrogen bonding with the aryl group. Here we synthesized a series of enaminones with the methylene protons on the opposite face. Comparing these results to those previously synthesized, we found that these current derivatives are quite selective compared to the 2-desmethyl derivatives (Eddington *et al.* 20[illegible]; Scott *et al.* 1993). The active compounds included **5e, [illegible] 5i, 5l, 5n,** and **5p**. **5d** was MES active with some scPTZ activity. Both **5a** and **5o** were MES and scPTZ active with notable toxicity. Protection was also noted in **5f, 5[illegible]** and **5p** during 6 Hz testing. These results are contrasted to earlier results with 5-methyl compounds (Eddington *et al.* 2003) where all compounds except the [illegible]-cyano compound were active. Table [illegible] shows the results in the VI test in rats via oral administration. As noted from the table, several of [illegible] compounds were moderately active. **5f**, [illegible], and 5[illegible] were submitted to this test. Also, several compounds were advanced to the 6 Hz test in mice (5[illegible], 5[illegible] and 5p). A pilocarpine induced status prevention (P[illegible]) seizure test was undertaken and proved negative for **5i**. The pharmacology results show the vinyl proton is not essential for anticonvulsant activity. Additionally, some compounds have long duration of binding at the receptors.

Acknowledgments

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Table 3. Anticonvulsant activity: maximal electroshock (MES) test in mice

Compound	Dose (mg/kg)	MES (30 min)[a]	MES (4 h)[a]	scPTZ (30 min)[a]	scPTZ (4 h)	Tox (30 min)[b]	Tox (4 h)[b]
5a	30	0/1	0/1	0/1	0/1	[illegible]	[illegible]
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	**1/1**	0/1	**1/1**	0/1	**4/4**	0/2
5b	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	[illegible]	0/2
5c	30	0/1	0/1	0/1	[illegible]	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	0/4	0/2
5d	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	**1/3**	0/3	0/1	0/1	[illegible]	0/4
	300	**1/1**	**1/1**	0/1	[illegible]	0/4	[illegible]
5e	30	0/1	0/1	0/1	[illegible]	0/4	0/2
	100	0/3	0/3	[illegible]	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	0/4	0/2
5f	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	[illegible]	0/4
	300	0/1	**1/1**	0/1	0/1	[illegible]	0/2
5g	30	0/1	0/1	[illegible]	[illegible]	0/4	0/2
	100	0/3	[illegible]	0/1	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	**1/4**	0/2
5h	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	[illegible]	0/1	0/8	0/4
	300	0/1	**1/1**	[illegible]	[illegible]	0/4	0/2
5i	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	[illegible]	0/3	[illegible]	0/1	0/8	0/4
	300	[illegible]	**1/1**	0/1	0/1	**2/4**	0/2
5j	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	0/1	[illegible]	0/1	0/4	0/2
5k	30	0/1	[illegible]	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	0/4	0/2
5l	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	[illegible]	**3/3**	0/1	0/1	0/8	0/4
	300	**1/1**	[illegible]	0/1	0/1	0/4	0/2
5m	[illegible]	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	[illegible]	0/1	0/1	0/1	0/1	0/4	0/2
5n	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/1	0/1	0/1	0/8	0/4
	300	**1/1**	0/1	0/1	0/1	**2/4**	**1/2**
[illegible]	[illegible]	[illegible]	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	**1/1**	0/1	**1/1**	0/1	**2/4**	0/2
5p	30	**1/1**	0/1	0/1	0/1	0/4	0/2
	100	**3/3**	0/3	0/1	0/1	0/8	0/4
	300	**1/1**	**1/1**	0/1	0/1	0/4	0/2

[a] Ratios where at least one animal was protected have been highlighted in bold for easier data interpretation. Data indicate the number of mice protected/number of mice tested. Pretreatment times of the test analogs vary from as early as 15 min up to 4 h. This is the Epilepsy Therapy Screening Program (ETSP) protocol to identify compounds with an early onset of action as well as a long duration of action. [b] Rotarod neurologic toxicity test (Tox).

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The Impact of Prescribed Fire on Moth Assemblages in the Boston Mountains and Ozark Highlands, in Arkansas

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Running title: Prescribed Fire Impacts Moth Assemblages

Abstract

In addition to the impacts of prescribed fires on forest vegetation, this ecosystem process also has dramatic impacts on associated insect assemblages. For herbivorous, terrestrial insects, fire predictably results in a cycle of initial insect population reduction followed by recovery and growth, in which these insect populations exceed pre-fire abundances. We sought to examine if fire-induced disturbance cycles make prescribed burned areas more or less suitable specifically for moths (order Lepidoptera), which is a major food source for, among others, multiple bat species. We surveyed moth assemblages at 20 burned and 20 unburned sites in the Boston Mountain and Ozark Highland ecoregions of Arkansas, to determine if biomass or abundance of moths differed between areas that had been burned in the past 10 years, and those areas that had never been burned. Samples were collected early (April to July) and late (August to November) in the growing season of 2017 (hereafter early season and late season, respectively). We compared biomass and abundance of all moths, and of five representative moth species, between burned and unburned sites. The five moth species were chosen and considered to be representative due to their high relative abundance, and ease of identification. The five chosen moth species included the banded tussock moth (*Halysidota tessellaris*), white-dotted prominent moth (*Nadata gibbosa*), ailanthus moth (*Atteva aurea*), grape leaffolder (*Desmia funeralis*), and painted lichen moth (*Hypoprepia fucosa*). Results from paired t-tests showed no significant difference in total biomass, or abundance of representative species between burned and unburned sites. However, generalized linear regression models showed significantly higher abundance of moths in areas with high basal area that had been previously burned ($\beta = -0.038 \pm 0.004$ SE, $p < 0.0001$). Lower number of snags ($\beta = -0.081 \pm 0.0044$; $p < 0.0001$) and more open canopy ($\beta = 0.001 \pm 0.0001$ SE; $p < 0.0001$), also increased abundance of moths in an area. Our results show that fire acts as an intermediate disturbance, driving moth populations in the Ozark Mountains of Arkansas.

Introduction

Fires, Then and Now

Fire is important in natural ecosystems. Fire regulates competition of vegetative communities, consumes litter and debris, cycles nutrients into the soil, controls insect pests and diseases, and facilitates fire-dependent species (USDA Forest Service Southern Region 1989). The history of the Ozark Mountains of Arkansas and Missouri exemplifies the importance of fire in a hardwood context. For the purpose of this study, we will use the term, "Ozark Mountains" to refer to the mountainous region encompassing the Boston Mountain and Ozark Highlands ecoregions. Studies on fire scars of trees in the Ozark Mountains revealed that on average, fire intervals for the study site occurred every 7.7 years from 1670-1820 but decreased to every 2 years from 1821-1880 (Stambaugh and Guyette 2006). Native peoples regularly used fire to clear land and limit vegetative growth, as did European settlers arriving in the 1800s (Waldrop and Goodrick 2012). Historic accounts exist that show the structure of forests, including the Arkansas Ozark Mountains, in the early 1800s when fires were frequent. Land surveyors of the time recorded that the Ozark Mountains were characterized by open woodlands and prairies (Foti 2004; Stambaugh and Guyette 2006; Jacobson and Primm 1997). As a source of ecological disturbance, fires kept these woodlands and prairies from maturing into dense, closed canopy forests.

Humans began to suppress fires in the 1900s. Fire-scar studies reveal that some sites in the Ozark Mountains had not been burned since 1972 (Guyette and Spetch 2003; Guyette *et al*. 2006; Stambaugh and Guyette 2006). Fire suppression has caused a large increase in tree density and allowed for the accumulation of leaf litter and debris in environments such as the Ozark Mountains. These closed-canopy forests offer less foraging opportunities for wildlife than the open woodlands and prairies that were present in the past (Ober and Hayes 2008; Bender *et al.* 2015). Although the effects of prescribed burning are not fully understood, natural resource agencies are using prescribed burns in order to restore forests to some of their former conditions (Dey and Hartman 2005).

Insects

Insect abundance declines during fires (specifically terrestrial insects), and for approximately two months after, due to the initial fire exposure, the loss of food sources, and the loss appropriate habitat (Swengel 2001). Mild surface fires consume vegetation, cause shoot dieback, and can kill trees 12 cm in diameter and smaller that are used by insects to live in and feed on (Dey and Hartman 2005). In addition, fire burns leaf litter that some insects live in and consume. The majority of the negatively impacted terrestrial insects recover within one year, and the rest recover within two years after a fire, after which these insect populations grow to exceed pre-fire numbers (Dajoz 1998; Swengel 2001; Evans *et al*. 2013). Thus, fire yields a cycle of insect population declines followed by periods of recovery and growth. It is not currently known if this cycle, which results from prescribed burning, makes burned areas more or less favorable for terrestrial insects than unburned areas over the long-term. This study seeks to determine if the effects of fire make prescribed burned areas more likely to support higher moth abundances, specifically compared to areas that had not been burned recently.

Whether moths are in higher abundance in burned areas or unburned areas may depend on their plant hosts' resilience to fire. We were interested in five moth species, which included: ailanthus moth (*Atteva aurea*), painted lichen moth (*Hypoprepia fucosa*), grape leaffolder (*Desmia funeralis*), white-dotted prominent moth (*Nadata gibbosa*), and the banded tussock moth (*Halysidota tessellaris)*. Of the moth species chosen for study, *H. fucosa* relies only on lichen and mosses that typically recover slowly after fire. *Halysidota tesselaris* uses multiple woody species, including alder (*Alnus* spp.), ash (*Fraxinus* spp.), and oaks as host plant species (Beadle and Leckie 2018). *Nadata gibbosa* relies on oaks, maples (*Acer* spp.) and cherry trees (*Prunus* spp.), among others, as hosts, whereas *D. funeralis* is associated with evening primrose (*Oenothera* spp.), grape, and redbud trees (*Cercis canadensis*) (Beadle and Leckie 2018). *Desmia funeralis* also relies on vegetative hosts such as grasses and flowers that proliferate after fire. Lastly *A. aurea,* depends primarily on ailanthus trees as hosts (Beadle and Leckie 2018), these trees are known to be hardy and shade-tolerant, and although not fire-resistant (Rebbeck *et al.* 2014), the plant has a tendency to regenerate quickly after fires (Fryer 2010).

Species of trees with thick bark can protect and insulate themselves from fire and tend to be fire resistant. Trees that have thick bark and are more fire resistant include *Juglans nigra* (black walnut), *Pinus* spp. (pines), *Salix nigra* (black willow), *Diospyros virginiana* (American persimmon), *Celtis* spp. (hackberry), *Crataegus* spp. (hawthorn), *Rhus* spp. (sumac), and *Quercus* spp. (oaks) (Onduso 2013, Karstenson 2010a). Fire resistant understory vegetation includes *Vaccinium* spp. (blueberry), *Rubus* spp. (blackberry), *Trifolium* spp. (clover), and *Vitis* spp. (grape) (Onduso 2013; Karstenson 2010a). Moth species that rely on fire resistant hosts such as those listed were expected to be present in higher abundance in burned sites or show no difference in prevalence between burned and unburned sites.

Since fire consumes litter and debris, and helps limit vegetative competition, it is expected that fire would allow groundcover such as grasses and flowers to proliferate. This could affect a moth species such as *D. funeralis* that relies on some grass species as host plants. In a high-intensity fire-study, grasses recovered within two to three years after the fire, and grass cover was much higher five years after the fire than it had been prior to fire (Ivanova *et al.* 2017). Moth species that rely on vegetative hosts are therefore expected to be more numerous in burned areas. For those moth species that rely on lichen and mosses as hosts, we expected the opposite trend. Lichens and mosses tend to decrease in fire-prone areas, with slow recovery after fire (Garrido-Benavent *et al.* 2015; Ivanova *et al*. 2017).

Due to a need for further investigation into the impacts of fire on forest moth communities, we chose to compare burned and unburned sites across the Ozark Mountains. We predicted that moth species which have fire resistant hosts would either show no significant difference in abundance in either burned or unburned sites, or show a preference for burned sites. Moth species with fire-susceptible hosts were predicted to have higher abundance in unburned sites. In general, since moths have been shown to recover after fires and exceed pre-fire numbers, moth biomass and abundance were hypothesized to be higher in the burned sites. To test this hypothesis we collected and compared moth samples from twenty burned and twenty unburned sites in the Ozark Mountains of Arkansas.

Methods

Location

Data was collected in the Ozark Highlands and Boston Mountain ecoregions in the Ozark Mountains of Arkansas. Elevation ranges from 122 m at the confluence of the Buffalo and White Rivers to 730 m in the Boston Mountains. The Ozark Mountains have a mesic temperature regime and receive 107 to 127 cm of precipitation per year (Karstenson 2010a; Karstenson 2010b). The region is characterized by second-growth forests recovered from timber and agriculture industries (Jacobson and Primm 1997). Lowland areas also include livestock farming and pastureland (Karstensen 2010a; Karstenson 2010b). Prescribed burns are implemented in the area, by the National Park Service, partly to increase numbers of valuable oak species. Oaks respond well to fire due to reduced competition and decreased canopy cover. The Ozark Mountains hardwood forests primarily consist of oak, short leaf pine (*Pinus echinate* spp.), beech (*Fagus* spp.) and hickory (*Carya* spp.) (Onduso 2013; Guyette *et al.* 2006).

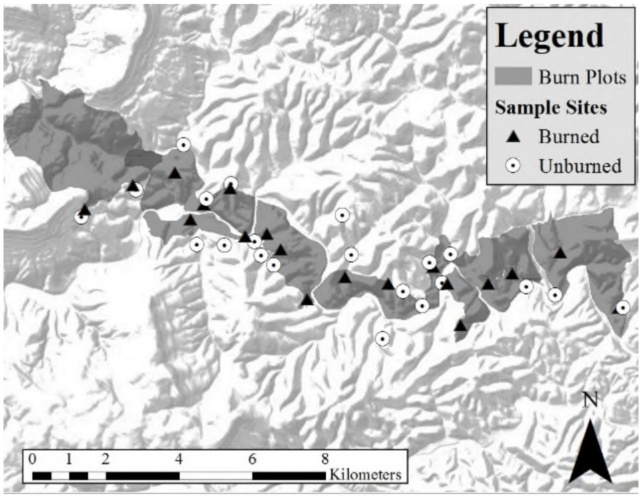

Fig. 1. Study area in Newton County, AR showing the forty sites surveyed and sampled from April-November 2017. The gray area is the prescribed burned area, the triangles are the burned sites, and the circles are the unburned sites (adapted from Blanco 2018).

Site Variables

Forty sites in the Erbie, Ozark, and Pruitt areas in the Buffalo National River, managed by the National Park Service, were chosen for sampling (Figure 1). Twenty of these sites have undergone prescribed burning, in the previous 3-5 years, by the National Park Service, and 20 of these sites have no record of being burned in the previous 10 years. All of the sampling sites were chosen at random. Each was located no less than 30 m from any road and was at least 1.5 km from any adjacent sites. Basal area, canopy cover, and the number of snags were all measured or recorded at each of the 40 sites. These variables were measured in a circular plot with a radius of 11.5 m at each site. Canopy cover was assessed using a spherical densiometer, at the plot center and in the four cardinal directions from the plot center, recorded as the percent "openness" of a site, and then averaged for each site. The total number of snags with a DBH of at least 16 cm were recorded in each of the 11.5 m circular plots.

Moth Collection

Moth samples were collected from April to November of 2017. This period was divided into two sampling intervals, and labeled accordingly as: early season (from April-July), and late season (from August to November). The aim was to sample each of the 40 sites once during each season; 35 of the sites were successfully sampled twice. Each site was sampled one time per season, and a total of two sites were sampled each night, one at an unburned site, and one at a burned site, for a total of 70 nights of moth sampling.

A Universal Black Light Trap with a 12-watt black light powered by a 12-volt battery was used to attract and trap all insects (Bioquip Inc., Rancho Dominguez, CA). A glass jar containing methyl acetate covered by a mesh cloth was used in the traps to kill the insects. Each trap was hung on a 1.5 meter-tall, metal shepherd's hook at a random point in the circular plot (Threlfall *et al.* 2012). The trap was set 30 minutes before sunset and taken down 30 minutes after sunrise. Batteries were tested to ensure longevity of the light source before each trapping event.

Sample Processing

After collection, the insect samples were stored in brown paper bags and then dried at air temperature for at least 4 days (samples were weighed every day, until weight stopped changing). All samples were reweighed every day following the initial drying period to ensure that mass stayed the same. After samples were dried moth species were separated from all other insects, weighed, and counted. The total moth biomass from any given trap was recorded (g). The five moth species that we were specifically interested in were separated from the combined insect and moth samples and counted, and weighed, as individual species.

Data Analysis

Statistical analyses were performed to compare total biomass of all moths, total abundance, and the abundance of the five representative species, between burned and unburned areas, and between early and late season. To investigate differences in abundance of the five selected moth species between the early and late season, a paired t-test was conducted (abundance from each species from each trap for each season, and for burned and unburned sites were compared). To compare total moth abundance and biomass among sites, a generalized linear regression model was developed. Errors associated with repeated measures (non-convergence of models) forced us to average data from the two sampling seasons; we then compared burned and unburned areas. Two generalized linear regression models were used to compare total moth abundance and biomass, each as a response variable, between burned and unburned areas. Each of the models included burn status (factor with 2 levels), basal area (continuous), number of snags (counts), and canopy cover (%) as explanatory variables. A Poisson distribution was used for count data. All statistical analyses was done using program R (R Core Team 2016).

Results

After accounting for bad weather, we ended up with 70 nights of insect trapping (35 trap nights in each of the seasons). Biomass of all moths, for both sampling seasons, was 362.55 g. Moth abundance varied between the 2 sampling seasons, with 13,471 individually countable moths in the early season and 6,613 individually countable moths in the late season. Of the 5 species of interest, in both burned and unburned areas, *H. fucosa* accounted for the most individuals (257 and 176 individuals respectively). Two of the moth species (*H. tesselaris* and *N. gibbosa*) were captured in slightly higher, albeit non-significant numbers, in unburned sites than burned sites (($t_{10} = 0.53$, $p = 0.606$; Figure 2). *Atteva aurea* accounted for the lowest recorded abundance for both burned and unburned areas (15 and 16 respectively). There was no statistically significant difference in abundance of any of the individual moth species between early season and late season ($t_{10} = 1.9$, $p = 0.08$; Figure 3).

Total biomass was similar between burned and unburned sites (175.7 g and 174.5 g respectively), resulting in no statistically significant differences, and high standard errors (unburned areas: 0.337 ± 0.814 g, $p = 0.681$).

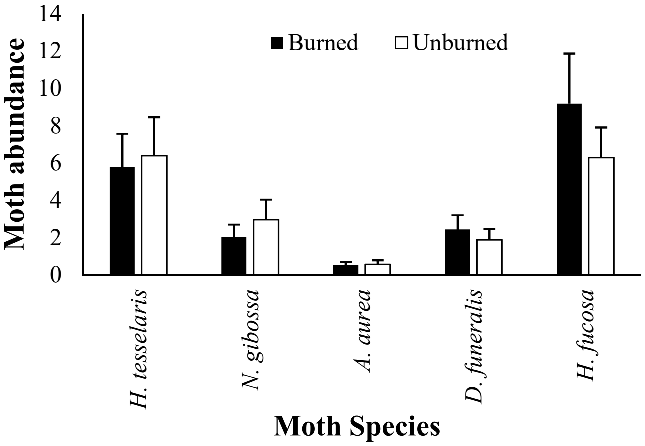

Fig. 2. Average abundance +SE of 5 representative moth species in burned sites compared and unburned sites. Data for the early summer season and late summer season is summed. Moths collected April-Nov 2017 in Newton County, AR.

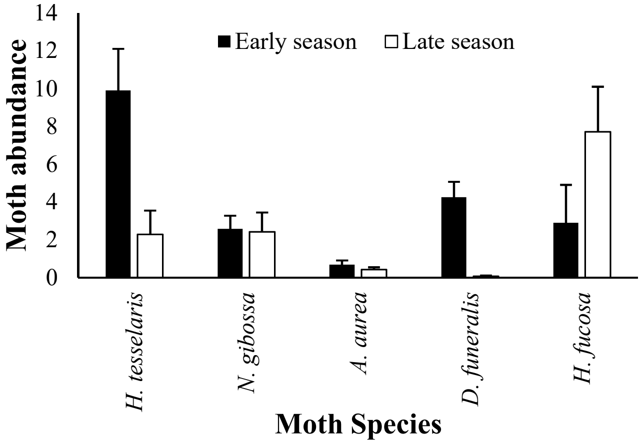

Fig. 3. Average abundance +SE of 5 representative moth species in the early sampling season and late sampling seasons. Data burned and unburned areas is summed. Moths collected April-Nov 2017 in Newton County, AR

Total moth abundance was lower in unburned areas (β = -0.022 ± 0.28 SE; Table 1), than in burned areas. Although these unburned areas generally had higher basal area, more moths were found at sites with higher basal area (β = 0.038 ± 0.003 SE; Figure 4). On average burned sites had lower basal area (3.4 ± 0.51 SE m^2/ha) than unburned sites (6.3 ± 1.43 SE m^2/ha) (Figure 5). Hence, although moths were more abundant in burned sites, they specifically had higher numbers at sites with greater basal area.

Table 1. Model output for the generalized linear model describing variation in moth abundance at 40 sites Newton County, AR. Variables presented in the model included basal area, number of snags, percent canopy cover, and whether or not a site was burned. Moths were collected April-Nov 2017 in Newton County, AR.

Variable	Estimate (β)	p-value
Basal area	0.039 ± 0.004	<0.0001
Snags	-0.078 ± 0.004	<0.0001
Canopy cover	0.001 ± 0.001	<0.0001
Unburned	0.442 ± 1.660	0.438
Unburned * basal area	-0.034 ± 0.004	<0.0001

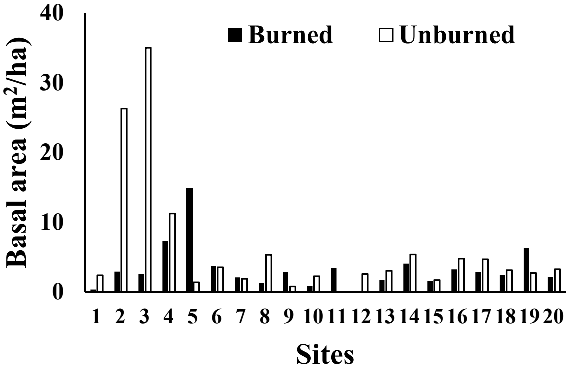

Fig 4. Basal area (m2/ha) of burned sites compared to unburned sites. Data collected in April –November 2017 in Newton County, AR.

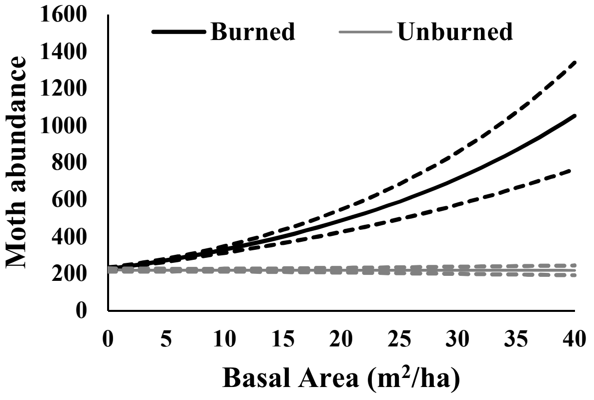

Fig. 5. Predicted moth abundance (solid lines) ±95% CI (broken lines) with increased basal area in burned and unburned areas (while keeping canopy cover and number of snags constant). Data collected in April –November 2017 in Newton County, AR.

Burned sites had more open canopies than unburned sites (74% open vs. 47% open; Figure 6), and our regression models showed that canopy cover played a significant role in predicting moth abundance; more open canopy areas generally had a higher abundance of moths (β = 0.001 ± 0.0001 SE; p < 0.0001).

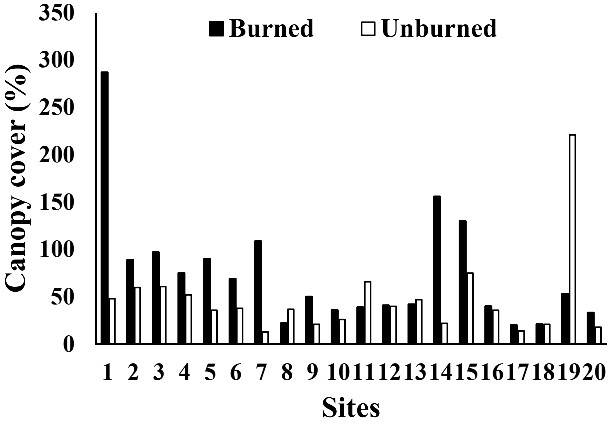

Fig. 6. The canopy cover (%) of burned sites compared to unburned sites. Data collected in April 2017 in Newton County, AR.

Although burned sites generally had more snags than unburned sites (3.1±0.48 SE and 1.7± 0.40 SE respectively), moth abundance was lower at sites with high snag numbers (β = -0.081± 0.0044; p < 0.0001) (Figure 7).

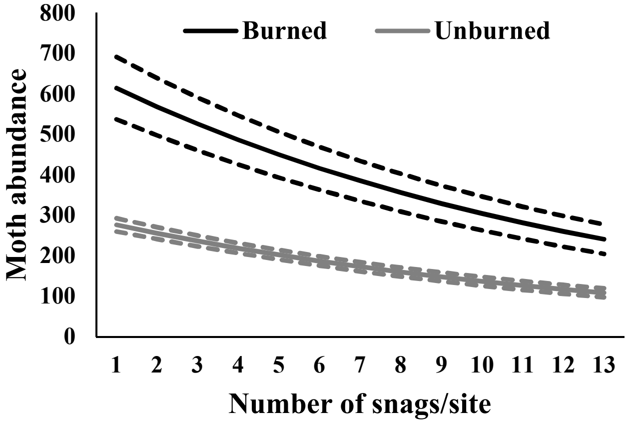

Fig. 7. Predicted moths abundance (solid lines) ±95% CI (broken lines) with increased number of snags in both burned and unburned areas, from data collected April-November in Newton County, AR.

Discussion

Similar abundances of *H. tessellaris*, *N. gibbosa*, and *D. funeralis* in burned compared to unburned sites was expected. These numbers can be explained by the fact that these species all have fire dependent hosts that likely did not differ between burned and unburned sites (Onduso 2013). The lack of difference in abundance of *H. fucosa* between burned sites and unburned sites suggests that prescribed fire in the Ozark Mountains does not significantly alter the moss and lichen stratum that *H. fucosa* relies on. The prediction that *H. fucosa* would prefer unburned sites because mosses and lichen decline after fire and recover slowly was not supported (Garrido-Benavent *et al.* 2015; Ivanova *et al*. 2017). This may be because the Ozark prescribed burns are too mild to significantly impact the moss and lichen stratum. Prescribed burns in the Ozark Mountains are low intensity surface fires, the least destructive type of fire (Dey and Hartman 2005; Onduso 2013). Surface fires like those used in prescribed burns do not always completely burn a site and can leave some trees unscarred (Guyette and Spetich 2002). Mosses and lichen may therefore remain in unburned patches, within the burned areas, providing resources for *H. fucosa* (Reinhard and Menges 2004; Calabria *et al.* 2016).

The fact that there was not a decrease of *A. aurea* in burned areas could indicate that the decrease in litter through prescribed fires favors the establishment of small, light seeds such as those of ailanthus trees (Onduso 2013). Although burning is frequently used to control some invasive species, it can often lead to the spread, and increase, of alien invasive plant species (Grace *et al.* 2001; Guthrie *et al.* 2016). In our case fire did not affect *A. aurea*, which could be indicative of fires not having an impact (positive or negative) on the ailanthus tree. Of course, more detailed analyses of other moth species would yield clearer results. Ideally we would have wanted to examine moth species that are specifically fire-sensitive or fire-resistant (either physiologically or because of characteristics of their plant hosts), but in the end ease of identification and larger numbers guided us to use the species that we have.

The lack of difference in biomass, but much higher abundance of moths, in prescribed burned sites indicate that prescribed burns in the Ozark Mountains are impacting moth assemblages. It is known that the density of generalist butterflies is higher in pine-oak barrens where burning occurs than in idle pine-oak barrens (Swengel and Swengel 2001). Prescribed burns in the Ozark Mountains are a management tool used to promote growth of beneficial herbaceous plants and to discourage growth of herbaceous plants that have little value to wildlife (Dey and Hartman 2005; Onduso 2013). Moth abundance may be higher in prescribed burned sites because prescribed burns encourage growth of vegetation, providing a food source for moths (Ivanova *et al.* 2017). Terrestrial insects (and specifically herbivorous insects) are known to frequent areas that have recently been burned, in preference over unburned areas, specifically to take advantage of new vegetative growth (McCullough *et al.* 1998; Swengel 2001). The low intensity, and infrequent application, of prescribed burns in the Ozark Mountains may not alter habitats enough to significantly change biomass of all moth species, but could positively affect specific moth species. This implies that perhaps specific lighter weight moths increased in abundance, and compensated for the loss of some of the heavier species. A future study should examine numbers of all moth species, and how those differ between burned and unburned areas.

Although forest-stand characteristics, such as basal area, play a key role in insect abundances and diversity, Dodd *et al*. (2008) showed that short term changes to these characteristics only alter moth assemblages temporarily and that of more important concern should be overall woody plant species richness. Dodd *et al.* (2012) found a negative relationship between Lepidopteran abundance and basal area, they also showed that Lepidopteran abundance was negatively correlated with disturbance. If fire acts as a large disturbance, the assumption is that there would be a decrease in Lepidoptera abundance or diversity, or both. Our data showed that moths responded positively to burned areas, and more so positively to areas with high basal area within burned stands.

Many moth species prefer early successional, open canopy, habitats (Grand and Mello 2004; Nöske *et al.* 2008), which is in line with our results, and pointed to characteristics of some of the burned areas. The main effects from burns may be an increase in the number of snags, a forest canopy that is more open, or a forest stand that is restored to an earlier successional stage.

The results from the present study support the intermediate disturbance hypothesis proposed by Connell (1978). The mild or intermediate disturbance caused by prescribed burns may be ideal for supporting higher moth numbers. Disturbance caused by prescribed burns may decrease basal area, increase the development of new snags, and open canopy cover (Drapeau *et al.* 2002; Boyles and Aubrey 2005; Peterson and Reich 2011). Without disturbance from prescribed burns, competition may be more intense for moths in unburned sites. Burned sites may be ideal habitats for moths because prescribed burns encourage the growth of vegetation that serves as a food source and habitat for moths. In addition, prescribed burns may be an agent of intermediate disturbance that decrease resource competition among moths and allow for greater moth abundance.

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Vertebrate Natural History Notes from Arkansas, 2019

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Running Title: Vertebrate Natural History Notes, 2019

Abstract

Important observations of natural history often go unreported because they are not part of larger studies, but small details can provide insights that lead to interesting questions about ecological relationships or environmental change. We have compiled recent important observations of distribution, deformities, foods, parasites, and reproduction of various vertebrates. Included are a new record of Lake Sturgeon (*Acipenser fulvescens*), a new size record of mudpuppy (*Necturus louisianensis*), and a second observation of bilateral gynandromorphism in a Northern Cardinal (*Cardinalis cardinalis*).

Introduction

Vertebrate field biologists constantly observe natural history parameters and relationships among organisms in human-altered environments. Introduction of non-native species may confound relationships and understanding of life history parameters of native species. Knowledge of distribution and natural history of many species within Arkansas is becoming better documented, but much remains to be discovered and reported. We continue to update the state of knowledge of Arkansas's vertebrates (see Tumlison *et al*. 2017 and references therein). Here, we include previously unreported records of distribution, food habits, parasitism and disease, size, and reproduction in vertebrates from Arkansas.

Methods

Fishes were collected with 3.1 × 1.4 m, 3.1 × 1.8 m, and 6.1 × 1.8 m seines (all 3.175 mm mesh), or with a backpack electrofisher. Fish specimens were documented either by a photovoucher or a specimen housed in the vertebrate collections at Henderson State University (HSU), Arkadelphia, AR. Voucher specimens of fishes or herps were fixed in 10% formalin and preserved in 50% isopropanol. Measurements such as total length (TL) not originally taken in metric units are reported first in English, as initially recorded. Bat records were based on catch/release surveys by expert chiropterologists, or from specimens sent to the Arkansas Department of Health and identified by D. Saugey.

Trematodes were fixed in nearly boiling tap water without coverslip pressure, stained in acetocarmine, cleared in methyl salicylate, and mounted in Canada balsam. Tissues from one fish were processed by standard histological methods following Presnell and Schreibman (1997) with sectioning at 8-10 µm followed by staining with hematoxylin and eosin. Vouchers (photovouchers or slides) of parasites were deposited in the Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln, NE.

Results and Discussion

CLASS PETROMYZONTIDA

Petromyzontidae - Lampreys

***Ichthyomyzon castaneus* Girard – Chestnut Lamprey**. A single adult (20 cm TL) was collected on the White River near Three Chutes Rapid (36.342935°N, 92.528952°W) on the border of Marion and Baxter Cos. on 13 February 2019 by J. Pyle. This individual was attached to the head of a Brown Trout (*Salmo trutta*; Fig. 1). This lamprey has been collected previously from the White River (Robison *et al*. 2006; Salinger *et al*. 2018); however, this is the northernmost record from the White River below Bull Shoals Dam. Furthermore, Salinger *et al*. (2018) reported this species parasitizing Rainbow Trout (*Oncorhynchus mykiss*) in Arkansas, but ours may be the first report from Brown Trout.

Figure 1. Wound on the head of a Brown Trout (*Salmo trutta*) caused by a parasitic Chestnut Lamprey (*Ichthyomyzon castaneus,* inset of lamprey mouth) collected from the White River, Marion/Baxter cos. on 13 February 2019. Photo by Jon Pyle.

CLASS ACTINOPTERYGII

Acipenseridae – Sturgeon

***Acipenser fulvescens* Rafinesque – Lake Sturgeon**. The Lake Sturgeon is a rare fish in Arkansas and only occasionally is caught by commercial fishermen in the larger rivers of the state. Robison and Buchanan (1988) reported only 3 records of this sturgeon in Arkansas. Buchanan *et al*. (1993) added 2 localities, 1 each in Desha and Prairie Cos. Later, Crump and Robison (2000) reported an historical record from the Caddo River in Pike County.

On 16 January 2019, Dwight Ferguson, a commercial fisherman from Black Rock, caught, photovouchered, and released a 3 ft. (1 m) long Lake Sturgeon (*Acipenser fulvescens;* Fig. 2) in a hoop net in the Black River near Powhatan, Independence Co., AR (36.067080°N, 91.117798°W) in about 8-10 ft. (2.4-3.0 m) of water. Mr. Ferguson (*pers. comm.*) said the Black River was fairly low at the time of the capture. The discovery of this specimen, small for the species, attests to the continued presence of this rare fish in Arkansas.

Figure 2. Lake Sturgeon (*Acipenser fulvescens*) captured from the Black River, Independence Co., on 16 January 2019. Photovoucher by Dwight Ferguson.

Anguillidae – Eels

***Anguilla rostrata* Lesueur – American Eel**. This interesting fish species is an uncommon inhabitant of Arkansas streams and rivers (Robison and Buchanan 1988). A single specimen of this species was caught on a trotline baited with sunfish in the Jimmy Creek arm of Bull Shoals Lake, Marion Co., AR on 25 June 2018 by Wilson Bowling. The fish weighed 5 lb., 5 oz. (2.4 kg), and was 40.5 in. (1.03 m) in TL. Mr. Bowling, Postmaster in Bull Shoals, caught the eel and weighed it on his certified postal scale in front of an AGFC biologist prior to Cody Wyatt, AGFC biologist, weighing it on the official scale at the AGFC office. The eel was 1 oz. over the current state record (https://www.agfc.com/en/fishing/state-records/) and is a new Arkansas state size record for the American Eel.

Cyprinidae – Minnows and Carps

***Cyprinella galactura* (Cope) – Whitetail Shiner**. A gravid female (112 mm TL) specimen was collected on 29 May 2018 by CTM at Crooked Creek in Yellville, Marion Co. (36.223053°N, 92.689181°W). Pflieger

(1997) reported this shiner to spawn from early June to mid-August in Missouri. Robison and Buchanan (1988) noted that breeding males are found in early July in northern Arkansas. Our observations indicate a slightly earlier onset of reproduction than was previously known in Arkansas.

***Luxilis chrysocephalus* Rafinesque – Striped Shiner**. A 92 mm TL male specimen collected by CTM on 21 December 2018 from Big Fork Creek off St. Hwy 8, Polk Co. (34.468100°N, 93.947686°W) exhibited bilateral exophthalmos, also known as bulging eyes or pop-eye (Fig. 3:A-B). This is the first time, to our knowledge, that this condition has been reported in *L. chrysocephalus isolepis* or any Arkansas fish. Hargus (1991) reported that exophthalmos is a common condition in finfish. He suggested that poor water quality, including gas imbalances and parasitemias may cause exophthalmos; immunological deficiencies and genetic factors are also involved. This fish did not harbor eye parasites (*Diplostomum*) and was not collected from visibly poor water quality. The fish did, however, have a buildup of clear, watery tissue fluid under the eye from an unknown pathology which likely caused the pop-eye condition.

Another *L. c. isolepis* (female, 107 mm TL) collected from the site was found to have lordosis (Fig. 3C). Lordosis is defined as an abnormal ventral curvature of the vertebral column, accompanied by abnormal calcification of the affected vertebrae (Kranenbarg *et al*. 2005). This is the second time an abnormal vertebral condition (scoliosis) has been reported in a Striped Shiner from this locality (Tumlison *et al*. 2018).

Figure 3. Eye and skeletal anomalies in 2 *Luxilus chrysocephalus isolepis*. A-B: Individual exhibiting bilateral exophthalmos, scale bar A = 10 mm, scale bar B = 5 mm. C: specimen with lordosis (arrow), scale bar = 20 mm. Photos by CTM.

Catostomidae – Suckers

***Hypentelium nigricans* (Lesueur) – Northern Hogsucker.** A 305 mm TL individual was collected on 19 November 2018 by CTM from Walnut Creek off US Hwy 270 at Charlton Recreation Area, Garland Co. (36.223052°N, 92.689180°W). This female contained a large complement of yolked ova in 2 egg sacs (Fig. 4). In Arkansas, *H. nigricans* has been reported to breed in April and May (Robison and Buchanan 1988). We document that eggs may be well into development as early as November.

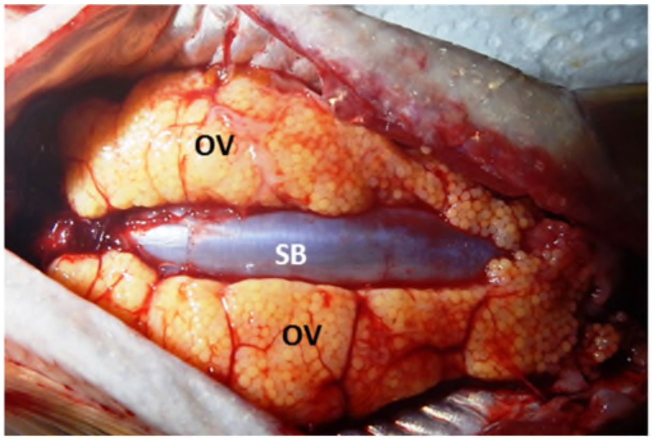

Figure 4. Female *Hypentelium nigricans* with yolked ova (OV) in 2 egg sacs. Note the central position of the swim bladder (SB). Photo by CTM.

Ictaluridae – Bullhead Catfishes

***Ameiurus melas* (Lesueur) – Black Bullhead**. Two adult (132, 203 mm TL) individuals were collected by CTM on 24 March 2018 from the White River, SE of Elkins off Co. road 49, Washington Co. (35.973855°N, 93.984183°W). The smaller one had a massive infection of the yellow grub trematode (*Clinostomum marginatum*) on the base of every fin (except adipose) and the right eye (Fig. 5:A-F). Yellow grub has been reported previously in *A. melas* (GenBank MF38191) from Benton Co. (McAllister *et al.* 2018), and a large number of *C. marginatum* (500) were reported from a Brown Bullhead (*A. nebulosus*) in Pennsylvania (Torres and Price 1971). In addition, hyperinfections of *C. marginatum* have been reported in both Smallmouth (*Micropterus dolomieu*) and Largemouth Bass (*M. salmoides*), in Arkansas (Daly *et al*. 1991). The present hyperinfection is noteworthy because there are no published reports of such an intense infection in this relatively small host with complementary photomicrographs.

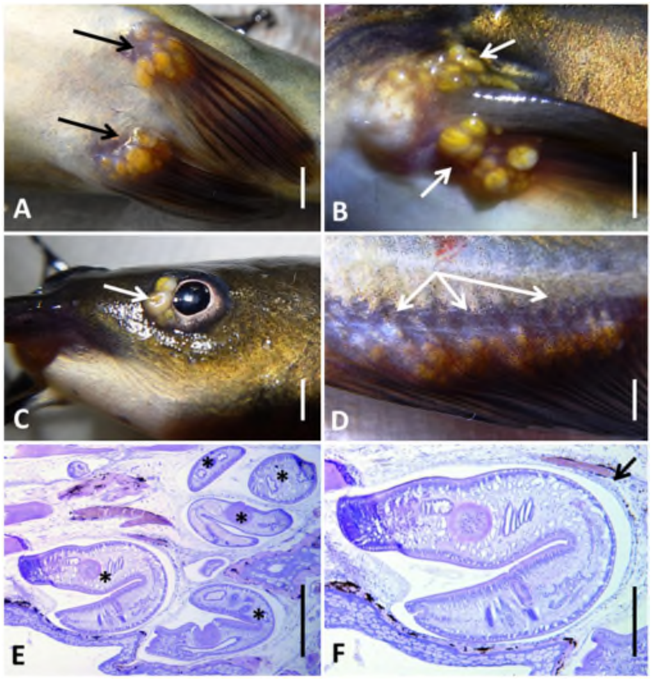

Figure 5:A-F. Hyperinfection of *Clinostomum marginatum* in Black Bullhead, *Ameiurus melas*. A. Encapsulated specimens in pelvic fins (arrows). Scale bar = 1.0 mm. B. Close-up of specimens in pectoral fin (arrows). Scale bar = 1.0 mm. C. Specimens encapsulated near left eye (arrow). Scale bar = 1.0 mm. D. Group of encapsulated specimens in anal fin (arrows). Scale bar = 1.0 mm. E. Photomicrograph of 5 specimens (*). Scale bar = 500 µm. F. Higher magnification of a single specimen showing host-derived capsule (arrow). Scale bar = 250 µm. Photos A-D by CTM, photos E-F by Stanley E. Trauth.

Centrarchidae – Sunfishes

***Ambloplites constellatus* Cashner and Suttkus – Ozark Bass**. A 165 mm TL specimen collected on 27 May 2018 from Crooked Creek, Yellville, Marion Co. (36.223053°N, 92.689181°W) contained 2 *Lebertia* sp. (Acari: Hydrachnida: Lebertiidae) water mites in its stomach. We know of no previous reports of water mites consumed by this fish.

***Lepomis megalotis* (Rafinesque) – Longear**. This sunfish is common throughout Arkansas (Robison and Buchanan 1988). A single specimen was collected by Paul Port on 23 June 2018, while spearfishing in Bull Shoals Lake (36.408194°N, 92.623972°W), Marion Co. This very large specimen was 8.75 in. (22.2 cm) in TL and weighed 9 oz. (0.255 kg). This fish was weighed on a digital hanging scale and TL was taken with a tape measure. Robison and Buchanan (1988) noted a maximum length for Longear at 7 in. (17.7 cm). The current official Arkansas state record weight for Longear is 1 lb., 2 oz. (0.51 kg), taken from Table Rock Lake on 22 April 1991; however, Ken Shirley (*pers. comm.*), retired fishery biologist for the Mountain Home District, commented that the current state record holder was actually a Redear Sunfish (*Lepomis microlophus*) and not a Longear, as some past AGFC fish records were certified by non-biologists (Wildlife Officers) that may have misidentified some fish. This Longear specimen from Bull Shoals is noteworthy because of its large size, both in length and weight.

Percidae – Perches

***Etheostoma collettei* Birdsong and Knapp – Creole Darter**. A 50 mm SVL female *E. collettei* collected 15 March 2018 from Ten Mile Creek at Lonsdale, Saline Co. (34.545274°N, 92.753888°W) contained 7 water mites in its stomach, including 2 *Lebertia*, 2 *Hygrobates*, 1 *Atractides*, and 2 *Woolastookia*. Tumlison *et al.* (2017) previously reported unidentified water mites from 2 *E. collettei* from this locality. It appears this darter may commonly ingest this food item at this specific locality in the state.

CLASS AMPHIBIA

Proteidae – Mudpuppies

***Necturus louisianensis* Viosca – Red River Mudpuppy**. Debate continues about whether this form is a subspecies (*N. maculosus louisianensis*) or a full species. In either case, it is the only form found in Arkansas. This large aquatic salamander occurs statewide in permanent water situations (Trauth *et al.* 2004). Specimens usually range between 180-230 mm in TL, and the record size recorded was 307 mm (Powell *et al*. 2016).

A large adult female specimen was captured from DeGray Lake in the Arlie Moore/ Oak Bower area, Hot Spring Co., on 11 February 2019. The fisherman, Robbie Morphew, was fishing with shiners as bait, by use of hook and line attached to floating noodles, in water about 1.5 m (5 ft.) in depth. The fresh specimen was 309 mm TL, and 207 mm snout-vent length (SVL). This individual (HSU1980) represents a new maximum length for Arkansas, and for the species (or subspecies).

CLASS AVES

Cardinalidae – Cardinals

***Cardinalis cardinalis* (Linnaeus) – Northern Cardinal**. Male Northern cardinals usually present bright red coloration whereas the female shows tinges of red but most of the body is drab. Very rarely, a condition known as bilateral gynandromorphism appears, where male coloration presents on one side of the body and female coloration on the other (Major and Smith 2016).

The first bilateral gynandromorph cardinal known to have been spotted in Arkansas was photographed near Roland in Pulaski Co. on 11 February 2018 (Tumlison *et al*. 2018). A bilateral gynandromorph cardinal was photographed 9 months later by Ed Laster on 7 November 2018, in Little Rock, Pulaski Co. (34.8063°N, 92.4895°W). The Roland cardinal was 15.3 km (9.5 mi.) NNE of this new observation. From the photograph (Fig. 6), it could not be determined with certainty whether this was the same bird. Both birds appeared to be male on the right side. The New York Times recently reported a similar bird from Pennsylvania (https://www.nytimes.com/2019 /02/09/ science/cardinal-sex-gender.html).

Figure 6. Image of bilateral gynandromorph Northern Cardinal (*Cardinalis cardinalis*) taken in Pulaski County, 7 November 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 Ed Laster.

CLASS MAMMALIA
ORDER EULIPOTYPHLA

Soricidae - Shrews

***Cryptotis parva* (Say) – Least Shrew**. An adult *Cryptotis parva* collected on 24 October 2018 on the campus of Northwest Arkansas Community College, Bentonville, Benton Co., was infested with 2 female *Leptinus americanus* beetles. *Leptinus americanus* has been reported previously from the Southern Short-tailed Shrew (*Blarina carolinensis*) and the Eastern Mole (*Scalopus aquaticus*) from Arkansas (Connior *et al*. 2014). *Cryptotis parva* is a new host record for this beetle. An adult *C. parva* collected at the same location on 2 October 2018 was infested with 3 female *Corrodopsylla hamiltoni*. This is only the second report of this flea in Arkansas, both occurring in the northwestern corner of the state (McAllister *et al*. 2017).

ORDER CHIROPTERA

Vespertilionidae – Vesper Bats

***Lasiurus cinereus* (Palisot de Beauvois) – Hoary Bat**. A rabid female Hoary Bat collected on 11 September 2018 from Siloam Springs, Benton Co., represents a new county record for Arkansas (Perry *et al*. 2018).

***Lasiurus seminolus* (Rhoads) – Seminole Bat**. Two new county records for the Seminole Bat have been obtained. On 11 August 2017, a Seminole bat was captured in a mist net in S30, T14S, R4W, on the Cut-Off Creek WMA in Drew Co. On 24 July 2018, an adult male Seminole Bat was captured in a mist net set over a road in S6, T14S, R25W, Hempstead Co. On the same night, a post-lactating adult female Seminole Bat was captured in a mist net set over a road in S21, T13S, R25W, on the Dr. Lester Sitzes III Bois D'Arc WMA. This observation may support other evidence of reproduction in Arkansas, as a newly volant juvenile Seminole Bat was captured on 26 July 2001 (Tumlison *et al*. 2002).

***Myotis austroriparius* (Rhoads) – Southeastern Myotis**. On 18 August 2018, an adult male Southeastern Bat was captured in a mist net set on a trail in S6, T6S, R5W, on the George H. Dunklin Bayou Meto WMA. This is a new county record for Jefferson Co. (Perry *et al*. 2018).

***Eptesicus fuscus* (Palisot de Beauvois) – Big Brown Bat**. On 8 August 2017, a post-lactating adult female Big Brown Bat was captured in a mist net set over a road in S3, T12N, R6E, on the St. Francis Sunken Lands WMA. This is a new county record for Poinsett Co., that helps fill a void of records in eastern Arkansas (Perry *et al*. 2018).

Mollossidae – Freetailed Bats

***Tadarida brasiliensis* (I. Geoffroy) – Brazilian Free-tailed Bat**. An adult male Brazilian Free-tailed Bat captured 16 August 2018 in a mist net set over a stream in S7, T5S, R6W, on the George H. Dunklin Bayou Meto WMA, is a new record for Arkansas Co., and the easternmost record in Arkansas (Perry *et al*. 2018).

ORDER RODENTIA

Sciuridae – Squirrels

***Sciurus carolinensis* Gmelin – Gray Squirrel**. Larvae of bot flies (*Cuterebra* sp.) cause myiasis in the animals they infest, and near maturity appear as large, darkened maggots visible through a hole in the skin of the host. Though bot flies are commonly reported as infesting gray squirrels, limited information has been reported in squirrels in Arkansas. On 14 July 2018, a single adult gray squirrel was collected near Mull in Marion Co., that contained a 3rd instar larvae of *Cuterebra emasculator* located on the abdomen near the right rear leg (Fig. 7). Although this botfly is rather common in squirrels, most of the records occur east of the Mississippi River (Sabrosky 1986). Skvarla *et al.* (2016) reported a single adult *C. emasculator* collected along the Buffalo National River in Newton County during a trapping survey. This is the first report of this species infesting a gray squirrel in Arkansas.

Figure 7. Botfly (*Cuterebra emasculator*) in a gray squirrel collected from Mull, Marion Co., on 14 July 2018. Photo by MBC.

***Sciurus niger* Linnaeus – Fox Squirrel**. On 6 March 2016, a single adult melanistic fox squirrel was seen by MBC at Tanyard Springs, Bella Vista, Benton Co. Though melanistic fox squirrels are not uncommon, they are seen most in the southeastern United States, and Benton County is the most extreme northwestern county in Arkansas from which they have been observed (Kiltie 1989).

***Glaucomus volans* (Linnaeus) – Southern Flying Squirrel.** Flying squirrels were recently discovered to fluoresce under ultraviolet (UV) light (Kohler *et al.* 2019). The study examined *G. volans* from several localities, but the closest specimen to Arkansas was from Tennessee, and no specimens were examined from the southwestern area of the species range in the United States (Arkansas, Louisiana, Mississippi, Missouri, Oklahoma, Texas). We examined 18 specimens (HSU 77-80, 82-87, 164, 173, 177, 178, 180, 185, 201, 457) from Clark and Pike Cos. in southwestern Arkansas under UV light, and 16 of them fluoresced to some degree with a pink coloration (Fig. 8).

Figure 8. Fluorescence in a Flying Squirrel from Clark Co., SW Arkansas. Under white light, the ventral hairs were white to tannish (left), but under UV light (right) about half of the pelage glowed pinkish. Photo by RT.

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De novo Development and Characterization of Tetranucleotide Microsatellite Loci Markers from a Southeastern Population of the House Finch (*Haemorhous mexicanus*)

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Running Title: House Finch Microsatellite Development

Abstract

Microsatellites are short tandem repeats (e.g. TAGATAGA) of base pairs in a species' genome. High mutation rates in these regions produce variation in the number of repeats across individuals that can be utilized to study patterns of population- and landscape-level genetics and to determine parentage genetically. In this project our objective was to develop microsatellite markers for the House Finch, *Haemorhous mexicanus*. This species has become one of the most well-studied species of songbirds due to its unique geographical, evolutionary, and epidemiological history. Using mist-nets we captured birds on the Arkansas Tech University campus and collected blood samples to obtain genomic DNA. Samples were processed in The Field Museum's Pritzker Laboratory for Molecular Systematics and Evolution, where we fragmented genomic DNA and isolated fragments that contained potential microsatellites using specially designed biotin labelled probes. These DNA fragments were transformed into competent *E. coli* cells which were then PCR-amplified and Sanger sequenced. After sequencing DNA fragments from approximately 500 *E. coli* colonies, we designed and characterized a set of 13 tetranucleotide microsatellite loci. The average number of alleles and heterozygosity found in 12 individuals from Arkansas was 8.69 and 0.80, respectively. This finalized set of microsatellites can be utilized by researchers to determine parentage and characterize genetic differences across House Finch populations.

Introduction

House Finches (*Haemorhous mexicanus*) are one of the most common and well-studied passerine species in North America. Their geographic range was originally restricted to the arid southwest, though in 1939 they were introduced to Long Island, NY (Aldrich and Weske 1978). Since this time the species' native (western) and introduced (eastern) ranges have each expanded dramatically to make them common breeders throughout most of the United States. The initial introduction caused a molecular founder effect (Hawley *et al.* 2006) and produced substantial variation in genetics (Hawley *et al.* 2006), morphology (Bock and Lepthein 1976; Shultz *et al.* 2016), and physiology (Bock and Lepthein 1976) across their range. Low genetic diversity among House Finches may decrease population fitness (Reed and Frankham 2003; Briskie and Mackintosh 2004) and their susceptibility to parasites and pathogens in these populations (Hedrick *et al.* 2001; Acevedo-Whitehouse *et al.* 2003). In fact, Hawley et al. (2005, 2006) suggested that reduced genetic diversity in the eastern population of House Finches may have contributed to their susceptibility to *Mycoplasma gallisepticum*. This poultry pathogen was first reported in Washington D.C. in the mid-1990s (Dhondt *et al.* 1998) and has since spread across eastern and western populations and caused dramatic losses in many House Finch populations (Hawley *et al.* 2006). In recent decades, this species has become a model organism for studies of population genetics, invasion biology, and disease ecology.

Studies of House Finches have also been instrumental in formulating our understanding of the evolution and maintenance of sexual signals in songbirds. Male finches express carotenoid-based pigmentation that can range from yellow to red (Hill 1993). Pigment concentration and corresponding extent and hue of colorful plumage varies widely across populations (Hill 1993; Inouye *et al.* 2001). While some individual- and population-level variation is explained by diet composition (Hill 1992; Inouye *et al.* 2001; Hill *et al.* 2002), correlative and experimental studies have demonstrated the complex nature of this connection and suggested an additional role for physiological and/or genetic mechanisms of control (Hill 1993, 2002). Multiple studies have demonstrated an association between male plumage coloration and reproductive success (Hill *et al*. 1999, Badyaev *et al*. 2001). Although this species forms socially monogamous pairs, up to 10% of their offspring may be the product of extra-pair matings (Oh and Badyaev 2006). Estimates of male reproductive success must therefore differentiate within-pair versus extra-pair offspring using genetic techniques.

To further advance our understanding of this species' population, landscape, disease, and evolutionary ecology we must utilize molecular markers to characterize genetic variation at the individual and population levels. In this study, we describe the development of microsatellite markers in an Arkansas population of House Finches. Microsatellites are regions of repetitive DNA containing short tandem repeats (e.g. AGATAGATAGAT). While the repeating sequence of base pairs is consistent across individuals, the number of times the sequence repeats can be highly variable across individuals. These non-coding regions are not thought to be under selection and thus can show non-selective patterns of evolutionary divergence.

Although microsatellites have been developed previously for this species (Hawley 2005, Oh and Badyaev 2009), the majority (18 of 25) were dinucleotide repeats which are generally more difficult to score than tetranucleotide due to the presence of shadow or stutter bands (Ginot *et al*. 1996; Daniels *et al*. 1998; Nater *et al*. 2009). Furthermore, few microsatellites have been developed from populations in the southeastern United States despite wide occurrence through this region. It is often preferable to utilize locally-developed microsatellites to avoid problems (e.g. low heterozygosity) resulting from rapid evolution of these loci. Here we describe the characterization of 13 tetranucleotide repeats that, when combined with previously developed microsatellites, will provide a robust microsatellite panel suitable for studies of paternity and population genetics.

Materials and Methods

Field methods

In late 2016 we captured 12 House Finches (3 females, 8 males, 1 unknown sex) using mist nests placed near bird feeders on the Arkansas Tech University campus (35.2945° N, 93.1363° W). We collected blood samples from each bird by puncturing the brachial vein with a hypodermic needle and collecting up to 60 µl upwelling blood in a heparinized capillary tube. Whole blood was applied to non-indicating FTA Elute micro cards (GE WB120410) which lyse cells and denature proteins while preventing DNA degradation. Samples were stored at room temperature (22 °C) until transfer to the Field Museum for genetic processing. Prior to release we aged and sexed the birds and collected basic morphological measurements. Birds were banded with a metal numbered band from the United States Fish and Wildlife Service as well as a passive integrated transponder (PIT) tag and a unique combination of plastic color bands (for related study objectives).

All birds were captured, handled, and released safely and in accordance with procedures approved by the Institutional Animal Care and Use Committee at Arkansas Tech University (approval no. 103116), Arkansas Game and Fish (permit no. 051020161), and United States Fish and Wildlife Service (permit no. 24044).

Microsatellite Enrichment

All laboratory methods were carried out in the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum in Chicago, IL. Microsatellite markers were developed following the enrichment protocol of Glenn and Schable (2005). Approximately 1 µg of genomic DNA (gDNA) from one individual was digested with RsaI and XmnI, and SuperSNX24 linkers were ligated onto the ends of gDNA fragments, which act as priming sites for polymerase chain reactions (PCR) in subsequent steps. Five biotinylated tetranucleotide probes [$(AAAT)_8$; $(AACT)_8$; $(AAGT)_8$; $(ACAT)_8$; $(AGAT)_8$] were hybridized with gDNA for 45 min. The biotinylated probe-gDNA complex was added to magnetic beads coated with steptavidin (Dynabeads® M-280 Invitrogen, Carlsbad, California). This mixture was washed twice with 2xSSC, 0.1% SDS and four times with 1xSSC, 0.1% SDS at 52 ºC. For the final two washes, the mixture was incubated for 1 min in a 52 ºC water bath. Between washes, a magnetic particle collecting unit was used to capture the magnetic beads which are bound to the biotin-gDNA complex. This allowed us to capture gDNA containing tetranucleotide repeats while other fragments (i.e. those not containing repeats) were washed away. Enriched fragments were removed from the biotinylated probe by denaturing at 95 ºC and precipitated with 95% ethanol and 3M sodium acetate. To increase the proportion of enriched fragments, a "recovery" PCR was performed in a 25 µl reaction containing 1X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.5 mM $MgCl_2$, 0.16 mM of each dNTP, 10X BSA, 0.52 μM of the SuperSNX24 forward primer, 1 U *Taq* DNA polymerase, and approximately 25 ng enriched gDNA fragments. Thermal cycling, performed in an MJ Research DYAD, was performed as follows: 95 ºC for 2 min followed by 25 cycles of 95 ºC for 20 s, 60 ºC for 20 s, and 72 ºC for 90 s, and a final elongation step of 72 ºC for 30 min. Subsequent PCR fragments were cloned using the TOPO-TA Cloning® kit following the manufacturer's protocol (Invitrogen). Bacterial colonies containing a vector with gDNA (i.e. white colonies) were used as a template for subsequent PCR in a 25 μl reaction containing 1X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.5 mM MgCl2, 0.12 mM of each dNTP, 10X BSA, 0.25 μM of the M13 primers, and 1 U *Taq* DNA polymerase. Thermal cycling was as follows: an initial denaturing step of 95 ºC for 7 min will be followed by 35 cycles of 95ºC for 20 s, 50 ºC for 20 s, and 72 ºC for 90 s. These PCR products were cleaned using MultiScreen-PCR Filter Plates following the manufacturer's protocol (Millipore, Billerica, Massachusetts). DNA sequencing was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California). Sequencing reactions were precipitated with ethanol and 125 mM EDTA and run on an ABI 3730 DNA Analyzer. We then developed primers flanking core microsatellite repeats using Primer3 (http://primer3.ut.ee). Forward primers were designed with M13-tails (5′-TGTAAAACGACGGCCAGT-3′) and reverse primers with a "pigtail" (5′-GTGTCTT-3′), the former to incorporate fluorescently labeled M13 primers via PCR (Schuelke 2000) and the latter to adenylate the 3′ end of the forward product (Brownstein et al. 1996).

Genotyping Individuals

Genomic DNA was extracted using DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany) following the manufacturer's instructions. Microsatellite loci were amplified separately in 10μl reactions using the following two-step thermal protocol: an initial denaturing step at 94 ºC for 4 min followed by 35 cycles of 94 ºC for 15 s, 58 ºC for 15 s, 72 ºC for 45 s, then 8 cycles of 94 ºC for 15 s, 53 ºC for 15 s, 72 ºC for 45 s and a final extension step at 72 ºC for 10 min. Each reaction had a final concentration of 10 mM Tris-HCL, 50 mM KCL, 0.5 mM dNTPs, 1.5 mM $MgCl_2$, 1 μg BSA, 0.16 μM fluorescently labeled M13 primer (6-FAM), 0.04 μM forward primer, and 0.16 μM reverse primer. PCR products were then combined with the ALEXA-725 size standard (Maddox and Feldheim 2014) and run on an ABI 3730 DNA analyzer. Allele sizes were determined using the Microsatellite Plugin (v1.4.6) in Geneious Prime (v2019.0.4) using the local southern sizing method. Loci were tested for Hardy-Weinberg equilibrium using GenAlEx (v6.5; Peakall and Smouse 2006, 2012) and linkage disequilibrium with Genepop (v4.2; Raymond and Rousset 1995; Rousset 2008).

Results and Discussion

A total of 12 House Finch individuals were screened using the 13 microsatellite loci developed here (Table 1). Across all loci, heterozygosity averaged 0.80 ± 0.05 SE (range: 0.58 to 1.00) and the number of alleles 8.69 ± 0.76 SE (range: 4 to 14). All loci were in Hardy-Weinberg equilibrium and no linkage disequilibrium or sex-linkage was detected. To determine the repeatability of our marker set we genotyped the blood sample of a recaptured individual. The sample was blindly processed exactly the same as the other samples and resulted in the same genotype as its original sample.

Even relatively minor scoring errors can affect paternity results. For example, Hoffman and Amos (2005) found that relatively minor error rates of 0.01 per allele could increase incorrect rates of paternity exclusion above 20%. Dinucleotide sequences like those from previously developed House Finch microsatellites (Hawley 2005; Oh and Badyaev 2009) are more difficult to score due to shadow or stutter peaks which can lead to scoring errors. The tetranucleotides used in our research will give more accurate and precise results in terms of paternity analysis. Ultimately, however, scoring error rates of known mother-offspring pairs will be needed to determine 'true' genotyping error rates.

Along with the two tetranucleotides developed by Hawley (2005) and five tetranucleotides developed by Oh and Badyaev (2009), these additional 13 loci will provide a robust market set that should minimize genotyping error rates. The microsatellites developed and characterized herein will enable researchers studying House Finches to more accurately determine genetic paternity and elucidate population- and landscape-level patterns of genetic diversity.

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Table 1. Characteristics of 13 tetranucleotide microsatellites loci isolated from a southeastern population of the House Finch (*Haemorhous mexicanus*). Sequences have been deposited in GenBank under the accession numbers listed.

Locus	Primer sequence (5'-3')[a]	Repeat motif	N_A	Size range (bp)	H_O	H_E	Accession no.
Haem001	F: TGGACATACCACAACATCTTAGGA R: TGCTCTAGCTTCCAGCCCTA	$(AACT)_{14}$	7	190-223	0.58	0.75	MN333897
Haem036	F: TAGCTGCTGTCAGGAAACCC R: CACAGCACAGCAGAGAGGAA	$(TAGA)_{12}$	8	179-199	1.00	0.81	MN333898
Haem086	F: ACAACATCAATGTCAGGTGATTCA R: ACCTCAAGGACTGGGACACT	$(GGAT)_{14}$	4	351-363	0.58	0.64	MN333899
Haem089	F: ACAGCAAAGAAGATTGTCATGCA R: AGAGAAGCTGAGGGGTCACA	$(AGAT)_{15}$	9	220-264	0.92	0.82	MN333900
Haem092	F: CCCAGAAGAGGGTCAGGAAA R: AGCCTACCCTCTTTAAATTTGAAACC	$(AGAT)_{16}$	9	286-326	0.92	0.79	MN333901
Haem110	F: CAGGAGTGCAGAAGTTGGCA R: ACTTCTGTTGCCATGTTTATCAAT	$(GATA)_{13}$	7	226-250	0.75	0.77	MN333902
Haem137	F: TGCAGAAGTTGGCACGTTTTT R: TACTTGATCCAATTGTGTGGTCT	$(AGAT)_{13}$	7	195-219	0.75	0.77	MN333903
Haem298	F: CGTACAAATGGAAGCTGTGCC R: TGGGTAGTAGCTTTGCTGCC	$(TAGA)_{14}$	10	246-294	1.00	0.85	MN333904
Haem309	F: TCCTGGTCTTTGCTGTTGTGT R: GTCTATGTCTCAGATGCAATGTGC	$(TAGA)_{14}$	8	266-280	0.75	0.84	MN333905
Haem325	F: TCAGTTGGAAGGGACCTAGTC R: TGAGCATCTGGAACATACTCCA	$(TCTA)_{12}$	14	250-350	0.92	0.85	MN333906
Haem326	F: TGATCTCATCTGCATTTATCTTCATTG R: GCTTAGCTACCATGAACCTTGC	$(TCTA)_{13}$	8	165-200	0.67	0.78	MN333907
Haem329	F: CTTCATGCCATGTCCTGCCT R: TGCTCCTCTGATTGACTCCAG	$(TCTA)_{16}$	8	213-245	0.58	0.84	MN333908
Haem330	F: CAGGAATCCCTCTTTTCAGCTG R: GCCTATGCTGTGATAATTGCAC	$(TCCA)_{14}$	14	204-328	1.00	0.90	MN333909

[a] TGTAAAACGACGGCCAGT and GTGTCTT were added to the 5' end for forward and reverse primers, respectively

F: forward primer, R: reverse primer; N_A: number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity

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Analyzing the Adoption Rate of Local Variable Type Inference in Open-source Java 10 Projects

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Running Title: Analyzing the Adoption Rate of LVTI in Java 10 Projects

Abstract

Type Inference is used in programming languages to improve writability. In this paper, we will be looking more specifically at Local Variable Type Inference (LVTI). For those unfamiliar with LVTI, we will also give an in-depth explanation of what it is and how it works. There is a lot of debate surrounding Type Inference in modern day programming languages. More specifically, whether the costs associated with LVTI outweigh the benefits. It has found its way into many higher-level languages including C#, C++, JavaScript, Swift, Kotlin, Rust, Go, etc. In this paper, we will look at the usefulness of LVTI and its popularity since the release of Java 10. Our study will show that LVTI in Java has not received widespread adoption. We will also explain a possible reason for this, based on the information we have gather from our empirical study which involved statically analyzing 6 popular open source Java 10 projects. We will also discuss different scenarios in which Type Inference can obscure different programming errors.

Introduction

Type Inference is a programming language feature that allows for compilers to, based on the context of a given procedure in a programming language, infer the type of a l-value or a r-value (Agarwal and Stoller 2004). Local Variable Type Inference (LVTI) is Type Inference restricted to the local scope of a program and only used for inferring the type of variables. In Java 10, a new LVTI operator was introduced which is the `var` operator. In order for it to be used, one simply uses the keyword `var` in the place of where the type would be specified. For an example of this, please refer to Figure 3. This allows for programmers to speed up their development process by not memorizing complex datatypes returned by different functions and operators and instead just use the `var` keyword. It can even be used in the place of basic datatypes such as int, char, float, etc. This helps improve the writability of a developer's code while programming, and dramatically increase the speed of the development process. There are, however, some drawbacks that come with LVTI that are most closely related to the readability of a programmer's code who uses LVTI. LVTI can lead to errors that are very difficult to debug as shown in Figure 4, and code that is very difficult to read if not well documented. One popular saying recalled by R. C. Martin in his book *A handbook of agile software craftsmanship* is "Code is read more than it is written", ergo a programs readability is far more important than it's writability in most cases (Danial 2018).

Though this debate has been around for as long as Type Inference has been around, there has yet to be studies performed on LVTI in the newly released Java 10. Additionally, due to Java's Eclipse IDE (Integrated Development Environment), programmers may find the readability of code using the `var` keyword may differ from most other static programming languages which lack a native IDE.

In order to obtain a greater understanding of the true popularity of Java 10's new LVTI feature, we turn to the true judge on the issue: the programming code of the greater Java community. In this project we statically analyzed the frequency of the LVTI feature in 6 popular opensource Java 10 projects.

The results of our study showed that the frequency of usage of Java 10's LVTI is very low to non-existent in most Java 10 projects. This may be due to just how new Java 10 is. Additionally, most of the projects we analyzed have been around for a few years at the least and are currently working on ensuring their projects compatibility with Java 10 before using some of its newer features. It could also be related to developers not wanting to use LVTI due to its drawbacks.

The following contributions will be made through this paper: An empirical study of Java 10's LVTI in real world open source projects.

- A discussion of why Java 10's LVTI has not reached widespread adoption.

- A discussion of the benefits and hinderances associated with LVTI in Java 10.
- An in depth look at different bugs and errors that may be caused by Java's LVTI.

Related Works

A number of articles have been written relating to the adoption of a new programming language feature. There are many static analysis tools for open source projects (Beller *et al.* 2016; Hellström 2009). Kim et al. conducted analyzing type inference in C# (Kim *et al.* 2013) with static analysis tool for open source projects. They showed the usage of `var` type with the number of developers in each open source project. Kim and Yi conducted the acceptance of programming language features commonly referred to as "syntactic sugars" (Kim and Yi 2014). They examined the acceptance of different features that had been around for quite some time in both C# and Java. But they did not examine Type Inference in particular within either of the languages. Smith and Cartwright proposed type inference algorithm that can calculate correct results because Java 5 algorithm fails (Smith and Cartwright 2008).

Background

There have been a few different versions of Type Inference released in Java and changes that have been made to each of these since Java 5. From the perspective of an outsider who is un-familiar with Java or a new Java 10 developer without past experience using Java, LVTI may not seem to be anything special. A feature common to most statically typed programming languages to help with especially complex data-types. However, to a veteran Java developer, LVTI is a game changer. Unlike previous versions of Type Inference features in Java, it infers the entire type of a variable, not just parameter types or generic types. To some developers, this seems like a very nice feature to have and makes the development process much faster and easier. However, to others it may seem like a cause for poorly written, hard to debug code. There is no performance difference between explicit type declarations and implicit ones because `var` keyword instructs the compiler to infer the exact type from the right side of the initialization statement at compile-time based on the type inference algorithm (Kim *et al.* 2013; Agarwal and Stoller 2004).

Knowing the significant impact that LVTI would on the Java ecosystem, we constructed 3 research questions that we sought to answer:

- Is LVTI used widely in Java 10 projects? Why? Or why not?
- Why was LVTI added to Java?
- Are LVTI related errors hard to debug? Why? Or why not?

Implementation

We analyzed 6 open source projects to answer the research questions we discussed within the background section. We selected projects that were within the Java 10 category, according to Github.com. Table 1 displays the name of each project, the lines of Java code within the project, and the total lines of code in the project measured by the opensource command line tool CLOC (Pierce and Turner 2000).

We modified an existing open source programming language analysis framework, written in Java, Python, and MySQL (Parnin *et al.* 2011). The framework statically analyzes code in the following steps:

1. Download the full history of each project from a remote git repository using the git command line tool
2. Store the different file revisions in an intermediate format
3. Transfer the information about each revision to a table within a database
4. Extract occurrences of LVTI from each file revision and store the occurrence in an intermediate format.
5. Store the number of occurrences in the database.
6. Generate graphs for each project analyzed using Octave.

Evaluation

Research Question 1: Is LVTI used widely in Java 10 projects? Why? Or why not?

Our first research question is whether LVTI is used frequently in opensource Java 10 projects. To answer

Table 1: The 6 open source Java projects under investigation

Project Name	Lines of Java Code	Total Lines of Code
d3x-morpheus	65,854	92,104
PMD	116,597	271,882
Jenkins	154,660	250,658
Netty	259,740	270,643
Kafka	228,655	349,444
Elastic Search	1,256,251	1,516,724

this we measured the number of occurrences of LVTI in the opensource projects we downloaded. Figure 1 shows a side-by-side comparison of the usage of LVTI in each of the projects we analyzed. Only two out of the six projects we analyzed had any occurrences of the *var* keyword leaving four projects not using the newly introduced feature at all. Figure 2 shows the number of instances of `var` in each project over time. One of the projects (d3x-morpheus) showed a complete conversion of regular typing to the `var` keyword where possible in the matter of a very short time as can be seen in Figure 2. This project turned out to have the greatest number of occurrences with a staggering 142 instances of LVTI. It can be noted that most of the Java 10 projects analyzed have yet to use LVTI at all.

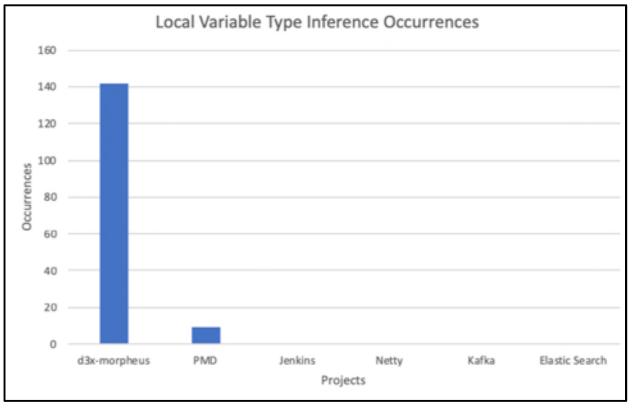

Figure 1: The occurrences of LVTI in the Java projects we analyzed.

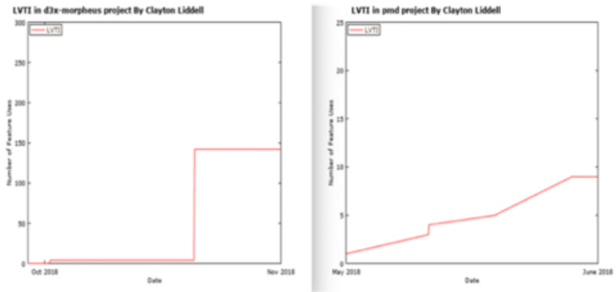

Figure 2: The occurrences of `var` in the two Java projects which used LVTI.

Research Question 2: Why was LVTI added to Java?

LVTI was added to Java in order to increase its writability as a programming language. It supports implicit typing of local variables, which offers succinct syntax, compared with explicitly typed variables (Goetz B. 2018; Marks S. 2018). In C#, the usage of implicit generics declaration (i.e., `var`) is relatively low and a small number of developers use `var`. However, developers would be more likely to use `var` when creating a long variable name with several parameters (Kim *et al.* 2013). It allows developers to not worry about keeping track of the types returned by different functions and dramatically speeds up the development process. It also helps decrease the redundancy of some code, such as when you create an object of a certain type and then have to re-specify the type for the l-value. This can be seen in Figure 3.

```
FileWriter writer = new FileWriter(file);
//becomes
var writer = new FileWriter(file);
```

Figure 3: Example usage of LVTI in Java 10.

Research Question 3: Are LVTI related errors hard to debug? Why? Or why not?

Using Type Inference can make code more concise. But, in some case, use of Type Inference can harm the readability of the program. Because the lack of a type being explicitly declared may make the code harder to read. An example of this can be seen in Figure 4.

```
// What does abd() return?
     var x = abc();

// The type returned from abc() is String!
     String x = abc()
```

Figure 4: The return type of `abc()` is unclear and so the type of x is also unclear.

As you can see in Figure 4, the use of explicit typing makes the code much easier to understand for someone new to a project. If it were not for explicit typing, a person new to the project may have to track down where the function `abc()` is declared just to determine its type.

This may not actually be a problem for someone who uses the Java Eclipse IDE though, since upon hovering over a function, the return type of that function is revealed to the user. So, in this case, the answer is both yes and no. Type Inference can make type related errors harder to debug, however the likelihood of type related errors due to LVTI is mitigated by a feature of the Java Eclipse IDE.

There is another case of an error being caused by Java's LVTI in conjunction with Polymorphism. This issue is addressed in Pierce and Turner's *Local Type*

Inference, however they never arrived at a solution to the issue. The issue is as follows: Imagine you have a parent class Vehicle created alongside child classes Bike and Car. If a variable is initialized with one of the child classes Bike or Car using Type Inference, it cannot be change to the other. Of course, this is an issue that arises in Polymorphism alone even without Type Inference and is often labeled as a feature rather than an issue. However, not being able to assign a variable to a child class of the same parent as the type the variable was first initialized with can be very confusing for an amateur programmer just starting out. And, the `var` operator only adds to the confusion if the programmer is mistaken on what type the variable was first initialized with. This can lead to a very difficult to debug error. For an example of this, see Figure 5.

```
class Vehicle {}
class Bike extends Vehicle {}
class Car extends Vehicle {}

var v = Bike();
//several lines of code later
v = Car();
```

Figure 5: Will result in an error due to incompatible types.

So, while LVTI in Java can make some errors hard to debug it's only in some uncommon cases.

Conclusion

We analyzed the usage of LVTI in 6 open source Java 10 projects and found that LVTI in Java 10 has yet to reach widespread adoption. It is a very handy feature that improves Java's writability with some drawbacks and seems to be a nice addition to Java 10. However, it is still too soon to tell whether it will become widely accepted within the Java community. Some reasons behind why it has yet to reach widespread adoption include: (1) LVTI is so new, that people have not had time to start using it. And, open source developers are still working to make sure their projects are Java 10 compatible, and (2) Java developers may be dissuaded from using LVTI due to the drawbacks we discussed.

We also discussed some Type Inference related errors and how LVTI can harm Java's readability and can also result in errors relating to incompatible typing in Java being more difficult to identify.

Future work may be needed at a later date in order to determine whether Java 10's LVTI becomes widely adopted given time.

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Distribution, Habitat, and Life History Aspects of the Dwarf Crayfishes of the Genus *Cambarellus* (Decapoda: Cambaridae) in Arkansas

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Running Title: *Cambarellus* Crayfishes of Arkansas

Abstract

The dwarf crayfishes of the genus *Cambarellus* are represented in Arkansas by only 2 species: *Cambarellus* (*Pandicambarus*) *puer* (Hobbs) and *C.* (*P.*) *shufeldtii* (Faxon). Both species are quite small and uncommonly encountered in the state. Between 1972 and 2018, we made 368 crayfish collections throughout the 75 counties of Arkansas. A total of 34 collections (our collections, plus museum specimens), and those previously collected by Reimer (1963) yielded a total of 304 specimens of *C. puer* and 12 collections of *C. shufeldtii* returned 54 specimens of *C. shufeldtiii*. Herein, we document these 2 dwarf crayfishes from primarily the Coastal Plain and Mississippi Alluvial Plain physiographic provinces of Arkansas. *Cambarellus puer* is documented from 24 counties whereas *C. shufeldtii* was recorded from only 12 counties. With regard to conservation status, both *C. puer* and *C. shufeldtii* should be considered as "Currently Stable" due to their widespread distribution and general abundance in Arkansas.

Introduction

Freshwater crayfishes of the families Astacidae, Cambaridae, and Parastacidae are native to every continent except Antarctica and Africa (Hobbs 1988). Cambarid crayfishes reach their greatest diversity in North America north of Mexico totaling 374 species with new species described each year (Taylor *et al.* 2007; Crandall and Buhay 2008). Crayfishes inhabit a variety of aquatic habitats including streams, rivers, lakes, reservoirs, swamps, roadside ditches, wet pastures, and fields (Bouchard 1978; Morehouse and Tobler 2013). Here, they function as important components of aquatic ecosystems (Huryn and Wallace 1987; Momot 1995; Usio and Townsend 2004).

The genus *Cambarellus* contains the smallest crayfishes found in Arkansas as fully adult individuals reach only 3.7 cm (1.5 in.). In Arkansas, 2 species of dwarf crayfishes of the genus *Cambarellus* occur, namely the Swamp Dwarf Crayfish, *C.* (*Pandicambarus*) *puer* (Hobbs) and the Cajun Dwarf Crayfish, *C.* (*P.*) *shufeldtii* (Faxon). The precise distribution of these 2 diminutive species in Arkansas is poorly known, and neither has had much published concerning their natural history and ecology, including reproductive biology, habitat characteristics, or general biology. This study was initiated to learn more about these species and to discern their geographical distribution in the state and aspects of their natural history and ecology.

Specific objectives of our study were to: (1) determine the relative abundance and precise distributional limits of the range of *C. puer* and *C. shufeldtii* in Arkansas, (2) gather data on life history aspects of both species, including information on habitat, reproductive period, and any other biological data available, (3) document data on their ecological and habitat characteristics, and (4) assess the current conservation status of each species based on the previously collected distribution data in the state.

Materials and Methods

Field work was conducted between 1972 and 2018 with a total of 368 collections made in all 75 counties in Arkansas. The bulk of the field work occurred during the fall, spring, and summer, which are prime collecting periods. A combination of aquatic dipnets, seines, and baited crayfish traps were employed to collect crayfishes, *C. puer* and *C. shufeldtii*. While most individual crayfish were released unharmed at the collecting site, voucher specimens of each *Cambarellus* species were preserved in 60% isopropanol or ethanol for later analysis. Total length (including chelae) was taken of all specimens in mm. The number of specimens in the Appendices represents the number of specimens preserved (historical data) or the total number collected at an individual site. Preserved vouchers were originally deposited in the Southern Arkansas University (SAU) Invertebrate Collection, the Illinois Natural History Survey (INHS) Crayfish Collection (INHS 2019), and the Smithsonian Institution (SI) Crayfish Collection. In addition to collections made during this survey, museum specimens housed at the United States National Museum of Natural History (USNM) (USNM 2019), INHS, and SAU were used to document the historic distribution of the 2 *Cambarellus* species in Arkansas. Published literature dealing with these crayfish species was also consulted. Both our survey and historical collection locations were converted to Section, Township, and Range (Sec, T, R) for mapping when known precisely.

Results and Discussion

Our survey produced 304 specimens of C. *puer* documented from Arkansas from 24 counties (Fig. 1, Appendix I). These include 162 specimens of *C. puer* taken in our personal collections, 81 specimens reported by Reimer (1963), and 61 specimens housed in museums not collected by us (Appendix I). This species was found in ditches, backwater areas of streams, and lakes. In addition, we documented 54 individuals of *C. shufeldtii* in 12 collections from 12 counties in Arkansas (Appendix II). *Cambarellus shufeldtii* was taken from roadside ditches, swamps, and backwater areas of streams and lakes.

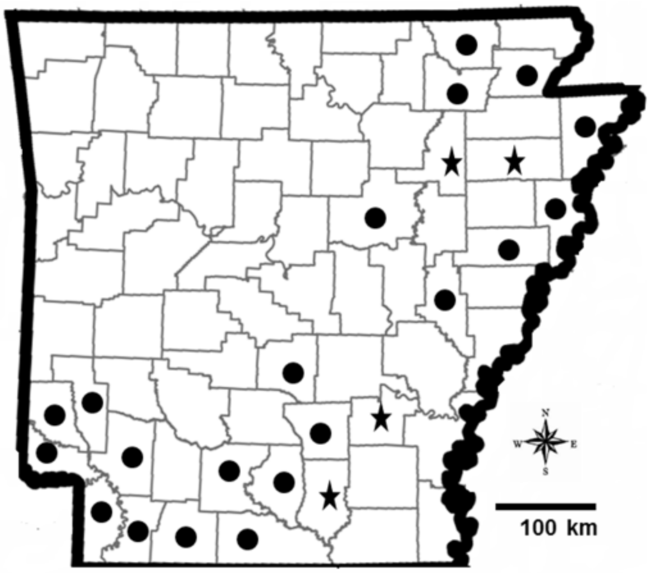

Figure 1. Counties with records of *Cambarellus puer*. Dots = previous county records; stars = new county records.

Recognition Characters of Cambarellus

Dwarf crayfishes of the genus *Cambarellus* Ortmann are small crayfishes ranging from 1.5 to 3.7 cm (0.6 to 1.5 in.). No species of *Cambarellus* in the United States is more than 3.7 cm (1.5 in.) in total length (Walls 2009). Form I males resemble those of several species of *Procambarus*, but always lack the cephalic process and caudal knob (Walls 2009). Males have copulatory hooks on the second and third legs. The hook on leg three is often split. Females have a characteristic annulus that looks like a wide tongue projecting from between the hind pair of legs; it is easily flexed up and down (Walls 2009).

CAMBARELLUS (PANDICAMBARUS) PUER HOBBS, 1945 - SWAMP DWARF CRAYFISH

Recognition Characters

This is a tiny crayfish under 3.7 cm (1.5 in.) in total length as a full adult with a reddish-brown to gray body coloration (Fig. 2). The ventral surface is white or cream-colored. The chelae have a long, cylindrical palm at least as long as the fingers; fingers and palm are smooth, without conspicuous tubercles. Tips of the chelae lack orange coloration. The surface of the rostrum of *C. puer* is flat, without the central trough-like depression found in many other crayfishes. The rostrum has lateral rostral spines and a well-developed acumen. The areola is present and broad. The antennal scales are large and broadest in the middle. Adults are generally 1.8 to 3.3 cm (0.7 to 1.3 in.) and rarely exceed 3.7 cm (1.5 in.) (Walls 2009). Females tend to be slightly larger than males. The carapace is laterally compressed and moderately arched dorsoventrally with strong cervical spines and the areola is open (Morehouse and Tobler 2013). The rostrum is flat and terminates in small lateral


G.A. Schuster 2013

Figure 2. *Cambarellus puer*.

spines at the base of the acumen. Chelae are small and slender with rather short fingers. In form I males, the gonopod has 3 terminal processes (mesial, central, and caudal) that are all strongly curved and about equal in length. The annulus ventralis of females is movable, subcircular, with a flattened or shallowly notched caudal edge, and has a strongly elevated central region (Taylor and Schuster 2004). Interestingly, there are 2 color phases in nature as follows: (1) the striped phase where the body is brownish with two broad, dark brown to black stripes running from above the edges of the telson and often outlined with dark, or (2) a spotted phase where 2 rows of dark brown spots run from above the eyes to the telson and are seldom outlined with dark pigment. Walls (2009) reports both patterns occur in both sexes. *Cambarellus puer* can only be consistently separated taxonomically from *C. shufeldtii* by the strongly curved terminal process on the male gonopod of *C. puer* whereas that terminal process is nearly straight in *C. shufeldtii* (Pflieger 1996). In addition, the rostrum of *C. puer* is broader and its lateral margins are slightly convex and not nearly straight as occurs in *C. shufeldtii*.

Relative Abundance

It appears that *C. puer* is a relatively uncommon to locally abundant crayfish within certain parts of Arkansas. Reimer (1963) made a total of 289 collections amassing 7,300 specimens among 33 species in 4 genera in his master's study (unpublished thesis) of the crayfishes of the state. Of his total of 7,300 specimens, he collected just 81 individuals of *C. puer* in 8 collections. In the present study, a total of 166 specimens of *C. puer* were taken in 15 collections out of 356 (0.4%) personal collections made in Arkansas since 1972. Thus, by combining our data with that of Reimer (1963) as well as existing museum specimens, we found that out of a total of 34 collections of *C. puer* made in Arkansas (1963 to 2017), 304 individuals of *C. puer* have been collected from the state. Most of these specimens are housed in museums, but a few were sent to Brigham Young University (Provo, Utah) for eventual DNA analyses. Collections of *C. puer* at individual sites in Arkansas ranged from 1 to 82 specimens (USNM 146051).

Habitat

In adjacent Oklahoma, *C. puer* is known from a single location: along the Little River in McCurtain County and has not been collected from the state since 1975 (Morehouse and Tobler 2013). In Louisiana, Walls (2009) found that *C. puer* was seldom found in permanent waters deep enough for predatory fish, but preferred shallow ditches, sloughs, and ponds with permanent vegetation. Most of Walls' collections were from habitats with mud or mud and sand bottoms and with little or no aquatic vegetation present. Penn (1950) took *C. puer* from lowland roadside ditches, cypress swamps, and swamp ponds. Pflieger (1996) collected this crayfish in Missouri from small intermittent creeks and the shallows of seasonally flooded sloughs and swamps. Interestingly, this crayfish species can survive drying conditions by finding refuge under woody debris and thick vegetation patches as it has been classified as a tertiary burrower (Pflieger 1996; Taylor and Schuster 2004).

In Arkansas, Reimer (1963) noted that his specimens of *C. puer* were from shallow roadside ditches with abundant aquatic vegetation, slow moving streams with clumps of vegetation, sloughs with mud bottoms, and streams with pebble-sized gravel bottoms and mud banks. In our 44 yr of collecting field data on crayfishes in all 75 counties in Arkansas, *C. puer* is established as an inhabitant of shallow, lowland roadside ditches, shallow sloughs with heavy vegetation, oxbow lakes, and along the margins of swamps. Substrates have typically been sand, mud, and/or clay. We found *C. puer* in shallow water with vegetation or deep leaf litter and usually a lack of predaceous fishes. Just as with *C. shufeldtii*, they do not truly burrow, but rather live just under the mud in small cells.

Distribution

Cambarellus puer occurs from southern Illinois and southeastern Missouri southward along the Mississippi River Basin to Louisiana and southwestern Alabama and westward to eastern and southern Arkansas, southeastern Oklahoma and southeastern Texas (Walls 2009; Morehouse and Tobler 2013). Data from our field trips (1972 to 2018) showed an absence of *C. puer* from the Ozarks and Ouachita Mountains physiographic regions as well as the Arkansas River Valley with a presence in the Coastal Plain physiographic province.

Reimer (1963) made 8 personal collections of *C. puer* which yielded 81 specimens and documented this crayfish from 8 counties in Arkansas including Cleveland, Grant, Lawrence, Little River, Miller, Ouachita, Randolph, and Union. In addition, Reimer (1963) added Greene and Howard counties based on collections by Dr. Horton H. Hobbs, Jr., with vouchers deposited at the USNM. Tumlison *et al.* (2016) added 6 collections and 6 new counties to the known distribution of *C. puer* in Arkansas. Our studies amassed a total of

15 new collections of *C. puer* from 4 additional counties in Arkansas (Fig. 2). These new county records include Bradley, Jackson, Lincoln, and Poinsett. Specific localities for *C. puer* (*n* = 304 specimens) are listed in Appendix I. *Cambarellus puer* was documented from a total of 24 counties throughout the Coastal Plain of Arkansas. Within the province, this crayfish was collected most in southwestern and eastern Arkansas and was less abundant in northeastern Arkansas. The highest number of *C. puer* specimens collected at one time in the state was 82 individuals (USNM 146051) collected by HWR on 23 August 1974 from a roadside ditch of Bodcaw Bayou 9.6 km (4 mi.) N of Lewisville, Lafayette County. Of all the 24 counties in which it was found, *C. puer* was most common in Lafayette County in southwestern Arkansas (Red River drainage) where 4 populations from 4 localities have been documented. At most of these other locations, however, *C. puer* proved to be uncommon.

In Louisiana, Penn and Fitzpatrick (1963) reported that *C. shufeldtii* appears to be extending its range at the expense of *C. puer* as sites formerly inhabited by the latter species now yield only *C. shufeldtii*. Under experimental conditions, *C. shufeldtii* was dominant over *C. puer* in most aggressive encounters and they tentatively concluded that competitive exclusion may be responsible for replacement of *C. puer* by *C. shufeldtii*. Interestingly, in Louisiana and Illinois, *C. puer* and *C. shufeldti* were rarely found in the same body of water (Penn 1950; Page 1985). In fact, we never found *C. puer* and *C. shufeldtii* together at the same Arkansas locality, nor have we documented any instance of replacement of *C. puer* by *C. shufeldtii* in the state.

Life History Aspects

In Louisiana, Form I males of *C. puer* have been collected from August to November (Walls 2009) which corresponds to the peak of their breeding activities according to Black (1966). In Missouri, form I males were collected from January to May. Males require 13 to 14 molts to achieve sexual maturity, and most will not breed during their first year of life (Pflieger 1996). Females with eggs were taken on 7 separate dates between 26 February and 12 May (Pflieger 1996). The latter reported 14 egg-bearing females ranging in length from 2.5 to 3.3 cm (1.0 to 1.3 in.) with the number of eggs per female varying from 43 to 110, averaging 79. The eggs were about 1 mm (0.04 in.) in diameter. Page (1985) reported ovigerous females and females carrying young were collected from March to May in Illinois. Juveniles have been found in Louisiana in late spring into early summer (Walls 2009). The life span of *C. puer* is approximately 15 to 18 months after hatching (Black 1966).

In Arkansas, Reimer (1963) collected Form I males (1 each) of *C. puer* during April, June, and July. Second form (II) males were collected in June (17 specimens) and July (2 specimens) and females in June (28 specimens) and July (4 specimens). One ovigerous female was taken in July by Reimer (1963) which had 45 olive brown eggs.

In our study, Form I males of *C. puer* were collected or documented on 26 April (USNM 1176846- 3), 31 July (USNM 117743-2; USNM 117744-6), 12 October (SAU-1), and 26 October (USNM 146704-1). Form II males were collected on 14 April (USNM 144583), 7 May (USNM 208635-2), and 11 October (SAU-2). We collected or documented single ovigerous females on 20 and 26 April, 23 May, and 26 and 28 July. Egg numbers varied from 49 to 82. No adult females with young attached were found during in our study. Regarding size, adult specimens of *C. puer* in the study ranged from 2.0 to 3.7 cm (0.8 to 1.5 in.) in length.

Decapod Associates

Decapod crayfish associates of *C. puer* in this study include the Digger Crayfish (*Fallicambarus fodiens*), Painted Devil Crayfish (*Lacunicambarus ludovicianus*), Ditch Fencing Crayfish (*Faxonella clypeata*), Twin Crayfish (*Procambarus geminus*), White River Crayfish (*P. acutus*), Swamp Crayfish (*P. clarkii*), and Vernal Crayfish (*P. viaeveridus*). Reimer (1963) reported 5 additional crayfish associates of *C. puer* in Arkansas including the Devil Crawfish (*L. diogenes*), Western Painted Crayfish (*Faxonius palmeri longimanus*), Shrimp Crayfish (*F. lancifer*), Ouachita River Crayfish (*P. ouachitae*), and Southern Plains Crayfish (*P. simulans*).

Conservation Status

In a report on the North American crayfish fauna, Taylor *et al.* (2007) estimated that 48% of species required some sort of conservation status and protection. Specifically, they designated *C. puer* (based on its entire species range) as a "Currently Stable" species, defined as a species or subspecies whose distribution is widespread and stable and is not in need of immediate conservation management actions. Our discovery of 304 individuals of *C. puer* across a 24 county area of Arkansas establishes this crayfish as uncommon in the state. However, we feel more concentrated collecting in eastern Arkansas would yield additional localities and individuals, even though this area has been heavily polluted with weed killing herbicides and various insecticides, particularly those targeting cotton destroying insects. Although NatureServe has not provided a state ranking, we nevertheless concur with Taylor *et al.* (2007) with the CS (Currently Stable) designation for *C. puer* in Arkansas.

CAMBARELLUS (PANDICAMBARUS) SHUFELDTII (FAXON, 1884) - CAJUN DWARF CRAYFISH

Recognition Characters

When fully adult, *C. shufeldtii* is a small crayfish 1.5 to 3.1 cm (0.6 to 1.2 in.) in length (Fig. 3). Females tend to be larger than males and have a maximum length of 3.3 cm (1.3 in.) (Pflieger 1996). The rostrum is flat above, with lateral spines and a well-developed acumen. The areola is broad. Males possess hooks on the second and third pereiopods. The chela has a long, cylindrical palm at least as long as the fingers; fingers and palm smooth, without obvious tubercles. The antennal scale is large and broadest in the middle. The male gonopod has 3 projections (mesial, central, and caudal) that are all straight or nearly so. The annulus ventralis of the female is tongue-like. Two distinct color patterns occur in *C. shufeldtii*, a spotted phase and a striped phase.In the former, the spots are arranged in lengthwise rows along the carapace and abdomen (Pflieger 1996) whereas in the latter, the spots are fused into uninterrupted bands. These color patterns occur in both sexes and no anatomical differences are associated with the phases. Volpe and Penn (1957) studied this situation extensively and they concluded that the color difference was due to a single gene, with the striped condition dominant over the spotted condition. *Cambarellus shufeldtii* can be consistently distinguished from *C. puer* by the male gonopod, which has straight terminal processes in *C. shufeldtii* rather than strongly curved terminal processes in *C. puer*. In addition, the raised portion of the female sperm receptacle is asymmetrical in *C. shufeldtii*, but not symmetrical as in *C. puer*.

Figure 3. *Cambarellus shufeldtii*. Modified from Robison *et al.* (2017).

Relative Abundance

Our data suggests that *C. shufeldtii* is a relatively uncommon crayfish in Arkansas. Of Reimer's (1963) total of 7,300 crayfish specimens collected among 289 collections, he collected just 3 individuals of *C. shufeldtii* in 1 collection. In the present study, a total of 20 specimens of *C. shufeldtii* were taken in 7 collections out of 356 collections (0.02%) made in Arkansas since 1972. By combining our data with Reimer (1963) and various museum specimens (*n* = 31), we found that of a total of 649 collections made in the state (1963 to 2018), only 54 individuals of *C. shufeldtii* have been collected. Many of these are housed in the USNM. Collections at individual sites ranged from 1 to 28 specimens (USNM 144583), although most collections yield low numbers (1 to 2 individuals).

Habitat

In Louisiana, Penn (1950) summarized the physical and biological factors affecting the habitats of *C. shufeldtii*. He found this species occurs primarily in clear, shallow, permanent water exposed to sunlight. Most of his collections were from habitats with mud bottoms with luxuriant growths of aquatic vegetation. Penn (1950) also reported that *C. shufeldtii* was not an obligate burrower, but rather survived short periods of drought in small "cells" several inches below the surface of the soil in which they survive until the next rain. No passageways connecting to the surface were found. Also in Louisiana, Walls (2009) reported *C. shufeldtii* is a species of shallow, rather muddy water habitats with either weed beds or deep layers of leaf-litter. Supportive of Penn (1950), Walls (2009) also found that rather than burrow when a slough or ditch dries up, this species does not actively burrow, but forms shallow cells just under the drying mud interface. In Tennessee, *C. shufeldtii* was found among decaying leaves and twigs in protected waters less than 15.2 cm (6 in.) deep along the edges of sloughs (Hobbs and Marchand 1943). In Missouri, Pflieger (1996) reported this species occurred in swamps, sloughs, and roadside ditches that were shallow, with no noticeable current, and choked with aquatic vegetation. Pflieger (1996) also reported that *C. shufeldtii* is not actually a burrower, but occupies cells as reported by other researchers.

In Arkansas, Reimer (1963) found *C. shufeldtii* in Bayou DeView (Monroe County) in clear, shallow water (less than 30.5 cm [12 in.] deep) devoid of vegetation and only slightly moving. Our study found similar habitat in Arkansas to that reported in Louisiana and Missouri studies. We collected *C. shufeldtii* from clear, shallow areas of vegetated backwaters of streams, swamps, and standing water in shallow roadside ditches.

Distribution

Cambarellus shufeldtii occurs along the Gulf Coast from southcentral Texas eastward to southwestern Alabama and northward in the lowlands along the Mississippi River to Illinois (Pflieger 1996; Walls 2009).

Little has been published on *C. shufeldtii* in Arkansas. The first actual collection of *C. shufeldtii* in Arkansas was apparently a single specimen by A. B. Leonard in White County (USNM 132713) and later reported by Williams (1954). In his unpublished thesis, Reimer (1963) made only 1 collection of *C. shufeldtii* in the entire state from Monroe County and referred to the White County specimen reported by Williams (1954). Later, Tumlison *et al.* (2016) added 5 new counties to the known range of *C. shufeldtii* in Arkansas including Columbia, Jackson, Lafayette, Lawrence, and Woodruff counties.

The present study documented a total of 12 collections of *C. shufeldtii* in Arkansas including 5 new county records in Clay, Crittenden, Desha, Miller, and Union (Fig. 4). Specific localities for all 12 collections of *C. shufeldtii* documented in the state (*n* = 54 specimens) are listed in Appendix II. *Cambarellus shufeldtii* was documented from 12 counties in the Coastal Plain ranging from Miller County in extreme southwestern Arkansas to Clay County in extreme northeastern Arkansas. At most of these locations *C. shufeldtii* was an uncommon crayfish. However, the highest number of specimens collected at one time was 28 (USNM 144583) individuals collected from Woodruff County on 14 August 1973 by H. H. Hobbs, Jr. Historic records by one of us (HWR) show an absence of *C. shufeldtii* from the Ozarks and Ouachita Mountains physiographic regions as well as the Arkansas River Valley.

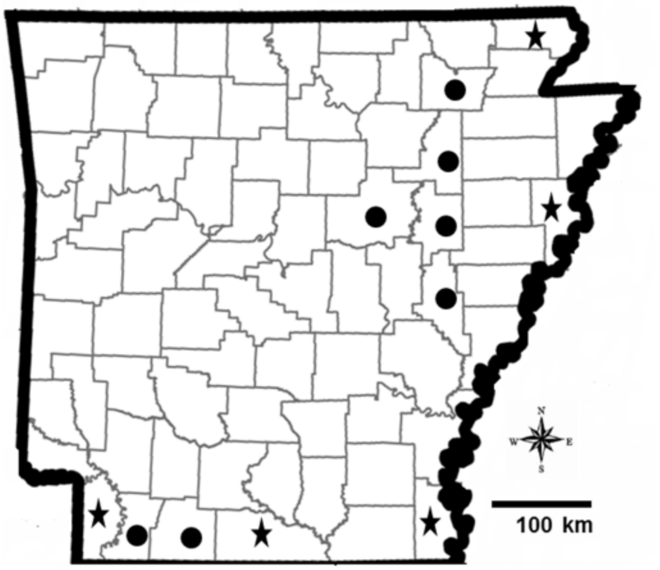

Figure 4. Counties with records of *Cambarellus shufeldtii*. Dots = previous county records; stars = new county records.

Life History Aspects

The biology of *C. shufeldtii* has been studied extensively in Louisiana (Penn 1942, 1950; Lowe 1956, 1961; Black 1966) and Missouri (Pflieger 1996). Penn (1942, 1950) found a continuous period of reproductive activity with 2 peaks in Louisiana. The first peak occurred in the late winter (shortest day length) while the second peak was in early spring and June which had the longest day length. Females mature at about 1.8 cm (0.7 in.) in length and may produce 2 broods during their life span of about 1 yr (Pflieger 1996). An average of 35 eggs per brood is produced by females (Penn 1950). Eggs hatch and they are carried by females beneath their tails. Young remain attached for about 3 weeks (Lowe 1961) until they molt. Males may breed twice by the time they are one year old, but may live 15 to 18 months. Males undergo 12 to 13 molts before maturity and they may breed during the first breeding season after the one in which they were spawned (Pflieger 1996).

In Louisiana (Walls 2009), Form I males have been collected year-round, and ovigerous females are also commonly encountered. Reproduction peaks in the cooler months and again in midsummer because females can produce 2 clutches of young per year. Form I males predominated in collections made in February, July, October, and November. Females with eggs were collected during February, March, and July. In Missouri, Pflieger (1996) found Form I males predominated in his collections in February, July, October, and November, while females with eggs were collected during February, March, and July. In Illinois, Page (1985) reported females with eggs or young were collected in April, May, and June. In 8 females ranging in lengths from 2.0 to 3.0 cm (0.8 to 1.2 in.) from Missouri, the number of eggs per female averaged 64, with a maximum of 108 (Pflieger 1996).

Little is known about the biology of *C. shufeldtii* in Arkansas. In our study, Form I males of *C. shufeldtii* has been documented in Arkansas on 14 April (USNM 144583-3) and 7 May (USNM 298635-3). Form II males were collected on 14 April (USNM 144583-1) and on 7 May (USNM 208635-2) in the state. We collected or documented ovigerous females from Arkansas from April to October (14 April [USNM 144583-1], 7 May [USNM 208635-1], and 11 October [SAU-1]). Egg numbers of female *C. shufeldtii* varied from 45 to 73. No adult females with young attached were found during our study. Regarding size, adult specimens of *C. shufeldtii* in our study ranged from 2.2 to 3.0 cm (0.9 to 1.3 in.) in length.

Decapod Associates

Nine decapod crayfish associates were collected in this study while searching for *C. shufeldtii*, including *F. fodiens*, *L. ludovicianus*, *L. diogenes*, *F. clypeata*, *P. geminus*, *P. acutus*, *P. ouachitae*, and Giant Bearded Crayfish (*P. tulanei*). Reimer (1963) reported 2 additional crayfish associates: *F. p. longimanu*s, and *F. p. palmeri*.

Conservation Status

Cambarellus shufeldtii is common along the Gulf Coast from southcentral Texas eastward to southwestern Alabama and northward in lowlands along the Mississippi River to Missouri (Lincoln County). This crayfish has a large distribution and is able to inhabit a wide range of habitats. There are no known threats currently impacting the population. Taylor *et al.* (2007) designated *C. shufeldtii* (based on its entire range) as a "Currently Stable" species. Our discovery of only 54 individuals of *C. shufeldtii* across a 12-county area in Arkansas, despite considerable effort, establishes this crayfish as uncommon in the state; however, we feel more concentrated collecting in appropriate habitat in southeastern and northeastern Arkansas would yield additional records, even though this area has been heavily polluted with weed killing herbicides and various insecticides. Although NatureServe doesn't provide a state ranking for this crayfish, we still concur with the range-wide status given *C. shufeldtii* by Taylor *et al.* (2007) of CS (Currently Stable).

In summary, within Arkansas, both *C. puer* and *C. shufeldtii* inhabit primarily the Coastal Plain and Mississippi Alluvial Plain physiographic provinces. Our research indicates that while both species are fairly widely distributed, both are rather uncommon in the state. The distributional range of *C. puer* includes 24 of 75 counties in the state and most populations are located principally in the Coastal Plain in southwestern and Mississippi Alluvial Plain in eastern Arkansas. At each location within these 24 counties, *C. puer* was typically uncommon. The distributional range of *C. shufeldtii* in Arkansas mimics that of *C. puer*, but populations only inhabit 12 counties in the Coastal Plain province in southwestern and Mississippi Alluvial Plain in eastern Arkansas. Within the state, *C. shufeldtii* was uncommon at each locality surveyed. Interestingly, these species were never found to occur at the same locality in the state.

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APPENDIX I. Collection locations of 300 specimens of *Cambarellus puer* in Arkansas (locality [township, section and range, when known]), date of collection, collector, number of specimens, and vouchers, if known). Includes 81 specimens of *C. puer* reported by Reimer (1963); however, he did not list any dates or number of specimens at his 8 collecting localities. Abbreviations: RWB (Raymond W. Bouchard); HHH (Horton H. Hobbs, Jr.); RR (Roland Reimer); HWR (Henry W. Robison).

Bradley County (*n* = 3)
(1) Roadside ditch, 5.1 km (3.2 mi.) SE of Banks on St. Hwy. 278. 19 Apr. 1986. HWR. (SAU, 3).

Calhoun County (*n* = 50)
(1) Ouachita River backwaters along US 167 N of Calion.26 Oct. 1974. HWR. (USNM 146704, 1 Form I male, 1 female, 47 juveniles = 49).
(2) Champagnolle Creek, 1.6 km (1.0 mi.) W of Hampton Oil Field (Sec. 3, T15S, R14W). 25 May 1975. S. Pelt. (USNM 147715, 1 ovigerous female).

Cleveland County (*n* = 11)
(1) Big Creek, 8.0 km (5 mi.) W of Pansy. No date. RR. (Reimer 1963).
(2) Stream at US 97, 4.8 km (3 mi.) NE of Jct. with St. Hwy. 15. 15 Aug. 1960. J. Bohlke. (USNM 116031, 1 Form II male, 3 females, 7 juveniles = 11).

Columbia County (*n* = 4)
(1) Roadside ditch, 9.7 km (6 mi.) S of Magnolia on US 79. 12 Oct. 2015. HWR. (SAU, 2 Form II males).
(2) Bayou Dorcheat at US 82, W of Magnolia. 11 Oct. 2015. HWR. (SAU, 2 females).

Crittenden County (*n* = 1)
(1) Big Creek, 17.9 km (11.1 mi.) N of Marion (35.3646°N, 90.2544°W). 14 Jun. 1977. HWR. (SAU, 1).

Grant County (*n* = not given)
(1) Ditch, 1.9 km (1.2 mi.) N of Saline River on US 167. No date. RR. (Reimer 1963).

Greene County (*n* = 9)
(1) Small stream, 7.9 km (4.9 mi.) NE of Paragould on St. Hwy. 1. 28 Jul. 1941. HHH. (USNM 117744, 6 form I males and 1 female specimens = 7).
(2) Ditch, 21.6 km (13.4 mi.) W of Paragould on St. Hwy. 25. 28 Jul. 1941. HHH. (2 females).

Howard County (*n* = 1)
(1) Roadside ditch, 20.1 km (12.6 mi.) W of Nashville on US 371. 20 May 1997. HWR. (SAU, 1).

Jackson County (*n* = 5)
(1) Village Creek at St. Hwy. 37 E of Tuckerman. 26 Jul. 1976. RWB. (1 male II, 3 females, 1 ovigerous female = 5).

Lafayette County (*n* = 97)
(1) Roadside backwater of unnamed tributary of Bodcaw Bayou, 6.4 km (4 mi.) N of Lewisville. 23 Aug. 1974. HWR. (USNM 146051, 36 Form II males, 46 additional specimens = 82).
(2) Unnamed tributary to Bodcaw Bayou, 5.6 km (3.5 mi.) from jct. of St. Hwy. 29 & Sunray Road on Sunray Road, 6.4 (4 mi.) N of Lewisville. 23 Mar. 1975. HWR. (SAU, 9).
(3) Tributary to Bodcaw Bayou,5.6 km (3.5 mi.) from jct. of St. Hwy. 29 & Sunray Road on Sunray Road, 6.4 km (4 mi.) N of Lewisville.26 April 1976. R. W. Bouchard. (USNM 176846, 3 Form I males, 1 ovigerous female = 4).
(4) Unnamed bayou, 1.3 km (0.8 mi.) NE of Buckner at St. Hwy. 82. 25 Oct. 1993. J. S. Rader. (INHS 11000, 2).

Lawrence County (*n* = not given)
(1) Unnamed creek, 3.2 km (2 mi.) SE of Hoxie off St. Hwy. 5 (36.0270°N, 90.9315°W). RR. (Reimer 1963).

Lincoln County (*n* = 2)
(1) Bayou Bartholomew off St. Hwy. 54 at Garrett Bridge (33.866647°N, 91.6562°W). 19 Aug. 1975. HWR. (SAU, 2 Form II males)

Little River County (*n* = 6)
(1) Slough, 0.2 km (0.1 mi.) S of Little River Bridge on Hwy. 71. RR. (Reimer 1963).
(2) Ditch, 23.7 km (14.7 mi.) S of Lockesburg on St. Hwy. 59. 31 Jul. 1941. HHH. (USNM 117743 - 2 Form I males 4 juveniles = 6) (Hobbs 1945, see p. 474).

Miller County (*n* = 2)
(1) Roadside ditch, 1.6 km (1.0 mi.) SW of Red River on US 71. No date. RR. (Reimer 1963).
(2) Roadside ditch, 3.2 km (2 mi.) SW of Red River on US 71. 16 Jun. 1979. HWR. (SAU, 2)

Mississippi County (*n* = 3)
(1) Pemiscot Bayou at US 61, ca. 3.2 km (2 mi.) N of Blytheville. 10 Jul. 1993. HWR. (SAU, 3 females).

Monroe County (*n* = 1)
(1) Stream at St. Hwy. 17, 14.5 km (9 mi.) S of jct. with US 70. 17 Aug. 1960. J. Bohlke. (USNM 116035, 1 female).

Nevada County (*n* = 1)
(1) Roadside ditch, Terre Rouge Creek at St. Hwy. 371. 6 Jun. 1980. D. Koym. (USNM 218558 - 1 Form II male).

Ouachita County (*n* = 1)
(1) Roadside ditch, 0.2 (0.1 mi.) S of Two Bayou Creek on St. Hwy. 4. No date. RR. (Reimer 1963).
(2) Roadside ditch, 4.8 km (3.0 mi.) S of Two Bayou Creek on St. Hwy. 4. 20 Apr. 1979. HWR. (SAU, 1 ovigerous female).

Poinsett County (*n* = 1)
(1) Roadside ditch, 6.4 km (4 mi.) W. of Harrisburg. 16 Aug. 1974. HWR. (SAU, 1 Form I male).

Randolph County (*n* = not given)
(1) Roadside ditch, 1.6 km (1.0 mi.) SW of Current River on US 67. No date. RR. (Reimer 1963)

Sevier County (*n* = 11)
(1) Little River backwater swamp, ca. 1.6 km (1.0 mi.) N of St. Hwy. 41. 12 Oct. 1974. HWR. (USNM 146696, 2 Form I males).

(2) Ditch southeast of Eagletown, OK. 29 Apr. 1972. W. Hayes. (USNM 146081, 1).
(3) Ditch, 19.8 km (12.3 mi.) S of Lockesburg on St. Hwy. 71. 31 Jul. 1941. HHH. (USNM 117743, 3 male juveniles, 5 female juveniles = 8) (Hobbs 1945).

St. Francis County (*n* = 1)
(1) Unnamed stream at US 70, 9.7 km (6 mi.) E of Godwin. 14 Aug. 1960. J. Bohlke. (USNM 116029, 1 Form II male).

Union County (*n* = 4)
(1) Tributary of Smackover Creek, 10.1 (6.3 mi.) N of El Dorado. RR. (1) (Reimer 1963).
(2) Ditch, 11.9 km (7.4 mi.) E of El Dorado. 19 Jun. 1980. HWR. (SAU, 2).
(3) Calion Lake spillway at Calion. 11 Jun. 1981. D. Cummings. (USNM 177606, 1 Form II male and 1 female = 2).

White County (n = 5)
(1) Ditch near Bayou Des Arc, 9.7 km (6.0 mi.) NE of McRae on US 67. 14 Aug. 1960. J. Bohlke (USNM 116030, 4 Form II males, 1 female = 5).

APPENDIX II. Collection locations of 54 specimens of *Cambarellus shufeldtii* in Arkansas (locality [township, section and range, when known]), date of collection, collector, number of specimens, and vouchers, if known). Abbreviations: RWB (Raymond W. Bouchard); RR (Roland Reimer), HWR (Henry W. Robison).

Columbia County (*n* = 3)
(1) Bayou Dorcheat at US 82, W of Magnolia. 11 Oct. 2015. HWR. (SAU, 2 Form II males, 1 ovigerous female = 3) (Tumlison *et al.* 2016)

Clay County (*n* = 1)
(1) Roadside ditch at Greenway. 19 Jun. 1979. HWR. (SAU, 1).

Crittenden County (*n* = 1)
(1) Unnamed creek, 20.1 km (12.5 mi.) N of Marion. 19 Jun. 1979. HWR. (SAU, 1).

Desha County (*n* = 1)
(1) Unnamed creek, 2.9 km (1.8 mi.) E of Dumas. 18 Aug. 1974. HWR. (SAU, 1 male).

Jackson County (*n* = 1)
(1) Village Creek at St. Hwy. 37, E of Tuckerman. 19 Feb. 1977. RWB. (1 female) (Tumlison *et al.* 2016).

Lafayette County (*n* = 11)
(1) Unnamed oxbow lake of the Red River, 1.0 km (0.6 mi.) SW of Boyd. 7 May 1982. HWR. (USNM 208635, 3 male I, 2 male II, 3 females ovigerous, 3 other females = 11).

Lawrence County (*n* = 1)
(1) Village Creek at Minturn. 19 Feb. 1977. RWB. (1 female) (Tumlison *et al.* 2016).

Miller County (*n* = 3)
(1) Roadside ditch, ca. 14.5 km (9 mi.) W of Garland. 12 May 1984. HWR (SAU, 3).

Monroe County (*n* = 1)
(1) Bayou DeView at St. Hwy. 17, S of Cotton Plant. No Date. RR. (Reimer 1963) (1).

Union County (*n* = 2)
(1) Roadside ditch, 2.4 km (1.5 mi.) E of Strong on US 82 (Sec. 35, T18S, R12W). 23 Jul. 2000. HWR. (SAU, 2).

White County (*n* = 1)
(1) Slough, 23.2 km (14.4 mi.) W of Augusta. 8 Sept. 1948. A. B. Leonard. (USNM 132713, 1).

Woodruff County (*n* = 28)
(1) Roadside ditch and culvert on US 64, 3.2 km (2 mi.) W of jct. of US 64 & St. Hwy. 39 at Fair Oaks.14 Apr. 1973. HHH. (USNM 144583, 18 Form I males, 1 Form II male, 1 female adult, 4 female ovigerous, 4 female juvenile = 28).

History and Current Status of the Inca Dove (*Columbina inca*) in Arkansas

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Running title: Status of Inca Doves in Arkansas

Abstract

Formerly a bird of Central America, Mexico, and the southwestern United States, the Inca Dove (*Columbina inca*) has expanded northward across North America in the past few decades. It first appeared in Arkansas on October 26, 1968 in Saratoga, Howard County. Since then, the statewide range has grown to include at least 36 of 75 counties and is expanding. With the use of Christmas Bird Counts, Breeding Bird Surveys, and 2 citizen science sources, eBird and AR-Birds, we compiled 368 records of the species in the state. Inca Doves were observed year-round in Arkansas and are expanding their range in the state at an average rate of about 1 new county every 7 years and an increase of about 1 new report of the species every year. An overview of its overall range in North America indicates that there is enough data to warrant a redrawing of the range map for the species to include most of Arkansas.

Introduction

Several bird species have expanded their global ranges, with anthropogenic habitat modifications a major causative factor (Hengeveld 1988; Fujisaki *et al.* 2010). There is also strong evidence that many bird species are extending their ranges poleward (Thomas and Lennon 1999; Hickling *et al*. 2006; Kannan and James 2009), ostensibly due to anthropogenic climate change (IPCC 2014). It is important, therefore, to monitor local bird populations for geographic shifts, since an accurate understanding of bird distributions is vital for conservation efforts (Remsen 2001; Kannan *et al.* 2018).

Here we document the rapid progression in the global range of the Inca Dove, with focus on its history and status in Arkansas. This study follows a previous review of the Eurasian Collared-Dove in Arkansas (Fielder *et al.* 2012), which is one of the world's most rapidly expanding bird species (Hengeveld 1993). Of the 7 species of pigeons and doves (Aves: Columbidae) reported to occur in Arkansas, 3 are relatively new to the state, having arrived following an expansion of their ranges northward. These are Inca Dove *Columbina inca*, Eurasian Collared-Dove *Streptopelia decaocto*, and White-winged Dove *Zenaida asiatica*.

The Inca Dove was considered a bird of Mexico and southwestern United States, but it has expanded northward across North America. Three decades ago it was regarded "a rare transient and winter visitor" in Arkansas (James and Neal 1986), but in recent years there has been a plethora of reports in the state. Globally, Inca Doves used to occur from northwestern Costa Rica to northern Mexico and parts of the United States bordering Mexico (Fig. 1; Mueller 2004). Arkansas is not included in the species' range in Cornell Lab of Ornithology's widely used online source of range maps for North American birds (Allaboutbirds.org 2019).

Despite the recent increase in reports of Inca Doves in Arkansas and elsewhere in North America, and the fact that the species has been spreading for at least 3 decades, no systematic review has been done to track its progression and monitor its status. Extralimital information on Inca Doves has only been anecdotal (Hardy 1958; Johnston 1965; Behle 1966; Felis 1976;

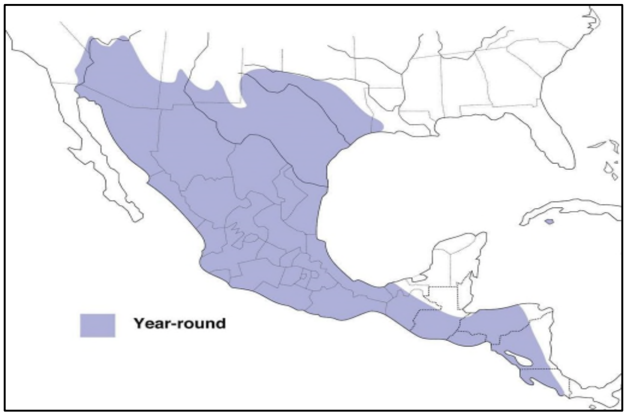

Fig. 1. Published global range of the Inca Dove (Allaboutbirds.org 2019 from Mueller 2004).

Bartnicki 1979; Paine 1988; Robbins and Easterla 1992). Therefore, we conducted this study to 1) assess the species' current global distribution, 2) review the species' history, current status, and distribution in Arkansas, and 3) investigate quantitative trends in the species' spread across the state.

Methods

We compiled a comprehensive historical account of Inca Doves in Arkansas using citizen science data in eBird (2019) and archives of the listserv for Arkansas birders, AR-Birds (AR-Birds-L 2019). Data from the eBird database include data from the archives of the Arkansas Audubon Society (2015). We compiled a total of 336 Inca Dove records from these two sources. We took care to avoid duplications between and within the 2 sources. For every report of Inca Doves in the state, we noted date, exact location, number encountered, observers, and general comments. We counted a record as an observation of the species in a particular location, regardless of the number of observers reporting, number of individual birds present, or number of days reported. However, reports that spanned multiple months were counted separately for each month.

We also compiled quantitative information from annual Christmas Bird Counts (CBC) in the state, conducted annually by National Audubon Society (2010) mid-December through mid-January (29 additional unduplicated records). Numbers observed per ten party hours were obtained as in Fielder *et al.* (2012) for Eurasian Collared-Doves in the state.

To get insights into the species' breeding status in the state, we obtained information from Breeding Bird Surveys (BBS) (Robbins *et al.* 1986; Pardieck 2018) in Arkansas (3 records). Altogether, we compiled 368 unduplicated reports of Inca Doves in Arkansas from eBird, AR-Birds, CBC, and BBS. Microsoft Excel was used to plot data and generate regression equations and correlations.

Results

Global Range Progression

Since the 1960s, Inca Doves have steadily expanded their range northward from Mexico and Central America to now include much of the southwestern and southcentral United States (Mueller 2004). Some reports have occurred as far north as Canada (eBird 2019, Fig. 2). In addition, the Inca Doves' range has expanded southward to include southern Costa Rica (Garrigues and Dean 2014) and perhaps Panama (Fig. 2).

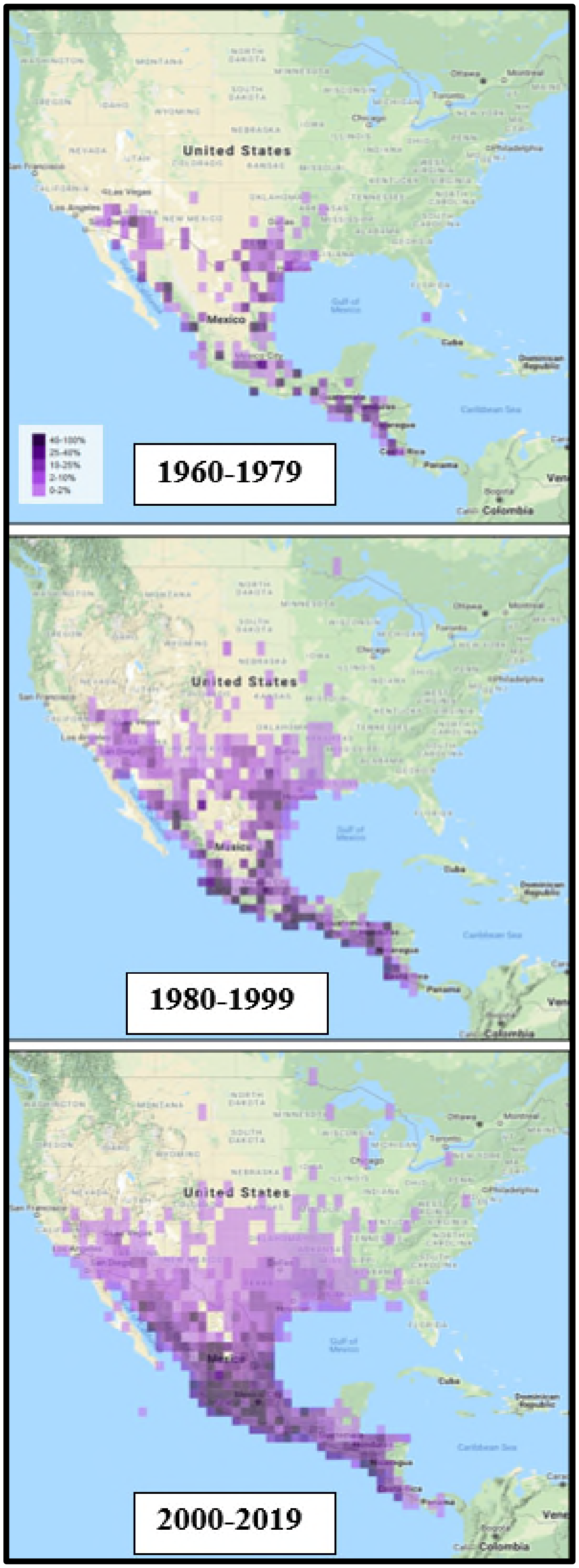

Fig. 2. Global Inca Dove range progression through the past 6 decades. Images generated using eBird (www.ebird.org) on March 11, 2019. The scale gives percent of checklists reporting the species.

Data in eBird from the most recent year (2019) indicate that the current range encompasses most of Arkansas plus portions of all neighboring states (Fig. 2).

History of Inca Doves in Arkansas

The first report of an Inca Dove in Arkansas was of a single bird sighted by Mr. and Mrs. Ira McJenkins in Saratoga, Howard County, from October 26, 1968 to March 24, 1969 (James and Neal 1986, eBird 2019). Two years later, from February 24, 1971 to March 28, 1972, 1 was seen at a feeding station in Pine Bluff, Jefferson County, followed by a recovery of a headless bird in Fayetteville, Washington County, on December 3, 1972 (James and Neal 1986). After a decade-long gap, sightings resumed in 1982 and have since continued almost every year (eBird 2019; James and Neal 1986).

Current Arkansas Range

There were few Inca Dove reports in Arkansas from the 1960s through the late 1970s, but by the late 1990s reports were common. Today reports show that the range covers most of the state (Figs. 2 and 3).

Inca Doves were reported in 36 of the 75 Arkansas counties in eBird/AR-Birds (Fig. 3). The areas without Inca Dove reports may not necessarily mean they are absent. It is possible that opportunistic birding ventures, if and when conducted, missed the species. There are some counties in the southcentral region without Inca Dove reports, but because of the recorded presence of Inca Doves in all the surrounding counties it is reasonable to assume that Inca Doves are at least occasionally present in the entire portion of the state south of the Arkansas River. Nevertheless, there is currently a significant area in the northern and northeastern parts of the state where the lack of Inca Dove reports indicates that this region may not yet be part of the established Inca Dove range (Fig. 3).

Inca Doves were reported in 20 of 33 counties with portions south of the Arkansas River. These reports from the southwestern portion of the state accounted for 261 (78%) of 336 reports. Overall, judging from the numbers and dates (Fig. 3 and Table 1), it appears that Inca Doves expanded radially from the southwest corner of the state in a northeasterly direction, as can be expected for a species whose original range is southwest of the state. A similar expansion has been documented for the Eurasian Collared-Dove in Arkansas (Fielder *et al*. 2012).

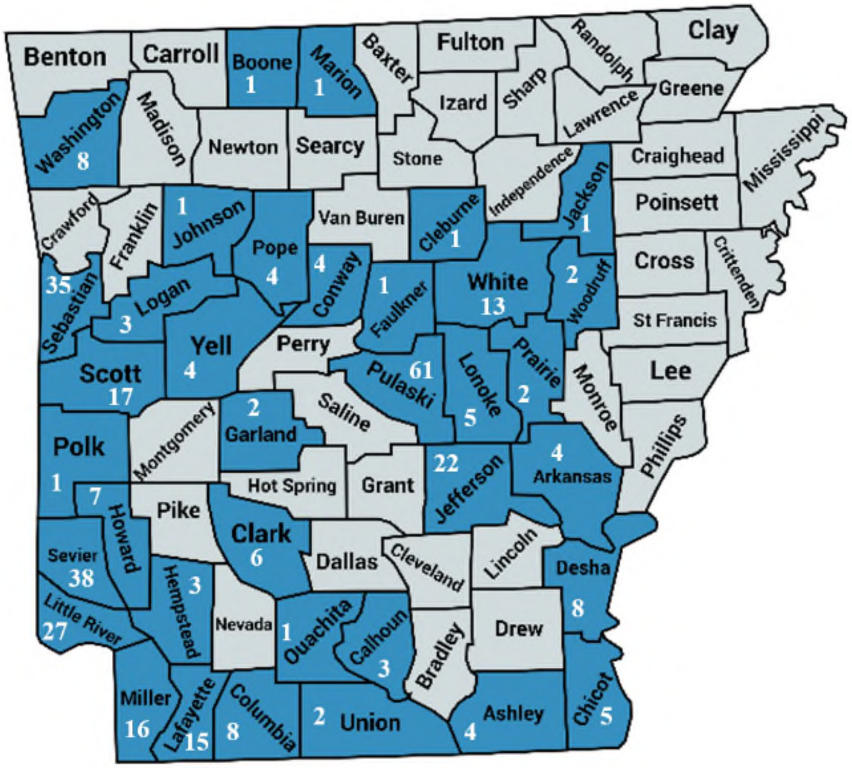

Fig. 3. Distribution of Inca Dove reports from Arkansas. Darker shaded counties indicate the documented presence of Inca Dove. Numbers indicate total number of reports from eBird and AR-Birds. Map created using https://mapchart.net.

Quantitative Trends in Arkansas

Of the 32 Arkansas counties that conducted CBC December 1967 to January 2018, only 8 (25%) reported Inca Doves (Table 1). Mean numbers of Inca Doves observed per 10 party hours across all 8 of these counties show an increasing trend with a weak linear correlation ($R^2 = 0.08$) from the first report in 1992 through present (Fig. 4). However, overall Inca Dove densities remain low. The mean number per 10 party hours for 2017 indicates an encounter rate of approximately 1 bird every 37 party hours in these 8 counties. This is near the average for the last 6 years (Fig. 4).

Table 1. Number of individual Inca Doves per 10 party hours from Christmas Bird Counts (CBC) in Arkansas counties reporting the species.

County	First CBC Observed Year	1992-2004	2005-2017
Chicot	1992	0.02	*
Miller	1995	0.10	0.96
Columbia	1998	0.59	0.46
Clark	1998	0.01	0.34
Washington	2011	0.00	0.01
Jefferson	2013	0.00	0.15
Ashley	2014	*	0.07
Sebastian	2016	0.00	0.12
8-county average		0.12	0.24

*There was no CBC done in these periods of time for these locations.

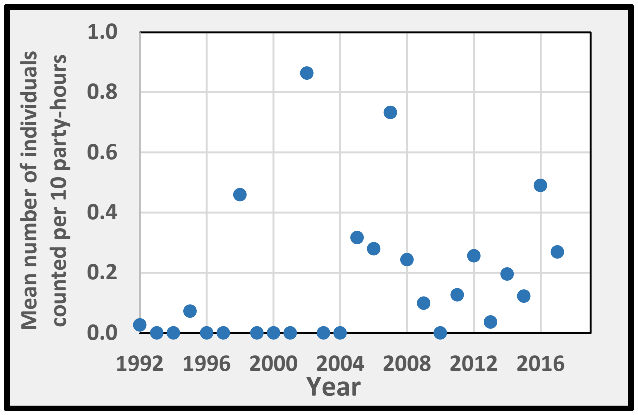

Fig. 4. Mean number of individual Inca Doves observed per 10 party hours in 8 reporting Arkansas counties by year (from CBC)

The average number of Inca Dove encounters per 10 party hours from CBC in these 8 reporting counties has been increasing over time, doubling from 0.12 in 1992-2004 to 0.24 in 2005-2017. Most of these counties show an increase in birds per party hour between the 1992-2004 and 2005-2017 time periods. In general, the southernmost counties experienced earlier first Inca Dove encounters and a higher encounter rate than more northern CBC counties (Table 1).

More Arkansas counties reported Inca Dove through time (Fig. 5). Using the eBird/AR-Birds datasets, we found a reasonably strong correlation between the number of counties that reported Inca Doves and time (Fig. 5). The trend line indicates an increase of about 1 new county every 7 years.

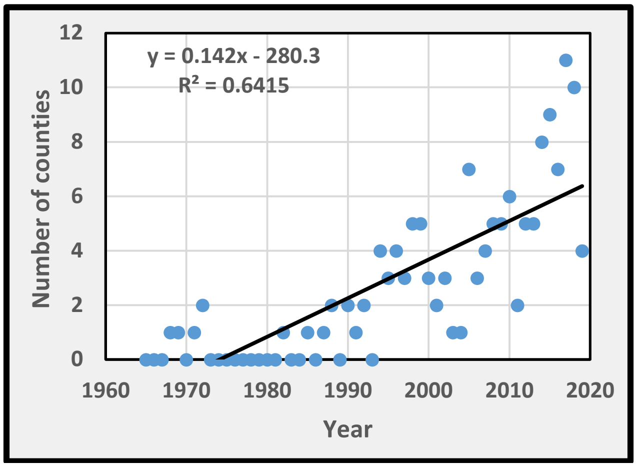

Fig. 5. Number of counties reporting Inca Doves in Arkansas by year (from eBird/AR-Birds)

The annual number of Inca Dove reports in Arkansas increased through time (Fig. 6). The trend line indicates that the number of annual reports will increase by 1 approximately every 3 years.

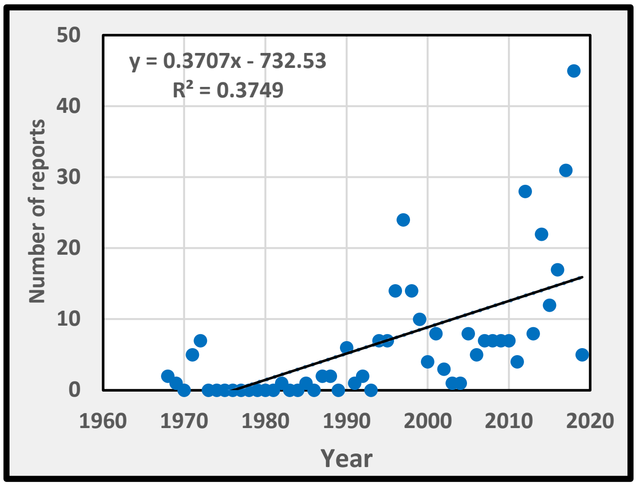

Fig. 6. Number of Inca Dove reports in Arkansas by year (from eBird/AR-Birds)

Counts of individual birds from eBird/AR-Birds also increased with time (Fig. 7). The trend line suggests that the annual number of individual birds will increase by about 1 per year (Fig. 7). An overview of Figs. 4-7 and Table 1 indicates that the species is steadily increasing its presence in the state and is becoming established.

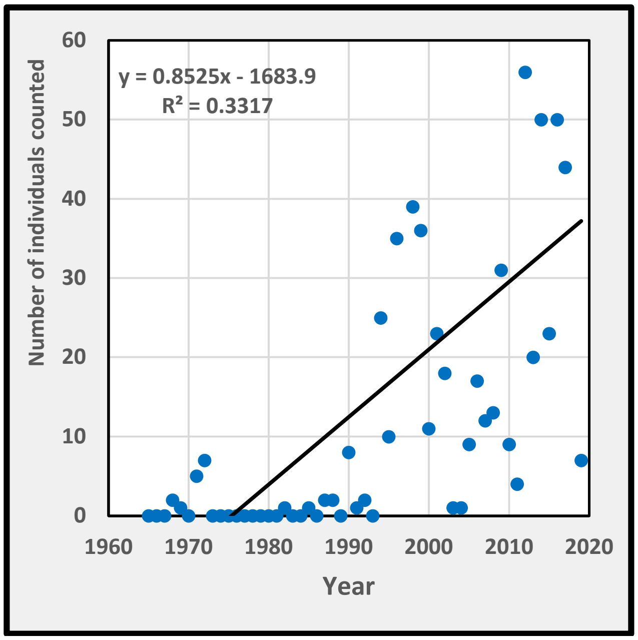

Fig. 7. Number of individual Inca Doves counted in Arkansas by year (from eBird/AR-Birds)

BBS reports in Arkansas were too limited to indicate any significance. Only 5 birds were reported in these surveys, and these reports came from 3 routes (2 in Hope, Hempstead County, in June 2003 and 2008, and 1 in Lockesburg, Sevier County, in June 2016). On May 20, 1995, the first recorded Inca Dove nest from Arkansas was observed in Ogden (Little River Co.), which on June 8 was confirmed to have a live nestling (eBird 2019). From July 10 to 24, 1997, an active nest with 2 fully feathered young was observed in De Queen, Sevier County. On May 28, 1998, the third nesting record was from Little River County (Arkansas Audubon Society 2015). One young bird was observed in a yard in Fort Smith Sebastian County (Sandy Berger, personal communication, month and year unknown).

Phenology of Occurrence in Arkansas

Inca Doves have been observed year-round in Arkansas. A phenology of total encounters in the combined eBird/AR-Birds dataset suggest that the species is sedentary and does not leave the state in winter (Fig. 8).

Discussion

This paper relies heavily on data collected from citizen science sources. We realize that much of this is not peer-reviewed. However, the collective and crowd-sourced nature of citizen science, wherein a multitude of observers report their sightings, increases sample size and dilutes the effect of any errors in reporting. Moreover, the fact that the Inca Dove is easily identifiable and unlikely to be confused with other species adds to the credibility of this data.

One factor that helps to explain the increased reports of the species in eBird and AR-birds is the increase in number of birders and the preponderance of tools currently available for locating, identifying, and reporting observations. Nevertheless, the data indicate a clear pattern of range expansion of the Inca Dove.

The data we assimilated indicate that the global range maps of Inca Dove need to be revised to keep up with the expansion across North America. We found no evidence to support the hypothesis (Mueller 2004) that the northward range progression of the species may be hindered by cold climates. In fact, there are reports of the species in Canada (Fig. 2), and there are winter (December-February) eBird reports from Montana (2006) and New York (2017). With behaviors like daytime "pyramiding", in which up to 12 birds stack up on top of each other in 2-3 rows, night-time huddling, and group-basking and sunning, the species seems adapted to tolerate the cold (Johnston 1960; Mueller 2004; Robertson and Schnapf 1987). In fact, the species

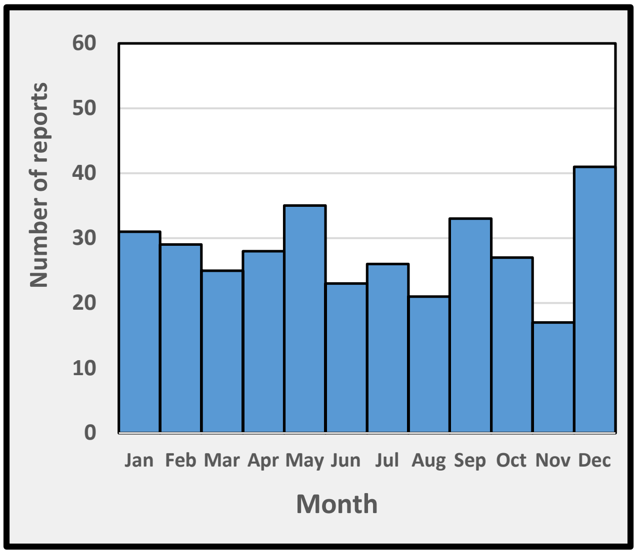

Fig. 8. Phenology of Inca Dove reports in Arkansas (from eBird/AR-Birds)

has been shown to have remarkable physiological flexibility to deal with extreme low temperatures, including reduced pulmocutaneous water loss by metabolic quiescence, and nocturnal hypothermia (Trost and MacMillen 1967a,b).

Mueller (1992) reported that Inca Dove population increased significantly in Texas and southwest United States 1966-1991. His review indicated that 66% of BBS routes in the central region showed increases. However, he also cautioned that the low abundance of Inca Doves (<1/route) made BBS data less reliable.

Our analyses of more recent data show that this expansion has continued to include Arkansas. Although our data strongly show the species' increased spatial and temporal presence in Arkansas, it is unclear at what rate overall densities have increased. This may be because of the relative low proportion of Arkansas counties participating in CBC. With more coverage, a better picture may emerge on population size and density of Inca Doves in Arkansas.

The reason behind the remarkable range expansion of Inca Dove remains unclear (Mueller 2004). While it has apparently benefitted from human settlements in parts of the new range (Phillips 1968; Gibbs *et al*. 2001), similar anthropogenic habitats elsewhere remain uncolonized (Hubbard 1971). Therefore, predicting future trajectories for this species is challenging. In any case, our review strongly indicates that the species is established and increasing its presence in Arkansas.

While the ultimate ramifications of the rapid expansion of Inca Dove are not clear, future studies should focus on the effects of Inca Dove, Eurasian Collared-Dove, and White-winged Dove range expansion on niches of closely related species established in Arkansas, like Mourning Dove *Zenaida macroura*.

Acknowledgments

The project was part of the Undergraduate Research course taken by Ethan Brooks at the Biology department of University of Arkansas—Fort Smith (UAFS). This article would not have been possible without publicly available citizen science data. Our thanks to all birders who reported their information into AR-Birds and eBird, and who participated in CBC and BBS. The UAFS College of Science, Technology, Engineering, and Mathematics supported conference travel and publication charges. Allan Mueller critiqued the manuscript. Dan Scheiman helped with eBird database. Sandy Berger provided anecdotal information. Comments by 3 anonymous reviewers improved the paper.

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Proteomics of Carbon Fixation Energy Sources in *Halothiobacillus neapolitanus*

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Running title: Sulfur oxidation as energy for carbon fixation

Abstract

Through the use of proteomics, it was uncovered that the autotrophic, aerobic purple sulfur bacterium *Halothiobacillus neapolitanus* displays changes in cellular levels of portions of its carbon dioxide uptake and fixation mechanisms upon switch from bicarbonate to CO_2(g) as carbon source. This includes an increase in level of a heterodimeric bicarbonate transporter along with a potential switch between form I and form II of RubisCO. Additional changes are seen in several sulfur oxidation pathways, which may indicate a link between sulfur oxidation pathways as an energy source and carbon uptake/fixation mechanisms.

Introduction

Halothiobacillus neapolitanus is an obligate aerobic chemolithoautotroph capable of utilizing the complete oxidation of inorganic sulfur compounds as its sole source of metabolic energy (Garrity *et al.* 2005). While formally classified within the Purple Sulfur Bacteria (PSB) (Kelly and Wood 2000; Ghosh and Dam 2009), *H. neapolitanus* does not perform anoxygenic photosynthesis as it lacks the necessary photosynthetic reaction centers and associated antenna pigments (Lucas *et al.* 2009). It does, however, possess carboxysomes, which allow for aerobic autotrophic growth (Kerfeld *et al.* 2010; Bonacci *et al.* 2012).

H. neapolitanus genome sequence (Lucas *et al.* 2009) indicates that this species contains genes for a diverse set of sulfur oxidation (sox) activities including; a) a sulfur oxygenase/reductase homologous to that found in archaea species (Veith *et al.* 2012), b) several genes for homologs of sulfide:quinone reductases found in green sulfur bacteria (GSB) species (Gregerson *et al.* 2011), c) genes for a complete thiosulfate oxidizing multi-enzyme system (TOMES) pathway similar to that found in *Paracoccus pantotrophus* GB-17 (Friedrich *et al.* 2005; Bardichewsky *et al.* 2006; Reijerse *et al.* 2007; Zander *et al.* 2011), d) a flavocytochrome based sulfide dehydrogenase homologous to FccA/FccB (9) and e) a unique tetrathionate forming thiosulfate dehydrogenase that appears to be hetero-oligomeric as opposed to the homo-dimeric enzyme from the PSB *Al. vinosum* reported by Denkmann *et al.* (Denkmann *et al.* 2012; Brito *et al.* 2014).

One distinguishing feature of the sox gene arrangement in *H. neapolitanus* is the unique arrangement of genes for the TOMES pathway. The majority of organisms studied to date display a general pattern of having a core set of enzymes (sox AXYZBCD) in either a single operon or at least closely spaced within the genome. *H. neapolitanus* shows no such arrangement with TOMES components widely dispersed through the genome and even on opposite strands (Figure 1).

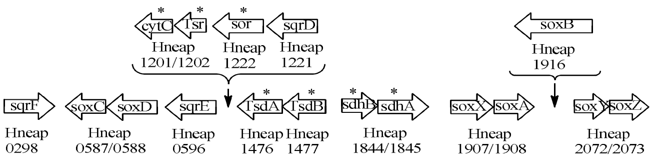

Figure 1. Relative arrangements of genes reported to be associated with oxidation of inorganic sulfur in *H. neapolitanus*. Note the lack of a single, contiguous sox operon.

Carbon fixation in *H. neapolitanus* appears to be primarily associated with a carbon concentration mechanism (ccm) and carboxysomes containing the carbon fixation mechanism. The ccm is composed of one heterodimeric bicarbonate transporter at Hneap_0211/0212 and a heterotrimeric transporter at Hneap_0907-0909 as shown in Figure 2. Both of these appear to be similar to those reported for *T. crunogena* by Scott and colleagues (Mangiapia *et al.* 2017). *H. neapolitanus* does not appear to possess genes for additional types of bicarbonate transporters (Scott *pers. comm.*).

In addition to the aforementioned carbon concentration mechanism, genes are present for a protein shell-enclosed carboxysome. These genes include shell proteins, a shell-based carbonic anhydrase

(CA) and cbbS, cbbM, cbbL subunits of RubisCO (Figure 3). The properties and role of carboxysomes in *H. neapolitanus* in carbon fixation have been well characterized by Heinhorst and colleagues (Kerfeld *et al.* 2010).

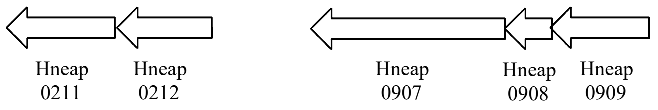

Figure 2. Gene arrangement for the predicted bicarbonate transporters in *H. neapolitanus*.

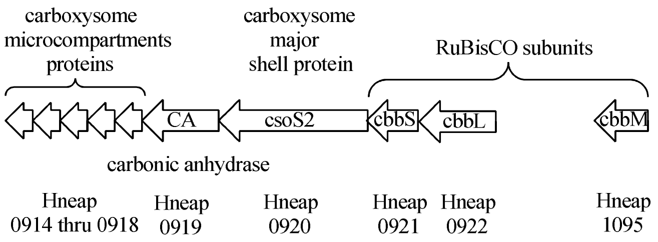

Figure 3. Relative gene locations for genes comprising the ribulose-1, 5-bisphosphate carboxylase-oxygenase (RubisCO) of *H. neapolitanus*.

While the structure and role of carboxysomes have been well studied in a wide variety of autotrophic microbes, the unique gene arrangement of the sulfur oxidation (i.e. energy producing) pathways of *H. neapolitanus* gives rise to questions as to the relationship between sulfur oxidation as an energy source and carbon fixation in this species. This report uses proteomics technologies to explore the relationship between changes in dissolved inorganic carbon speciation and energy producing pathways in the obligate autotroph *H. neapolitanus*.

Materials and Methods

Halothiobacillus neapolitanus, DSM 15147, was obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) in Braunschweig, Germany. Cells were cultured at a constant pH of 7.0 +/- 0.2 in the media previously described by Heinhorst and coworkers, using thiosulfate as energy source (Kerfeld *et. al.* 2010). All cultures were performed at 30°C in a continuous flow chemostat using a dilution rate of 0.25. Constant aeration at 3L/min per liter of culture volume was used in order to maintain aerobic growth conditions.

The effect of different forms of dissolved inorganic carbon (DIC) was assessed by dividing replicate growths into two separate groups with carbon sources as follows: a) 5mM sodium bicarbonate supplemented into the growth media accompanied by aeration with CO_2 free (i.e. scrubbed) air and b) aeration with 5% (v/v) CO_2 in air.

Growths for each carbon source were performed in triplicate. Harvested cell mass from individual growths was flash frozen at -77°C and stored at -80°C until submitted to the University of Arkansas for Medical Sciences Proteomics Core facility for quantitative analysis.

Proteins were reduced, alkylated, and purified by chloroform/methanol extraction prior to digestion with sequencing grade modified porcine trypsin (Promega). Tryptic peptides were labeled using tandem mass tag isobaric labeling reagents (Thermo) following the manufacturer's instructions and combined into one multiplex sample group. The labeled peptide multiplex was separated into 36 fractions on a 100 x 1.0 mm Acquity BEH C18 column (Waters) using an UltiMate 3000 UHPLC system (Thermo) with a 40 min gradient from 99:1 to 60:40 buffer A:B ratio under basic pH conditions, and then consolidated into 12 super-fractions. Buffer A was composed of 0.1% formic acid and 0.5% acetonitrile in water. Buffer B was composed of 0.1% formic acid in 99.9% acetonitrile. Both buffers were adjusted to pH 10 with ammonium hydroxide. Each super-fraction was then further separated by reverse phase XSelect CSH C18 2.5 um resin (Waters) on an in-line 150 x 0.075 mm column using an UltiMate 3000 RSLCnano system (Thermo). Peptides were eluted using a 60 min gradient from 97:3 to 60:40 buffer A:B ratio. Eluted peptides were ionized by electrospray (2.15 kV) followed by mass spectrometric analysis on an Orbitrap Fusion Lumos mass spectrometer (Thermo) using multi-notch MS3 parameters. MS data were acquired using the FTMS analyzer in top-speed profile mode at a resolution of 120,000 over a range of 375 to 1500 m/z. Following CID activation with normalized collision energy of 35.0, MS/MS data were acquired using the ion trap analyzer in centroid mode and normal mass range. Using synchronous precursor selection, up to 10 MS/MS precursors were selected for HCD activation with normalized collision energy of 65.0, followed by acquisition of MS3 reporter ion data using the FTMS analyzer in profile mode at a resolution of 50,000 over a range of 100-500 m/z.

Data Analysis

Proteins were identified and reporter ions quantified by searching the UniprotKB *Halothiobacillus* *neapolitanus* database (2,353 entries) using MaxQuant (Max Planck Institute) with a parent ion tolerance of 3 ppm, a fragment ion tolerance of 0.5 Da, a reporter ion tolerance of 0.001 Da, fixed modifications including carbamidomethyl on C, TMT-10 plex on K and the peptide N-terminus, and variable modifications including oxidation on M, and acetylation of the protein N-terminus. Scaffold Q+S (Proteome Software) was used to verify MS/MS based peptide and protein identifications (protein identifications were accepted if they could be established with less than 1.0% false discovery and contained at least 2 identified peptides; protein probabilities were assigned by the Protein Prophet algorithm (Nesvizhskii *et al.* 2003) and to perform reporter ion-based statistical analysis. Quantitative comparisons between samples grown using $CO_{2(g)}$ versus the bicarbonate control were done using the Mann-Whitney test with the Benjamini-Hochberg correction in order to compare triplicate samples grown using either $CO_{2(g)}$ or bicarbonate as DIC source.

Results

A change in DIC species from 5mM bicarbonate ion to dissolved $CO_{2(aq)}$ appears to induce changes in cellular levels of a number of proteins including bicarbonate transporters, several enzymes in sulfur oxidation pathways and specific terminal oxidases. Only slight changes of less than 0.5 log2fold are seen in cellular levels of traditional stress response proteins in both cytoplasmic and extracytoplasmic spaces (Figure 4). Since 0.5 log2fold change is the generally accepted minimum to be considered a significant change, these low levels of change indicate that the observed changes in cellular levels of other proteins are probably not linked to a general stress response type of mechanism. It is interesting to note that those chaperones traditionally associated with the cytoplasm display a general trend of increases with log2fold increases of +0.37, +0.31 and +0.38 for groL, groS and dnaK respectively, while those associated with extracytoplasmic activities display decreases of -0.12 and -0.36 for surA and ompH.

Changes that are more significant are seen in the cellular levels of proteins associated with carbon uptake and fixation. Figure 5 shows that the dimeric bicarbonate transporter located at gene loci Hneap_0211/0212 displays a log2fold increase of +1.16 and +1.26 for the two respective subunits. The two detected subunits of the trimeric transporter show no significant change in level with log2fold changes between -0.05 and +0.17. These results indicate that only one of the two bicarbonate transporters appears to be sensitive to CO_2 as carbon source. No information is available on the relative sensitivity of either transporter to bicarbonate concentrations.

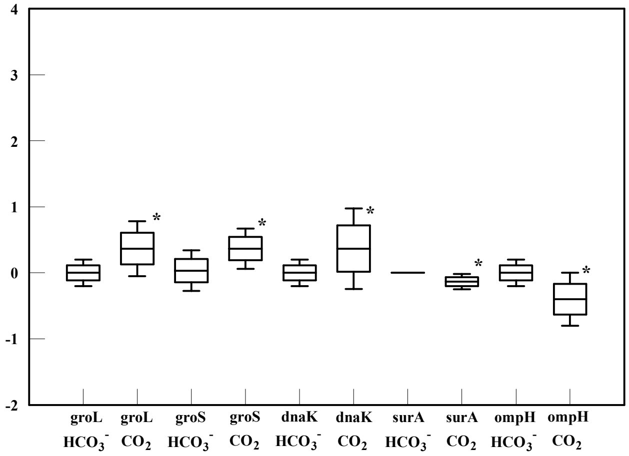

Figure 4. Changes in cellular levels of chaperones associated with changes in DIC species. * p-values <0.05.

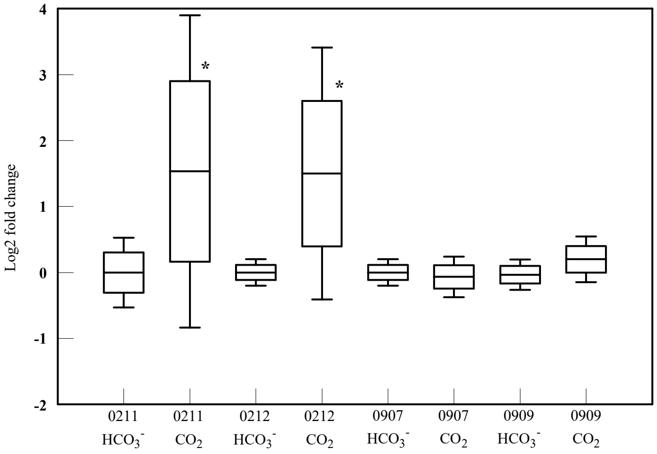

Figure 5. Changes in cellular level of the dimeric bicarbonate transporter (Hneap_0211/0212) and the trimeric bicarbonate transporter (Hneap_0907/0908/ 0909). * p-values < 0.05

In addition to the carbon concentration mechanism, minor changes in the relative levels of individual peptides associated with the RubisCO and the carboxysome were detected. A general trend of increased levels of peptide associated with form I was detected with log2fold changes of +0.49 and +0.56 for carboxysome shell protein 1 (csS1) and the RubisCO small subunit (cbbS) respectively. Other proteins displayed increases less than 0.25. Changes in level of subunit cbbM, form II, are not as clear. While log2fold change is calculated at 0.02, the p-values of < 0.05

coupled with the wide error of means in the HCO_3- reference sample makes interpretation difficult (Fig. 6).

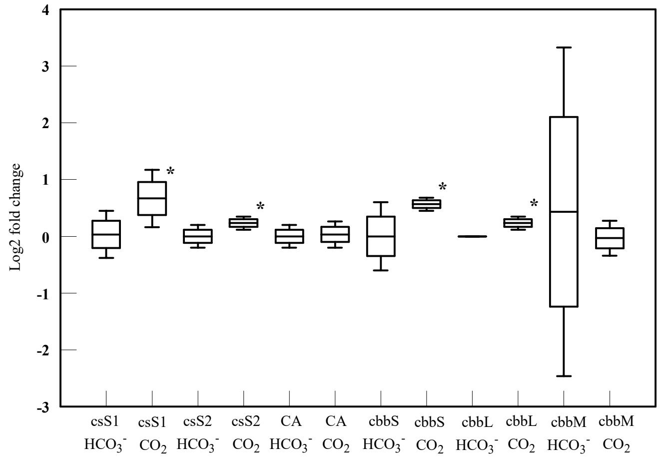

Figure 6. Changes in cellular levels of proteins associated with the carboxysome and RubisCO. * p-values < 0.05.

Changes in levels of proteins involved in cellular energy production include both those involved with substrate level oxidations and terminal oxidases. Figure 7 shows changes in the levels of proteins involved in sulfide oxidation. The sulfide:quinone reductase homologs show several significant changes with sqrF increasing 0.92 log2fold and the sqrD and sqrE decreasing by 0.70 and 0.37 log2fold respectively. Subunit B of the cytochrome linked sulfide dehydrogenase only increased by 0.34 log2fold. It is interesting to note the general trend for these sulfide oxidation proteins. Those that show slight increases are homologs of proteins in the green sulfur bacteria (GSB), which are predicted to face the extracytoplasmic space increase while those that display decrease would be predicted to face the cytoplasm in GSB (9).

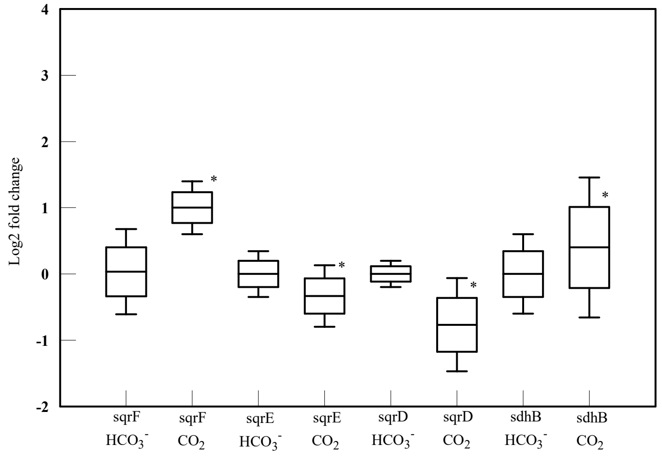

Figure 7. Sulfide:quinone oxidoreductases and sulfide dehydrogenase levels show a general trend of increases for those protein predicted to face the extracytoplasmic space. * p-values < 0.05.

No significant changes in cellular levels of either the tetrathionate forming (tsdA and tsdB) or TOMES (soxY and soxB) thiosulfate oxidizing pathway proteins appears to occur with log2fold changes ranging from -0.20 to 0.01. Although the remainder of the sox pathway proteins (Z, AX, CD) are not shown for clarity, they follow the same trend with log2fold changes ranging from -0.24 to 0.08. The sulfur oxygenase-reductase however, shows a 0.75 log2fold decrease as shown in Figure 8.

In addition to sulfur oxidation proteins, changes in levels of caa3, bd and cbb3 terminal oxidases were also detected (Figures 9 and 10). Figure 9 shows a clear trend in decrease in levels of the majority of subunits of the caa3 type terminal oxidase with decrease ranging from 2.26 to 0.83 log2fold. Other terminal oxidases display a lower sensitivity to the DIC source (Figure 10). While the bd-quinol oxidase at Hneap_1294/1295 displays a decrease of 0.57 log2fold in the detected subunit, the cbb3 type terminal oxidase displays log2fold changes ranging from -0.06 to +0.20 for the Hneap_1876 through 1880 subunits respectively, indicating a slight sensitivity to DIC species for the bd- quinol oxidase and little or no sensitivity for the cbb3 type terminal oxidase.

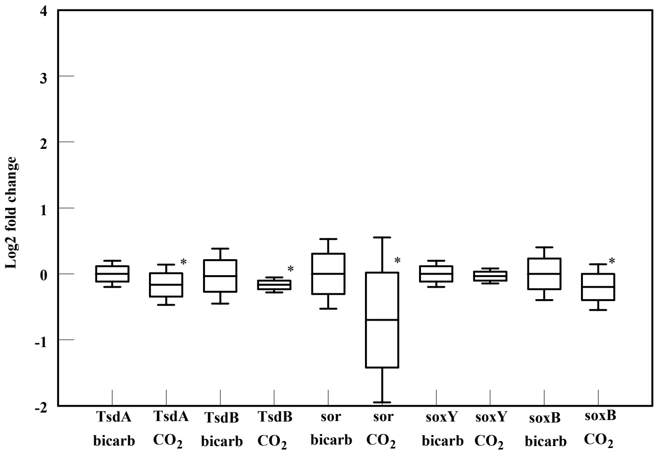

Figure 8. Thiosulfate oxidation by thiosulfate dehydrogenase (tsdAB) and TOMES (soxY and soxB). Sulfur oxidation (sor) is included for comparison. * p-values < 0.05.

Discussion

The low level of change in cellular levels of stress response related proteins indicates that the shift in DIC

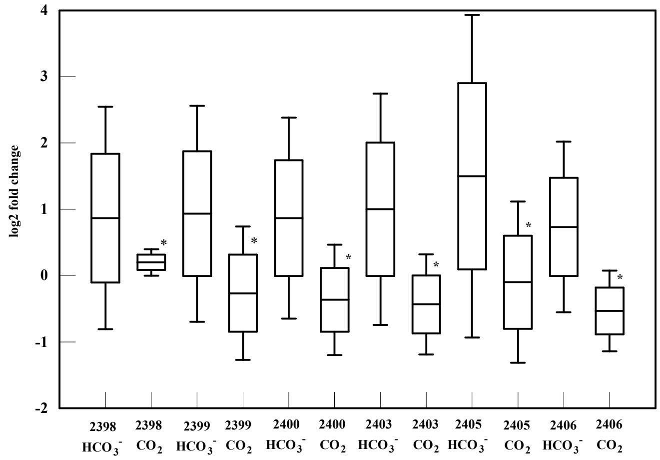

Figure 9. Changes in level of the caa3 type terminal oxidase upon DIC species change. * p-values < 0.05

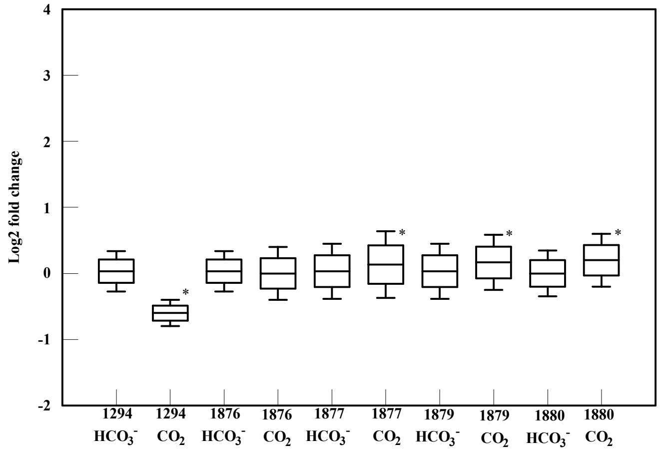

Figure 10. Changes in the cellular levels of the bd-quinol and cbb3 type terminal oxidases. * p-values < 0.05

species from bicarbonate to carbon dioxide does not trigger a strong stress response. This supports the observed changes in levels of other protein systems as being a result of selective sensitivity to dissolved inorganic carbon species and not a general stress response. The changes in levels of carbon concentration mechanisms and carbon fixation mechanisms are remarkably similar to those reported for *Thiomicrospira crunogena* cultured under low DIC conditions (Mangiapia *et al.* 2017). The *T. crunogena* heterodimeric bicarbonate transporter, Tcr_0853/0854 is increased, while the trimeric version Tcr_ 1081/1082/1083 shows no significant changes. In addition to changes in proteins involved in the carbon concentration mechanism, T. *crunogena* is reported to display a higher abundance of cbbL peptides when cultured under DIC limitations (Mangiapia *et al.* 2017). *H. neapolitanus* displays a slight increase in cbbL (log2fold = +0.25) upon switching from 5mM bicarbonate to CO_2(g) as carbon source indicating that the CO_2(g) may represent a carbon limitation situation for *H. neapolitanus* when compared to 5mM bicarbonate. In addition, the changes in levels of cbbM may be indicative of a change from form II to form I RubisCO in response to carbon limitation.

Changes in levels of proteins involved in energy producing pathways are more difficult to interpret since there has been no report from other sulfur oxidizing species of such changes based on changes in carbon species. The complexity of interpretation is especially true in the case of sulfide:quinone reductases such as sqr D, E and F and the sulfide dehydrogenase sdhAB since the sulfur substrate in the growth media being thiosulfate and not sulfide. Even if the thiosulfate in the media were to undergo decomposition during autoclaving, it is expected that only trace amounts of sulfide would be produced. That being the case, the increase in level of sqrF and sdhB proteins upon change from bicarbonate to CO_2(g) would be involved in processing only a fraction of the available substrate. Other than the decrease in sulfur oxygenase-reductase (sor), no significant change is seen in the levels of other sulfur oxidizing systems.

Changes in levels of terminal electron acceptors seem to fit a general pattern amongst other bacteria. The cbb3 terminal oxidase is the primary oxidase under aerobic conditions. This is consistent with there being no significant changes observed. The bd-quinol oxidase is a minor contributor. Several reports (Zhou *et al.* 2013; Osamura *et al.* 2017) indicate that caa3 type oxidases serves as a survival mechanism under substrate starvation conditions. The decrease in caa3 related proteins seen in Figure 9 may therefore represent an overall 'favor' of CO_2(g) as dissolved carbon species.

Conclusions

Changes in DIC species does appear to elicit changes in levels of proteins with a total of 99 out of 1990 detected proteins showing log2fold change >0.5. The tandem-mass-tagged MS (TMT-MS) technique proved sufficient to identify and quantitate peptides associated with both carbon uptake/fixation and energy production. Since the changes witnessed in bicarbonate uptake and carbon fixation mechanisms are similar to those previously reported for *T. crunogena*, this supports the validity of the results seen within both the carbon concentration mechanism and the energy producing pathways.

Acknowledgements

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Parasites (Trematoda, Nematoda, Phthiraptera) of Two Arkansas Raptors (Accipitriformes: Accipitridae; Strigiformes: Strigidae)

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Running Title: Parasites of Arkansas Raptors

Abstract

Very little is known about the helminth parasites of hawks and owls of Arkansas. We had the opportunity to salvage 2 road-killed raptors, a red-shouldered hawk (*Buteo lineatus*) and a great horned owl (*Bubo virginianus*) from the state and examine them for ecto- and endoparasites. Found were chewing lice (*Degeeriella fulva*) and a nematode (*Porrocaecum angusticolle*) on/in *B. lineatus*, and 3 digenean trematodes (*Echinoparyphium* sp., *Strigea elegans*, *Neodiplostomum americanum*), and nematode eggs (*Capillaria* sp.) in *B. virginianus*. We document 6 new distributional records for these parasites.

Introduction

Birds of prey or raptors make up an invaluable portion of the avian fauna of Arkansas (James and Neal 1986). In adjacent Oklahoma, novel information on the parasites of raptors has been gained recently by our research group from examination of salvaged road-killed specimens (McAllister *et al.* 2017, 2018). However, we are aware of only a single previous report (Richardson and Nickol 1995) on parasites (acanthocephalans) from great horned owls (*Bubo virginianus*) in Benton and Madison counties of the state, and nothing is available on parasites from any hawks of Arkansas. Here, we report new state records for 6 parasites found on/in 2 salvaged raptors of Arkansas.

Materials and Methods

An adult red-shouldered hawk (*Buteo lineatus*) was found dead on the road (DOR) on 9 February 2018 at 3.2 km E of Lockesburg off US 371, Sevier County (33°57'30.04"N, 94°07'53.78"W). In addition, an adult *B. virginianus* was found DOR on 28 May 2018 off St. Hwy. 14 in the vicinity of Caney, Marion County (36°06'26.18"N, 92°37'53.90"W). These specimens appeared to be recently killed and their bodies showed no sign of putrification. They were examined for most parasites but not examined for subcutaneous helminths or *Trichinella* sp. Their feathers were brushed for ectoparasites and those found were placed in a vial of 70% (v/v) ethanol; specimens were cleared in 10% potassium hydroxide, dehydrated through an ethanol series, further cleared in xylene, and slide-mounted in Canada balsam (Price *et al.* 2003). A mid-ventral incision was made to expose the viscera and blood samples were taken directly from the heart, smeared onto a microscopic slide and allowed to dry, then fixed in absolute methanol for 1 minute, stained in Giemsa for 30 minutes, and rinsed in neutral buffered phosphate saline. The gastrointestinal (GI) tract from the throat to cloaca was removed, rinsed in 0.9% saline, and organs placed in individual Petri dishes. Several 100 mm sections of the GI tract were cut, split lengthwise, and examined under a stereomicroscope for endoparasites. Feces from the rectum from both raptors were collected and placed in 2.5% potassium dichromate. Fecal flotations were accomplished with Sheather's sugar solution (sp. gr. 1.30). Trematodes were rinsed in saline, fixed in hot tap water without coverslip pressure, preserved in 70% ethanol, stained in acetocarmine, cleared in methyl salicylate, and coverslip mounted in Canada balsam. Nematode ova from the fecal flotation were placed on a microscopic slide, coverslip mounted, and photographed.

Photovoucher hosts were deposited in the Henderson State University (HSU) collection, Arkadelphia, Arkansas. Voucher specimens of ectoparasites were deposited in the General Ectoparasite

Collection in the Department of Biology at Georgia Southern University, Statesboro, Georgia. The trematodes and nematodes were deposited in the Harold W. Manter Laboratory (HWML) of Parasitology, University of Nebraska, Lincoln, Nebraska, or retained for molecular analyses.

Results and Discussion

A nematode and chewing louse was recovered from *B. lineatus* and 3 digeneans, and a nematode egg was found in *B. virginianus*. An eimerian coccidian was also found in *B. virginianus* and was reported in a recent report that included molecular analyses (McAllister *et al.* 2019). The blood smears were negative and no cestodes or acanthocephalans were found. Data is presented below in annotated format.

TREMATODA: DIGENEA: ECHINOSTOMATIDAE

***Echinoparyphium* sp.** – Very young juvenile specimens of an *Echinoparyphium* sp. was collected from the intestinal tract of *B. virginianus*. *Echinoparyphium* species are common, widely distributed intestinal parasites causing disease in animals worldwide. Intermediate hosts include a variety of taxa, including freshwater snails, mussels, planarians, fish, frogs, and aquatic turtles, whereas the definitive hosts are mainly birds and mammals (Huffman and Fried 2012). *Echinoparyphium recurvatum* (von Linstow) has been reported in *B. virginianus* from Alberta, Canada (Ramalingam and Samuel 1978). We report the genus from Arkansas for the first time.

STRIGEIDAE

***Strigea elegans* Chandler & Rausch, 1947**. – A single specimen was taken from the intestinal tract of *B. virginianus*. This digenean was originally described from 6 of 22 (27%) *B. virginianus* from Wisconsin (Chandler and Rausch 1947). It was later redescribed by Dubois and Rausch (1950) from the same host and locale. The life cycle is a 4-host obligatory one that involves snails as first intermediate hosts, bufonid and ranid tadpoles as second intermediate hosts, watersnakes and ducks as third intermediate hosts (with tetracotyles), and owls as final hosts (Pearson 1959; Miller *et al.* 1965). Kinsella *et al.* (2001) reported *S. elegans* in *B. virginianus* from Florida. We document *S. elegans* from Arkansas for the first time.

DIPLOSTOMIDAE

***Neodiplostomum americanum* Chandler and Rausch, 1947.** – Three specimens were recovered from the intestinal tract of *B. virginianus*. This trematode has been reported previously from *B. virginianus* from Connecticut, Florida, Mississippi, and Wisconsin (see Woodyard *et al.* 2017). It has also been reported from other raptors, including *Accipiter* spp., *Asio otus, Athene cunicularia, Buteo* spp., *Megascops asio,* and *Strix varia* from Connecticut, Florida, Louisiana, Mississippi, and Wisconsin, and Ontario, Canada (Woodyard *et al.* 2017). We report a new state record for *N. americanum* as well as the first report of this parasite from west of the Mississippi River.

NEMATODA: ANISAKIDAE

***Porrocaecum angusticolle* (Molin, 1860) Baylis and Daubney, 1922.** – Five (4 male, 1 female) *P. angusticolle* (HWML 110402) were found in the stomach of *B. lineatus*. *Porrocaecum angusticolle* was originally described as *Ascaris angusticollis* by Molin (1860) from specimens collected from the intestines of common buzzard (*Buteo buteo*) and osprey (*Pandion haliaetus*) from Europe. It was transferred to the genus *Porrocaecum* by Baylis and Daubney (1922). This nematode has been reported from 6 species of hawks from the Nearctic Realm (Table 1). We document the first report of *P. angusticolle* from Arkansas.

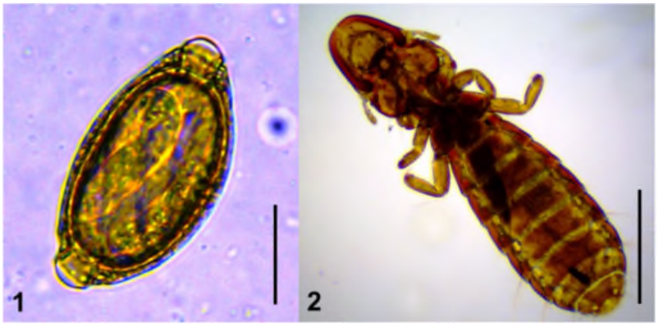
TRICHURIDA: CAPILLARIIDAE

***Capillaria* sp.** – Ova of a *Capillaria* sp. (Fig. 1) were recovered from the feces of *B. virginianus*. Two capillarid species have previously been reported from *B. virginianus*, including *C. falconis* (Rudolphi) from Florida, Wisconsin, and Alberta, Canada, and *C. tennissima* (Rudolphi) from Florida (Read 1949; Ramalingam and Samuel 1978; Kinsella *et al.* 2001) and Connecticut (Richardson and Kinsella 2010). This is the first time *Capillaria* sp. ova have been reported from any owl from the state.

ARTHROPODA: INSECTA: PHTHIRAPTERA: ISCHNOCERA: PHILOPTERIDAE

***Degeeriella fulva* (Giebel, 1874).** – Several *D. fulva* (Fig. 2) were taken from *B. lineatus*. This louse has been previously reported from *B. lineatus* as well as 27 other species of raptors belonging to 9 different genera (Price *et al.* 2003). We document a new state record for this louse in Arkansas.

In conclusion, we document several new distributional records for parasites of *B. lineatus* and *B. virginianus*. Most importantly, additional parasites are reported for the first time from an Arkansas *B. virginianus* and others from *B. lineatus* from the state.



Figures 1 and 2. Parasites of *Bubo virginianus* and *Buteo lineatus*. (1) Embryonated capillarid egg from feces of *B. virginianus*. Note characteristic bipolar plugs. Scale bar = 250 µm. (2) Chewing louse, *Degeeriella fulva* from *B. lineatus*. Scale bar = 1.0 mm.

Although this survey included only 2 specimens, it continues to illustrate the significance of salvaging road-killed raptors which can yield knowledge on their parasites that could not be obtained otherwise because of state and federal restrictions on collecting and euthanizing migratory birds.

Acknowledgments

The Arkansas Game and Fish Commission and U.S. Fish & Wildlife Service issued Scientific Collecting Permits to CTM. We thank Ethan T. Woodyard (Mississippi St. University) for examining some trematodes. We dedicate this paper to the memory of Drs. Douglas A. James (1925–2018) and Kimberly G. Smith (1948–2018), renowned ornithologists of the University of Arkansas.

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Table 1. Records of *Porrocaecum angusticolle* in hawks from the Nearctic Realm.

Common Name	**Species**	**Locality**	**Reference**
Cooper's hawk*	*Accipiter cooperi*	North America*	Morgan and Schiller (1950)
Sharp-shinned hawk*	*A. velox*	North America*	Morgan and Schiller (1950)
Red-tailed hawk	*Buteo borealis*	North America†	Canavan (1931)
		E. North America*	Morgan and Schiller (1950)
Red-shouldered hawk	*B. lineatus*	E. North America†	Canavan (1929)
		Arkansas, USA	This report
Broad-winged hawk	*B. platypterus*	North America*	Morgan and Schiller (1950)
		Florida, USA	Kinsella *et al*. (1995)
Marsh hawk	*Circus hudsonius*	North America*	Morgan and Schiller (1950)

*Locales not given.
†Captives from Philadelphia Zoo.

Long-tailed Weasel (*Mustela frenata)* Distribution Survey in Arkansas: Challenges in Detecting a Rare Species

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Running Title: Long-tailed Weasel Distribution

Abstract

Long-tailed weasels (*Mustela frenata*) have one of the widest distributions of mustelids in the western hemisphere and were distributed across a majority of the American continents ranging from Canada through the contiguous United States, Mexico, and into northern South America. However, on a local scale they are considered uncommon and rare. We assessed the distribution of long-tailed weasels across Arkansas to determine occupancy in each ecoregion of Arkansas, and determined the detectability on two local, adjacent sites. No long-tailed weasels were detected within the ecoregions, but the species was detected with intensive sampling on one local site. It should be emphasized that although the species was not detected within the ecoregions, this does not indicate it does not occur broadly across the state.

Introduction

Long-tailed weasels (*Mustela frenata*) have one of the widest geologic and ecologic ranges of mustelids in the western hemisphere (Fagerstone 1987) and historically were distributed across a majority of the American continents (Sheffield and Thomas 1997). Ranging from Canada through the contiguous United States, Mexico, and into northern South America, the species exhibits a broad distribution. However, on a local scale they are considered uncommon and rare due to low densities and their secretive nature (King and Powell 2007; Fagerstone 1987). Densities for most populations are unknown, though some states, such as Kansas, estimate local densities range from 1 to 32 per km^2 (Timm *et al*. 2019) depending upon prey availability and habitat. Knowledge of status and distribution at the regional scale of long-tailed weasels is lacking. Conservation and management of *M. frenata* would benefit from an increased understanding of the species and its distribution at the regional scale.

Status of long-tailed weasels in Arkansas is currently not known. Sealander and Heidt (1990) report the species as widespread, but rare in Arkansas; similar to its continental range. Availability of prey, such as pocket gophers (*Geomys spp.*), and suitable habitat, notably presence of permanent water sources (King and Powell 2007), likely dictates presence and density of long-tailed weasels. Listed as a species of least concern by the International Union for Conservation of Nature (IUCN 2014), it may be listed differently in individual states (Reid and Helgen 2008). In Arkansas, the long-tailed weasel is listed as a Species of Greatest Conservation Need (State Rank – S3 (Fowler 2015)); however, hunting and trapping seasons for the species are still open (Sasse 2012). Some neighboring states also list the species as protected or list it as a species of special concern. For example, Missouri lists *M. frenata* as Vulnerable and a Species of Conservation Concern (MDC 2014), and Louisiana considers the species rare (LDWF 2014).

Our goal was to assess the distribution of long-tailed weasels across Arkansas. Specifically, our objectives were to: (1) determine occupancy in each ecoregion of Arkansas, and (2) determine the detectability on two local, adjacent sites. These efforts were intended to better inform managers and biologists of where the species occurs and how to most effectively sample for the species.

Study Areas

Statewide

The study was conducted across the state of Arkansas in each of the ecoregions and included wildlife management areas, national wildlife refuges, and national forest properties. Four ecoregions (Figure 1), including the Ozark Mountains (Ozark Mountains, Boston Mountains, and Arkansas Valley), Ouachita

Mountains, Gulf Coastal Plain, and Mississippi Alluvial Valley (including Crowley's Ridge) occur in Arkansas. The Ozark Mountains region has narrow valleys walled by steep mountains, except the Arkansas River Valley which has broad valleys. Geology in this region is primarily sandstone and limestone with some dolostones in the oldest surface rock (Arkansas Geological Survey 2015). Cover types in the Ozark Mountains include savanna, prairie, and oak-hickory or oak-hickory-pine forests at higher elevations. At lower elevations, bottomland hardwood forests dominate (Omernik and Griffith 2014). Additionally, cedar glades are also present where soil is shallow (30 - 61 cm; Arend and Collins 1949).

The Ouachita Mountains are an east-west trending range with valleys that vary from narrow to broad and surface geology that is equally variable including shale, sandstone, and chert (Arkansas Geological Survey 2015). Oak-hickory-pine forests with open, grassy woodlands on south-facing slopes are the dominate cover type in the Ouachita Mountain region (Omernik and Griffith 2014).

The Gulf Coastal Plain is characterized by gently rolling hills made of sand, silt, clay, and gravel (Arkansas Geological Survey 2015). The Gulf Coastal Plain has oak-hickory-pine forests in the upland areas and hardwoods dominate the bottomlands (Omernik and Griffith 2014).

The Mississippi River shaped the eastern portion of the state, known as the Mississippi Alluvial Plain, depositing sediments such as sand, silt, clay, and gravel. Additionally, portions of this region have loess hills that provide the only topographic relief of the eastern section of Arkansas (Arkansas Geological Survey 2015, Omernik and Griffith 2014). Bottomland hardwoods and agricultural croplands dominate the Mississippi Alluvial Valley (Omernik and Griffith 2014).

Camp Robinson Special Use Area-Stone Prairie Wildlife Management Area

The Camp Robinson Special Use Area (CRSUA) and the Stone Prairie Wildlife Management Area (SPWMA) are located in Faulkner County, Arkansas. Camp Robinson Special Use Area is approximately 4,029 acre in size and the Stone Prairie WMA is approximately 898 acres in size (Figure 2). These two areas were chosen because of the perceived likelihood of the species occupying the sites. The CRSUA is managed intensely for bird dog training and field trials. As such, prescribed burns were conducted each winter. The SPWMA was recently purchased (March 2017) as a wildlife management area for the purpose of Northern Bobwhite (*Colinus virginianus*) restoration.

Both areas are within the Ouachita Mountains and have a sandstone, chert, shale, and novaculite parent material for soils (Arkansas Geological Survey 2015). Open fields and oak-savannahs dominate the CRSUA, while open grasslands and oak-pine woodlands are the dominate cover types on the SPWMA.

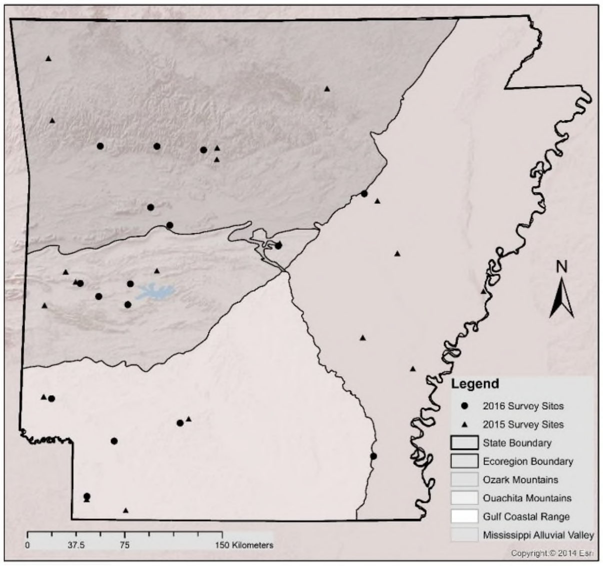

Figure 1. Sites sampled in Arkansas for long-tailed weasels (Mustela frenata) from 8 March to 15 June 2015 (circles) and from 24 February to 8 May 2016 (triangles) to determine occupancy and detectability.

Figure 2. Camp Robinson Special Use Area and adjacent Stone Prairie Wildlife Management Area (formally owned by the Nature Conservancy) sampled to assess methods to detect long-tailed weasels in winter 2017.

Methods

Data Collection

Long-tailed weasels are found in association with waterways adjacent to fields and forests (Schwartz and Schwartz 2001). As such, we created a sampling frame of potential sites based on those habitat features modeled in a GIS. Locations that were sampled (Figure 1) were randomly selected from the sampling frame. ArcMap® 10.2.1 was used to develop a sampling frame. Habitat covariates thought to be important to long-tailed weasels included open and forest cover types, proximity to permanent water, soil type (based on pocket gopher preferences identified by Kershen (2004)), and past presence of pocket gophers. These habitat layers were intersected in a GIS. A 200 m buffer was placed around the output of the data intersection; this distance represents the average distance traveled for foraging by weasels (Gehring and Swihart 2004). Finally 100 random points were generated as the sampling frame. We randomly selected 20 sampling points, five in each physiographic region, from the sampling frame as survey sites for each year of sampling (Figure 1). Publicly-owned land, such as state and federal lands (e.g., Arkansas Game and Fish Commission (AGFC), United States Forest Service (USFS) or National Wildlife Refuges (NWR)), was largely represented in the sampling scheme, but privately-owned property was also surveyed. We collected data from late February to mid-June because long-tailed weasel movement is increased during that time of year (Downey 2004).

The single species, single season approach (Mackenzie *et al.* 2006) was used with *a priori* assumptions for probability of detection, probability of occupancy, and variance set at $p = 0.1$, $\Psi = 0.1$, and $var(\Psi) = 0.2$, respectively. Values for probability of detection and occupancy were set low to account for the difficulty in detecting this species. Variance was liberal to account for the expected, sporadic detection of the species. The optimum number of surveys per site selected for this project was 14 (MacKenzie *et al.* 2006). Therefore, 14 stations per site were established. Stations were separated by $\geq$ 0.5 km and placed at least 50 m from roads and trails.

A station consisted of 2 track stations, 1 camera trap, and 2 hair traps to detect long-tailed weasels. Track stations are considered the most effective and inexpensive method of sampling weasels (Downey 2004). Track stations were housed in a small, wooden cubby, 60 cm in length that is open at both ends (Figure 3). On either side of the bait (approximately 20 g of raw chicken), a 20-cm aluminum plate covered with a fine layer of toner (King and Edgar 1977) was placed with contact paper on the innermost third of the plate for track collection. In addition to bait, we sprayed predator attractant (Wildlife Research® Paws and Claws, Ramsey, MN, USA) at the site. We replaced bait, contact paper, and toner as needed, but predator scent was refreshed at the station daily. All tracks were identified to species.

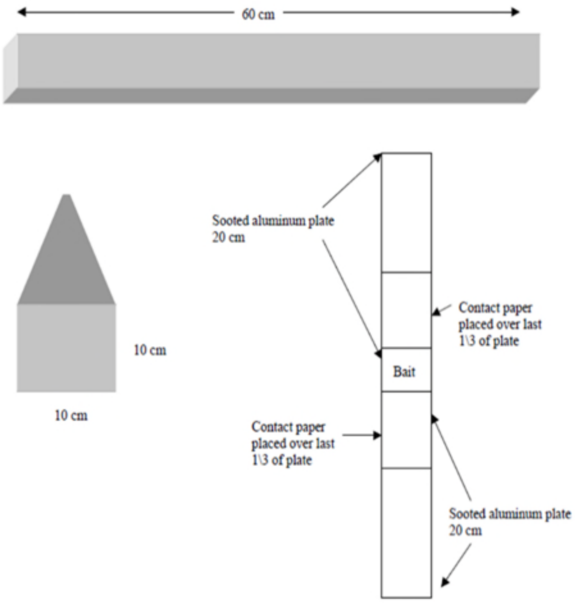

Figure 3. Cubby design used to house track plates to Arkansas 8 March - 11 June 2015 and 24 February - 8 May 2016.

The second detection technique used was a camera trap (Browning® Strike Force HD camera, Morgan, UT, USA). One camera was placed at each station to record images at a rate of 3 frames/sec for 2 sec when triggered by movement. At each station, cameras were mounted 0.5 - 1.0 m above ground on natural vegetation (i.e., trees) and 2.5 - 3.0 m from the cubby. We identified photos of animals to species as quality allowed (O'Connell and Bailey 2011). Hair traps, the third detection technique, were made of wooden dowels mounted on each side of the bait in the cubby, 3.75-cm from the floor of the cubby, rolled with packing tape (Henry *et al.* 2011). We collected tape with hair and stored it in envelopes with desiccate until returning to the lab (Kendall and McKelvey 2008). In the lab, hair was removed from the tape, placed on a glass slide and identified to species using a dichotomous hair key (Debelica and Thies 2009).

We checked stations daily for three nights at each site; bait, batteries, and memory cards were replaced as needed. During 2016, randomly selected sites were sampled additional nights to assess the effect of longer sampling duration. The same methods were applied to the local, intensive sampling on the CRSUA and the SPWMA. We randomly selected locations across each area using a GIS. Research followed guidelines of the American Society of Mammalogists (Sikes *et al.* 2011) and was approved by the Tennessee Technological University Institutional Animal Care and Use Committee (#2014-001).

Results

Statewide

Data collection to estimate the distribution and status of long-tailed weasels in Arkansas occurred from 8 March to 11 June 2015, and from 24 February to 8 May 2016. Due to extensive flooding, one survey site in the Gulf Coastal Plain was not sampled in 2015; additionally, one survey site in the Ouachita Mountains and three sites in the Mississippi Alluvial Plain were not sampled in 2016.

During the 2015 season, 18 of 19 sites were on public land and all 16 sites in 2016 were on publicly-owned land. A total of 14 mammalian species was detected in 2015 (Table 1) and 18 species were detected in 2016 (Table 2). Species detected most often both years included northern raccoons (*Procyon lotor*), Virginia opossums (*Didelphis virginiana*), bobcat (*Lynx rufus*), and coyotes (*Canis latrans*). Most detections occurred with the infrared-triggered cameras, but some Virginia opossum detections were from tracks or hair traps. Northern raccoons and Virginia opossums were the most detected species in both years. Species detected the least were spotted skunk (1 site, both years) and striped skunk (1 site, 2016 only).

Of the 35 sites sampled, none resulted in long-tailed weasel detections. Previously, based on examination of hair with a microscope, we falsely detected long-tailed weasels in 2015. This was attributed to the similarity of morphology and hair size of long-tailed weasel and Virginia opossum hair. Genetic analyses confirmed that suspected long-tailed weasel detections were actually *D. virginiana*. Although this is not a promising result, it should be emphasized that although the species was not detected, this does not indicate it does not occur in the state.

Camp Robinson Special Use Area-Stone Prairie Wildlife Management Area

A total of 35 sites, 16 sites on CRSUA and 19 sites on SPWMA, was sampled. Sites were sampled from 7 February through 31 March on the CRSUA because of public events (e.g., field trials) being held, and sites were sampled from 7 January through 31 March on the SPWMA. Cameras were operable an average of 32.5 (± 17 (1 s.d.)) trap days on CRSUA and for 57.2 (± 22 (1 s.d.)) trap days on the SPWMA; a total of 1,606 trap days were surveyed for both sites. A total of 14,626 images of animals was taken on the CRSUA, and 35,319 images were taken on SPWMA; the total number of images taken on these two adjacent sites was 49,945. Cameras were set to capture three images per second for two seconds, and 8,324 events were captured.

Deer, northern raccoons, Virginia opossums, rabbits (Eastern cottontail and swamp) and squirrels (gray and fox) were the five most common species captured on the cameras (Table 3).

Only one (1) long-tailed weasel was captured by a camera (Figure 4). That image was captured 21 days after the site was established. The site where the image was captured had 44% canopy cover, 60% litter, 40% herbaceous cover, 10% visual obstruction at 1 m above the ground, was adjacent to a stream (~3.5 m) and 240 m from the nearest road. The overall success rate, based on the number of events, was 0.002%. No evidence of long-tailed weasels was observed with track plates or hair traps.

Discussion

Statewide

Although long-tailed weasels were not detected in Arkansas in the statewide portion of the study, the lack of detection does not indicate the species is absent from the state. We expected the species to be difficult to detect, thus the *a priori* assumption of a low detection rate. However, in occupancy studies such as the current study, false absences can occur with no solution to account for this issue (MacKenzie *et al.* 2006). There are several possible explanations for the absence of *M. frenata* detections in this study; some were the result of logistical constraints, some resulted from unpredictable and extreme abiotic events, and some were likely due to the absence of the species.

We followed the recommendations of Downey (2004) to determine the optimum time of year for this survey. In previous studies, surveys for *M. frenata* have occurred in the late winter and early spring months, approximately March through May (Downey 2004; Fowler and Golightly 1994). This time frame has been determined to be a period of increased activity for the species; thus, increasing the likelihood of detection. The studies by Downey (2004) and Fowler and Golightly (1994) both occurred at northern latitudes (Alberta and Placer County, California). Timing of high activity in

Table 1. Five most numerous species detected by site between 1 March and 15 June 2015 in Arkansas.

Site	Species				
	Northern Raccoon	Virginia Opossum	Bobcat	Coyote	American Mink
Camp Robinson WMA	X	X			
Miller Farms	X	X			
NACA	X	X		X	
Devil's Den SP	X	X	X		
Ouachita NF 45	X	X		X	
Ouachita NF 39	X	X		X	
Ouachita NF 30	X	X		X	
Pond Creek NWR	X	X			X
Poison Springs WMA		X			
Sulphur River WMA					
Lafayette WMA	X	X	X		X
Ozark NF 4	X		X	X	
Ozark NF 19	X		X	X	
Hurricane Lake WMA	X	X	X		
Dagmar WMA	X	X	X		
White River NWR	X	X		X	X
Ouachita NF 34	X	X			
Bayou Meto WMA	X	X	X		
St. Francis NF	X	X	X		
Total Sites Detected	17	16	8	7	3

the species may differ in the Southeast compared with the locations of these previous studies. Adjusting the survey time frame to encompass the winter months of December to March as Gehring and Swihart (2004) did in their northern Indiana trapping efforts may increase the likelihood of detecting long-tailed weasels in Arkansas. However, the current Arkansas trapping season for furbearers, including weasels and similarly-sized furbearers (e.g., American mink (*Neovison vison*)), is open during these months (Sasse 2014). In the last 25 years, very few captures, including incidental captures, of *M. frenata* have occurred during the Arkansas furbearer trapping season (Sasse 2012). Moving the survey time frame to late-spring and early- or mid-summer may be an option considered by managers and future researchers.

Most carnivore surveys implement sites for at least 10 to 14 days and often much longer periods of time to increase the likelihood of detection (Kendall and McKelvey 2008). During the current study, 14 stations at each site were left out for three nights on most occasions. Duration of stations ranged from 2 to 8 nights. During the 2015 season, some sites required up to 2.5 to 3 hours of driving time to reach the next closest site and time to set stations was limited by daylight. During the 2016 stations, the maximum driving time between sites was approximately 2 hours, so all sites were set in the first day of each 4-day trip. However, on one occasion, flash flooding prompted the early removal at two sites in southwestern Arkansas. Additionally, four sites remained out for 7 to 8 nights. Although the sites with longer duration did not produce *M. frenata* detections, increasing the duration of surveys across all sites may increase the probability of detection if long-tailed weasels are present. In a study by Foresman and Pearson (1998) of forest carnivores in southwestern Montana, American marten (*Martes americana*) latency to detection (LTD) ranged from 2.3 to 24.0 days. The mean LTD from this study across two methods (sooted-track plates and remote cameras) and three species was 13.5 ± 4.9 days; they indicated a range of 8.6 to 18.4 days was required to detect American marten, fisher

Table 2. Five most numerous species detected by site between 24 February and 8 May 2016 in Arkansas.

Site	Species				
	Northern Raccoon	Virginia Opossum	Coyote	American Black Bear	Bobcat
Cut-Off Creek WMA	X	X			
Bald Knob NWR	X				
Bois D'Arc Creek WMA	X	X			
Camp Robinson WMA	X				X
Ouachita NF 28	X				
Ouachita NF 33	X				
Ouachita NF 37	X	X			X
Ouachita NF 41	X	X			
Ozark NF 3	X	X	X	X	
Ozark NF 11		X		X	
Ozark NF 22		X			
Ozark NF 23	X	X	X	X	
Petit Jean River WMA	X	X	X		X
Poison Springs WMA		X			
Pond Creek NWR	X	X	X		
Sulphur River WMA	X				
Total Sites Detected	13	11	4	3	3

(*Pekania pennant*), and wolverine (*Gulo gulo*, Foresman and Pearson 1998). A similar time period may be required to detect long-tailed weasels because of their secretive nature, low populations densities, and small size (King and Powell 2007). We compensated for the temporal aspect by spatially sampling at 14 locations within a single site.

Long-tailed weasels occupy a wide variety of habitats and inhabit more ecoregions than any other member of the mustelids. The generalist nature of this species is the primary reason for their occupancy of low- to high-elevation ecoregions (Fagerstone 1987; Pasch and Pino 2013). Cover types the species occupies range from open areas such as prairies, marshes, meadows, alpine, and agricultural areas to fencerows, thickets, brushlands, open woodlands (e.g., oak savannas), swamps, and to some extent, forests (Fagerstone 1987; Sealander and Heidt 1990; Schwartz and Schwartz 2006; LDWF 2014). The lack of knowledge regarding specific habitat preferences of long-tailed weasels makes it difficult to select areas where opportunity for detection is increased. Ultimately, habitat preference is likely driven by prey availability and areas with diverse habitat patches tend to provide higher prey biomass and diversity (Gamble 1981).

Obtaining access to large tracts of privately-owned property proved difficult in 2015; therefore, this survey was restricted to surveying publicly-owned property for most of 2015 and all of 2016. Due to a randomized survey design, some sites were located in large tracts of forest owned by federal or state agencies, often in pine (*Pinus spp.*) plantations. Late seral stage forests and pine plantations exhibit lower biodiversity, including species that may be considered prey for long-tailed weasels; as such several survey sites had a reduced likelihood of habitation by *M. frenata* (Estades and Temple 1999, Gamble 1981). Additional access to privately-owned property would have increased the opportunity to survey portions of the state and habitat surrounding pasture, old fields, and hay fields, including favored prey species such as voles (*Microtus spp.*) and pocket gophers (*Geomys spp.*, Gamble 1981). Increased ecotones (i.e., edge habitats) should exhibit increased prey availability and future research may benefit from

Table 3. Number of sites that were visited by different mammalian species of the total sampled on the Camp Robinson Special Use Area (n = 16) and Stone Prairie Wildlife Management Area (n = 19) from 7 January to 31 March 2017.

Species	Camp Robinson SUA	Stone Prairie WMA
Northern Raccoon	13	15
Virginia Opossum	12	13
Squirrel (grey and fox)	12	11
Rabbit (Eastern cottontail and swamp)	12	12
White-tailed Deer	10	16
Armadillo	8	9
Coyote	7	8
Fox (Gray or Red)	6	3
Rat (*Rattus* spp.)	4	7
Bobcat	4	4
Feral Cat	3	0
Otter	2	0
Striped Skunk	2	8
Mouse (Species unknown)	1	1
Long-tailed Weasel	1	0
Unknown	1	2

diversifying land ownership, and thus habitat types, of survey sites for long-tailed weasels.

During both survey seasons, sites in the southern and eastern portions of Arkansas experienced extensive and, in some cases, long-term flooding. Flooding prevented surveys at 5 sites over the 2 years and reduced time spent at 2 sites. The effect of flooding on small mammals is generally in the form of displacement or death and little is known about recolonization rates (Triska *et al.* 2011). In a study of fisher in North Dakota riparian habitat, an extreme flood event occurred in the spring of 2009 with >95% of suitable habitat inundated for 7-8 weeks. Researchers expected detection rates to be greatly reduced from the 2008 surveys to the 2009 surveys. However, they found that fisher returned to the area 15 days after the river receded to below flood stage and 75 days after initial flooding (Triska *et al.* 2011). These findings suggest that medium-sized, highly-mobile mammals can recolonize an area relatively quickly after extreme flooding events. Conversely, Wijnhoven *et al.* (2006) found that it may take 9 months or more for small mammals to recolonize an area after flood water recedes. Although long-tailed weasels are a highly-mobile species, they are also considered a small mammal. Additionally, they depend on a high abundance of small mammals (namely rodents) due to their high metabolism. Long-tailed weasel populations displaced by extreme flooding events may take as long, or longer, than their prey base to return to habitat occupied prior to flooding. Because of the high probability of floods occurring in portions of Arkansas, detecting long-tailed weasels is further complicated due to sporadic fluxes in local distribution and potential prey abundance.

Land use changes over the last few decades, coincidental with the absence of long-tailed weasels in the trapping harvest, likely played a role in the lack of evidence of the species in the surveys. A very large percentage of the state has been in agriculture (41%) or saw-log production (57%), and the number of acres in agriculture and saw-log production increased by 100,000 acres and 300,000 acres, respectively, from 2010 to 2013 (University of Arkansas 2011, 2014). With a growing percentage of the state being managed for agriculture and timber, it is likely that our findings are, in fact, correct in that there were no long-tailed weasels where we randomly sampled. It should be noted that it is not possible to demonstrate the absence of a species with certainty.

Camp Robinson Special Use Area-Stone Prairie Wildlife Management Area

The local, intensive survey of two adjacent sites yielded the only observation of a long-tailed weasel during the study. The assumption of 14 trap days being required to observe an individual on a site was not met

Figure 4. Long-tailed weasel image captured 3 March 2017 on the Camp Robinson Special Use Area.

(MacKenzie *et al*. 2006). On our sites it appeared no less than 21 days were required to observe one individual; this is based on the fact that the site at which the image of the weasel was captured (#39) was operational for 21 days when the image was taken. However, there were 28 other sites operational during the same period as site #39. Based on the collective effort across the CRSUA and SPWMA and just the period site #39 was operational, the minimum number of "trap days" required to capture that image was 580; some sites were operational for more than a month prior to the site that captured the image was established. Moreover, many of the sites were operational for up to 3 weeks following capture of the image without any other instances of a long-tailed weasel being observed.

Given the images of the long-tailed weasel that were captured, there was no apparent interest in the cubby, lure, or bait that was at the station; the image appeared to be captured randomly. This observation is in comparison to the other furbearers, such as northern raccoons, Virginia opossums, coyotes, otters, and skunks, which were captured in images. Typical behavior includes some investigation of the cubby at least. While this may have occurred during the time delay following the six images that were taken, the individual did not remain in the area long enough for additional images to be captured. While use of cubbies are productive in the northern extent of the species distribution, their use in Arkansas was not. This is an area of interest that will require further investigation.

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A Tale of Two Sylamores: Understanding Relationships Among Land Use, Nutrients, and Aquatic Communities Across a Subsidy-Stress Gradient

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Running Title: Subsidy-Stress Gradients in the Sylamore Creek Watershed

Abstract

Agricultural land use can negatively impact primary producers, macroinvertebrates, and fishes. Small-scale changes in land use can subsidize an aquatic ecosystem, where an increase in nutrients allows nutrient-limited biota to flourish, and minor increases in sedimentation may help support populations of collector-filterers. The stimulation in performance caused by small disturbances is part of the subsidy-stress gradient, where increasing perturbation subsidizes an ecosystem until a certain threshold is reached, at which a decline in performance and increased variability starts to occur. The North and South Sylamore watersheds provide a useful template to investigate the subsidy-stress gradient in relation to land use. North Sylamore flows through the Ozark National Forest and had a heavily forested catchment, while South Sylamore flows through mostly private land, some of which was pasture (23%). Physicochemical, macroinvertebrate, and fish data were collected from four sites within each watershed to determine if South Sylamore was exhibiting a response to pasture/agriculture characteristic of a subsidy-stress gradient. Sites within South Sylamore had significantly higher nitrate levels, larger macroinvertebrate populations dominated by collector-filterers, and greater abundance of algivorous fish, suggesting South Sylamore may be subsidized by the surrounding pastoral lands. However, South Sylamore also had a significantly lower proportional abundance of sensitive macroinvertebrate taxa and more unique tolerant fish taxa, suggesting South Sylamore is experiencing stress as well. Habitat quality of South Sylamore could be improved by restoration of trees within the riparian zone. Monitoring aquatic systems for subsidy-stress responses can inform restoration/management decisions and guide intervention prior to watersheds and aquatic communities becoming overly stressed.

Introduction

Civilization has long been understood to both enhance and impair natural environments depending on the magnitude and scale of impact, yet the idea of the "subsidy-stress gradient" has only recently gained prevalence in ecological fields since its conception in Odum *et al.* (1979). The subsidy-stress gradient describes a response where increasing perturbation stimulates performance until a certain threshold is surpassed, at which a decline in performance and increased variability starts to occur (Odum *et al.* 1979). Numerous studies document subsidy-stress responses within aquatic ecology, such as macroinvertebrate community biomass responses to phosphorus gradients (King and Richardson 2007), bacterial abundance responses to salinity gradients (Kefford *et al.* 2004), and periphyton responses to water velocity gradients (Biggs *et al.* 1998). Land-use gradients in catchments have also been shown to induce subsidy-stress responses in stream communities, whereby invertebrate density and algal biomass were higher in catchments with more pastoral land cover (Niyogi *et al.* 2007).

Subsidy-stress responses are crucial to understand given land-use alteration could reach a threshold level inducing substantial change to stream ecosystems. Identifying ecological thresholds is important for conservation but difficult to quantify. However, increased forest loss led to increased variance in community metrics of some Neotropical forest animal communities (Roque *et al.* 2018). Resultant losses in biodiversity could in turn affect ecosystem processes, particularly in ecosystems that experience fluctuations on seasonal time scales (Keddy and Reznicek 1982). Thus, catchment systems with relatively little agricultural land-use could provide an excellent model to study the subsidy-stress hypothesis in relation to land-use gradients. Whereas small increases in pastoral land-cover and subsequent abiotic shifts may subsidize stream biodiversity, increases in heteroscedasticity of community metrics may be indicative of an approaching threshold (Barnosky *et al.* 2012; Roque *et al.* 2018).

Agricultural land use has been recognized as degradative to streams because of its nonpoint-source pollution and alterations to stream channel flows, riparian zones, and instream habitat (Allan 2004). Higher agricultural land use is associated with higher inputs of nutrients, sediments, and pesticides into streams. High nutrient loads support more algal growth in streams (Smith *et al.* 1999), which can lead to hypoxic conditions in localized areas of slow-moving water (Allan 2004). High sediment loads have also been shown to negatively impact primary producers, benthic macroinvertebrates, and fish through a number of mechanisms, including light obstruction, impaired respiration, and reduced suitability of substrate habitat (Wood and Armitage 1997; Piggott *et al*. 2015; Waite *et al*. 2019).

Conversely, elevated nutrient and sediment loads from increased agriculture could subsidize stream communities if they do not surpass a stress threshold. For example, nitrogen and phosphorus inputs could support biotic diversity due to their limited availability (Johnson *et al.* 2009), as shown in a study where higher numbers of macroinvertebrate taxa were found in pastoral lands with higher nutrient loads (Riley *et al.* 2003). Excessive sedimentation may interfere with feeding by collector-filterers if nets are buried by fine sediment, but minor increases in organic matter sedimentation may support their populations (Angradi 1999).

The North and South Sylamore watersheds in north Arkansas may provide a useful comparison by which to investigate the subsidy-stress gradient in relation to land use. North Sylamore Creek is located in the Springfield-Salem Plateau section of the Ozark Plateau in north-central Arkansas, and flows southeast into the White River (Mast and Turk 1999). South Sylamore Creek is located just south of North Sylamore Creek, and confluences with North Sylamore Creek just upstream of its confluence with the White River (Figure 1). North Sylamore Creek has remained mostly within a protected area (Ozark-St. Francis National Forest) since the early 1900's (Strausberg and Hough 1997); therefore, its land cover consisted primarily of forest and habitat surrounding North Sylamore Creek was relatively undisturbed (Mast and Turk 1999). Land cover in South Sylamore Creek catchment had more agricultural activity, with ~20% of the land cover consisting of pastoral land, but the catchment remained heavily forested (~ 75%). Based on these land-use patterns, it is possible to see a subsidizing effect of pastoral land on the South Sylamore stream community, as influenced by the physicochemical factors associated with agricultural land-use.

We hypothesized that in comparison to North Sylamore Creek, South Sylamore Creek would exhibit indictors of subsidy and/or stress based on responses to increased pasture land use. Increased runoff and nutrients from pasture land use may have resulted in increased basal food subsidies (*e.g.*, fine particulate organic matter and periphyton); therefore, we expected to find higher macroinvertebrate abundance, higher proportions of filter-feeding invertebrates, and higher proportions of scraping/grazing invertebrates and fish in response to food subsidies. In contrast, increased pasture may have led to harsher abiotic conditions (*e.g.*, lower dissolved oxygen, higher temperatures, higher contaminants, etc.) resulting in decreases in taxa richness and relative abundance of sensitive macroinvertebrates and fishes and increases in tolerant taxa in South Sylamore relative to North Sylamore. Through measuring overall habitat quality and community metrics in each system, we assessed whether South Sylamore Creek appears to be subsidized and/or stressed by moderate increases in pasture land use relative to the more forested North Sylamore Creek.

Methods

Study Site

Sylamore Creek is a spring-fed stream that flows east through the Springfield Plateau in the Ozark Highlands ecoregion of northern Arkansas. It is a 5th order tributary to the White River composed of two 4th order branches or subcatchments, North Sylamore Creek (NS) and South Sylamore Creek (SS). The two confluence to form Sylamore Creek approximately 0.8 river kilometers upstream of the White River. The NS flows through a highly forested, public land riverscape while SS flows through mainly private land. According to the National Land Cover Database 2011 (NLCD), land cover in NS is composed of 95% forest, 2% pasture, and 3% development. In the SS system, land cover consists of 72% forest, 23% pasture, and 5% development. We selected eight total sites on the longest perennial reach of each system: four sites on NS and four sites on SS (Figure 1). All four sites on NS were on the NS main stem: Barkshed (BD), Gunner Pool (GP), Blanchard Springs (BS), and Rosa Hole (RH). Four sites were selected along the Roasting Ear Creek/South Sylamore continuum as it represented the longest contiguous perennial segment: Roasting Ear/Mill Creek (RM), Roasting Ear Clearwater (RC), Double Bridge (DB), and

Angler's Resort (AR). All sites were sampled once during fall of 2018 (14-15 September or 12-13 October) (Table 1).

Land Use/Land Cover (LULC)

Upstream catchments of each site were delineated using StreamStats (United States Geological Survey). Using ArcGIS Desktop 10.6 and the 30-meter resolution National Land Cover Dataset (NLCD) 2011 raster file, land cover percentages (forest, pasture, and development) were calculated for each site's upstream catchment. Wooded riparian buffer width (up to 200 m) was calculated at each site using Google Earth satellite imagery and the measuring tool. Ten measurements (five equidistant measurements per bank) were made corresponding to reach length and averaged for comparison. Measurements greater than 200 m because of contiguous forest were recorded as "200" when calculating means.

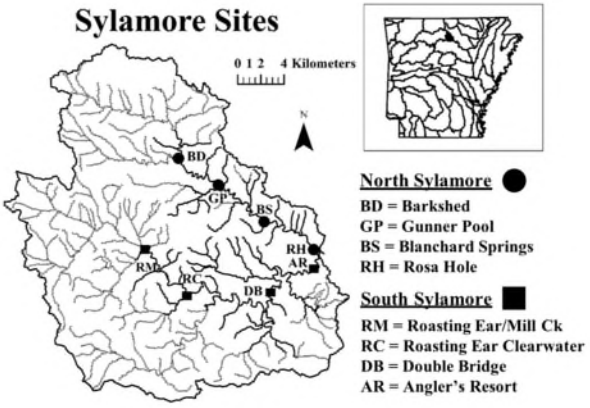

Figure 1. Sylamore Creek watershed with sites labelled corresponding to which system they are in. Dotted grey lines represent intermittency. Inset map in the top right represents the watershed shaded black.

Physicochemical

At each site we measured dissolved oxygen (DO; mg/L), specific conductivity (μS/cm), and temperature (°C) using a YSI 85 handheld water quality meter (Yellow Springs Instruments Inc., Yellow Springs, OH). Turbidity (NTU) was measured with a Hach 2100P Turbidimeter. Within the selected stream reach, we chose two representative riffles and depending on its length, created two or three transects perpendicular to the riffle, giving a total of four to six transects per site. We measured the width of each transect in meters. At each of five points distributed evenly across each transect, we measured depth and substrate (modified Wentworth Scale [Cummins 1962]: bedrock, boulder, cobble, pebble, gravel, sand/silt). At least one transect per riffle, we measured stream velocity using a Marsh-McBirney flow meter (FloMate 2000, Marsh-McBirney Inc., Frederick, MD) at five equidistant points to calculate discharge.

Filtered (500 ml) water samples were collected upstream at each site for both nitrate and phosphate. Water samples were kept on ice until laboratory analysis. Nitrate was analyzed following the Cadmium Reduction Method (Hach 2015; Method #8192). Phosphate was analyzed using the Ascorbic Acid Method (Hach 2015; Method #8048).

Unfiltered (1 L) water samples were collected upstream at each site for total dissolved solids (TDS) and total suspended sediments (TSS). Water samples were kept on ice until laboratory analysis. To measure TSS, the unfiltered water sample was filtered through an ashed, pre-weighed Whatman® glass microfiber filter (4.7 cm diameter; 1.6 microns pore size) and then weighed again after drying in the oven at 60 °C. Difference in dry filter weight before and after filtration was TSS (mg/L). TDS was measured by collecting the filtrate from TSS in a pre-weighed container. Containers were placed in the drying oven at 60 °C for the liquid to evaporate, and the container was re-weighed to get TDS (mg/L).

Chlorophyll a

Six periphyton samples per site were collected using a divot sampler (sample area equaled 4.91 cm^2) following Lamberti and Steinman (1997) and Burgad *et al.* (2018). Periphyton samples were collected at approximately 25%, 50%, and 75% of wetted width. Specific sampling locations were determined by haphazardly tossing a 0.5 m^2 polyvinyl chloride frame and sampling the largest rock within the frame. Divot samples were primarily taken on cobble in riffles at depths of 2-55 cm. A spherical crown densiometer was used to measure canopy cover corresponding with each periphyton sample. Samples (periphyton slurries) were filtered in the field with pre-weighed filters and kept on ice until laboratory analysis.

The laboratory analysis followed Havel (2016), where filters were heated in a 95% ethanol solution (5 minute exposure at 78°C), kept in the dark (24 hrs), and centrifuged. A 0.25 M HCl acid addition was used to correct for pheophytin (degradation products) in samples. Concentrations of chlorophyll *a* (μg/L) were determined using the volumetric formula described in Havel (2016). In some instances, the entire sample extracted with the divot sampler could not be filtered; therefore, we recorded volume filtered in the field for each sample and used the volumetric formula.

Macroinvertebrates

Standard benthic macroinvertebrate collections were made using a 25.4 cm x 30.5 cm D-frame dip net having 500 micrometer mesh generally following Barbour *et al.* (1999). A composite sample for each site consisted of six kick sets stratified across riffles similar to periphyton samples. Substrate was disturbed by foot ~ 0.5 m upstream of the dip net, and any large substrates were rubbed by hand. Samples were preserved in 70% ethanol. All individuals were sorted and identified in the laboratory using Merritt and Cummins (1996), McCafferty (1998), and Voshell (2002). Identifications were to the family level with the following exceptions: order level for Amphipoda, Isopoda, and Oligochaeta, genus level for *Hexatoma* sp. and *Corbicula* sp., and Chironomidae were separated into either sub-family Tanypodinae or non-Tanypodinae.

Functional feeding group designations were made following Barbour *et al.* (1999) and Cummins *et al.* (2005). Taxa assigned to multiple feeding groups were split evenly across groups following Greathouse and Pringle (2006). Tolerance values were assigned to taxa based on multiple sources in an attempt to assign values that best fit taxa in our watershed. Preference was given to values reported from systems in proximity to Arkansas. Tolerance values were predominantly taken from Bressler *et al.* (2006) since they dealt with macroinvertebrates in Mississippi. Taxa not listed in Bressler *et al.* (2006) were taken from Lenat (1993), as those values reflected macroinvertebrates in North Carolina. Lastly, tolerance values for any remaining unassigned taxa were taken from Barbour *et al.* 1999. Tolerant taxa had values equal to or greater than 7, and intolerant taxa had values ≤ 3 (Hotz 2010). Hilsenhoff Biotic Index values were calculated for each site (Hilsenhoff 1987). Biotic index values ranged from 0 to 10, where 0 indicated excellent water quality and 10 indicated very poor water quality.

Fishes

Fishes were sampled in riffles, runs, and pools within a 174 to 317 m reach at each site. Fish were sampled in different riffles than macroinvertebrates and periphyton. Composite fish samples per macrohabitat were collected using a 1.2 x 4.6 m seine (pools and runs) and a 1.2 x 2.4 m seine (riffles), both having a mesh size of 0.48 cm. Macrohabitats were exhaustively sampled with kick sets and hauls as described by Matthews (1986 and 1990) and Burgad *et al* (2018). Larger individuals collected were identified, counted, and released in the field. Smaller fishes were fixed in 10% formalin and returned to the lab for identification (UCA IACUC Protocol # 17-004). All specimens were identified to species, stored in 70% ethanol, and catalogued into the UCA Fluid Vertebrate Collection. Fishes were classified as tolerant or sensitive following Dauwalter *et al.* (2003) for Ozark fishes. Percent relative abundances of Centrarchidae and grazing fishes (*Campostoma* spp.) were calculated as common indicators of disturbance in Ozark streams (Dauwalter *et al.* 2003).

Data analysis

Most statistical tests were performed in R (version 3.4.4; R Core Team 2018). Means of abiotic and biotic variables comparing SS (n=4) versus NS (n=4) were evaluated with Student's t-test (Welch's adjustment). A Log_{10} transformation was performed with data that did not meet assumptions of normality. If normality could not be attained, means were tested with Wilcoxon Rank Sums Test. Spearman's rank correlation (rho) examined relationships between key continuous variables of interest (Chl *a* versus canopy cover, nutrients, and grazers). Macroinvertebrate FFGs and fish assemblage structures were evaluated with Nonmetric Multidimensional Scaling (NMDS) ordination performed in PCORD Version 6. Relative abundances of FFGs and fishes were arcsine square root-transformed. Distance matrices were constructed using Bray-Curtis dissimilarity. Multi-response permutation procedures (MRPP) tested null hypotheses that FFG composition and fish community structure were equal between NS and SS. Significance was determined using an alpha level of 0.05.

Results

Land use/land cover

Upstream catchments of sites in NS ranged from 83.9 to 197.9 km^2 and those in SS 119.2 to 367.8 km^2 (Table 1). Larger catchment sizes in SS were confounded by much of the upstream portions of the watershed being intermittent (Figure 1). All sites in NS had nearly 95% forest cover within upstream catchments. All sites in SS had at least 20% less forest cover in their upstream catchments. Conversely, SS catchments had 19-25% pasture while NS had less than 3%. There was a small portion of development in every upstream catchment, but most of the SS sites had slightly more development from sprawl of Mountain View in the southeast corner of the watershed.

Mean wooded riparian buffer was highest at NS sites (146.4 m) relative to SS sites (37.1 m) ($p = 0.03$). Mean riparian buffer across NS sites ranged from

200.0 m at Barkshed to 103.0 m at Rosa Hole. Mean wooded riparian buffer across SS sites ranged from 67.0 m at Roasting Ear/Clearwater to 21.9 m at Angler's Resort.

Physicochemical

Pebble was the dominant substrate at all sites except Blanchard Springs (NS), where bedrock was dominant (Table 1). Riffle depth (0.09-0.23 m), riffle width (7.8-16.9 m), and discharge (0.1-0.4 m^3/s) were relatively similar across sites; however, values were higher at Angler's Resort (SS) (discharge was 0.7 m^3/s). Temperature and dissolved oxygen were mostly typical for the region, but DO level at Angler's Resort (6.12 mg/L) was lower than expected and was somewhat lower at Double Bridge (7.09 mg/L) relative to measurements at other sites. Mean canopy cover tended to be greater at NS (54.4%) compared to SS (37.1%), but the difference was not significant (p = 0.11).

Average total suspended sediment (TSS) tended to be greater in SS (71.3 mg/L) compared to NS (57.5 mg/L), but the difference was not significant. (p = 0.34) (Table 1). Roasting Ear at Clearwater had highest TSS (88.8 mg/L), and Blanchard Springs had lowest TSS (42 mg/L). Mean turbidity was not different at SS (2.19 NTU) and NS (1.54 NTU) sites (p = 0.80).

Mean conductivity was significantly greater in SS (317.9 µS/cm) compared to NS (272.3 µS/cm) (p = 0.01). Roasting Ear at Mill Creek had highest conductivity (340.1 µS/cm), and Rosa Hole had lowest conductivity (252.7 µS/cm) (Table 1). Mean total dissolved solids (TDS) tended to be higher in SS (99.6 mg/L) compared to NS (76.5 mg/L), but the difference was not significant (p = 0.24). Blanchard Springs had highest TDS (120.8 mg/L), and Barkshed had lowest TDS (48.4 mg/L).

Mean nitrate concentration was significantly higher at SS (1.375 mg/L) compared to NS (0.75 mg/L) sites (p = 0.03). Roasting Ear at Mill Creek had highest nitrate concentration (1.7 mg/L) and Gunner Pool had lowest (0.6 mg/L) (Table 1). Mean phosphate concentration tended to be higher at SS (0.22 mg/L) compared to NS (0.07 mg/L) sites, but this difference was not significant (p = 0.30).

Chlorophyll a

Mean chlorophyll *a* concentrations were highly variable and not significantly different at SS (771.0 ± 232.70 SD µg/L) and NS (1862.5 ± 431.34 SD µg/L) (p = 0.20) sites (Table 1). Rosa Hole had highest average chlorophyll *a* concentration (3592.0 ± 1043.1 SD µg/L), and Double Bridge Loop had lowest (175.5 ± 74.3 SD µg/L). Chlorophyll *a* concentrations were not associated with canopy cover (r_s = 0.14), nitrate concentrations (r_s = - 0.33), phosphate concentrations (r_s = 0.03) or sampling date (r_s = 0.34, p = 0.40). However, chlorophyll *a* was positively associated with macroinvertebrate grazer relative abundance (r_s = 0.71, p = 0.04). Interestingly, there was a trend for a negative association between chlorophyll *a* and relative abundance of the algivorous fishes *Campostoma* spp. (r_s = - 0.42, p = 0.29).

Macroinvertebrates

We collected and identified 9,750 individuals that encompassed 47 taxa. In total, SS sites contained 6,192 individuals representing 39 taxa, and total abundance at NS sites was 3,558 individuals and 40 taxa (Table 2). Mean number of individuals tended to be higher at SS (1,548.0 ± 497.3 SD) sites relative to NS sites (889.5 ± 252.3 SD), but this difference was not significant (p = 0.07). Most abundant taxa were Psephenidae, Elmidae, Amphipoda, Heptageniidae, and Hydropsychidae. These taxa were mostly ubiquitous across sites; however, Amphipoda was predominately collected at Roasting Ear/Mill Creek. Both catchments had 7 unique taxa, and taxa richness did not vary between catchments (p = 0.94). The nonnative Asian clam (*Corbicula fluminea*) was only collected in SS.

Both NS and SS each had 11 sensitive taxa. The most abundant sensitive taxa were Perlidae, Corydalidae, and Helicopsychidae (Table 2). NS (0.61) had a higher mean proportional abundance of sensitive taxa relative to SS (0.40) (p = 0.02). Both NS and SS each had 7 tolerant taxa. The most abundant tolerant taxa were Caenidae, Coenagrionidae, and predatory Chironomidae. Both NS (0.06) and SS (0.08) had similar mean relative abundances of tolerant taxa (p = 0.65). All sites in both systems had biotic index values that ranged from 3.2 - 4.3 (Table 1) and were therefore classified as "Excellent" water quality (HBI = 0.00 - 3.50) to "Very Good" water quality (HBI = 3.51 - 4.50) (Hilsenhoff 1988). Mean HBI of NS sites (3.37) was within the "Excellent" range while mean HBI of SS sites (3.85) was within the "Very Good" range.

Relative abundance patterns of functional feeding groups differed somewhat between NS and SS. SS tended to have higher proportional abundance than NS of filtering collectors (23.3, 8.8) and shredders (6.9, 0.4). NS tended to have higher proportional abundance than SS of predators (11.6, 7.8) and scrapers (59.5, 41.8). Proportion of collector gatherers was about 20% of total for both systems. Further, NMDS indicated NS and SS sites segregated in multivariate space based on functional feeding

Table 1. Land use and physicochemical characteristics of all sites sampled in the Sylamore watershed during 2018.

	Barkshed 14 Sept	Gunner Pool 13 Oct	Blanchard Springs 15 Sept	Rosa Hole 15 Sept	REC/Mill Creek 12 Oct	REC/ Clearwater 12 Oct	Double Bridge 16 Sept	Angler's Resort 15 Sept
Catchment Size (km²)	83.9	130.0	182.3	197.9	119.2	297.8	328.9	367.8
Reach Length (m)	317.0	287.0	143.0	142.2	148.0	201.0	160.0	191.6
GPS Coordinates	36.0195 -92.2495	35.9955 -92.2126	35.9681 -92.1689	35.9433 -92.1236	35.9488 -92.2866	35.9135 -92.2466	35.9119 -92.1662	35.9354 -92.1219
Mean Riffle Depth (m) (± 1 SD)	0.09 (0.07)	0.12 (0.04)	0.11 (0.06)	0.10 (0.05)	0.23 (0.9)	0.14 (0.05)	0.14 (0.10)	0.14 (0.07)
Dominant Riffle Substrate	Pebble (40%)	Pebble (35%) & Cobble (30%)	Bedrock (40%)	Pebble (67%) & Gravel (37%)	Pebble (73%)	Pebble (67%) & Cobble (30%)	Pebble (67%)	Pebble (64%)
Water Temperature (°C)	28.5	16.1	25.8	27.2	15.6	17.8	24.6	24.4
Specific Conductivity (µS/cm)	277.9	293.8	264.9	252.7	340.1	318.6	309.1	303.9
Dissolved Oxygen (mg/L)	7.5	8.4	7.9	9.2	8.3	8.2	7.1	6.1
Mean Canopy Cover (%) (± 1 SD)	53.3 (14.1)	37.9 (6.3)	45.8 (8.2)	80.4 (12.1)	39.1 (14.7)	43.4 (16.6)	11.0 (8.6)	34.8 (9.4)
Nitrate Concentration (mg/L)	0.80	0.60	0.70	0.90	1.7	1.5	1.2	1.1
Phosphate Concentration (mg/L)	BDL	0.12	BDL	0.15	0.21	0.02	0.10	0.56
Total Dissolved Solids (mg/L)	48.4	74.0	120.8	62.8	94.0	96.0	106.8	101.6
Total Suspended Sediment (mg/L)	50.8	73.2	42.0	64.0	84.9	88.8	66.2	45.2
Mean Chlorophyll *a* (µg/L) (± 1 SD)	2223.4 (2134.7)	1405.0 (1483.8)	229.5 (216.4)	3592.0 (2557.0)	261.8 (271.4)	1386.0 (1654.3)	175.5 (182.2)	1263.8 (1285.3)
% Forest	96.5	95.9	94.7	94.8	76.9	78.0	70.6	72.2
% Pasture	1.0	1.4	2.0	2.0	19.2	18.4	24.9	22.7
% Developed	2.5	2.7	3.3	3.2	3.9	3.6	4.5	5.1
Mean Wooded Buffer (m) (± 1 SD)	>200.0 (0.0)	128.7 (72.6)	153.9 (57.2)	103.0 (60.1)	23.8 (6.4)	67.0 (46.7)	35.7 (35.5)	21.9 (21.7)
Hilsenhoff Biotic Index (HBI)	3.6	3.2	3.3	3.4	3.6	3.3	4.2	4.2

Table 2. Aquatic macroinvertebrate taxa counts in North and South Sylamore. Amphipoda and Elmidae were classified under multiple FFGs (Greathouse and Pringle 2005). Chironomidae taxa were separated into Tanypodinae (T) or Non-Tanypodinae (NT).

Taxon	North Sylamore	South Sylamore	Tolerance Value
	Shredders		
Amphipoda (1/3)	0.33	505.67	
Capniidae	7	-	Sensitive
Haliplidae	2	-	Tolerant
Leuctridae	4	4	Sensitive
Tanyderidae	2	1	
Tipulidae	2	20	Sensitive
	Filtering Collectors		
Corbicula	-	3	
Hydropsychidae	143	806	
Isonychiidae	135	474	
Philoptamidae	5	6	Sensitive
Polycentropodidae	3	-	
Simulidae	27	7	
Sphaeridae	-	5	Tolerant
	Gathering Collectors		
Amphipoda (1/3)	0.33	505.67	
Baetidae	17	63	
Caenidae	39	270	Tolerant
Chironomidae - NT	60	27	
Elmidae (1/2)	594	210.5	
Ephemeridae	3	-	
Ephemereliidae	-	1	Sensitive
Hydrophilidae	2	-	
Hydroptilidae	1	3	
Isopoda	4	44	Tolerant
Leptophlebiidae	4	1	Sensitive
Limnichidae	-	1	
Oligochaeta	37	13	
Siphlonuridae	1	-	Tolerant
Tricorythidae	7	47	
	Scrapers		
Amphipoda (1/3)	0.33	505.67	
Elmidae (1/2)	594	210.5	
Helicopsychidae	1	92	Sensitive
Heptageniidae	515	675	
Petrophila	4	-	
Physidae	-	1	Tolerant
Planorbidae	1	1	Tolerant
Pleuroceridae	191	257	
Psephenidae	727	1,029	
	Predators		
Aeshnidae	7	2	Sensitive
Calopterygidae	3	6	
Chironomidae - T	46	35	Tolerant
Coenagrionidae	54	29	Tolerant
Corydalidae	42	113	Sensitive
Gerridae	-	1	
Gomphidae	2	6	Sensitive
Gyrindae	-	1	
Hexatoma	9	-	
Perlidae	235	196	Sensitive
Perlodidae	1	1	Sensitive
Tabanidae	4	12	
Veliidae	22	1	

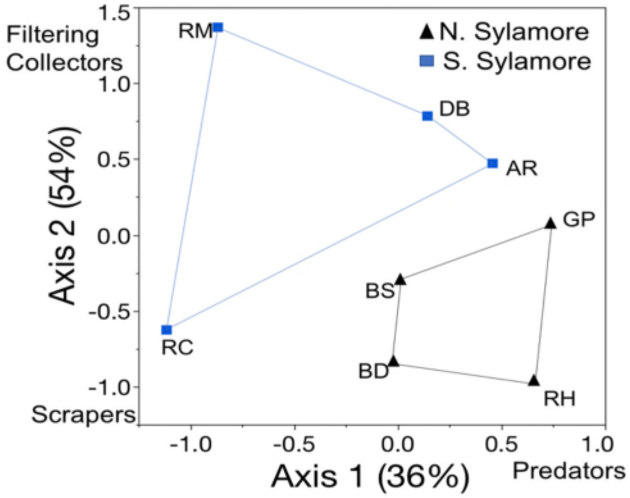

Figure 2. NMDS plot of aquatic macroinvertebrate relative abundance with sites in Functional Feeding Group space for North Sylamore (triangles) and South Sylamore (squares). For Site ID see Figure 1. Correlated functional feeding groups are labeled on each axis.

group relative abundances (Figure 2). NMDS Axis 1 (r_s = -0.04, p = 0.93) and Axis 2 (r_s = 0.38, p = 0.35) were not associated with sampling date. MRPP indicated there was not a significant grouping of sites based on functional feeding group composition, but there was a strong trend (p = 0.07). Filtering collectors were more associated with SS sites, and scrapers and predators were more associated with NS sites. Functional feeding group composition seemed to vary more among SS sites, particularly REC/Mill Creek and REC/Clearwater (Figure 2).

Fishes

Seining of riffle, run, and pool habitats within NS system yielded 1,333 individuals (23 species) and 2,364 individuals (31 species) in SS (Table 3). *Luxilus pilsbryi*, *Notropis nubilus*, *Lepomis megalotis*, and *Etheostoma caeruleum* were abundant in both systems. In total, SS had more tolerant taxa (12) than NS (7). Tolerant fishes *Luxilus chrysocephalus*, *Ameiurus natalis*, *Labidesthes sicculus*, *Lepomis cyanellus*, *Lepomis macrochirus*, and *Percina caprodes* were not detected in NS. *Semotilus atromaculatus* was the only tolerant species unique to NS (Table 3). Mean relative abundance of tolerant fishes in NS (0.29) was similar to SS (0.33). Total number of sensitive taxa was about equal between NS (16 species) and SS (19 species). *Noturus albater* was unique to NS, and *Chrosomus erythrogaster*, *Cottus immaculatus*, *Ambloplites constellatus*, and *Etheostoma zonale* were unique to SS.

Mean relative abundance of sensitive taxa in NS (0.71) was not significantly different from SS (0.66) (p = 0.39).

Mean relative abundance of centrarchids was not different between NS (0.09) and SS (0.08) (p = 0.48). Over five-fold more stonerollers were collected in SS (Table 3), but mean relative abundance of *Campostoma* spp. was not significantly different in NS (0.03) relative to SS (0.05) (p = 0.30).

NMDS suggested NS and SS sites had different fish assemblage compositions given the segregation in the ordination (Figure 3), but this difference was not significant (MRPP, p = 0.13). NMDS Axis 1 ($r_s = 0.26$, $p = 0.53$) and Axis 2 ($r_s = 0.67$, $p = 0.07$) were not associated with sampling date. NS sites and Angler's Resort tended to be associated with *Fundulus catenatus*, *Lepomis megalotis*, and *Etheostoma caeruleum*. Tolerant taxa *Notropis nubilus* and *Gambusia affinis* were most associated with SS sites Double Bridge and Angler's Resort. Based on distances observed in the ordination, there was more variation in fish assemblages across SS sites than NS sites (Figure 3).

Table 3. Total abundances of fishes collected in North and South Sylamore. * indicates a tolerant species (Dauwalter *et al*. 2003).

	North Sylamore	South Sylamore
Scientific Name	Total	Total
Campostoma. spp.*	31	163
Chrosomus erythrogaster	-	214
Cyprinella galactura	1	9
Hybopsis amblops	2	1
*Luxilus chrysocephalus**	-	6
Luxilus pilsbryi	391	828
Nocomis biguttatus	11	9
Notropis boops	1	40
*Notropis nubilus**	253	185
Notropis percobromus	2	78
Notropis telescopus	161	84
*Semotilus atromaculatus**	2	-
*Ameiurus natalis**	-	1
Noturus albater	4	-
Noturus exilis	3	1
*Labidesthes sicculus**	-	79
Fundulus catenatus	139	52
*Fundulus olivaceus**	59	64
*Gambusia affinis**	17	112
Cottus carolinae	10	7
Cottus immaculatus	-	22
Ambloplites constellatus	-	7
*Lepomis cyanellus**	-	1
*Lepomis macrochirus**	-	3
*Lepomis megalotis**	89	205
Micropterus dolomieu	24	8
Etheostoma blennioides	1	1
Etheostoma caeruleum	110	98
Etheostoma flabellare	14	43
Etheostoma juliae	4	13
*Etheostoma spectabile**	4	8
Etheostoma zonale	-	21
*Percina caprodes**	-	1
Total Abundance	1,333	2,364
Total Species	23	31

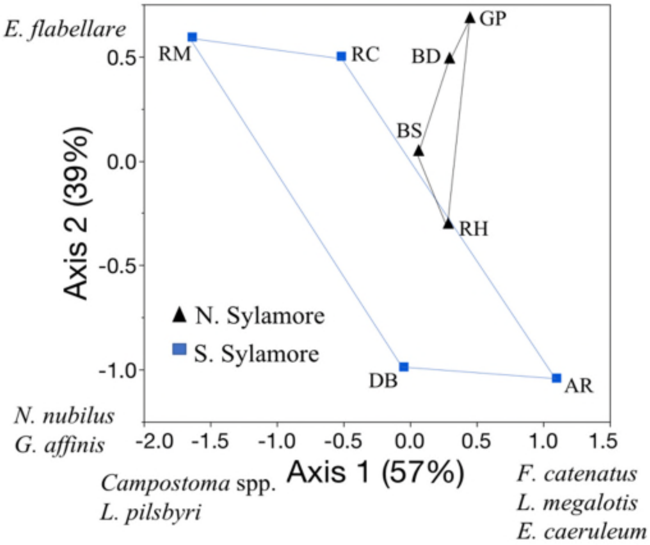

Figure 3. NMDS plot of sites in fish species space for North Sylamore (triangles) and South Sylamore (squares). For Site ID see Figure 1. Correlated species are labeled on each axis.

Discussion

Overall, physicochemical and biotic variables indicated both NS and SS are high quality Ozark streams that warrant attention from natural resource managers. For example, the macroinvertebrate-based HBI indicated both had very good to excellent water quality. Presence of 19 to 25% pasture in upstream catchments, in combination with a narrower wooded riparian buffer, created the potential for SS to have received more perturbations through time compared to NS. Indeed, some water quality parameters measured were indicative of watershed alteration in SS, providing the opportunity to study biotic responses to relatively low levels of disturbance within a subsidy-stress framework.

Physicochemical

In accordance with predicted responses to increased pasture land use within catchments, SS had higher concentrations of nitrate and tended to have higher concentrations of phosphate, TSS, and TDS. Similar results have been found in numerous other studies, where higher agricultural land use is associated with higher nutrient runoff into surrounding water bodies due to fertilizer inputs (Vitousek *et al.* 1997; Carpenter *et al.* 1998). Some of the nitrogen and phosphorous values measured in Sylamore, particularly SS, were similar to mean total phosphorous (~ 0.2-0.3 mg/L) and total nitrogen (~ 1.5-2.0 mg/L) measured in nutrient enriched Central Plains streams (Evans-White *et al*. 2009). Our sampling protocol was not ideal to detect alterations to dissolved oxygen; however, we did measure moderately low values for the Ozarks at two SS sites. Animal waste from domestic animals is a potential source of increased carbon and nutrients to SS. Higher rates of erosion and runoff predictably increase TSS and TDS as observed in SS (Hudson-Edwards 2003). A recent study in India found turbidity and TSS increased by 8.41% and 4.17% respectively, with every one percent decrease in forest cover (Singh and Mishra 2014). Chase *et al.* (2017) used experimental mesocosms to show that nutrient subsidy increased macroinvertebrate abundances in all taxa; however, when added with increased TSS, there was a negative stress effect in less tolerant species. Further research is needed to determine sources of increased nutrients and other solids in SS.

Chlorophyll a

Higher rates of nutrient runoff were predicted to occur in association with agriculture/pasture, and SS was therefore predicted to support higher algal growth (Smith *et al.* 1999; Dodds *et al*. 2002), potentially having a subsidizing effect on SS food webs. To our surprise, there were no differences in chlorophyll *a* values, and they even trended to be lower in SS despite elevated nutrients. Low canopy coverage and high nutrients are typically associated with higher algal abundance, as documented in previous studies (Hill and Knight 1988). However, we found no indications that chlorophyll *a* was related to canopy cover or nutrients.

Benthic algae appeared to be an important food source in both NS and SS, and biotic factors may have played a larger role than abiotic factors in its pattern of abundance during our sampling. Autotrophy can be an important carbon source in headwater streams (Minshall 1978), and chlorophyll *a* was higher than expected in headwaters of NS and SS based on predictions of the river continuum concept (Vannote *et al*. 1980) and published values. For Sylamore chlorophyll *a* divot samples that could be expressed as biomass per unit area, five were greater than 10 μg/cm^2 and seven samples were between 5 and 9 μg/cm^2; values greater than 10 μg/cm^2 fall within the "excessive" range for temperate streams (Dodds *et al*. 2002). Sylamore stream reaches had relatively open canopies, low turbidity, and stable substrates, and nutrients did not appear to be limiting, particularly in SS. Relative abundances of scraper macroinvertebrates, particularly Psephenidae, were relatively high (40-60%) in both headwater systems. Macroinvertebrate grazers across all sites seemed to be responding positively to increased benthic algae (as indicated by chlorophyll *a*). Hillebrand and Kahlert (2001) found that grazer presence had a stronger effect on algal biomass than nutrient enrichment, and Gregory (1980) observed that high (13.3 g/m^2) and intermediate (6.7 g/m^2) densities of grazers resulted in significant decreases in chlorophyll *a* concentrations compared with low (1.1-2.2 g/m^2) grazer densities. Abundance of the algivorous fish *Campostoma* was moderately negatively associated with chlorophyll *a* across our sites and this fish tended to be more abundant in SS. This pattern further represents evidence of the potential for top-down influence during our study. Seemingly elevated benthic algal production in the headwaters of both NS and SS need further study.

Macroinvertebrates

Trends for increased total number of macroinvertebrates in SS, particularly the filtering collectors, were indicative of a subsidy-related response. Pasture runoff has likely directly and/or indirectly resulted in increased or enriched food resources in SS (mechanisms reviewed by Evans-White *et al*. 2009). For example, high abundances of Hydropsychidae and Isonychiidae indicated SS must have an abundance of fine particulate organic matter in transport. Enhanced autotrophic and detrital resources and its consumption in SS probably lead to more fine organics in transport. Pasture land use has probably subsidized basal food resources for macroinvertebrates in SS, but insight here is limited without increased spatial and temporal sampling.

Similarity in overall macroinvertebrate richness and number and abundances of tolerant and sensitive taxa between NS and SS is consistent with a subsidy-stress gradient where nutrient input is still usable by the primary producers, and not yet enough to cause a stress response in the aquatic ecosystem (Odum *et al* 1979). Abundances of tolerant and sensitive species present in a system are often used, among other metrics, as indicators of water quality (Resh and Unzicker 1975). As water quality decreases, the amount of tolerant taxa increase while the amount of sensitive taxa decrease

(Lenat and Crawford 1994). Though proportional abundance of tolerant taxa was almost the same between the two watersheds, NS had a higher proportional abundance of sensitive taxa than SS, suggesting there could be some level of stress to sensitive taxa in SS. In support, Hilsenhoff Biotic Index (HBI) values were also slightly lower in SS relative to NS. Both study systems had good water quality, and alterations from increased pasture land use have probably had a subsidizing effect on many macroinvertebrates in SS but caused a degree of stress to some sensitive taxa.

Fishes

Similar to macroinvertebrates, fish assemblage data from NS and SS suggested responses to subsidies in SS and some evidence of stress. *Campostoma* spp., and to some extent *Notropis nubilus*, predominately feed on periphyton (Pflieger 1997) and tended to have greatest abundances in SS. These species probably benefit from increased food resources in SS. Scott and Helfman (2001) described a common phenomenon across the southeastern United States of lowland fishes expanding ranges into upland reaches of streams degraded by land use change (*e.g.*, increased pasture). These fishes tolerate aspects of the perturbation (*e.g.*, increased fine sediments) and benefit from enhanced/increased food resources, often feeding in the water column. We collected a number of fishes that are denizens of lowland habitats, often feeding in the water column (Pflieger 1997), that were unique to or most associated with SS: *Labidesthes sicculus*, *Gambusia affinis*, *Lepomis cyanellus*, and *Lepomis macrochirus*. These fishes were captured at the lowermost sites in SS, and therefore, had access to Rosa Hole in lower NS but were not detected there except for *Gambusia affinis* (in much lower abundance). Resource subsidies in SS seemed to have benefited fishes that feed directly on basal resources and probably have allowed expansion of some native lowland species.

Some sensitive fishes had interesting patterns of abundance and occurrence in NS and SS. *Chrosomus erythrogaster* and *Cottus immaculatus* were only detected in SS at either Roasting Ear/Mill Creek or Roasting Ear Clearwater. Both of these species tend to be associated with springs (Pflieger 1997), and a large spring occurs on Mill Creek that probably influenced these collections. Large springs were not present in or near our study reaches on NS, and we know from previous research that *Chrosomus erythrogaster* occurs further upstream within the intermittent reaches of NS (Walker *et al*. 2013) and in smaller tributaries (Mitchell *et al*. 2012). *Noturus albater* was detected at three out of the four sites in NS, and was not detected at SS sites. Two individuals were collected at Blanchard Springs, one was collected at Barkshed, and one was collected at Rosa Hole. *Noturus albater* is a sensitive taxon (Dauwalter *et al*. 2003) endemic to the Ozark region of southern Missouri and northern Arkansas (Pflieger 1997). This species is typically associated with clear, high-gradient streams, in gravel riffles (Pflieger 1975), and it may be vulnerable to conditions in SS. However, our study is limited by only sampling during a single season. More extensive collecting, both temporally and spatially, is needed to better understand fish distribution patterns in Sylamore Creek.

Conclusions

The subsidy-stress framework helped to understand the current ecological status of two subcatchments within the same watershed but having different land use patterns. Relative to NS, SS showed evidence of enhanced biological response consistent with inputs of usable resources (*e.g.*, nutrients and probably organic matter) related to increased pasture land use (*sensu* Odum *et al*. 1979). Macroinvertebrate and fish data suggested the amount of perturbation in SS has become a stressor to some taxa, but the overall communities remain healthy. Amounts of pasture land use within upstream catchments of SS (19-25%) are less than thresholds reported to significantly stress macroinvertebrates (> 30%; Quinn and Hickey 1990) and fishes (> 50%; Wang *et al*. 1997) in other studies. However, land use alterations in SS are approaching these thresholds, and the watershed could be vulnerable to persistent effects of pasture. An additional future concern is higher amounts of development in SS and the subsequent potential for multiple stressors and their interactions impacting this stream. Most sites in SS, and a few specific locations in NS, had narrow or sparsely wooded riparian zones. A potential way to limit inputs from pasture into the streams is to enhance forest buffer strips, particularly along SS. These strips act like fences to effectively mitigate the movement of sediment, contaminants, and nutrients during surface runoff (NRCS 2010). Using the hypothetical performance curve predicted by the subsidy-stress hypothesis (Odum *et al*. 1979), land use change in SS may have moved this system outside the "normal operating range" and into the "subsidy effect" range based on comparisons with NS. However, this is difficult to establish without comparisons with historical conditions and more extensive sampling. Additional sampling will allow improved evaluation of the variance and stress components of the hypothesis predicted to increase with increased perturbation.

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Review

Cadmium Sulfide-buffered Photovoltaic Systems: Assessing the Environmental, Health, and Economic Impacts

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Running Title: Cadmium Sulfide-buffered PV Systems

Abstract

As the world's population continues to grow, it is expected that global energy demand will also continue to rise in the future. This projected growth in energy demand coupled with increasing awareness on carbon emissions and global climate change associated with the use of fossil fuels has accelerated the demand for various renewable energy technologies, including solar photovoltaics (PV). PV technology is currently undergoing a transformation with development of several thin film technologies such as perovskite solar cells which not only offers higher efficiency and scalability, low-cost production, but also non-toxicity and stability. Cadmium sulfide (CdS) is a widely used buffer material in thin film PV, which has significant advantages over other alternate buffer materials in terms of efficiency and low-cost of production for large-area processing of thin films. However, the potential environmental risks associated with the use of cadmium are of concern. In this paper, we assess and monetize environmental, health, and socio-economic externalities associated with the use of CdS. We quantified the environmental, human health, and socio-economic impacts of cadmium emissions from CdS-buffered PV system. In addition, this paper provides a comprehensive outlook of the past, current, and future global market growth rate of thin film PV technologies.

Introduction

Solar PV technology converts solar energy directly into electrical energy using the optoelectronic properties of the suitable semiconductor materials. Buffer layers are commonly used in optimization of thin film solar cells by forming a reliable p-n junction with the absorber layer and allowing maximum transmission of light (minimum absorption loss) to the junction region and the absorber layer (Friedlmeier *et al*. 2017). Buffer layers passivate the junction material, providing a layer of appropriate thickness and index of refraction that reduces the overall reflectance, while avoiding shunts between the absorber and the front electrode (Mughal *et al*. 2015; Wennerberg 2002).

Figure 1 illustrates the structure of the solar cell that utilizes n-type CdS and p-type cadmium telluride (CdTe) as buffer and absorber materials respectively. The buffer layer is sandwiched between the absorber layer and the anti-reflection coating (ARC). A rear contact (highly transparent conducting oxide, such as tin oxide) and a front contact (metal electrode, such as nickel or aluminum) are used to carry excited electrons in the conduction band, across the junction from the n-type to the p-type semiconductor, to an external load. These electrons then dissipate their energy into an external circuit and returns to the rear contact of the

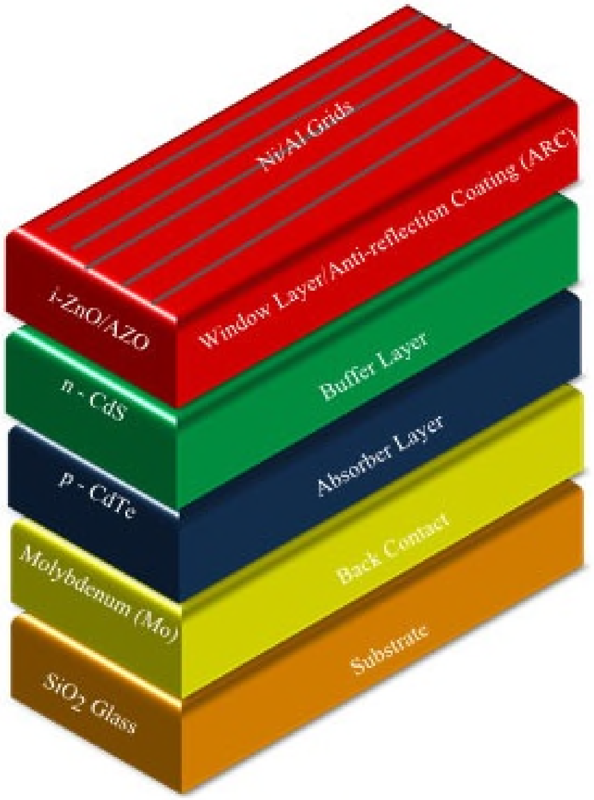

Figure 1. n-CdS/p-CdTe PV cell structure.

PV cell (Oladeji 2000; Mughal *et al*. 2014). The absorber layer constitutes the core of any PV device; however, the junction interface properties between the absorber and buffer layers are also quite significant to the performance of the device (Hultqvist 2010). The interfacial properties of buffer layer affect efficient charge separation and transfer.

CdS is the oldest and most widely known buffer material which was initially used in solar cells for aerospace applications back in the 1950's (Reynolds *et al*. 1954). It is an important n-type semiconductor material with an optical bandgap of 2.42 eV (Naghavi *et al*. 2010). The commercially available CdTe and copper indium gallium diselenide/copper indium diselenide (CIGS) technologies utilize CdS buffer layer to form a heterojunction interface (Rix *et al*. 2015). Chemical bath deposited (CBD) CdS-based PV devices yield good performances, however, there are drawbacks concerning industrial-upscaling from the use of the carcinogen thiourea and hazardous cadmium (Cd) in large amounts (Paris and Gmbh 2005). CBD encompasses a variety of routes for synthesizing thin films at a relatively low temperature by immersing a substrate in a liquid solution (Feitosa *et al*. 2004). The process involves generation of S^{-2} ions in the presence of an aqueous alkaline bath containing a Cd salt, which results in the precipitation of CdS. Deposition of CdS is based upon the reaction between the precursors in a metastable condition allowing large area processing with low fabrication costs (Mughal *et al*. 2015). Other depositions techniques such as atomic layer deposition (ALD) and sputtering are quite expensive as compared to the CBD. Several studies have emphasized upon the replacement of CdS in chalcopyrite PV devices due its toxicity (Hultqvist 2010; Naghavi *et al*. 2010; Paris and Gmbh 2005). However, the PV industry is reluctant to replace CdS, as it is a proven cost-effective material which can reach energy conversion efficiencies of 23.3% for a 0.5 cm^2 laboratory cell (Green *et al*. 2017). Although research is underway to evaluate alternatives for CdS, the most efficient heterojunction PV devices still utilize a CdS buffer layer (Ward *et al*. 2014). Table 1 summarizes the operational performance of CdS-buffered PV systems synthesized by different deposition techniques.

The toxicity of Cd compounds (CdTe, CdS, etc.) is well documented (T.E.P.A.T.C.O and European Union 2003). They can enter the environment from many different sources such as manufacturing site, landfills, incinerators, etc. These chemicals can move through air, soil, and water contaminating the environment. Human exposure to these hazardous chemicals by inhalation, ingestion, or skin contact poses a great risk to human health and social conditions (Tchounwou 2012). The toxicity of Cd first came to light with the outbreak of "itai-itai" disease in Japan in 1950's (Kasuya *et al*. 1992). This disease caused severe pain, and discomfort in bones and joints. This happened when the runoff water from the mines containing large concentration of Cd was used in irrigation of various crops. Cd was absorbed by the crops and passed on to the humans resulting in various diseases including kidney failure (White and Chaney 1980). Since that time there has been a gradual increase in awareness regarding adverse

Table 1. Operational performance of cadmium sulfide (CdS)-buffered TFP devices utilizing different deposition techniques.

Deposition technique	**Efficiency [%]**	**Current density, J_{sc} [mA/cm^2]**	**Open circuit voltage, V_{oc} [mV]**	**Fill factor, FF [%]**	**Area (cm^2)**
Atomic Layer Deposition (ALD) (Hultqvist *et al.* 2007)	16.7	32.8	671	75.8	0.5
Chemical Bath Deposition (CBD) (Green *et al.* 2017)	23.3	32.98	621	74.7	0.5
Physical Vapor Deposition (PVD) (Rusu *et al.* 2005)	14.1	31.4	610	73	0.5
Sputtering (Gupta and Compaan 2004)	14	23.6	814	73.25	0.3
Ultrasonic Spray Pyrolysis (USP) (Fella *et al.* 2010)	12.5	30.3	570	73	0.3

impacts of Cd to human health.

In 1993, the International Agency for Research on Cancer (IARC) classified Cd as Category 1 human carcinogen (IARC 1987). Later, the National Toxicology Program (NTP) conducted an independent assessment and concluded that Cd and Cd-compounds are human carcinogens (Huff *et al*. 2007). As PV production ramps-up, this will speed-up the mining process for the extraction of these compounds, increasing emissions and metal leakage from waste dumps into the air, soil, and water. Clean and sustainable energy is the enabler for the welfare and economic development of a society. Solar PVs have the potential to meet these energy requirements and we've seen rapid growth during past two decades. With large scale adoption and deployment of the new PV energy systems, it is imperative to comprehend their impacts on environment and human health.

In this paper, the primary goals of our study were threefold: (1) investigate emissions from CBD CdS buffer layers during the life cycle of PV cells, and the potential for Cd release into the environment; (2) examine potential environmental and health risks related to manufacturing and disposal of CdS-buffered PV systems; and, (3) assess and monetize environmental, health, and socio-economic externalities associated with use of CdS-buffered PV systems.

Global PV Market Share and Growth

The sun emits 3.8 x 10^{26} Watts (W) of power, with a corresponding amount of energy produced each second equaling 3.8 x 10^{26} joules (J) (University of Minnesota 2014). The amount of energy that the sun produces in an hour can meet the annual energy needs of the whole planet, whereas the energy stored in the earth's known reserves of fossil fuels corresponds to only 20 days of sunshine (Forsberg 2009). In economic terms, we have already wasted a huge amount of the energy resource.

PV production has increased dramatically since 2005 in the United States, reaching 40 gigawatts (GW) of installed PV capacity in 2016 from five gigawatts (GW) in 2005, with global installed capacity reaching 320 GW (Philipps *et al*. 2017). According to the International Energy Agency's (IEA) Technology Roadmap on Solar Photovoltaic Energy (2014 Edition, the PV share in global electricity productions could reach 16% by the end of 2050 with installed PV capacity reaching 4.512 TWs. While world's energy growth in consumption is expected to reach 33 terawatts (TWy) (IEA 2014). Figure 2 shows the trend of increase in installed global PV capacity.

PV is the fastest growing market with compound annual growth rate (CAGR) of PV installations of 40% between 2010 and 2016 (Philipps *et al*. 2018). In 2016, Europe's contribution to the cumulative PV installations amounted to 33% compare to 26% for China and Taiwan (Philipps *et al*. 2017), however, China is expected to take the lead soon after 2020. Latin America, Africa and the Middle East, and OECD Pacific will continue to increase their PV market share. From 2030 to 2050, the PV share of India and other Asian countries is expected to rise from 13% to 25%. In contrast, the U.S. share is expected to remain near 15% from 2020 onwards, and Europe's share to decrease constantly from 44% in 2015 to 4% in 2050 mainly due to growing PV capacity in other parts of the world. By the end of 2050, Africa and the Middle East will have the largest share of global PV production (IEA 2008).

Thin Film Photovoltaics Share and Growth

TFP technologies are subdivided into three main families: (1) amorphous (a-Si) and micromorph silicon (a-Si/µc-Si); (2) cadmium-telluride (CdTe); and, (3) Copper-Indium-Diselenide (CIS) and Copper-Indium-Gallium-Diselenide (CIGS).

After early years of steady increase in TFP technology share, in 2016, the global PV market production share of all TFP technologies amounted to about 6% (4,900 MWp) of the total annual production falling from 13.2% in 2010 and 11.5% in 2012 (Philipps *et al*. 2017; Philipps *et al*. 2018). Figure 3 illustrates the timeline of global PV market share of TFP technologies from 2000 to 2016. This also reflects the challenges faced by TFP technology given significant cost reductions and efficiency improvements experienced by crystalline silicon (c-Si) in 2011 and 2012. Of that 6%, the market share for CdTe and CIGS technologies is

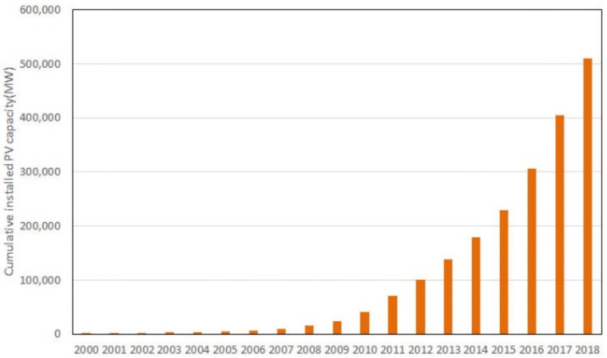

Figure 2. Cumulative installed global PV capacity 2000-2018 (Data adapted from Statista 2019).

respectively 3.9% (3,100 MWs) and 1.8% (1,300 MWs) (Philipps *et al*. 2017; Philipps *et al*. 2018) in which CdS is deployed as a buffer material (GBI 2012). Global PV cumulative installed capacity for CdS-buffered PV systems installation between 2000 and 2016 was 20,746 MWs (Table 2).

First Solar Corporation has approximately 90% of the market share of CdTe technology, with majority of market share being in North America, as the usage of Cd in the European Union (EU) is highly regulated. Hanergy Thin Film Power has about one-third of the market share of CIGS technology and a majority of its share is in Asia-Pacific and European markets (Research and Markets 2014). Amorphous silicon's (a-Si) share within TFP had rapidly increased in past (Campillo and Foster 2008); however, the focus is gradually shifting towards CIGS, a relatively novel thin film technology that has gained significant attention from stakeholders across the globe. With its efficiency expected to surpass that of CdTe in the next few years and its potential to overcome challenges associated with CdTe and a-Si, the market for CIGS technologies is expected to grow at a relatively higher rate (Research and Markets 2017). Figure 3 shows the trend in growth of thin film technologies.

TFP technologies are currently not as efficient as those of c-Si (Research and Markets 2014) and therefore, more thin film modules are required to generate the same amount of energy, but they are strongest in the utility scale market because the cost of the panels outweighs the cost of land and labor.

Considering that there is a huge potential for efficiency improvements in TFP technology, this sector could see the growth again with expected production share to exceed 20% of the PV market by 2020 (GBI 2012; U.S. Energy Information Administration 2017).

Towards Sustainable PV

Solar PVs will contribute extensively to satisfy ever-increasing global energy needs. Therefore, issues of sustainability and cost needs to be addressed with increased urgency. The search for sustainable PV materials that combine lower costs, lower toxicity, and effective/efficient energy manufacturing processes is becoming increasingly important. There is a clear need to focus upon the externalities related to the use of PV materials and the evaluation of their impacts.

The PV industry should not just focus upon fabricating high efficiency PV modules, but also focus upon several other issues that are critical for its progress towards large industrial-scale PV production. These issues include: (1) long-term stability; (2) environmentally benign and low-energy manufacturing process; (3) use of abundant, non-toxic materials; and (4) improved disposal/recycle techniques.

Table 2. Global PV market share of TFP technologies ([a]Weckend, *et al.* 2016. [b] IEA 2014, [c] Fraunhofer Institute for Solar Energy Systems 2018).

	2000	2005	2010	2011	2012	2013	2014	2015	2016
Global PV cumulative installed capacity (GWs) [a,b]	1.3	5.1	40.3	70	100	140	182	242.7	320
Global thin film photovoltaic (TFP) technology production share in PV market [c]									
Percentage (%)	10	5	13.2	13	11.5	9.4	6.8	7.3	6
Gigawatts (MWs)	14	100	2,300	4,200	3,220	3,500	3,615	4,200	4,900
Global CdS-buffered TFP technology market share in PV market									
Percentage (%)	1	1	10	8.5	8	8	6.1	6.6	5.7
Gigawatts (MWs)	1.4	20	1,950	2,900	2,500	2,750	2,875	3,550	4,400
Global cumulative CdS-buffered PV installation, 2000–2016 (MWs)	20,746								

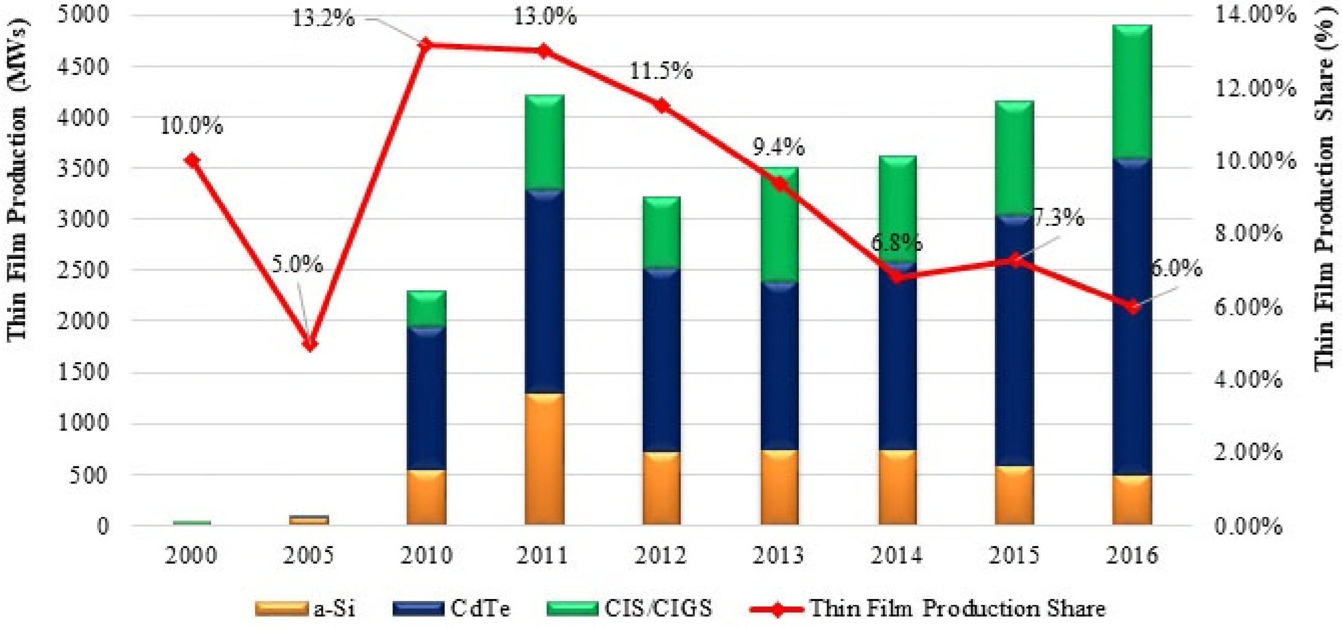

Figure 3. Global thin film PV production.

Assessment and Monetization

Emissions from CdS Buffer Layer

Cd is utilized in two different ways in the process of fabricating PV modules. CdS is used as a buffer layer and CdTe is used as an absorber layer. There are several deposition techniques that are employed in fabricating CdS/CdTe-based PV systems (Green *et al*. 2017; Hultqvist *et al*. 2007; Rusu *et al*. 2005; Gupta and Compaan 2004; Fella *et al*. 2010). Some of these techniques, such as physical vapor deposition (PVD) and atomic layer deposition (ALD), do not possess any significant risk of Cd exposure, whereas techniques such as CBD (on industrial scale) may lead to Cd emissions (Paris and Gmbh 2005). The potential impact upon the environment and human health is from gaseous and aqueous Cd emissions from the CBD process. The synthesis of precursors for CBD is the primary source of Cd emissions to the environment. Indirect Cd emissions are released into the water due to electricity consumed (for heating solution) during the process and for recycling the used bath in the process (Fthenakis and Kim 2007). Figure 4 illustrates the total Cd emissions into the environment from depositing an 80-nm thick CdS film over an area of 1 m^2 using CBD. The deposition technique in the process emits 6.31 mg of Cd into the air, soil, and water (Philipps *et al*. 2017).

For a 15.7% efficient module of a CdS–buffered PV system, it would require a PV cell area of 6.4 m^2 to generate a kW of energy on a clear day when solar irradiance for a surface perpendicular to the sun's rays at sea level is about 1000 Watt/m^2. Therefore, to generate the CdS-buffered PV share (5.7%, 4,400 MW in 2016) in the global TFP market, 28,000,000 m^2 of film area is required, which could potentially release 176.85 kg of total Cd into the environment from the manufacturing process. Environmentally friendly PV materials and deposition techniques are needed to avoid these toxic emissions. This explains the regulatory and policy concerns about the amount of Cd utilized in PV systems and the efforts to replace CdS with an alternate buffer material.

Manufacturing Costs

The minimum quantified material and energy required to deposit 1 m^2 CdS (~80 nm thick layers) buffer layer for a CdS-buffered PV system using CBD method is shown in Table. 3 (Raugei and Fthenakis 2010). The associated calculated manufacturing cost of CBD CdS was $2.80 over an area of 1 m^2. The prices of the chemicals listed in Table. 3 were for retail customers. These prices may be considerably lower for industrial customers. [All the prices were recorded for Alfa Aesar, a Thermo Fisher Scientific Brand as of

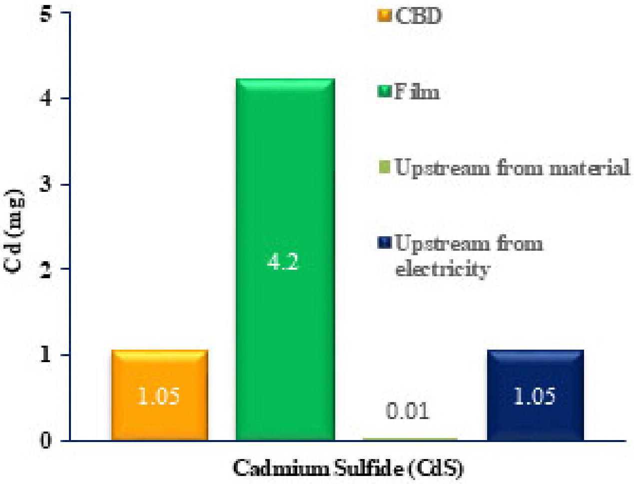

Figure 4. Total Cd Emissions (air, water, soil), from the chemical bath deposition (CD) of a 1-m^2 CdS thin films, 80 nm thick (Fthenakis and Kim 2007).

December 19, 2018, https://www.fishersci.com/us/en/home.html].

Environmental Costs and Issue

In general, the potential environmental impacts associated with solar PV includes land use and habitat loss, use of water and other natural resources, use of hazardous materials, and the life cycle emissions (Hope 2004). The impact varies greatly depending upon the technology type, scale and size of the PV systems, site location, etc. The size of a PV system ranging from small, distributed rooftop PV modules to large utility-scale PV systems will determine the level of environmental impact. Large utility-scale PV systems (range from 3.5 to 10 acres per megawatt) will have higher environmental impact and can raise concerns over land degradation and habitat loss (Edenhofer *et al.* 2011). TFP systems contain a number of toxic materials more than those used in traditional Si-based PV systems. While there are no global warming emissions associated with operation of PV systems (Raugei and Fthenakis 2010), there are emissions associated with other stages of the PV life cycle, including manufacturing, materials transportation, installation, maintenance, and disposal (Hope 2004). Most estimates of life cycle emissions for PV systems are between 0.07 and 0.18 lbs of CO_2E/kWh. This is far less than the life cycle emission rates for natural gas (0.6 - 2 lbs of CO_2E/kWh) and coal (1.4-3.6 lbs of CO_2E/kWh) (IEA 2014). Cd emissions from the life cycle of a PV system are 90-300 times lower than those from coal-powered plants (Hope 2004; Fthenakis 2004).

The environment is exposed to certain amount of Cd naturally through erosion and abrasion of rocks and soils, forest fires, and volcanic eruptions (ICdA 2005). The environmental impact due to Cd emissions from CdS-buffered PV systems during their operation is going to be almost non-existent, since the CdS buffer layer is not only stable, but also encapsulated between other layers with an overlaid glass film (Raugei and Fthenakis 2010; Hope 2004; Fthenakis 2004). First Solar Inc. CdTe technology received various International Electrotechnical Commission (IEC) certifications, and it complies with ISO 9001 and ISO 14001 standards and have a Class B fire rating (Class A Spread of Flame) according to UL and ULC 1703 standards. The glass plates surrounding CdTe material sandwiched between them (as in all commercial modules) seal during a fire and Cd release was negligible (Rix *et al.* 2015). However, a significant portion of scientific community believes that the CdS-based PV modules undergoing serious mechanical damage and chemical changes could cause Cd vapors to escape (Hope 2004; ICdA 2005; European Commission DG ENV 2011), thus harming the environment and life in and around these huge PV arrays. These deadly vapors forced by uncontrolled wind currents might travel to populated areas and cause a catastrophic event. However, end-of-life risks associated with the PV systems are of biggest concern since policies and systems regarding disposal or recycling appear to be inadequate globally (Klugmann-Radziemska 2012). Dollar costs of environmental externalities are difficult to evaluate and depend upon assumptions that can be subject to wide interpretation and discretion. Although, environmental impacts and associated dollar costs are often included in economic comparisons between renewable and conventional energy, investors rarely include such environmental costs in the bottom line used to make decisions.

The environmental problems linked with CdS-buffered PV systems include the release of Cd emissions in air, soil, and water at the manufacturing facility, the installation site and the disposal or recycling facility (Fthenakis 2009). The environmental impacts of Cd emission could result in real cost to society, in terms of human health (loss of workdays, health care costs), infrastructure decay (from acid rain), declines in forests and fisheries, and perhaps ultimately, the costs associated with the climate change (Edenhofer *et al.* 2011). The growing concern about the Cd in the environment is that, if not properly handled after the end-of-life of the PV systems, Cd may escape from landfills and leach into the ground water, contaminating

Table 3. Materials (g) and energy (kWh) required for the chemical bath deposition of 1m^2 CdS thin films (Raugei and Fthenakis 2010).

Material inputs [a]	**Minimum quantity req. (g)**	**$ Price (dollars/gram; most quantities are 500g)**	**Manufacturing cost ($)**
Cadmium sulfate ($CdSO_4$)	0.61	4.42	2.69
Thiourea (NH_2CSNH_2)	0.2	0.074	0.02
Ammonia (NH_4OH)	1.3	0.03	0.04
Electricity (kWh)	0.4	0.1152	0.05
Total manufacturing cost			**2.80**

streams, lakes, and rivers, and changing their acidic balance, and putting not just human health, but aquatic organism lives also on risk (Plachy 2003; National Research Council (US) Subcommittee on Zinc Cadmium Sulfide 1997). Furthermore, Cd is bound to particulate matter and can easily be taken up by bottom-dwelling animals as food (National Research Council (US) Subcommittee on Zinc Cadmium Sulfide 1997). According to a study by BIO Intelligent Service, Cd leaching is, on average, at 7% of the volume of Cd contained in a PV module condition to no change in the pH value of the module. However, Cd leaching in landfill settings could potentially increase by 29% if exposed to a lower pH such as nitric acid or acid rain (European Commission DG ENV 2011).

Cd is a major component of CdTe PV modules and a secondary component in CIGS PV module, with approximately 4.60 g and 0.368 g of Cd present in an average CdS-buffered CdTe and CIGS PV module (that weighs about 12 kg/m^2). Hence, the potential for Cd leaching into the environment is between 0.03 and 0.32 g per about 12 kg/m^2). Hence, the potential for Cd leaching into the environment is between 0.03 and 0.32 g per PV module (Fthenakis 2004). The external cost of environmental pollution linked to Cd leakages, respectively, is reported to be $76,852.2 per U.S. ton (European Commission DG ENV 2011), which means that the environmental cost for the installed CdS-buffered PV capacity (2000-2016) could total $5.73 million (see Table. 4). However, this cost will vary over time and will decrease with improved technologies to safely dispose/recover Cd. PV systems have a lifetime of 25 years, and considering the last two decades of significant production, the recycling will begin in earnest by 2030 for PV capacity installed in 2005.

Healthcare Costs and Issues

The potential adverse health issues linked with exposure to Cd are primarily at the PV manufacturing facility and disposal, or recycling facility, whereas Cd emissions are substantially below human health evaluation levels during the life of the PV system (Raugei and Fthenakis 2010).

Cd is considered to be among the most toxic materials used in the PV industry. It is carcinogenic with a biological half-life of 30 years and is known to have long-term effects upon the kidneys (Wennerberg 2002, Butterman and Plachy 2002). Cd could enter and harm the human body through several pathways. It can be ingested through contaminated food and water, but the more potent method could be through inhalation of Cd vapors (National Research Council (US) Subcommittee on Zinc Cadmium Sulfide 1997). Inhaling Cd can cause pneumonitis, pulmonary edema, and death. Intense exposure may lead to more serious effects resulting from severe bronchial and pulmonary irritation. Its effect on liver and kidneys can manifest as various diseases including cancer (Hope 2004). Cd was one of the eleven metals among 53 chemicals on the Persistent Bioaccumulative Toxic (PBT) list targeted by the environmental protection agency (EPA) for a 50% reduction in 2005 (Butterman and Plachy 2002). People who work in PV manufacturing settings, where Cd-containing materials are mostly used in powder form, are at most risk as its dusts can easily be inhaled. Despite Cd exposure well below the threshold limit value (TLV), workers who are involved in the manufacturing process could potentially be at risk for significant exposure, well in excess of the TLV (Spinazzè *et al.* 2015). Persons who survive such acute exposure episodes may recover without permanent damage, but it is possible that repeated episodes of acute or subacute pneumonitis may result in development of lung emphysema. Cd exposure is also believed to cause cancer and high blood pressure (Sinha *et al.* 2014).

The external cost related to human health damage from Cd emissions/leaching is approximately $54,431.6 per U.S. ton (European Commission DG ENV 2011), which means that the cost of human health damage from the installed CdS-buffered PV capacity (2000-2016) could total $4.1 million (see Table. 4). These costs are based upon not only the improper disposal of Cd from residues from CdTe and CIGS technologies, but also PV modules in ambient use allowing uncontrolled Cd leaching into soil or emissions into air.

Recycling and Disposal Costs and Issues

Recycling and disposal of PV systems is difficult due to the decades-long period between the installations and end-of-life of PV modules, a relatively low concentration of the PV materials, and geographical dispersion (Heath *et al*. 2017; Fthenakis 2000). This requires proper scheduling and sustainable recovery methods. PV waste is expected at two levels: (1) manufacturing; and (2) end-of-life PV module. With recycling to start in significant volume by 2030, the total quantity of disposed PV system waste mostly consists of glass and could total 70.8 million MT globally by 2050 (see Figure. 5). If this waste was fully injected back into the economy, the recovered material could worth more than $15 billion, thus increasing the efficacy for producing future PV systems or other raw-material-dependent technologies, since costs for material recycling are usually lower than the costs for new technical-grade material. This amount also equates to the raw materials required to synthesize two billion modules and produce 630 GW of energy (Heath 2017), with potential to decrease the energy payback time period to 0.6 - 1.14 years, which is currently between six and eight years (Fthenakis 2009). Thus, economics alone is likely to generate interest in recycling. Companies like First Solar, SunPower, Panasonic, SolarCity, Trina Solar, etc. have already implemented recycling programs, however, these initiatives should be driven by environmental responsibility rather than the economic benefits (Fthenakis 2000). By the end of 2016, 0.25 million MT of PV waste was generated, representing 0.6% of the total mass of the globally installed PV systems, which stands at 4 million MT (Weckend *et al*. 2016). Figure 5 shows the projected PV system waste from the disposed modules, its value creation, and potential to produce PV systems and equivalent energy.

Industrial processes to recycle TFP modules are already established, and can retrieve substances like glass and Al, as well as semiconductor-related materials (Heath *et al*. 2017). The typical composition of a TFP module is: 84% glass, 12% of Al frame, 3% polymer encapsulant, and the most essential materials forming the PV layers (Mo, Cu, In, Ga, Se, Cd, Zn, S) are only a very small fraction of about 23 g in a 12 kg square meter ($1m^2$) sized module (Klugmann-Radziemska 2012). The mass of recovered semiconductor materials for 1 m^2 PV module is approximately 5.23 g and 8.62 g for Ga and In, and 8.98 g and 9.15 g for Cd and Te (Klugmann-Radziemska 2012). A number of recycling techniques are under development globally for PV modules. These recycling and treatment options vary by producer and type of technology. However, policy actions are needed to address the global challenges associated with increasing volumes of PV modules waste going forward. Frameworks that enable efficient waste management tailored to the needs of each country or state are essential. China, Germany, and Japan are expected to be the top three countries for solar PV panel waste by 2030. By 2050, China is still expected to have the highest amount of waste. The U.S. will overtake Germany in the second place with Japan expected to remain in third place (Heath *et al*. 2017). EU countries have pioneered electronic waste regulations that cover PV module collection, recovery, and recycling targets. The EU WEEE Directive requires that all solar PV module suppliers finance the end-of-life collection and recycling costs (European Commission DG ENV 2011). In contrast, many countries classify PV modules as either general or industrial waste. In Japan and the U.S., general waste regulations may include testing these modules for hazardous content and prescribing and prohibiting specific shipment, treatment, recycling and disposal methods (Weckend *et al*. 2016). First Solar recycles CIGS and CdTe TFP modules with recovery rates of 90% for glass and 95% for semiconductor materials (Krueger 2009). With the purchase of each First Solar module, funds are set aside to cover the estimated future costs of collection and recycling. These funds pay for all packaging and transportation costs associated with the collection and recycling of the modules. This program follows a three-step process: register each module, collect each module once dismantled, and recycle the modules for material recovery (Krueger 2010). An efficient recycling method can diminish the environmental impacts of manufacturing waste as well as end-of-life module waste, while economically recovering the materials for future use. Ideally, the design and production process of PV components should incorporate the end-of-life dismantling of components into the parts that can be reused or recycled. The manufacturers also have a strong financial incentive to ensure that these highly valuable and often rare materials are recycled rather

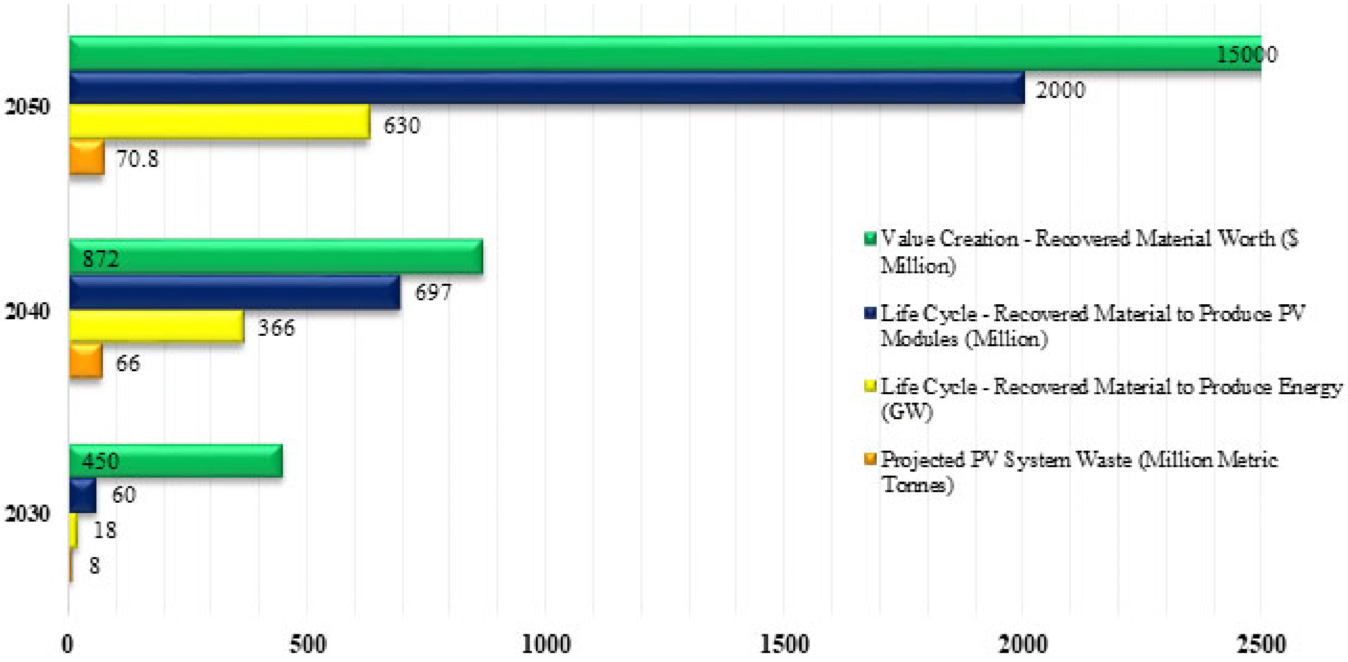

Figure 5. Projected PV system waste from disposed modules; value creation, and potential to produce PV systems and equivalent energy from recovered PV material (Heath *et al.* 2017).

than discarded. The recycling methods and procedures for Cd at present are similar to those for NiCd batteries and LCDs (Fthenakis 2000). Additionally, if all of the aqueous waste containing Cd compounds from rinsing, plate stripping, and ion exchange regeneration can be converted to Cd and Cd salts through precipitation and filtration, the industry could reduce both the manufacturing cost plus Cd emissions into the environment since most of the Cd will be recovered from the waste bath (Fthenakis 2004). A study at Japan Storage Battery Association (JSBA) revealed that the price of the material has an inverse relation with the quantity of recycled materials (Scoullos *et al* 2001).

The total estimated cost of recycling in TFP modules is approximately 10 ¢/W including transportation and collection costs (Fthenakis 2000). Therefore, the estimated total recycling costs for CdS-systems to incur for capacity installed from 2000 to 2016 could total $2.08 billion (Table 4). [Estimated Cd content from TFP waste was calculated using following formula: (number of modules required to generate the TFP market share x cadmium content in the module)/(weight of the module (12 kg)). The result was then converted into U.S. tons. Estimated Cd leaching into the environment was calculated using following formula: (number of modules required to generate the TFP market share x potential for Cd leaching into the environment (0.32 g/module). The result was then converted into U.S. tons. Estimated Cd emissions from the manufacturing process was calculated using following formula: (number of modules required to generate the TFP market share x Cd emissions from CBD of CdS thin film (6.31 mg/m^2)].

Safe disposals of various components of TFP module suggests the decommissioning of the modules should be done in a way that no hazardous material is released into the environment. If the toxic material ends up in landfills, it could leach into the ground water, or in incinerators, burning materials resulting in emission of toxic to the air (Sinha *et al.* 2014; Weckend *et al.* 2016). Although the cost of landfill disposal of PV modules is still lower than the cost of recycling the modules (European Commission DG ENV 2011), recycling is environmentally profitable. Additionally, with improved recovery methods, recycling costs are expected to decrease, whereas the landfill disposal costs are constantly increasing due to increased environmental regulations associated with the disposal of hazardous material to protect the environment (T. E. P. A. T. C. O. and European Union 2003). The cost of landfill disposal is 1 ¢/W for large quantities of non-hazardous waste and 23 ¢/W for hazardous waste excluding packaging and transportation costs, respectively (Fthenakis 2000). Therefore, the estimated total disposal

Table 4. Monetization: External costs related to use of Cd in CdS-buffered System.

Cadmium-containing TFP technology market share (MWs) by type:		
Year of production (Philipps *et al* 2018)	**CdTe**	**CIS/CIGS**
2000	-	1
2005	20	-
2010	1,400	350
2011	2,000	900
2012	1,800	700
2013	1,650	1,100
2014	1,850	1,025
2015	2,450	1,100
2016	3,100	1,300
Total TFP market share in the PV market (MWs)	14,270	6,475
	20,745	
Number of modules (100 watt) required to generate the market share	1,712,400,000	777,000,000
Estimated cadmium content present in TFP Waste (in U.S. tons) (Fthenakis 2004)	723	23
Environmental Costs		
Estimated Cd leaching into the environment (in U.S. tons) (Fthenakis 2004)	50	23
Estimated Cd emissions from manufacturing process (U.S. tons)	1	0.45
Estimated total environmental costs (in million dollars) @ \$76,852.2/U.S. ton of Cd leaching (European Comission DG ENV 2011)	5.73	
Healthcare Costs		
Estimated total human health damage costs (in million dollars) @\$54,431.6/U.S. ton of Cd leaching (European Comission DG ENV 2011)	4.1	
Recycling and Disposal Costs		
Estimated Recycling Costs (10¢/watt) in billion dollars (Fthenakis 2000)	2.08	
Estimated Disposal Costs (23¢/watt) in billion dollars (Fthenakis 2000)	4.77	

costs for CdS- buffered PV systems to incur for capacity installed from 2000 to 2016 could total \$4.77 billion. Due to uncertainty and limited information on the extent of the future recycling and disposal costs from potential technological shifts, we used fixed rate while calculating these costs based upon prices available in the literature.

Conclusion

The impact of any new technological advance/ material upon human health and the environment must be carefully examined before it can be adopted on a large scale. Issues of sustainability and cost needs to be addressed with increased urgency, and there is a clear need to focus upon the externalities related to the use of PV materials and the evaluation of their impacts. CdS-buffered TFP technology has now matured and it is now important to assess its impact before it is widely adopted. The external costs related to environment, human-health damages, and disposal from use of Cd will outweigh the high price of other alternate PV materials. These external costs will exponentially increase as demand for energy increases in the future.

If researchers from diverse scientific disciplines can work together with support from manufacturers and monitoring by governmental agencies, nearly any technology can be utilized in a smart and profitable manner with minimal-to-no harm to the humans, thus, avoiding socio-economic burdens. If government, industry, and research institutions each play their respective parts, the potential payoff is significant, given recycling PV modules is expected to represent a $15 billion opportunity worldwide by 2050. Potential environmental impacts of minimizing Cd leaching and resource loss due to non-recovery of valuable conventional resources and rare metals in PV modules are significant. Until these issues are properly addressed, a shadow of doubt will hang over the true environmental impacts of solar energy.

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A Dobsonfly, *Corydalus cornutus* (Megaloptera: Corydalidae), from Arkansas with Aberrant Mandibles

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Running title: An Aberrant Dobsonfly

Adult dobsonflies, *Corydalus cornutus* (L.), demonstrate strong sexual dimorphism in mandible configuration and length (Contreras-Ramos 1998; Bowles *et al*. 2007). Males typically have greatly elongated and robust mandibles that are tubular in cross-section and generally lack subapical teeth (Fig. 1A). In contrast, female mandibles are unmodified, much shorter, subequal in length to the head, and dorsoventrally flattened with prominent subapical teeth (Fig. 1B) (Contreras-Ramos 1998; Bowles *et al*. 2007). Contreras-Ramos (1998, 2004, 2011) presented phylogenetic analyses that indicated short mandibles among other *Corydalus* species is the plesiomorphic character state while elongated mandibles represent the apomorphic state. The phenological expression of shortened mandible length thus resides within the genome of the genus. As noted by Contreras-Ramos (1998), long mandibles are most common among male *C. cornutus*, but those of some males are intermediate between those of females and males with long mandibles.

Gynandromorphism and intersexes are conditions where an organism expresses both male and female characteristics simultaneously. Gynandromorphs exhibit both genetically male and genetically female tissues (Narita *et al.* 2010). In other words, they are sexual mosaics where some parts of the specimen are clearly female while other parts are male. Gynandromorphs have been commonly reported among insects (see Narita *et al*. 2010 for review). A similar term, gynomorphism, has been used to describe males that have a morphological resemblance to females (e.g., coloration) but not necessarily having female sexual characters. In contrast to gynandromorphs and gynomorphs, intersexes are genetically uniform but sexually ambiguous (Narita *et al*. 2010). Some intersex individuals may be genetically intermediate between typical male and female genotypes, while others may be genetically purely male or female, but some parts of their bodies show a sexual phenotype that is opposite to their genetic sex (feminization or masculinization of body parts) (Narita *et al.* 2010).

Bowles *et al*. (2007) reported on two male *C. cornutus* collected from Boone County, Missouri that display shortened mandibles similar to those exhibited by females, although they had genitalia consistent with those of typical males. Those specimens were suggested to be gynandromorphs or possibly gynomorphs (Bowles *et al.* 2007) based solely on their physical appearance, but it is possible they represented intersex specimens. Furthermore, it is unclear if the reduced mandible length in some male *C. cornutus* truly represents a gynandromorphic/intersex condition, an ancestral trait being expressed, or due to environmental factors.

During July 2017, three male and 100 female *Corydalus cornutus* were collected as by-catch in blacklight traps from several locations along the Buffalo River, Arkansas. One of the male specimens collected at the junction of Clabber Creek and Buffalo River (see collection data) was small bodied and had greatly reduced, symmetrical mandibles (Fig. 1C). This specimen is small having a forewing length of only 39.5 mm, each mandible was only 7.2 mm long, and the head capsule was only 5.5 mm wide. The genitalia of this specimen were consistent with that of typical males. The head/mandible length ratio of the aberrant male (0.76) is the same as the ratio of an aberrant male reported by Bowles et al. (2007) from Jefferson County, Missouri. The mandibles of the Missouri specimen are flattened with two discreet subapical teeth similar to that of females, while the mandibles of the Arkansas specimen are more tubular-shaped, greatly reduced in length and diameter, and with a single subapical tooth (Fig. 1C). The Arkansas specimen has a mandible configuration somewhat intermediate between those depicted in Bowles *et al*. (2007; figs 4, 5 and 7).

Two additional males collected at Toney Bend on the Buffalo River, only a few kilometers upstream from the previous site, are much larger bodied and the mandibles are typical. One specimen has a forewing length = 56 mm, left mandible length = 29 mm, right mandible length = 30 mm, and head/mandible length

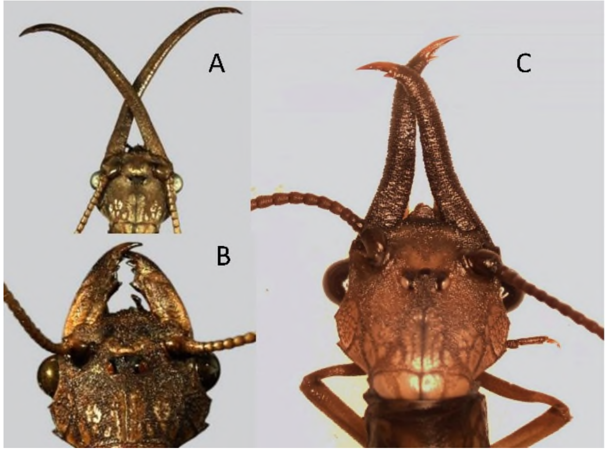

Figure 1. Dobsonfly, *Corydalus cornutus*. A. Typical male, B. Female, C. Aberrant male.

ratio = 0.33. The second specimen has a forewing length = 47 mm, left mandible length = 28.5 mm, right mandible length = 28.5, and head/mandible length ratio = 0.28). Among the females collected, wing length of measured specimens (n=52) ranged from 38 to 54 mm (mean= 47.6 mm), which falls within the previously reported range for this species (Contreras-Ramos 1998, Bowles *et al*. 2007).

The basis for shortened male mandibles in *C. cornutus* remains unclear. The roles of genetics, phylogeny and environmental factors presently cannot be distinguished. It is unknown if the specimen presented here represents a gynandromorph, a gynomorph, an intersex, the expression of an ancestral character (Narita *et al.* 2010) or phenotypic plasticity due to environmental conditions (e.g., temperature, nutrition, pollution) experienced during development. *Corydalus* mandibles are a secondary sexual trait, and as such, their development is modulated by trade-offs and condition dependence (Liu *et al*. 2015). Typically, increased mandible size in adult male megalopterans is a trade-off with reduced wing size and vice versa (Liu *et al.* 2015). That trade-off was not apparent with the aberrant male reported here whose entire body was smaller than that of a typical male. The mandibles of the aberrant specimen resemble the shortened mandibles of a closely related species, *Corydalus texanus* Banks, giving some plausibility to an ancestral expression.

Acknowledgements

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Collection data

All specimens were collected with blacklight traps at Buffalo National River, Arkansas on July 13-14, 2017, collectors D.E. Bowles and C.R. Cheri. Coordinates are UTM eastings, northings (15S).

Marion Co., Buffalo River @ Clabber Creek, UTM 540894, 3998226 , 1 male, 72 females; same, but Buffalo River @ Toney Bend, UTM 540382, 3994907, 2 males, 3 females; same, but Newton Co., Buffalo River @ Carver, UTM 496551, 3982104, 1 female; Buffalo River @ Davis Creek, UTM 504197, 3984942, 15 females; same, but Searcy Co., Buffalo River @ Tyler Bend, UTM 520982, 3982722, 9 females.

Reference specimens are deposited in the collection of the Heartland Inventory and Monitoring Network, National Park Service, Springfield Missouri

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Plains Spotted Skunk Pelt Purchase Trends in the Ozarks and Ouachitas, 1943-1990

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Running Title: Plains Spotted Skunk Purchase Trends

The plains spotted skunk (*Spilogale putorius interrupta*) was previously considered a common animal across much of the central United States. However, this subspecies has undergone a severe population decline and the current rarity of this subspecies has led to it being petitioned for protection under the Endangered Species Act (Gompper and Hackett 2005, U.S. Fish and Wildlife Service 2012). While difficult to find across most of its range, it can still be found, though uncommon, in the Arkansas Ozarks and Ouachitas (Hackett *et al*. 2007; Lesmeister et al. 2009; Perry *et al.* 2018; Sasse and Gompper 2006; Sasse 2018).

The Arkansas Game and Fish Commission requires buyers of fur pelts to report on the number and species of pelts purchased each year and records from 1943-1990 were summarized by region. Pelt purchases from the Delta and Gulf Coastal Plains as well as all post-1990 spotted skunk pelt purchases were negligible and are not included (Sasse and Gompper 2006). Data on spotted skunk pelt purchases in the Ozark Plateau region of Missouri is from Sampson (1980). In order to allow for comparisons across regions data was standardized to harvest/1000 square kilometers (Figure 1).

Purchases in all three regions were relatively high in the 1940s but began a steep decline in the Missouri Ozarks in the mid-1940s and in the Arkansas Ozarks and Ouachitas in the early 1950s. While purchases in the Missouri Ozarks continued to decline slowly the Arkansas Ozarks saw a modest recovery in the early 1960s and in the mid-1970s nearly returned to 1940s harvest levels. Curiously, there was no increase in Ouachitas purchases in the mid-1960s however it too returned to 1940s levels in 1978. Although Missouri Ozarks purchases increased 460% from 1971 to 1974 it was still much lower than seen in earlier years.

Arkansas spotted skunk pelt purchases have been shown to be dependent on pelt price and these spikes in purchases occurred in years with higher spotted skunk values (Sasse and Gompper 2006), however, in other states this relationship with price has not always been as strong, especially in recent decades as harvest has become incidental to take of other species (Clark *et al.* 1985; Gompper and Hackett 2005; Sasse 2018; Sasse and Gompper 2006).

Like other mountainous areas of spotted skunk range, total pelt purchases in these regions of Arkansas and Missouri were relatively small. The increased trapper effort associated with high fur prices in the 1970s did not result in similar increases in spotted skunk harvests in formerly-prairie lands that had been converted to agriculture and that previously had extremely dense spotted skunk populations (Gompper and Hackett 2005). Since fur purchases in the Ozarks and Ouachitas in the mid-1960s and 1970s were similar to those observed in the 1940s, and lacking any other data upon which to assess spotted skunk population trends, this suggests that populations in these regions did not decline from the 1940s through the 1970s as they did elsewhere (Gompper and Hackett 2006).

A significant amount of research in recent years has focused on similar ecosystems (Wilson *et al.* 2016; Thorne *et al.* 2017; Sprayberry and Edelman 2018), but may not be particularly useful in illuminating the causes of, or solutions for, declines seen in Oklahoma, Kansas, Iowa, and northern Missouri. Their current rarity may therefore represent their historic status and implies that the factors, which are not well understood, that allowed very dense populations to develop in the Great Plains in the first half of the 1900s are not relevant to mostly forested mountain regions such as the Ozarks and Ouachitas.

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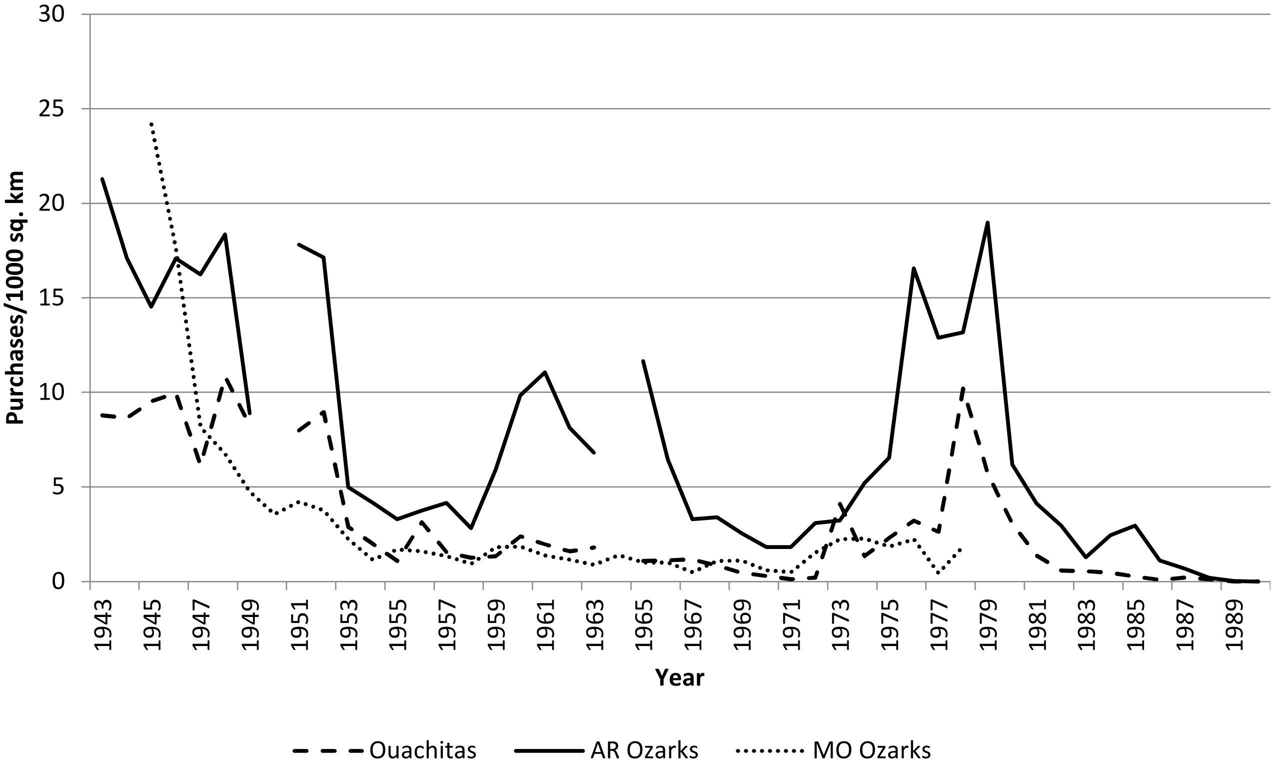

Figure 1. Spotted Skunk Pelt Purchases by Region, 1943-1990.

Observations of an Alligator (*Alligator mississippiensis*) Nest and Behavior of Hatchlings in Clark County, with Anecdotal Observations of Other Alligator Nests in Arkansas

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Running Title: Observations of an Alligator nest in Arkansas

Historically, native populations of the American alligator (*Alligator mississippiensis)* extended throughout the southern portion of Arkansas (Trauth *et al*. 2004). By the early 1900s, populations range-wide had declined due to unregulated hunting, commercial exploitation, and habitat loss (McIlhenny 1935). In 1961, the Arkansas Game and Fish Commission (AGFC) implemented protection of this species, and in 1967 the U.S. Fish and Wildlife Service (USFWS) listed the alligator as an endangered species (USFWS 1967; Watt *et al*. 2002). The AGFC conducted a restocking program from 1972-1984, in which 2,841 alligators were released mostly in the southern portion of the state, and about 80% of the alligators were placed on private lands (Irwin and Wooding 2002). Some of the stock originated from Grassy Lake, a privately owned ~1200 hectare floodplain cypress swamp in Hempstead Co., Arkansas, but the majority came from Rockefeller Refuge and Sabine National Wildlife Refuge in Louisiana (Watt *et al*. 2002). The species made a full recovery throughout the historic range of Arkansas and was removed from the endangered list in 1987 (USFWS 1987).

Trauth *et al*. (2004) noted nesting or observation of young in Arkansas, Calhoun, Chicot, Hempstead, and Miller counties. In July of 2005, the first reported alligator nest was documented at the Red Slough Wildlife Management Area in McCurtain County, Oklahoma (Arbour and Bastarache 2006), though records of alligators in southeastern Oklahoma exist from the 1800s (Heck 2006). In northeast Texas, areas of the Sulphur River bottoms and Red River tributaries display healthy juvenile populations indicating nesting and breeding, and within the southern portion of Arkansas, alligators have confirmed breeding populations in Millwood Lake and Grassy Lake (Arbour and Bastarache 2006).

Still, little is known about reproductive biology and behavior of hatchling alligators in Arkansas. McCallum *et al*. (2003) reported a nest found on 7 August 2001 at Arkansas Post National Memorial, Arkansas Co., and reported seeing 22 hatchlings at the site 10 months later. In contrast, a second pod of hatchlings was found in the same area but were absent the following spring. Pods of hatchlings observed by Smith *et al*. (2016) in southern Arkansas were in vegetation near the shore (no other information about numbers of pods, nests, or specific localities were provided, as records were kept only for harvestable animals over 1.22 m (4 ft.) in length). A nest in nearby southeastern Oklahoma produced 19 hatchlings (Arbour and Bastarache 2006). Juvenile alligators were rarely observed during surveys in Arkansas habitats, perhaps because cold temperatures kill young alligators (Irwin and Wooding 2002), though smaller individuals also are more difficult to locate during surveys. These few observations demonstrate the need for better understanding of nesting success and post-hatching survival at the northern limit of the range of the species.

Most knowledge about alligator mating and reproductive behaviors that may relate to Arkansas are from studies conducted in Louisiana (Joanen 1969; Joanen and McNease 1970, 1971, 1975). Initiation of courtship typically begins with vocalizations and bellowing in early April. Male alligators have a 2-week peak of spermatogenesis, which decreases after mating, and by mid-June 90% of spermatogenic activity ceases (Joanen and McNease 1989). The female alligator develops mature ova during May-early June, and lays eggs about 3.5 weeks after ovulation (Joanen and McNease 1989).

Females attend and protect the nest. The average incubation period is 65 days, and through the period of 30-45 days, temperature of incubation determines the sex of the hatchlings (Lang and Andrews 1994), although incubation history can set this period a little earlier (McCoy *et al*. 2015). Nests can have varied temperatures, but eggs incubating experimentally at

31.5°C or less developed into females, while those incubating at 32-33.5°C produced all or mostly males and higher temperatures again produced females (Lang and Andrews 1994). As hatching begins, "peeping" vocalizations from the newborn alligators alert the mother to uncover the top of the nest. During this process, females may assist hatchlings and transport the young to the water (Hunt and Watanabe 1980).

On 3 April 2018, we observed an approximately 2 m long alligator on the western side of a small pond near Arkadelphia, Clark Co. The pond is approximately 14 meters off a 2-lane road and is surrounded on the other three sides by lowland forest, briars, and brushy vegetation. Aquatic vegetation in the pond was sparse and occurred primarily around the banks. At the western end of the pond is a small island approximately 7 m long x 5 m wide. Approximately 50 m southeast of the pond is a creek that runs beneath a bridge and allows wildlife to access farmland and other marshy areas, including Clear Lake.

Daily visits revealed that the alligator typically stayed on or near the island but occasionally occupied the middle of the pond. On 18 April, we observed a second alligator, approximately 2.5 m. in length, swimming near the smaller one. We continued observations between April and October. Although we never observed courtship behaviors, vocalizations, or mating, we noticed possible mate-guarding behavior as the larger alligator consistently positioned itself between the smaller alligator and us. Both alligators remained together until 18 May, after which the larger alligator was no longer seen.

Throughout June, sightings of the smaller alligator were unremarkable. On 31 July 2018, we took a canoe into the pond and found no evidence of alligators, although they could have been present and hidden on the banks. However, we discovered an alligator nest on 7 August, in an area on the eastern bank of the pond where a patch of ground had been cleared of vegetation. Most of the rest of the pond was surrounded by a more wooded landscape, and there we observed signs of heavy use by feral pigs *(Sus scrofa*). As egg predators (McIlhenny 1935; Elsey *et al*. 2012), activity of pigs might have destroyed the nest had it been constructed on that side of the pond, but we have no evidence that nest site selection was based on predator avoidance.

Most of the vegetation surrounding the nest site was Roundleaf green briars (*Smilax rotundifolia* L.). A heap of sticks and dead plant material were mounded on top of a higher part of the bank, producing a structure 1.6 m long x 1.3 m across and 43-56 cm in height (Fig. 1). A slide of bare ground extended from the mound to the water. A nest described in southeastern Oklahoma was slightly larger, at 2 m wide x 1.8 m long, and about 60 cm tall, and was comprised of bulrush (*Scirpus* sp.) (Arbour and Bastarache 2006).

Figure 1. An alligator (*Alligator mississippiensis*) nest in Clark County, 15 August 2018. Photo by RT.

Upon our approach to the mound, the smaller alligator, presumed to be the nesting female, appeared in the water near the bank approximately 1 meter from the nest. Alligators reach sexual maturity at about 2 m (Joanen and McNease 1975), so this was possibly her first nesting attempt. We set up a Spypoint Link-S infrared game camera to monitor nest activity.

On 11 September, we heard "peeping" coming from the nest, and the unhatched alligators responded to vibration when we tapped the sticks on top of the mound. Five days later at 22:14 h, the game camera captured the female alligator on top of the mound. This was the only time the game camera captured female attendance directly on the nest (Fig. 2). We inspected the nest the next day, and although we found no alterations, peeping still could be heard.

From 21-25 September, Arkadelphia received approximately 10.2 cm (4 in.) of rain, and a significant drop in daily air temperature from 35.6°C (96.1°F) to 17.8°C (64.0°F), with prediction of lows in the 50s later in the week (it did reach 14.4°C (57.9°F); (www.usclimatedata.com/climate/arkadelphia/arkansas/united-states/usar0016/2018/9). We were concerned about the effect of appreciable temperature change, as the effect of thermal shock to a clutch of unhatched alligators is not understood. Lang and Andrews (1994) noted that embryos tolerate temperatures 1-3°C different from their viable range for 1-2 days.

Survival of alligators at the northern limit of the

Figure 2. The female alligator checking her nest at 2214 hr on 16 September 2018, caught by a game camera

range is limited by chilling temperatures. Partial freezing of ponds may trap smaller alligators under ice and cause them to suffocate, and the smallest individuals may die due either to lesser physiological tolerance to thermal shock or lesser ability than larger individuals to withstand lower temperatures (Spotila *et al*. 1972; Brandt and Mazzotti 1990; Lee *et al*. 1997). Spotila *et al*. (1972) noted that alligators enter water to avoid thermal stress induced by warmer or colder air temperatures.

The nest provides insulation whether or not it contains decaying vegetation. Because eggs and hatchlings still in a nest cannot access water, they may be more susceptible to changes in air temperatures they cannot escape, especially if the nest is not lined with a lot of decaying vegetation, whose decomposition incubates the eggs (McIlhenny 1935; Chabreck 1973). In Louisiana, maximum and minimum temperatures taken at the position of the eggs in a successful nest ranged from 38.9-28.9°C (102-84°F), with the highs occurring in very early development (McIlhenny 1935). The embryo does not survive past developmental stage 18 if temperatures remain above 36°C (96.8°F; Lang and Andrews 1994). In another study, mean temperatures taken in nests during late August averaged between 26.6-31.7°C (79.9-89.1°F) over 3 years (Chabreck 1973). Thus, we were concerned about effects of our lowering temperatures in late September.

Further, hatching occurs between late August and early September in Louisiana (Joanen and McNease 1975), and in eastern Texas most nests hatched in the first week of September (the latest date of hatching was 21 September; Saalfeld 2010). We excavated our nest on 27 September at 1630 h because the shift in temperatures, increased precipitation, presence of a second adult alligator in the pond, and the delayed hatching caused concern. We observed behaviors of hatchling alligators at the time of hatching from a wild nest, and later the development of feeding behaviors in the lab.

During collection of eggs and hatchlings, we observed neither of the adult alligators in the pond. We gently brushed the surface material, comprised mostly of small sticks and dirt, from the top of the nest and encountered eggs at a depth of approximately 15 cm. By use of a VWR® handheld digital thermometer, we determined the temperature of the nest to be a cool 24.2°C (75.6°F).

The nest contained 33 eggs. In Louisiana, clutch sizes averaged 38.9 eggs (Joanen 1969). Without changing their orientation (to prevent possible damage, see Ferguson 1985; Woodward *et al.* 1989), unhatched eggs were moved carefully to a plastic container lined with original nesting material and were transported to the vertebrate lab at Henderson State University, and were incubated in original nesting material at a temperature of 30°C. We continued incubation for 6 days to see if any other eggs would hatch.

Only those eggs in the process of hatching in the nest had survived. Eight were hatched within the nest at the time of opening, and 4 others were in the process of hatching. We measured (mm) the 21 unhatched eggs (mean ± STD, minimum-maximum): length was 60.5 ± 2.1, 57-65, and width was 37.3 ± 5.0, 33-38. Trauth *et al*. (2004) noted that alligator eggs are usually about 75 x 45 mm, and McIlhenny (1935) recorded usual measurements of 66 x 41 mm, so ours appear to be smaller than normal; possibly due to being from one of the first nests constructed by a young female.

We opened the dead eggs to determine developmental status of each. Eight eggs contained almost fully developed embryos, which we suspect may have died due to the rapid changes in temperature experienced a few days before we opened the nest. Nest temperature at the time of collection had dropped well below the tolerance suggested by Lang and Andrews (1994). McIlhenny (1935) similarly described a nest opened in late September, in which most of the young were dead due to lack of heat in the nest. Alternatively, Joanen (1969) observed a nest no longer attended by the female, in which half of the young had managed to hatch and liberate themselves whereas the other half were matured but died in the egg.

Three additional eggs from our nest showed development to the point of some bone and scale, but these had died early in incubation. The remaining 10 eggs appeared to have been infertile.

One day after hatching, we measured total length

(TL), snout-vent length (SVL), and mass of each of the 12 hatchlings. Mean ± STD, and minimum-maximum measurements were: TL (mm) 213.4 ± 5.1 (205-222); SVL (mm) 97.0 ± 3.0 (90-102); and mass (g) 25.4 ± 2.8 (20.9-29.6). In Louisiana, McIlhenny (1935) noted that hatchlings usually measure 9 in. (229 mm) or more, so ours may be a little small, consistent with the smaller eggs.

During hatching in the nest, hatchlings were aggressive and bit debris and each other while exiting their shells. Biting and wiggling helped the emerging alligators get their front legs free from the shell so they could pull themselves from, and shake off, their housing (Fig. 3). Only the snout protruded from 1 hatching egg brought to the lab, but the alligator did not exit overnight. The next morning, RT touched the snout of this baby with his finger, eliciting a biting response. The alligator was allowed to bite, and it held on while wriggling free from its eggshell. The aggressive biting behavior of the new hatchlings diminished within a few hours, and we observed little antagonistic interaction afterwards although all 12 hatchlings were kept in constant contact.

We placed hatchlings in a dry holding tank for 3 days until their umbilici were closed (to decrease chances of infection). Then, they were transferred to a 90 cm X 44 cm glass tank filled with dechlorinated water to a depth of 3 cm. On 3 October, 6 days after collection from the nest, we began attempts at feeding. Because smallest alligators feed largely on invertebrates and small fishes (McIlhenny 1935, Delaney 1990), our hatchlings were fed aquatic insects and worms collected locally, as well as commercial crickets and small shiners. Video of feeding behaviors was recorded by use of cell phones.

Figure 3. A hatchling alligator with only its head free from the eggshell often bit debris or siblings to help get its front legs out of the shell, Clark Co., 27 September 2018. Photo by RT.

We first offered earthworms and crickets, and the hatchlings attempted capture usually by sideways thrusts of their heads as potential prey came within reach. Within a few days, the hatchlings pursued cricket prey by taking a few steps in the direction of nearby prey, and attempted capture by use of side thrusts of the head. When we introduced earthworms to the tank, foraging behavior consisted of an individual placing its snout against the bottom of the tank and walking while moving the head side-to-side, at a rate of checking both sides about each second. When worms were caught, hatchlings were inept at prey handling and mostly repetitiously bit the prey, with the earthworm either escaping or being broken apart. Within a few days, capture of worms became aggressive and the hatchling shook the worm vigorously and began forward head thrusts to move the worm toward the back of the throat for ingestion.

Occasionally, two alligators grabbed the same earthworm, and attempted to pull it from each other. On 9 October, 12 days after recovery from the nest, we first observed the spin motion characteristic of crocodilians that attempt to separate meat from a prey item. More aggressive foraging behavior had developed along with this behavior, including pursuit and shaking of captured prey.

We introduced Golden Shiners (*Notemigonus chrysoleucas*) as prey on 8 October. Alligators initially had difficulty catching this more elusive prey, and pursued more vigorously and with more misses than with previous prey. When prey were caught, alligators had difficulty swallowing. Some individuals carried the catch in their mouths for some time, and eventually began pressing their heads against the aquarium glass to push the prey further into their mouths for consumption. As foraging skills improved, capture was more successful but competition became apparent as individuals commonly grabbed prey held in the mouths of siblings. Rolling behavior had improved and was employed more quickly and efficiently as the alligators had developed strength and agility, and most captured shiners were sheared and quickly became partial meals of 2 alligators.

After strong feeding behaviors had developed, we transferred the hatchlings to the alligator farm at Hot Springs on 16 November 2018.

Anecdotal Observations of Other Alligator Nests

Successful nests in eastern Texas had larger circumferences, were closer to water but farther from trees, and had less canopy cover, and the primary causes of failure were predation and flooding (Saalfeld 2010). The nest we report was at the bank of a pond on the opposite side from activity of feral pigs, which reduced the chances of predation. McCallum *et al*. (2003) and Trauth *et al*. (2004) noted a nest at Arkansas Post National Memorial that appeared to have been comprised of herbaceous vegetation and constructed at the base of a tree.

Grassy Lake, in Hempstead Co., contains a population of alligators representing the original stock of native alligators in Arkansas (Irwin and Wooding 2002). During 2003, 5 nests were located in the area. Two of the nests were built at the base of bald cypress (*Taxodium disticum*) trees and had incorporated Redvine (*Brunnichia ovata*) material in the construction of the nest, and 2 were found with females attending the nest. Nests were constructed away from nearby human activity, and wallows were present. Four of these nests were constructed with heavy reliance on woody debris ranging from sticks to branches, 50-100 mm wide (Fig. 4). In southern Louisiana, where herbaceous marsh plants are more available, alligators used branches torn from bushes of larger diameter (such as alders 64-76 mm, 2.5-3.0 in.) for use in building nests, but discarded the trunks (McIlhenny 1935). Nests in the more wooded areas around Grassy Lake incorporated such materials, however.

Figure 4. An alligator nest near Grassy Lake, Hempstead Co., comprised of larger sticks and branches mounded with other debris and dirt at the base of a bald cypress (*Taxodium distichum*), 30 August 2003. Photo by Lisa K. Irwin.

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Bilateral Diaphyseal Chondrodysplasia and Polymorphic Osteodysplasia of the Tibiofibulas in a Southern Leopard Frog, *Lithobates sphenocephalus* (Amphibia: Anura: Ranidae)

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Running Title: Tibiofibula Osteochondrous Dysplasia in a Southern Leopard Frog

Much attention has been focused on limb malformations in anurans following the startling discovery in 1995 of major limb deformities in Northern Leopard Frogs (*Rana pipiens*) from Minnesota in 1995 (Ouellett *et al*. 1997; Ouellet 2000; Blaustein and Johnson 2003; Lannoo 2008). Ouellet (2000) and Lannoo (2008) provided in-depth summaries on the widespread occurrence of anuran limb malformations in the United States. Blaustein and Johnson (2003) described the complexity of these malformations. The numerous causes for these malformations can be attributed to a number of natural phenomena, or they can be considered as being manmade (Lannoo 2008).

In Arkansas, Thigpen *et al.* (2014) documented limb abnormalities in a population of Fowler's Toads (*Anaxyrus fowleri*). They also provided a short description of the complexity of each observed abnormality. McCallum (1999) reported on the malformed Southern Leopard Frogs (*Rana sphenocephala* = *Lithobates sphenocephalus*) in Illinois and summarized the literature on amphibian abnormalities to date. McCallum and Trauth (2003) reported the Arkansas distribution and growing frequency of abnormalities among Blanchard's Cricket Frogs (*Acris blanchardi*) over four decades. However, the frequency of amphibian abnormalities in Arkansas and the species afflicted is an understudied segment of their ecology. Below, we report on a previously undescribed type of limb abnormality (Meteyer 2000) in the Southern Leopard Frog (*Lithobates sphenocephalus*) from Arkansas.

While road cruising on Turkey Pond Loop (35.2147195N, 92.7567921W) on the night of 21 September 2018 in Conway County, SET collected 5 *Lithobates sphenocephalus* (Dodd 2013) specifically for histological study of the urogenital anatomy in this species. All frogs were returned to Trauth's histo-herpetology laboratory in Morrilton and sacrificed using a dilute chloretone solution. Upon closer examination of the frogs prior to fixation in 10% neutral buffered formalin (NBF), SET observed that one female (SVL = 63 mm) exhibited unusual swellings in the diaphyseal region of both tibiofibulas. This frog was tagged with a personal identification tag (SET 4625) and immediately photographed (Fig. 1). (Later, this specimen was re-tagged with an Arkansas State University Museum of Zoology tag—ASUMZ 3370).

We removed the swollen portion from the diaphyseal region of the left tibiofibula of our specimen along with the tibiofibula proper into 10% NBF. Then, we treated the tissue with a decalcifying solution (1% hydrochloric acid) for 48 h. Next, we prepared the tissue for light microscopy and staining using standard histological methods (Presnell and Schreibman 1997). We dehydrated the tissue mass in a graded series of ethanol solutions, cleared the mass with xylene, and infiltrated the mass in paraffin in a 56°C oven overnight. We then placed the tissue into an embedding mold for hardening, which was followed by block trimming and mounting for sectioning using a rotary microtome. We histosectioned the tissue into 10 µm serial ribbons and adhered these ribbons to microscope slides with Haupt's adhesive. We transferred the slides through staining dishes containing hematoxylin (eosin counterstaining) —H&E to reveal general cytology or Pollak trichrome stain (Pollak) to enhance connective tissue components. SET photographed tissue images using a Leica M80 stereomicroscope and/or a Leica DM 2000 LED light microscope. All slides are currently in the possession of the senior author.

Histological examination of the tissue mass (Figs. 2, 3) revealed a complete disruption of the normal diaphyseal bone structure in this adult frog. The tibiofibula was separated into two poorly ossified and mostly fragmented bony shaft regions (Fig. 2A) on opposite sides of the lesion. These peripheral segments of compact bone were surrounded by hypertrophic regions of hyaline cartilage intermingled with complexes of dysplastic bone (Fig. 2B). Polymorphic bone is the name we have chosen for this unusual dysplastic bone type, because of its aberrant physical appearance and configuration. This abnormal bone type shares some structural features with cancellous (spongy) bone of caudates (Castanet *et al.* 2003) by exhibiting a

Figure 1. A. Ventral view of female *Lithobates sphenocephalus* (ASUMZ 33750) possessing bilateral diaphyseal dysplasia (arrows) of the tibiofibulas. B. Magnification of the hindlimbs of A with arrow pointing to the bulbous enlargement of the left tibiofibula.

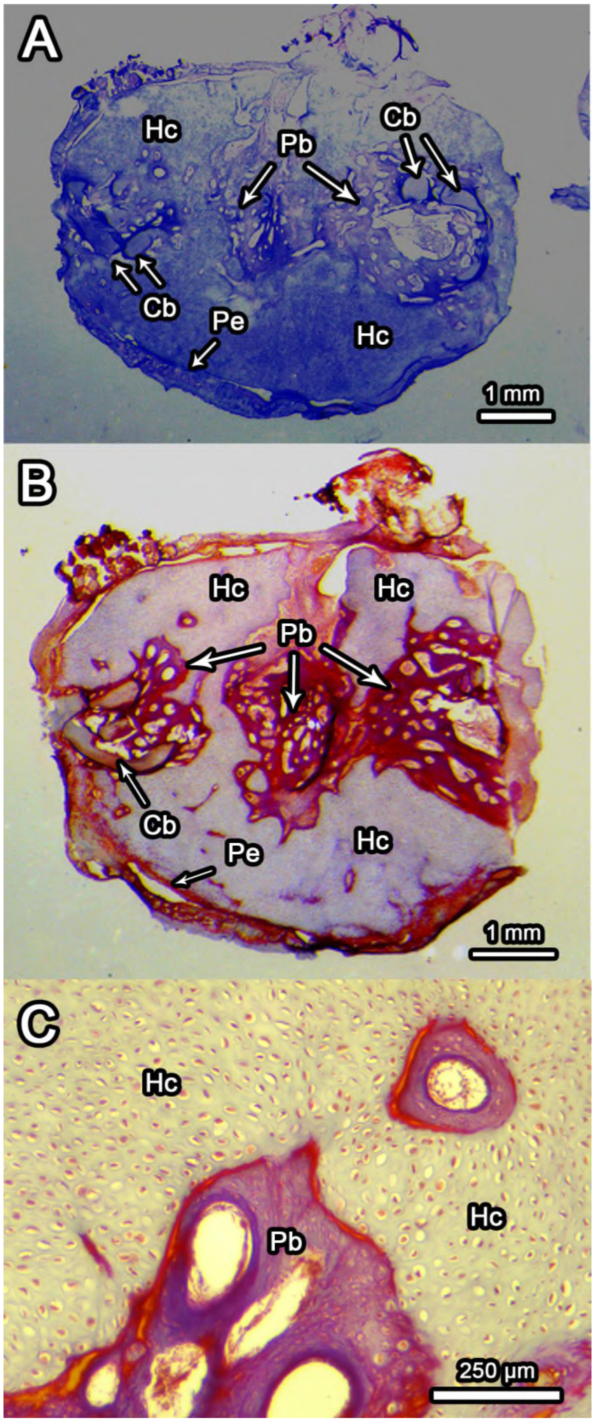

Figure 2. A. Transverse section through the bulbous tissue mass of the left tibiofibula of ASUMZ 33750 revealing original diaphyseal bone shaft fragments of compact bone (Cb) along with a preponderance of hyaline cartilage (Hc) interspersed around regions of polymorphic bone (Pb). Pe = perichondrium. H&E. B. Transverse section of bulbous mass showing the circumferential arrangement of hyaline cartilage (Hc) surrounding three regions of polymorphic bone (Pb). The perichondrium (PE) lines the exterior of the hyaline cartilage. Pollak. C. Magnification of hyaline cartilage and polymorphic bone. A Pb nodule is isolated at the upper right. Pollak.

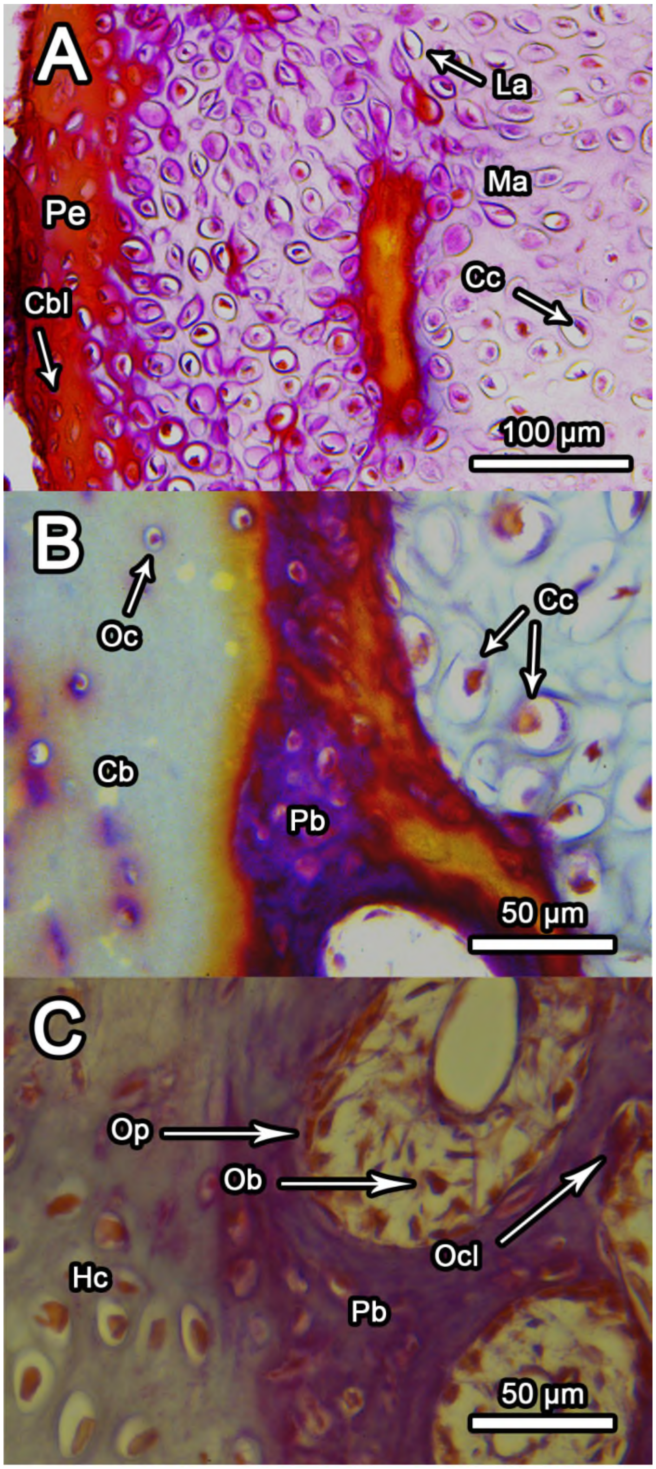

Figure 3. A. Magnification of the perichondrium (Pe) shown in Fig. 2B illustrating the proliferation of chondrocytes of hyaline cartilage. A chondrocyte (Cc) can be seen within its lacuna. Pollak. La = lacuna; Ma = matrix; Cbl = chondroblast. B. Magnification of the compact bone /hyaline cartilage interface of Fig. 2B separated by a zone of polymorphic bone. Abbreviations as before. Pollak. Oc = osteocyte. C. Magnification of a hyaline cartilage/polymorphic bone boundary zone showing an osteoprogenitor cell (Op), an osteoblast (Ob), and an osteoclast (Ocl) within pocket-like nodules. Pollak.

trabeculae-like fretwork whose inner linings are comprised of endosteal cell layers of osteoprogenitor and osteoblast cells (Fig. 3C); however, these trabeculae lack any evidence of extensive calcification even though osteoprogenitor cells appeared to be scattered about within the dark-staining matrix. Also, the trabeculae of polymorphic bone exhibited signs of continuous remodeling as evidenced by the numerous pocket-like nodules (Fig. 2C) scattered throughout much of the chondrodysplastic regions that dominate this tumor-like growth. The presence of osteoclasts supports this hypothesis (Fig. 3C).

We observed three major polymorphic bone aggregates (Fig. 2B). The overall design of these osteogenic regions can best be described as an arachnoid-like patchwork of numerous pockets, channels, spaces, and nodules separated by trabeculae containing a matrix embedded with subperiosteal bone cells.

Mizgireuv *et al.* (1984) reported a high incidence of tumor-like osteochondrous dysplasia affecting the hindlimbs of the Inkiapo Frog, *Rana chensinensis*, and the lesions were associated with larval development in paper factory and sewage effluents. The authors concluded that the anomalies in adult frogs were attributed to the cytotoxic effect of teratogenic agents.

At present, we are unaware of any environmental conditions that could account for the osteochondrous dysplasia in our specimen. Moreover, the remarkable bilateral placement of the 2 lesions in our specimen of *Lithobates sphenocephalus* suggests the possibly of a genetic factor leading to a pairing of hindlimb developmental anomalies during embryonic bone growth.

Acknowledgments

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Age Estimation using Phalangeal Skeletochronology in Northern Crawfish Frogs, *Lithobates areolatus circulosus* (Amphibia: Anura: Ranidae), from Arkansas

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Running Title: Phalangeal Skeletochronology in the Northern Crawfish Frog

As an obligate crayfish burrow dweller, crawfish frogs have historically occupied a relatively narrow ecological niche throughout their distribution in the tall grass prairies and grasslands of the central and south-central United States (Redmer 2000; Powell *et al.* 2016; Lannoo *et al.* 2018). Habitat loss and shifting climate patterns pose as major threats to the continued existence of this species (Lannoo and Stiles 2017). In Arkansas, the Northern Crawfish Frog, *Lithobates areolatus circulosus*, occurs in only 19 of its 75 counties (Trauth *et al.* 2004; Trauth and Holt 2017). Because of their secretive nature, late winter-early spring breeding season, and current protected status by the Arkansas Game and Fish Commission, this species remains a rarity in most museum collections in the state (Trauth *et al.* 2004). Moreover, only anecdotal information exits regarding any aspect of their natural history in Arkansas (Trauth *et al.* 1990).

In the present study, we chose to conduct a phalangeal skeletochronological investigation of the Northern Crawfish Frog utilizing museum specimens (n = 10) deposited in the herpetological collection (ASUMZ 13900, 14150, 31084-86, 33746-49, 33611) housed in the Arkansas Center for Biodiversity Collections located at Arkansas State University. Four frogs included in this sample were recently collected by SET while road cruising on Turkey Pond Loop (35.2147195N, 92.7567921W) in Conway County on the 20th and 27th February, 2018. Our goals were to estimate the age of individuals within this small Arkansas frog sample by counting annular lines of arrested growth (LAGs) and compare these results with the age estimates found for this frog by Redmer (2000), who utilized the same histological technique on a crawfish frog population in southern Illinois.

The distal phalanx (Fig. 1) of the 4th toe of the left hind foot from each frog was removed and placed into either 70% ethanol (historic specimens) or 10% neutral buffered formalin for fresh toes. Then, we treated all toes with a decalcifying solution (1% hydrochloric acid) for 48 h. Next, we prepared the phalanxes for examination by light microscopy using standard histological methods (Presnell and Schreibman 1997). We dehydrated the toes in a graded series of ethanol solutions (70-100%), cleared each toe with xylene (50-100%), and infiltrated the toe segment in paraffin in a 56 °C convection oven overnight. We then embedded each toe into a paraffin mold for hardening, which was followed by block trimming and mounting for sectioning using a American Optical rotary microtome. We histosectioned each toe phalanx transversely into 10 µm serial ribbons and adhered these ribbons to microscope slides with Haupt's adhesive. We transferred the slides through staining dishes containing hematoxylin and eosin (H&E). One of us (SET) photographed the toes using a Leica DM 2000 LED light

Figure 1. Photomicrograph of a transverse section through the distal phalanx of *Lithobates areolatus circulosus* (ASUMZ 33748) revealing phalangeal bone (Pb). Sm = striated muscle.

microscope. All slides are currently in the possession of SET.

The toes of 8 males and 2 females were examined and yielded the following results (ASUMZ no., collection date, snout-vent length—SVL, gender, and estimated age in yrs.): 1) ASUMZ 13900, 7 March 1987, 71, male, 1; 2) ASUMZ 14150, 14 March 1989, 80, male, 2; 3) ASUMZ 31085, 29 April 2008, 96, male, 3; 4) ASUMZ 31086, 29 April 2008, 92, male, 3; 5) ASUMZ 33746, 20 February 2018, 75, male, 3; 6) ASUMZ 33748, 27 March 2018, 88, male, 4; 7) ASUMZ 33749, 20 February 2018, 88, 4; 8) ASUMZ 33747, 20 February 2018, 105, female, 5; 9) ASUMZ 31084, 1 January 1966, 111, female, 4, and 10) ASUMZ 33611, 28 February 2017, 120, male, 8.

Our skeletochronological results were generally similar to the age-body length distributions reported by Redmer (2000) for his crawfish frog population from southern Illinois. Except for our largest male (10), older males (6, 7; Fig. 1A, B) exhibited 4 LAGs, and this estimated age matched well with most of the body sizes of 4-year-old males found in Illinois. Two of our 3-year-old males (3, 4) had slightly larger body sizes compared to the Illinois sample (71 – 86 mm SVL). These larger body sizes possibly indicate growth following reproductive activity. Redmer (2000) had no 1-year-old males in his sample. Our 1-year-old male matched in size to his 2-year-old males. Our oldest female (8) was 5 years old (Fig. 1C). Except for our oldest male (Fig. 3), all body sizes were comparable to values found for frogs reported by Redmer (2000). In addition, 8 years, as observed in our oldest male, was near the maximum lifespan of 9 years documented for this species (Mike Lannoo *pers. comm.*).

Skeletochronology can show increments of annual growth as well as cyclic growth patterns in bones. These patterns often reflect seasonal changes in feeding activity resulting from dramatic shifts in climatic events. For example, the male in Fig. 2B exhibited a large growth increment in year 1, which was then followed by a much smaller increment in year 2. The growth rings seen in years 3 and 4 are, in turn, even smaller than year 2 but similar to one another. In contrast, the variations in the growth pattern of the female shown in Fig. 2C are striking with substantial growth in year 2 followed by greatly reduced growth in years 3 and 4. Then, growth in year 5 showed a two-fold increase compared to the two previous years.

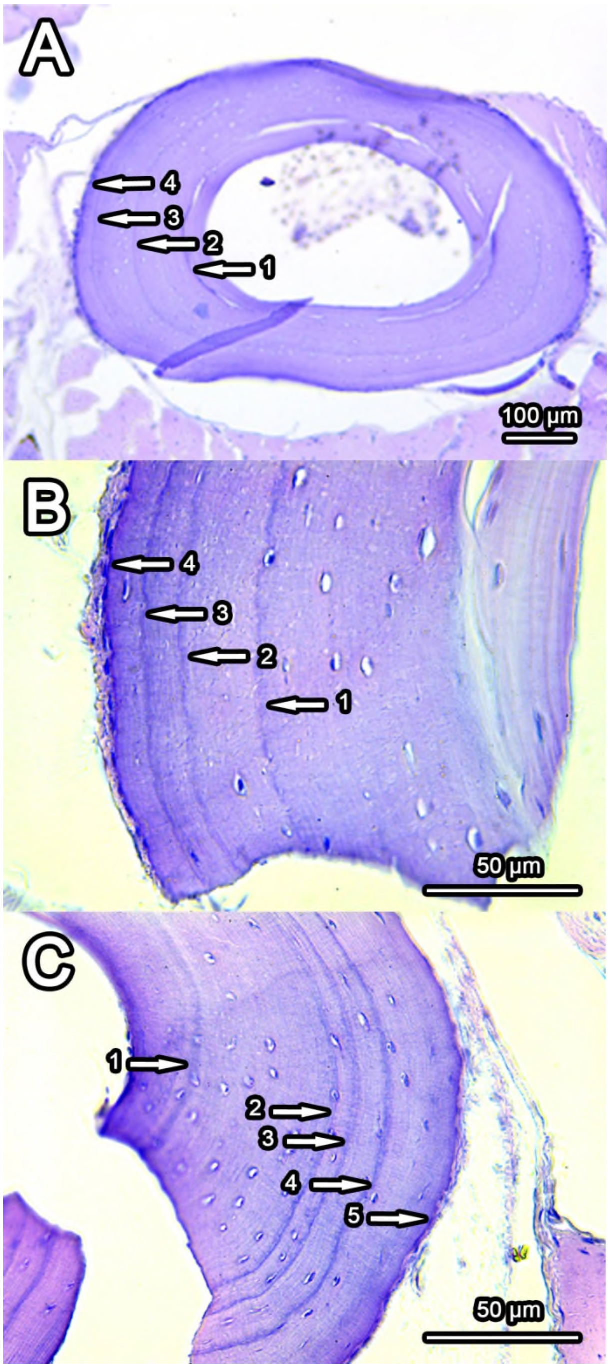

Figure 2. Photomicrographs of transverse sections through the distal phalanxes of 3 *Lithobates areolatus circulosus* revealing lines of arrested growth (ends of arrows). A. ASUMZ 33748. B. ASUMZ 33749. C. ASUMZ 33747.

Acknowledgments

We thank the Arkansas Game and Fish Commission for issuing the scientific collection permits to SET (nos. 012020161 and 020520182).

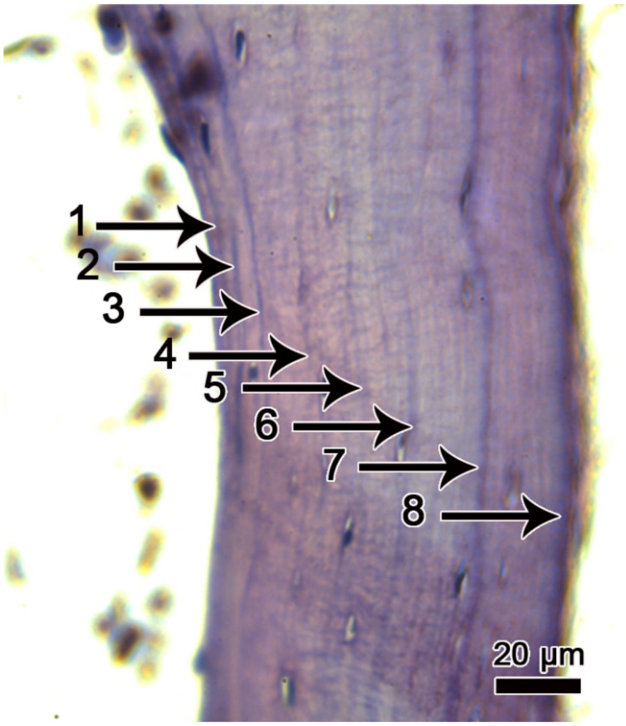

Figure 3. Photomicrograph of a transverse section through a distal phalanx of *Lithobates areolatus circulosus* revealing 8 lines of arrested growth (ends of arrows) in ASUMZ 33611.

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Notes on the Natural History of Selected Invertebrates from Arkansas, 2019

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Running Title: Natural History of Invertebrates

The invertebrate fauna are a large component of ecosystems. Yet, invertebrate biologists are few and scattered in Arkansas; thus, the invertebrate fauna of much of the state is poorly known. Herein we document new records of distribution and provide notes on the natural history of selected invertebrates from Arkansas. Field observations and collections were made by the authors and students at Henderson State University (HSU) and Southern Arkansas University (SAU). Invertebrate specimens were preserved in 70% or 90% isopropanol and housed at HSU, SAU, or the United States National Museum (USNM). Digital photography also was used to document species within their habitats (images available from RT).

CLASS TURBELLARIA

Bipalium kewense Moseley 1878. Land Planarian. This land planarian is easily identified by its diagnostic spade-like head and bi-colored body. McAllister *et al*. (2018) provided an updated distribution map of this exotic planarian in Arkansas.

On 4 April 2010 a single adult specimen of *B. kewense* was collected from a under a stepping stone in an urban yard in El Dorado, UNION CO. by MBC. Other specimens were collected around this time at the same location. This represents a new county record for Union County; however, these planarians have been reported from adjacent Ashley, Columbia, and Ouachita counties (McAllister *et al*. 2018).

CLASS CRUSTACEA

Cambarus diogenes Girard. Devil Crayfish. Reimer (1963) reported this primary burrower as *C. diogenes diogenes* from 18 counties in Arkansas. While this burrowing crayfish is fairly common in Arkansas, we report 2 new county records in LEE CO.: Burrow ca. 11 km. (6.8 mi.) NE. of Marianna, AR (Sec. 35, T3N, R4E), 20 May 1983, HWR (1 specimen), and ST. FRANCIS CO.: Burrow in a roadside ditch along St. Hwy. 149, 4 km. (2.5 mi.) S. of Shell Lake, AR (Sec. 31, T16N, R6E), 25 May 1982, HWR (1 ovigerous female).

Cambarus ludovicianus Faxon – Painted Devil Crayfish. Reimer (1963) reported this primary burrower as *C. diogenes ludovicianus* from 28 counties in Arkansas. Tumlison *et al*. (2016) provided additional collections of *C. ludovicianus* from Columbia Co. and the Lafayette-Columbia county line. McAllister *et al*. (2018) added 3 counties (Bradley, Clark, and Dallas) to its range in Arkansas. This primary burrower is common in southwestern and southeastern Arkansas, but herein we report 2 new county records from LINCOLN CO.: Roadside burrow along St. Hwy. 81, 11.3 km. (7 mi.) S. of Star City, AR (Sec. 20, T10S, R7W), 5 May 1977, HWR (1 specimen), and LITTLE RIVER CO.: Burrow ca. 6.4 km. (4 mi.) S. of Foreman, AR on St. Hwy. 41 (Sec. 10, T13S, R32W), 6 June 1979, HWR (1 specimen).

Procambarus clarkii (Girard) - Red Swamp Crayfish. Faxon (1914) was the first to report this species from Arkansas listing the species from Pulaski County. Later, Reimer (1963) collected *P. clarkii* from 9 counties in eastern Arkansas including Clay, Chicot, Craighead, Crittenden, Desha, Greene, Jackson, Mississippi, and Monroe counties. McAllister *et al*. (2018) recently added 4 counties to its state range, notably Lee, Phillips, St. Francis, and Union counties. We report first county records for LINCOLN CO.: Roadside ditch at Garrett Bridge, AR (Sec. 6, T10S, R5W), 23 March 1980, HWR (1 specimen) and POINSETT CO.: Roadside ditch along U.S. Hwy. 63, ca. 6.4 km. (4 mi.) S. of Trumann, AR (Sec. 7, T11N, R6E), 17 April 1978, HWR (1 specimen).

Procambarus tulanei Penn - Giant Bearded Crayfish. Reimer (1963) reported *P. tulanei* from only 2 counties in southern Arkansas including Columbia and

Ouachita. Hobbs and Robison (1988) documented 25 collections of *P. tulanei* from 10 counties including Ashley, Columbia, Drew, Hot Spring, Jefferson, Lafayette, Montgomery, Nevada, Ouachita, and Union counties. Tumlison and Robison (2010) added new county records from Bradley and Clark counties. Herein, this crayfish is documented for the first time from DALLAS CO.: (1) Ditch along St. Hwy. 7, ca. 7.2 km. (4.5 mi.) NW of Sparkman, AR (Sec. 6, T9S, R17W), 22 April 1989, HWR (1 specimen) and LINCOLN CO.: Burrow along St. Hwy. 114, 1.6 km. (1 mi.) E of Palmyra, AR (Sec. 15, T10S, R8W), 24 March 1979, HWR (1 specimen).

CLASS CHILOPODA

Hemiscolopendra marginata (Say). Eastern bark centipede. This centipede occurs throughout the Southeastern United States with a northern range limit occurring in northwestern Arkansas (Shelley 2002). Herein, we provide 3 new county records (Fig. 1) to augment the previously known Arkansas distribution map provided by McAllister *et al*. (2012). BENTON CO.: St. Hwy 12 in Gentry, 8 Oct 2012, LK Ramey; CARROLL CO.: Berryville, 27 Apr 2017, MBC; and MARION CO.: vic. Mull, 13 Apr 2018, MBC.

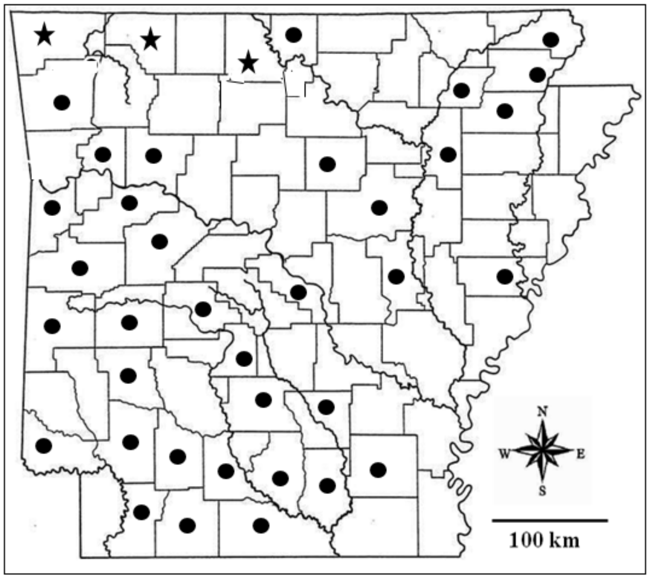

Figure 1. Records of *Hemiscolopendra marginata* in Arkansas. Symbols: dots = historic records; stars = new records.

CLASS ARACHNIDA

Nephila clavipes (Linnaeus 1767) – the golden orbweaver, golden-silk orbweaver, or banana spider. *Nephila* is primarily a neotropical genus, and *N. clavipes* is the only species that occurs in North America. It ranges from Central America into United States along the Gulf Coast, occurring primarily in the warmer portions of the subtropical regions (Comstock 1948; Levi 1980; Evans 2007). Preferred habitat tends to be damp areas with open forest and humidity above 80% (Moore 1977).

Nephila clavipes was first documented in Arkansas during 2009 in the Ouachita River bottoms, Felsenthal National Wildlife Refuge (FNWR), in Ashley and Union counties of southeastern Arkansas (Tumlison and Robison 2010). They reported 4 individuals at that time, but by 2015 the population observed in the area increased to 38 (Tumlison *et al.* 2016). A visit to the site in September 2018 revealed no individuals of *N. clavipes*. A part of the winter of 2017 was particularly cold in the area, with lows between -3 and -9°C (16 to 27 °F) for 4 consecutive days between 7-10 January (https://www.usclimatedata.com/climate/crossett/arkansas/united-states/usar0133/2017/1). Range expansion by this large tropical spider northward from the southern coastal US has been attributed to climate change (Bakkegard and Davenport 2012), with warming temperatures allowing northward expansion. It appears that several days of significant cold may recede the northern limit of range. Thus, expansion and contraction of the range of *N. clavipes* in southern Arkansas may serve as an index of effects of climate change in the state.

Acknowledgments

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Photographic Record of a Greater Roadrunner (*Geococcyx californianus*) from Drew County, Arkansas

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Running title: Greater Roadrunner (*Geococcyx californianus*) from Drew County

The geographic range of the greater roadrunner (*Geococcyx californianus*) extends from central Mexico to north-central California in the west and to western and central Arkansas in the east (Hughes 2011). This range has been expanding to the north and east during historical times, including in Arkansas (Hughes 2011). Greater roadrunners were first recorded in southwestern Arkansas in 1936 (Baerg 1950). By 1950, they were recorded in Crawford County and Pulaski County (James 1960), and by the mid-1950s, greater roadrunners occurred in Washington and Benton counties, where they were considered to be numerous (Brown 1963). The published range of the greater roadrunner in Arkansas currently extends across the state, except in a north-south band adjacent to the Mississippi River (James and Neal 1986; Hughes 2011). Herein we provide the first published record and photograph (Fig. 1) of a greater roadrunner from Drew County.

In June 2018, the authors began hearing rumors of a greater roadrunner being sighted within the city limits of Monticello. Several residents claimed to have seen the bird hanging around the edges of the local golf course. On July 10, the owner of a home just to the northeast of the golf course alerted us to the presence of the bird on his property, and allowed us access to investigate. Although we were unable to find the greater roadrunner on that day, we returned on July 11 and were able to observe and photograph the bird (Fig 1). The greater roadrunner, which appeared to be an adult, was

Fig 1. Greater roadrunner (*Geococcyx californianus*) photographed on 11 July 2018 at Monticello, Drew County, Arkansas.

visible for nearly an hour, during which it wandered along the edge of a wooded area, hunted for food, and took a dust bath. We continued to hear reports of the bird throughout the summer. One observer claimed to have seen two greater roadrunners at one time, but we were unable to confirm more than one individual. In June and July 2019, a greater roadrunner, presumably the same individual, was seen and photographed on the same property where we photographed the bird in 2018.

Greater roadrunners have previously been reported from near Crossett in Ashley County in 1978 (James and Neal 1986), and from the western part of the state of Mississippi in 1982 (Maxon 2005). Although the geographic range is expanding, possibly in part due to changes in habitat and warming climate, it is unclear whether the individual observed in Drew County is part of a trend or merely another extra-limital record. We intend to monitor the area for additional observations.

Acknowledgment

We thank Bennie Ryburn III for access to his property.

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Haemogregarina sp. (Apicomplexa: Eucoccidiorida: Adeleorina) from Eastern Spiny Softshell, *Apalone spinifera spinifera* (Testudines: Trionychidae), from Arkansas

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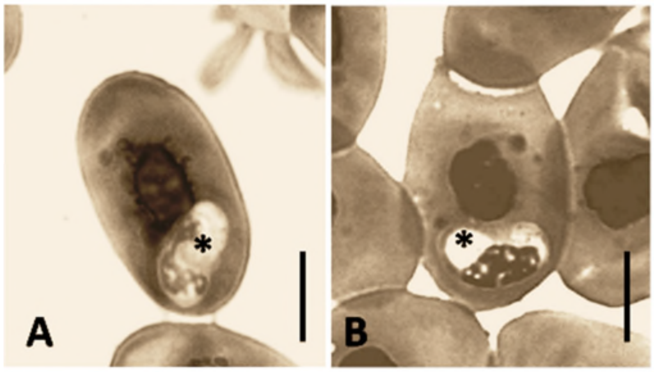
Running Title: Haemogregarine from *Apalone spinifera*

Haemogregarines are intraeythrocytic parasites that infect various vertebrates but are most commonly reported from aquatic turtles with leeches serving as the only known invertebrate hosts and vectors (Telford 2009). Numerous turtles from all the surrounding states of Arkansas (except Mississippi) have been reported to serve as hosts, including some from Louisiana (Degiusti and Batten 1951; Herban and Yaeger 1969; Acholonu 1974), Missouri (Smith *et al.* 1983) Oklahoma (McAllister 2015), Tennessee (Edney 1949) and Texas (Wang and Hopkins 1965). Hematozoan parasites have been identified in Arkansas turtles (McAllister and King 1980; McAllister *et al.* 1995, 2014, 2016), but nothing is known about those of spiny softshell turtles in the state. Here we report a new host record and the first photomicrographs of a haemogregarine from a common softshell turtle in Arkansas.

A single juvenile eastern spiny softshell. *Apalone spinifera spinifera* (carapace length = 145 mm) was collected by hand on 21 April 2017 from Crow Creek at Madison, St. Francis County (35°00'45.12"N, 90°44'16.71"W). It was killed by an intraperitoneal injection of sodium pentobarbital (Nembutal®) following accepted guidelines (SIH 2004). A bone saw was used to remove the plastron to expose the heart. Blood was obtained by making a small incision in the heart and taking a sample using an ammonium heparinized (75 mm long) capillary tube. Thin films were smeared onto glass slides, air-dried, fixed for 1 min. in absolute methanol, stained for 20–30 min. with Wright-Giemsa stain, and rinsed in phosphate buffer (pH = 7.0). Slides were scanned at 100× or 400× and when infected cells were found, photographs were taken. Length and width (L × W) measurements on gamonts of an intraerythrocytic parasite ($n = 20$) using a calibrated ocular micrometer under a 1,000× oil immersion lens are reported in micrometers (µm) as means ±SD followed by the ranges in parentheses. A photographic voucher of the host was deposited in the Henderson State University Vertebrate Collection, Arkadelphia, Arkansas. A voucher slide was deposited in the Harold W. Manter Laboratory (HWML) of Parasitology, University of Nebraska, Lincoln, Nebraska.

The red-blood cells of the *A. s. spinifera* was found to possess intraerythrocytic hematozoans (HWML 216010) thought to represent a *Haemogregarina* sp. Gamonts were kidney-bean shaped with a length of 12.5 ± 0.5 (11.5–13.0) µm and width of 8.0 ± 0.2 (7.8–8.2 × 5.8–6.0) µm (Figs. 1A–B). The nucleus of the parasite was elongate-ellipsoidal and measured 7.3 ± 0.2 × 5.4 ± 0.2 (7.0–7.5 × 5.3–5.6) µm (Fig. 1B). Intensity of infection revealed 1–2 gamont(s) infected erythrocyte(s)/20 microscopic fields.



Figures 1A–B. Gamonts of *Haemogregarina* sp. from *Apalone spinifera*. (A) Kidney-bean shaped gamont (*). (B) Another kidney-bean shaped gamont (*) showing dark-staining ellipsoidal-elongate nucleus. Scale bars = 10 µm.

Compared to other aquatic turtles, little has been published on haemogregarines of spiny softshell turtles (Ernst and Ernst 1979). Apparently the first to report a haemogregarine was Edney (1949) who reported *H. stepanowi* Danilewsky in 3 of 4 (75%) *A. s. spinifera* from middle Tennessee. The life cycle of this parasite involves transmission by leeches as described by Reichenow (1910). Later, Wang and Hopkins (1965) reported a *Haemogregarina* sp. in a single Texas spiny softshell, *A. s. emoryi* from eastcentral Texas, and Herban and Yaeger (1969) found *H. stepanowi* in 3 of 5 (60%) western spiny softshells, *A. s. hartwegi* from Louisiana.

To date, 8 species of turtles have been reported from Arkansas to harbor hematozoans (McAllister and King 1980; McAllister *et al.* 1995, 2014, 2016) including: common snapping turtle (*Chelydra serpentina*), alligator snapping turtle (*Macrochelys temminckii*), southern painted turtle (*Chrysemys dorsalis*), eastern river cooter (*Pseudemys concinna*), red-eared slider (*Trachemys scripta elegans*), common map turtle (*Graptemys geographica*), Mississippi mud turtle (*Kinosternon subrubrum hippocrepis*), and stinkpot (*Sternotherus odoratus*). There are 17 species and subspecies of aquatic turtles within 4 families in Arkansas (Trauth *et al.* 2004) and 9 species are yet to be reported as hosts of hematozoans. Obviously, more work needs to be done in surveying additional turtles, including smooth softshell (*A. mutica*) in the state (and elsewhere), for these apicomplexan parasites.

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Cotylogaster occidentalis (Aspidogastrea: Aspidogastridae) from Freshwater Drum, *Aplodinotus grunniens* (Actinopterygii: Sciaenidae), in Northeastern Oklahoma

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Running Title: *Cotylogaster occidentalis* in Oklahoma

Introduction

Freshwater Drum, *Aplodinotus grunniens* Rafinesque, 1819 are endemic to freshwater environs of the Americas, and their distributional range extends as far north as the Hudson Bay of Canada and reaches as far south as the Usumacinta River Basin of Guatemala (Fremling 1980). In the United States, eastward distribution includes the southern Great Lakes, eastern Appalachians and the entire Mississippi basin westward as far as Kansas, Oklahoma, and Texas (Page and Burr 2011). This fish appears to have the greatest latitudinal range of any freshwater fish in North America. It feeds mostly on small crustaceans, clams, snails, insect larvae (especially chironomids) and small fish (Miller and Robison 2004). In Oklahoma, *A. grunniens* occurs throughout the state, mainly in the larger lakes and rivers but uncommon to absent in the northwest (Miller and Robison 2004).

Freshwater Drum have been reported to harbor over 66 taxa of parasites, including protistans, aspidogastreans, monogeneans, trematodes, cestodes, nematodes, acanthocephalans, leeches, molluscs, and crustaceans (Hoffman 1999). One of these parasites, an aspidogastrean, *Cotylogaster occidentalis* Nickerson, 1902, was originally described from *A. grunniens* in the Minnesota River, Minnesota (Nickerson 1902). To our knowledge, *C. occidentalis* has also been reported from *A. grunniens* from Iowa, Louisiana, Mississippi, Tennessee, and Lake Erie, Canada (Simer 1929; Bangham and Venard 1942; Sogandares-Bernal 1955; Dechtiar 1972; Stromberg 1970; Hoffman 1999), and from freshwater mussels from Iowa (Kelley 1927), Michigan (Fredericksen 1972), North Dakota and Manitoba, Canada (Carney 2015). In addition, a report by Huehner and Etges (1972) describing *Cotylogasteriodes barrowi* from freshwater mussels (*Lampsilis* spp.) from Ohio was found to represent an immature stage of *C. occidentalis* by Fredricksen (1972). The life cycle of *C. occidentalis* normally takes place in snails but can also involve fishes, including experimental infections of *A. grunniens* (Dickerman 1948).

Nothing is known about *C. occidentalis* in Oklahoma. Here we present data on specimens of *C. occidentalis* obtained from 1 individual *A. grunniens* from the state, including new information on the parasite from scanning electron microscopy.

Materials and Methods

A total of 17 juvenile and adult *A. grunniens* (110 to 475 mm total length) were collected by boat electrofisher or 9.1 m seine from the Mississippi River at Osceola, Mississippi County, Arkansas (*n* = 4), the Red River, Marshall County, Oklahoma (*n* = 6), the Red River at St. Hwy. 37, McCurtain County, Oklahoma (*n* = 1), and the Verdigris River, Wagoner County, Oklahoma (*n* = 6). Specimens were placed on ice and processed within 24 hr for parasites.

Of the 7 aspidogastrean trematodes taken from the posterior intestine of one host; 5 were fixed in hot 10% buffered formalin and stored in 70% ethanol. Two of these specimens were stained with acetocarmine, dehydrated in an ethanol series, cleared in xylene or methyl salicylate, and mounted in Damar gum on slides, as permanent preparations. Three specimens chosen for scanning electron microscopy (SEM) studies were post-fixed in 1.0% osmium tetroxide, dehydrated through a graded ethanol series, infiltrated with hexamethyldisilizane (HMDS), mounted on stubs, and sputter coated with gold. Coated specimens were scanned using a Hitachi TM3030+ scanning electron microscope. Two specimens were fixed in 100% molecular grade ethanol for future molecular studies.

A voucher of *A. grunniens* was deposited in the Henderson State University Vertebrate Collection (HSU), Arkadelphia, Arkansas. A voucher specimen of *C. occidentalis* was deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of

Nebraska, Lincoln, Nebraska as HWML 139990.

Results and Discussion

One of 17 (6%) *A. grunniens* from the Verdigris River was found to harbor 7 specimens of *C. occidentalis*. Worms were found free in the lumen of the posterior one-fourth of the intestine; some worms were intermixed with pelecypod (bivalve) mollusc remains.

Whole mounts of 2 stained specimens were compared to the detailed redescription of *C. occidentalis* by Fredericksen (1972) and agreed with it in all essential details. SEM studies showed the retracticle (telescopic) neck (Figs. 1 A–B), pentalobate oral disc, elongate ventral adhesive disc with transverse ridges and marginal alveoli (Figs. 1 A–C), and a dorsal cone (Fig. 1C), all characteristic of this species.

SEM observations in this study add new information on the morphology of this parasite. The mouth opening is round, simple, and surrounded by small papillae (Figs. 2 A–B) and the anterior lobe of the oral disc bears an apical slit-like pit (Fig. 2C). The gonopore, was visible on the telescopic neck of one fully extended specimen (Figs 1B, 2D); it is a sunken ovoidal opening surrounded a plicate tegument and in the specimen examined partially occluded by what appears to be a clump of sperm (Fig 2D). These features were not discussed in a previous SEM study of *C. occidentalis* (Ip *et al.* 1982) likely because the specimens used in that study were contracted and the oral disc was also retracted. As described by Ip *et al.* (1982), the marginal organs bordering the marginal loculi end in ducts that are either inverted/retracted into pore-like cavities (Figs. 3 A–B) or everted (Fig. 3C). The margins and ventral surface of the adhesive disc are covered with small papillae (Figs. 3A–B). A protrusible pharynx reported by Ip *et al.* (1982) was not observed in our study.

Cotylogaster occidentalis appears to be the only bonafide fish parasitic aspidogastrean in the Nearctic region (Hoffman 1999; Choudhury *et al.* 2016) and the Freshwater Drum is its principal fish host. However, there is a relationship between the diet of *A. grunniens* and infections with *C. occidentalis*; the aspidogastrean is usually found in larger specimens that are capable of feeding on molluscs (Fredericksen, 1972). The infected fish in this study also had a considerable amount of crushed bivalve shells in its intestine and several worms were found intermixed with these remains.

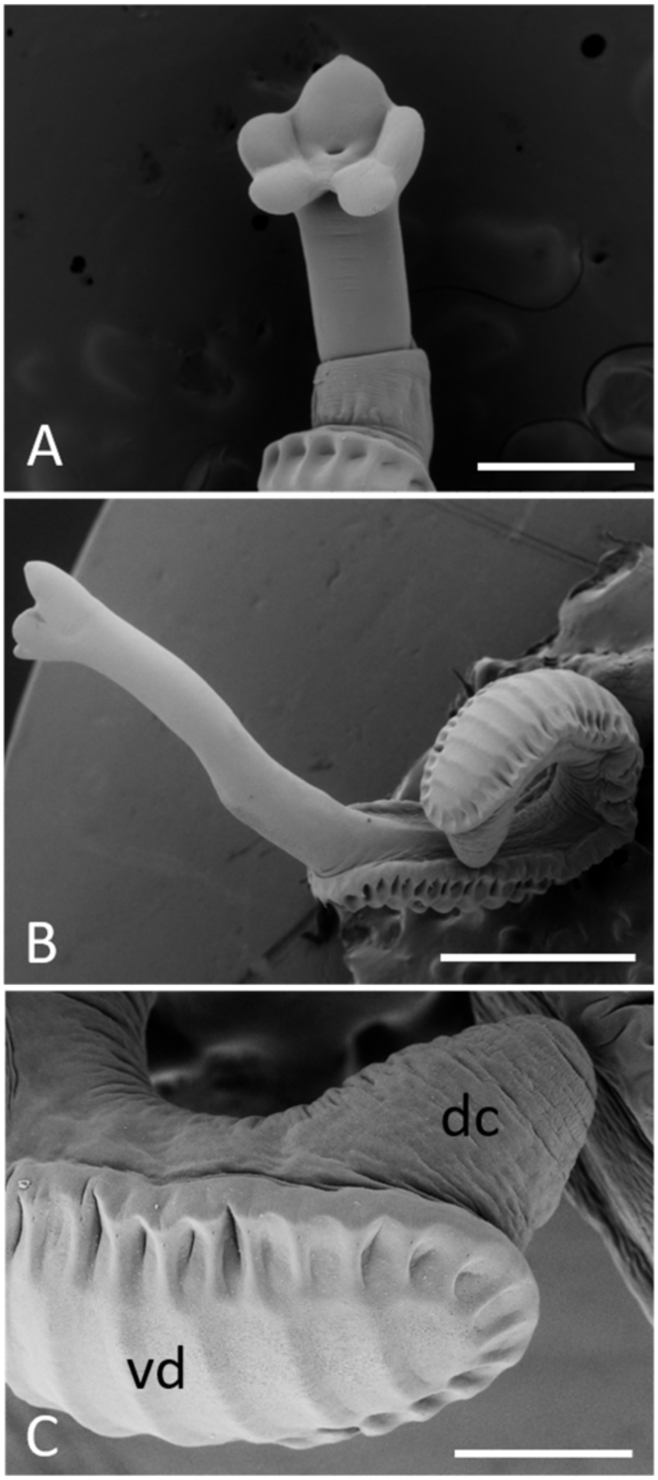

Figure 1. Scanning Electron Microscope (SEM) images of *C. occidentalis*. (A) Anterior end with partially retracted neck and expanded pentalobate oral disc; scale bar = 500 µm. (B) Whole mount with fully extended neck and partially opened oral disc; scale bar = 1 mm. (C) Terminal dorsal body cone (dc) and posterior end of ventral adhesive disc (vd); scale bar = 250 µm.

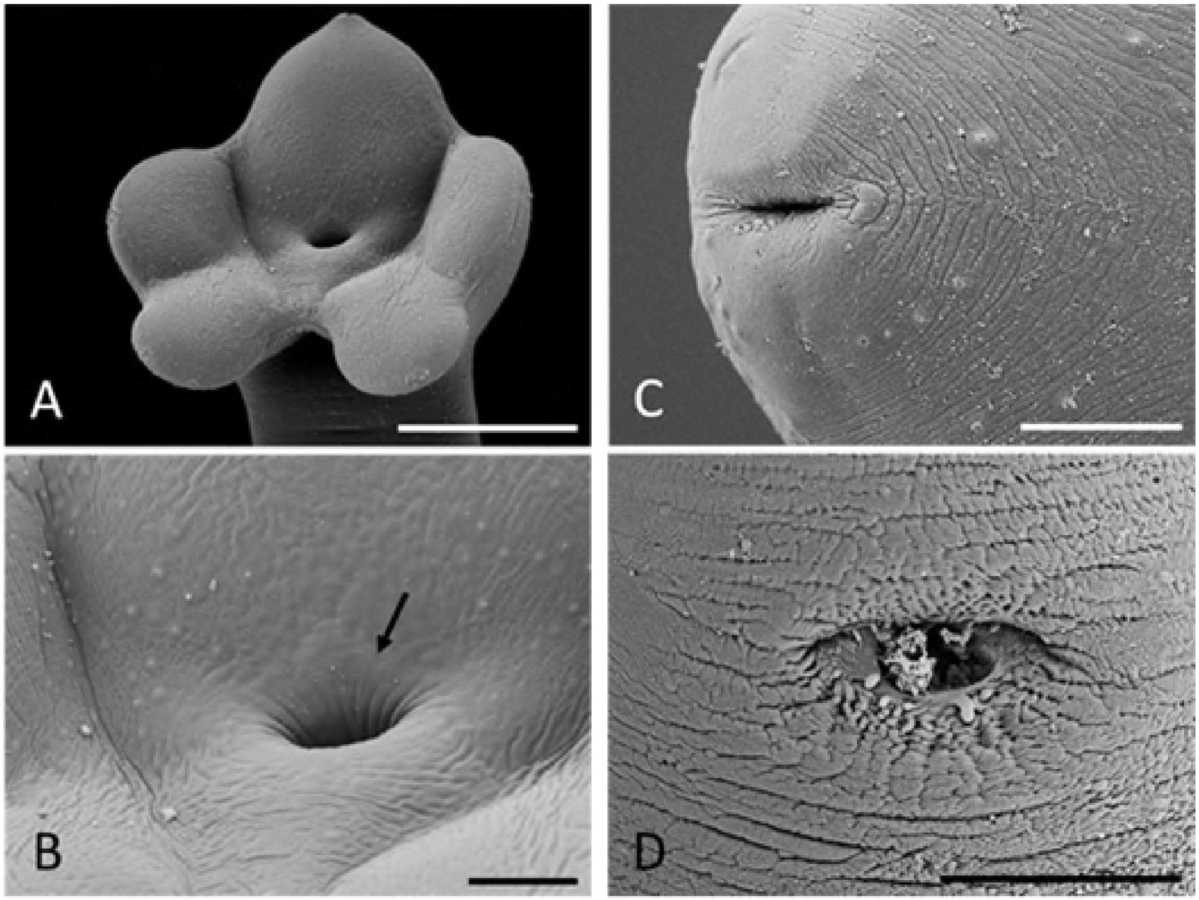

Figure 2. *C. occidentalis* (A) Close-up view of expanded pentalobate oral disc; scale bar = 250 µm. (B) Mouth (arrow) with papillae on either side; scale bar = 50 µm. (C) Terminal slit-like apical pore on anterior lobe of oral disc; scale bar = 25 µm. (D) Sunken gonopore surrounded by plicate tegument and opening containing a clump of sperm; scale bar = 50 µm.

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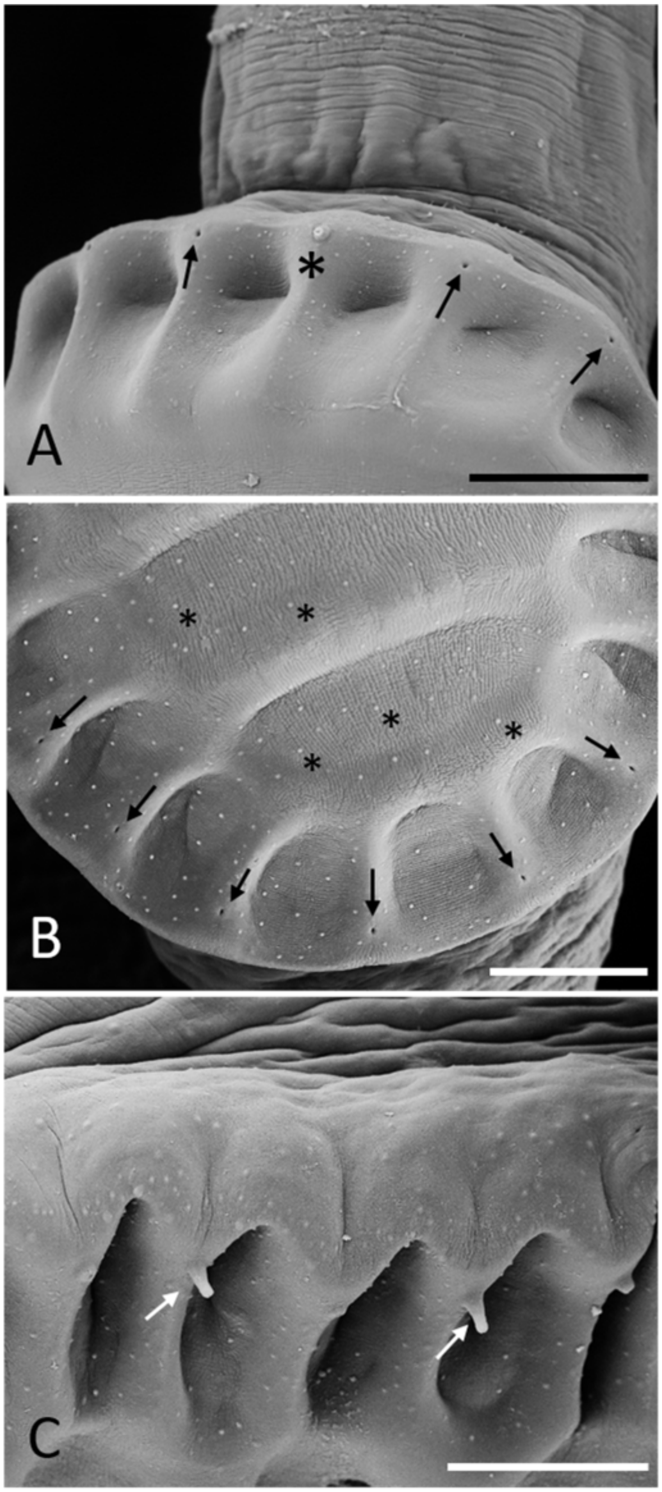

Figure 3. *C. occidentalis*. (A) Pore like cavities (arrows) of the marginal organs with one partially everted terminal duct (asterisk); scale bar = 150 µm. (B) Surface of adhesive disc with papillae (asterisks beside some papillae) and pores of the marginal organs (arrows); scale bar = 100 µm. (C) Everted ducts (arrows) of the marginal organs; scale bar = 100 µm.

More Host Records for Acanthocephalan Parasites from Arkansas Fishes (Aphredoderidae, Catostomidae, Centrarchidae, Cyprinidae, Esocidae, Percidae)

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Running Title: Records of Acanthocephalans from Arkansas Fishes

Over the last few years, our research consortium has provided a good deal of novel information on the acanthocephalans of Arkansas fishes (McAllister *et al.* 2014a, b, 2015, 2016a, b, 2018a, b). Here, we continue to document new host records for acanthocephalans from select fishes of the state.

During November 2017 and between March and October 2018, we collected fishes with a backpack electroshocker (DC current) and/or boat electrofisher from 9 sites on their river drainages/basin and 8 counties (Fig. 1). They were placed in aerated habitat water and necropsied within 24 hr. Fish were overdosed with a concentrated solution of tricaine methanesulfonate and measured for total length (TL). A mid–ventral incision from their anus and, anterior to the level of the stomach, was made to expose the gastrointestinal tract and other internal viscera (including gallbladder, gonads, and liver) which was removed and placed in a Petri dish containing 0.9% w/v saline. A stereomicroscope was used to scan tissues and locate acanthocephalans and, when found, were transferred to Petri dishes containing distilled water overnight to completely evert their proboscides. Specimens were fixed in 70–95% v/v DNA-grade ethanol, stained with acetocarmine and mounted entire in Canada balsam. Select 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.

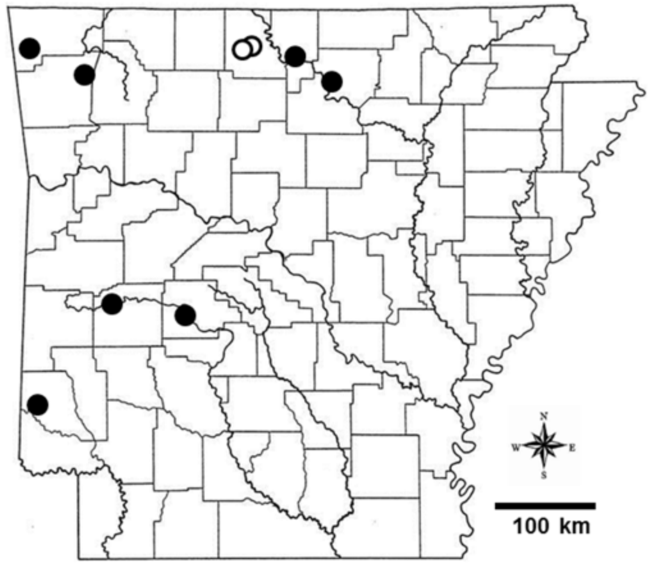

Figure 1. Eight Arkansas counties and major river drainages showing approximate location of 9 different sites (dots) where host fishes were collected.

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

The following taxa of acanthocephalans were found in Arkansas fishes:

EOACANTHOCEPHALA: NEOECHINORHYNCHIDA: NEOECHINORHYNCHIDAE

Neoechinorhynchus sp.

Hosts and localities: 1 (210 mm TL) Largemouth Bass (*Micropterus salmoides*), Red River drainage, Mill Creek at Horatio, Sevier County (33°56'23.47"N, 94°21'39.15"W), 20 Mar. 2018. 2 (155, 190 mm TL) Northern Hogsucker (*Hypentelium nigricans*), White River drainage, White River at Rim Shoals, Baxter County (36°15'27.56"N, 92°28'28.72"W), 30 May 2018; 1 (325 mm TL) Golden Redhorse (*Moxostoma erythrurum*), Ouachita River drainage, Caddo River at Caddo Gap, Montgomery County (34°23'56.40"N, 93°37'17.66"W), 25 Aug. 2018. 1 (128 mm TL) Spotted Sucker (*Minytrema melanops*), same locality as above, 18 Oct. 2018.

Prevalence and intensity: 1/1 (100%) *M. salmoides*, 3 males, 1 female; 2/3 (67%) *H. nigricans*, 4 male and 2 immature females; 1/6 (17%) *M. erythrurum*, 1 immature female; 1/1 (100%) *M. melanops*, 1 immature female.

Remarks: Unfortunately, since only immature female specimens were recovered, specific identification was not possible. Three species of *Neoechinorhynchus* have been previously reported from *M. erythrurum*, including *N. crassum* Van Cleave, 1919, *N. cylindratus* (Van Cleave, 1913) Van Cleave, 1919, and *N. strigosus* Van Cleave, 1949 (see Hoffman 1999). However, to date, only *N. cylindratus* has been reported from Arkansas fishes (McAllister *et al.* 2016b). This present finding represents the first acanthocephalan reported from an Arkansas *M. erythrurum*. In addition, it is the second time a *Neoechinorhynchus* sp. has been reported from *M. melanops* from the state; the first report was from a specimen collected in Union County (see McAllister *et al.* 2018b). The Pirate Perch has never been reported, to our knowledge, to harbor a *Neoechinorhynchus* sp., and we document a new host record herein for that parasite genus.

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

Hosts and localities: 1 (162 mm) Green Sunfish (*Lepomis cyanellus*), White River drainage, Crooked Creek at Yellville, Marion County (36°13'27.71"N, 92°40'59.01"W), 26 May 2018. 2 (130, 208 mm TL) Ozark Bass (*Ambloplites constellatus*), White River drainage, Crooked Creek at Pyatt, Marion County (36°14' 44.685", 92°50'4.5708"), 27 May 2018.

Prevalence and intensity: 1/1 (100%), 1 female; 2/9 (22%), 8 females.

Remarks: Unfortunately, the proboscides of these specimens were contorted; however, all other morphological characters fit those of *N. cylindratus* (Van Cleave 1919). Several other centrarchids from Arkansas and Missouri have been previously reported as hosts of *N. cylindratus* (McAllister *et al*. 2016b, 2018a); however, *A. constellatus* is a new host record for the parasite. This acanthocephalan has previously been reported from *L. cyanellus* (Hoffman 1999).

PALEOACANTHOCEPHALA: ECHINORHYNCHIDA: ECHINORHYNCHIDAE

***Acanthocephalus* sp.**

Hosts and localities: 1 (63 mm TL) Bigeye Shiner (*Notropis boops*), White River drainage, White River at Elkins, Washington County (35°58'25.60"N, 93°59'03.47"W), 24 Mar. 2018; 1 (82 mm TL) Steelcolor Shiner (*Cyprinella whipplei*), same locality and date; 2 (75 and 104 mm TL) *Ambloplites ariommus* (Shadow Bass), Arkansas River drainage, Flint Creek at Gentry, Benton County (36°14'33.95"N, 94°29'14.71"W), 23 Mar. 2018; 1 (175 mm TL) Grass Pickerel (*Esox americanus*), Red River drainage, Mill Creek at Horatio, Sevier County (33°56'23.47"N, 94°21'39.15"W), 14 May 2018; 1 (112 mm TL) *H. nigricans*, White River drainage, Crooked Creek at Yellville, Marion County (36°13'27.71"N, 92°40'59.01"W), 27 May 2018; 1 (190 mm TL) *H. nigricans* (same specimen herein for *Neoechinorhynchus* sp.); 2 (72 and 83 mm TL) *Aphredoderus sayanus* (Pirate Perch), same Horatio locality herein, 20 May 2018 and 20 Aug. 2018.

Prevalence and intensity: 1/1 (100%) *N. boops*, 2 males and 6 immature females; 1/3 (33%) *C. whipplei*, 2 immature females; 2/2 (100%) *A. ariommus*, 5 and 1 immature females; 1/4 (25%) *E. americanus*, 1 immature female; 1/5 (20%) *H. nigricans*, 1 immature female and 1 male and 2 immature females; 2/9 (22%) *A. sayanus*, 1 immature female each.

Remarks: Unfortunately, only males and immature females were found in hosts, which precluded specific identification. *Acanthocephalus dirus* (Van Cleave, 1931) Van Cleave and Townsend, 1936 (reported as syn. *A. jacksoni* Bullock, 1962) had been previously reported from *H. nigricans* (see summary in Hoffman 1999). However, we document a new host record for the genus *Acanthocephalus* from *A. sayanus*. McAllister *et al.* (2016b, 2018a) had previously reported *A. dirus* from 2 darters, 1 sunfish, and 1 shiner from Arkansas. Compared to its North American congeners, *A. dirus* has the widest distribution and diversity of hosts (Amin 1985). Here, we document the first acanthocephalan and only the second helminth from *A. ariommus*. In addition, we report the initial acanthocephalan from *C. whipplei*.

Acanthocephalus tahlequahensis Oetinger and Buchner, 1976

Host and locality: 1 (570 mm TL) Walleye (*Sander vitreus* syn. *Stizostedion vitreum*), White River drainage, White River at Rim Shoals, Baxter County (36°15'27.56"N, 92°28'28.72"W), 30 May 2018.

Prevalence and intensity: 1/1 (100%); 7 males, 29 females.

Remarks: This acanthocephalan shows little host specificity as it has been previously reported from a wide suite of fishes and families, including catostomids, ictalurids, cyprinids, cottids, centrarchids, and percids from Arkansas, Missouri, and Oklahoma (McAllister *et al*. 2016b, 2018a). Walleye have been reported to harbor numerous acanthocephalans, including those in the genera *Echinorhynchus*, *Leptorhynchoides*, *Neoechinorhynchus*, and *Pomphorhynchus* (Hoffman 1999). We document *A. tahlequahensis* in *S. vitreus* for the first time, and the initial species of *Acanthocephalus* documented from this host.

POMPHORHYNCHIDAE

Pomphorynchus bulbocolli Linkins in Van Cleave, 1919

Hosts and localities: 1 (290 mm TL) *H. nigricans*, White River drainage, Crooked Creek at Yellville, Marion County (36°13'27.71"N, 92°40'59.01"W), 29 May 2018; 1 (mm TL) *H. nigricans*, Ouachita River drainage, Bear Creek at Bear, Garland County (34°32'02.1588"N, 93°17'02.7492"W), 20 Nov. 2017. 1 (119 mm) Duskystripe Shiner (*Luxilis pilsbryi*), White River drainage, Calico Creek at Calico Rock, Izard County (36°07'24.4128"N, 92°08'38.6088"W), 31 May 2018.

Prevalence and intensity: 1/5 (20%) and 1/1 (100%) *H. nigricans*, 1 male; and 1/1 (100%) *H. nigricans*, 1 specimen; 1/1 (100%) *L. pilsbryi*, 2 specimens.

Remarks: This acanthocephalan has been previously reported from *H. nigricans* from Kentucky (Gleason 1984) and other North American fishes (Amin 1987; Hoffman 1999). It has also been reported from cyprinid fishes of the state, including *L. pilsbryi* from the same locality (McAllister *et al.* 2016b), but this is the first time *P. bulbocolli* has been reported from an Arkansas Northern Hogsucker.

Here we have provided new information (host records) on acanthocephalan parasites of some Arkansas fishes from the Arkansas, Ouachita, Red, and White river drainages of the state. We suggest surveys on more fishes of the Red and Mississippi-St. Francis river drainages, where little work has been done on their parasites in general. This will undoubtedly increase our knowledge of the acanthocephalans and other parasites of fishes in Arkansas.

Acknowledgments

The Arkansas Game and Fish Commission (AG&F) issued Scientific Collecting Permits to CTM and HWR. We thank Donald G. Cloutman (Burdett, KS), T. J. Fayton (Cornell University, Ithaca, NY), Nikolas H. McAllister (Lukfata Elementary, Broken Bow, OK), and James T. McAllister, III (University of Arkansas) for assistance in collecting. We also thank Dennis M. Richardson (Quinnipiac University, Hamden, CT) for use of the electroshocker and Christy Graham (Trout Program Supervisor-AG&F) for fish collections on the White River in Baxter County via a boat electrofisher. Scott L. Gardner and Gabor Racz (HWML), and R. Tumlison (HSU) provided expert curatorial assistance. Lastly, Michael A. Barger (Peru St. College, Peru, NE) provided assistance in the identification of acanthocephalans and his expert help is greatly appreciated.

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Dumortier's Liverwort, *Dumortiera hirsuta* (Sw.) Nees (Hepaticophyta: Marchantiales: Dumortieraceae) in Arkansas

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Running Title: *Dumortiera hirsuta* in Arkansas

Dumortier's Liverwort, *Dumortiera hirsuta* (Sw.) Nees is a relatively common thalloid liverwort that is widely distributed in the tropics of both hemispheres and also in the more humid and warmer regions of the temperate zones of India, Nepal, Japan, Brazil, México, Jamaica, North and South America, Europe, British Isles, New Zealand, Hawaii and Africa (O'Hanlon 1934; Nair *et al*. 2005). In the Western Hemisphere, it ranges southward to southcentral México and further south to Argentina; it is also found in some Caribbean islands (Stotler and Crandall-Stotler 1977). The species is found in several states in the USA including: Alabama, Arkansas, Florida, Georgia, Hawaii, Illinois, Kansas, Kentucky, Louisiana, Maryland, Mississippi, Missouri, North Carolina, Oklahoma, Pennsylvania, South Carolina, Tennessee, Virginia, and West Virginia (CNABH 2019). It is generally found in shaded damp forests, on rocks near watercourses, on submerged rocks, on exposed roots of higher plants, and in semi-evergreen, evergreen and shola forests. *Dumortiera hirsuta* is likely a species complex, comprising several genetically distinct siblings (Bischler-Causse *et al.* 2005; Forrest *et al.* 2011). We follow Piippo and Koponen (2013) in treating *D. hirsuta* as a single species. *Dumortiera hirsuta* is the only species currently recognized in the genus (Stotler and Crandall-Stotler 2017).

Apparently, Branner and Coville (1891) were the first to report *D. hirsuta* in Arkansas when they reported specimens from the vicinity of Salado Creek near Batesville (Independence County). Since then, several have reported this liverwort in the state, including Evans (1919), Wittlake (1950a,b, 1954), Redfearn (1964, 1966, 1968, 1970, 1979), Schuster (1992), and Timme and Redfearn (1997, 2005). Here, we provide a summary of vouchered collections of *D. hirsuta* as well as an interesting new population we discovered in the Ouachitas of the state.

We searched the Consortium of North American Bryophyte Herbaria (CNABH 2019) for previous records of *D. hirsuta* from Arkansas. In addition, collections from the herbarium at Henderson State University (HEND) were obtained. Field observations and collections were done by the authors in Montgomery County between 1972 and 1979 and again between 2010 and 2019. Vouchers are housed in the plant collections of the University of North Alabama (UNAF). Latitude and longitude (WGS 84) or township, section, and range are reported when known for all samples.

Description (derived from Schuster 1992)

One of the most distinguishing features of the genus is that the thalli are large, dark green, and translucent. Plants occur in large patches of overlapping thalli, individual thalli usually 7.0 to 15 cm long × 1.0 to 1.5 cm wide. Thalli are sparsely dichotomously branched with a broad midrib projecting ventrally, thallus margins hirsute; dorsal surface sometimes with a faint network of pale ridges; ventral scales hyaline, vestigial. Plants in the genus are monoicous, androecial disks and archegonial disks are borne at the thallus apex on short stalks, the female disk eventually elevated by an elongated stalk, stalk with 2 rhizoid furrows; capsule ovate.

Populations of *D. hirsuta* are known from 21 of 75 (28%) counties of the state, including Baxter, Benton, Cleburne, Conway, Crawford, Faulkner, Franklin, Garland, Hot Spring, Independence, Johnson, Madison, Marion, Montgomery, Newton, Pike, Pope, Pulaski, Searcy, Stone, and Van Buren counties (Fig. 1).

It appears this liverwort is mostly restricted to the uplands of the state being concentrated in the Ozarks with numerous other populations in the Ouachitas, particularly Montgomery County (Fig. 1, Appendix). There are no records from the West Gulf Coastal or Mississippi Alluvial plains of the state. Select field notes from collectors list deciduous woods (oak-hickory) with

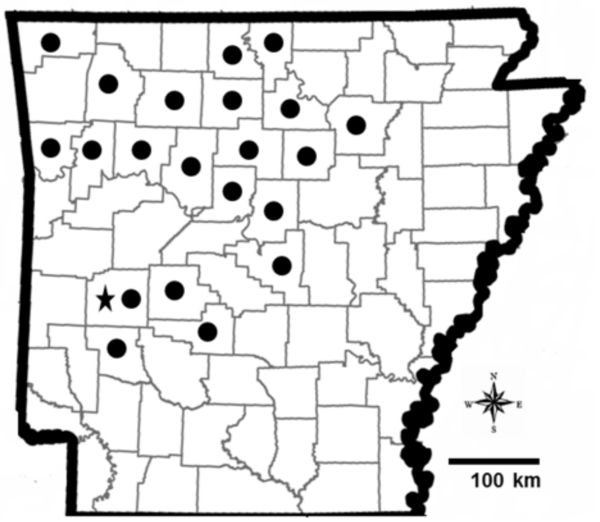
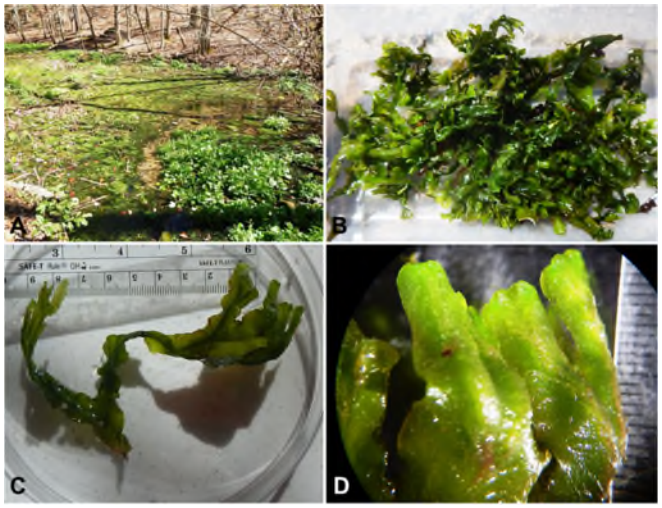


Figure 1. County map of Arkansas showing 21 counties supporting populations of *Dumortiera hirsuta*. Some dots in counties equal more than 1 population (see Appendix). Star = new locale.



Figures 2. *Dumortiera hirsuta*. (A) Boxx Spring study site looking to the northeast showing extensive cover of liverworts. (B) Mass of *D. hirsuta* from spring. (C) Individual thallus of *D. hirsuta* in Petri dish. Note mm scale. (D) Stereoscopic view of *D. hirsuta*. Note mm scale. Photos by CTM on 15 Mar. 2019.

shale and sandstone near creek, common on rocks in deep canyons, riparian habitat, seepy hillsides, abundant on shale near seep, near tributaries, slopes near or underside of sandstone ravines, and at various springs.

History of Discovering New Population

An interesting population of *D. hirsuta* was found on 6 Mar. 1972 by one of us (HWR) at Boxx Spring, Montgomery County (Sec. 33, T4S, R26W). Immediately upon arriving at this locale, HWR noticed the abundance of the liverwort and eventually told Dr. Daniel L. Marsh (DLM), late bryologist of Henderson State University (Tumlison and Robison 2010), of the site. However, it was more than 7 yr later on 16 Jun. 1979 that HWR and DLM went to Boxx Spring and observed the liverwort. On that eventful visit, DLM immediately identified the large liverwort as *D. hirsuta*. Later, another one of us (PGD) went to Boxx Spring and noted the population was of particular interest because the plants formed large, buoyant masses of several square meters in the shallow spring (Figs. 2A–D). Sexual reproductive structures were not observed in this population, and we are unaware of any other populations of *Dumorteira* that occur as extensive, buoyant mats in springs.

The study site covers 9.92 ha (24.8 acres) and is a mixture of pines (*Pinus* spp.) and upland hardwood forest including white oak (*Quercus alba*), black oak (*Q. velutina*), and southern red oak (*Q. falcata*) together with mockernut hickory (*Carya tomentosa)* and shagbark hickory (*C. ovata*) in the Ouachita National Forest. The surface geology of the area is closely folded ridges and valleys of Ordovician and Silurian novaculite, sandstone and shale (Branner 1937).

A summary of the collection parameters of 53 previous collections of *D. hirsuta* from Arkansas is provided in the Appendix. We expect additional populations of this bryophyte to be discovered in the state, particularly in the western Ouachitas.

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Appendix. County locations of 53 vouchered specimens of *Dumortiera hirsuta* from 21 Arkansas counties (locality, latitude/longitude in decimal degrees or township, section, and range [if known], date of collection, collector name and number, herbarium code. Abbreviations: Paul L. Redfearn, Jr. (= PLR), herbarium codes follow Thiers (2019).

BAXTER COUNTY (N = 1)

(1) N. Sylamore Creek. 22 May 2001. P. E. Hyatt 10299. UNAF

BENTON COUNTY (N = 2)

(1) Martins Bluff. May 1948. E.B. Whittlake 89. UARK.

(2) Above Beaver Reservoir, ca. 12.9 km NE of Springdale (Sec. 12, T18N, R29W). 3 Mar. 1965, PLR 16830. MO.

CLEBURNE COUNTY (N = 1)

(1) Above Red River at Shirley. Dec 1967; W.L. Graham s.n. MICH

CONWAY COUNTY (N = 1)

(1) Along small creek leading to Carpet Rock, Petit Jean State Park (Sec. 33, T6N, R18W). 8 Jun. 1965. PLR 17457. MO.

CRAWFORD COUNTY (N = 1)

(1) Wittlake (1954), no specimen located.

FAULKNER COUNTY (N = 1)

Boston Mts., UARK From: Timme and Redfearn (1997), (specimen not located)

FRANKLIN COUNTY (N = 3)
(1) Cass. (35.687581°N, 93.819361°W). 15 Jul. 1938. E.L. Braun s.n. CINC.
(2) Lower end of Spy Rock Hollow (Sec. 20, T12N, R26W). 14 Jun. 1973. PLR 28663, MO.
(3) Summit of White Rock Mountain (Sec. 22, T12N, R28W). 24 Apr. 1988. PLR. 34520. MO.

GARLAND COUNTY (N = 6)
(1) Hot Springs. 15 Feb. 1942. D. Demaree 22718. TENN.
(2) Hot Springs National Park. 26 Mar. 1942. D. Demaree 22742. TENN.
(3) Bonanza Springs. Apr. 1949. E.B. Whittlake 390. UARK.
(4) Ca. 8.0 km NE of Hot Springs, vicinity of Thousand Dripping Springs. 19 Mar. 1965. PLR 17028. MO.
(5) 4.8 km S of Lake Hamilton along Sorrells Creek W of St. Hwy 7. 2 Jul. 1968. PLR 24339. MO.
(6) 0.6 km N Charlton Recreation Area, Ouachita National Forest. 4 Jul. 1968. PLR 24520. MO.

HOT SPRING COUNTY (N = 5)
(1) Magnet Cove. 21 May 1938. D. Demaree 17477. TENN.
(2) Ca. 6.4 km NE of Bismarck along tributary of Prairie Bayou (Sec. 27, T4S, R20W). 1 Jul. 1968. PLR 24228. MO.
(3) Tributary of Valley Creek, Needle Eye Mt. (Sec. 26, T4S, R21W). 7 Nov. 1988. T. Bennett s.n.. HEND.
(4) Trap Mts., W/SW of Needle Eye Mt. "Needle Eye Branch" tributary of Valley Creek (Sec. 26, T8S, R21W). 7 Nov. 1988. B.W. Cowling s.n. HEND.
(5) Trap Mts., W/SW of Needle Eye Mt. (Sec. 26, T4S, R21W). 7 Nov. 1988. A. McKinnon s.n. HEND.

INDEPENDENCE COUNTY (N = 1)
(1) Salado Creek. 30 Jul. 1887. F.V. Corville 1. YU.

JOHNSON COUNTY (N = 3)
(1) Along Ozarks Highlands Trail near Hurricane Creek, Hurricane Creek Wilderness, Ozark National Forest (35.71624°N, 93.22375°W). 6 Jul. 2013. J.C. Brinda 4630 MO.
(2) Ca. 8.0 km NE of Fort Douglas, tributary to Big Piney River (Sec. 12, R21W, T12N). 19 Feb. 1965. PLR 16697. MO.
(3) Ca. 0.8 km E of Dillen (Sec. 12, T12N, R21W). 13 Jun. 2013. PLR 19025. MO.

MADISON COUNTY (N = 2)
(1) 3.7 km SE of St. Hwy 16, at Beech Hurricane Ravine below Muddy Gap on co. rd. 105, Ozark National Forest(35.8°N, 93.6°W). 9 Jun. 2000. B. Allen 22285. MO.
(2) 3.7 km SE of St. Hwy 16, at Beech Hurricane Ravine below Muddy Gap on co. rd. 105, Ozark National Forest (35.8°N, 93.6°W). 9 Jun. 2000. W.R. Buck 37347. NY.

MARION COUNTY (N = 1)
(1) Buffalo Point, Buffalo National River. R.M. Schuster 83-1945. F.

MONTGOMERY COUNTY (N = 10)
(1) Joplin. Apr. 1949. E.B. Whittlake 455. UARK.
(2) Joplin. 10 Jan. 1963. D. Demaree 47051. TENN.
(3) Vicinity of Collier Spring (Sec. 17, T3N, R24W). 20 Mar. 1965. PLR 17242. MO.
(4) Albert Pike Recreational Area, Little Missouri River (Sec. 27, T4S, R27W). 25 Mar. 1967. PLR 21215. MO.
(5) Ca. 0.6 km upstream of low water bridge, Little Missouri River, Camp Albert Pike. 16 Apr. 1980. R. Davis 16. MO.
(6) Albert Pike campground (Sec. 27, T4S, R27W, 34° 22'37"N, 93° 52'48"W). 19 Jun. 1983. M.L. Sargent 3221. ILL.
(7) On creek, near road FR 517, east end of Sulphur Mt. (Sec 23 T43 R26W), 20 Sept. 1986. T. Sligh s.n. HEND.
(8) Bluff Mt., FR 517A (S Section 25, N Section 36, TFS R27), 16 Mar. 1997. S. King s.n. HEND.
(9) Caney Creek Wildlife Management Area, Blocker Creek, N edge, Pryor Mts. (Sec. 27, T4S R27W), 20 Sept. 1986. K. Golden s.n. HEND.
(10) Boxx Spring off St. Hwy 8 (Sec. 33, T4S, R26W). 9 Jul. 2011. P.G. Davison & H.W. Robison 8130. UNAF.

NEWTON COUNTY (N = 5)
(1) Mt. Judea (35.921466°N, 93.060174°W). 2 Aug. 1913. W.H. Emig 1013. YU.
(2) 4.0 km NW of Boxley (Sec. 4, T16N, R23W). 4 Jun. 1968. PLR 23756. MO.
(3) 1.6 km SE of Ponca, Leatherwood Creek (Sec. 30, T16N, R22W). 2 May 1971. PLR 27389. MO.
(4) 0.6 km E of jct. St. Hwys 43 and 74 at Ponca, Leatherwood Creek (Sec. 30, T16N, R22W). 2 Feb. 1974. P.A. Thomas 169. MO.
(5) 0.6 km E of jct. St. Hwys 43 and 74 at Ponca, Leatherwood Creek (Sec. 30, T16N, R22W). 2 Feb. 1974. W.A. Weber 74-168. MO.

POPE COUNTY (N = 1)

(1) 4.8 km SW of Sandgap, tributary of Indian Creek (Sec. 16, T21N, R12W). 7 Mar. 1968. PLR 21485. MO.

PIKE COUNTY (N = 1)

(1) Bell Bluff area (Sec. 8, T9S, R24W). 3 Dec. 1988. A. McKinnon s.n. HEND.

PULASKI COUNTY (N = 1)

(1) Ferndale. Apr. 1950. D.M. Moore 1750. UARK.

SEARCY COUNTY (N = 1)

(1) 2.4 km of Morning Star, vicinity of Scott's Springs. 24 Jun. 1964. PLR 15237. MO.

STONE COUNTY (N = 7)

(1) Blanchard Springs Recreation Area, Ozark National Forest (Sec. 5, R11W, T15N). 6 Jul. 1964. PLR 15381, 15541. MO.
(2) Barkshed Recreation Area (Sec. 15, R12W, T16N). 27 Jun. 1966. PLR 19336. MO.
(3) 6.4 km N of Allison at Partee Springs (Sec. 26, T16N, R11W). 16 Apr. 1968. PLR 23324. MO.
(4) Vicinity of Gunner Pool Recreation Area, Ozark National Forest (Sec. 25, T16N, R12W). 7 Mar. 1968. PLR 22816, 22820. MO.
(5) Blanchard Springs Trail. 5 Apr. 1996. P. Majestyk s.n. HEND.

VAN BUREN COUNTY (N = 1)

(1) Bluffs S of Shirley. No date. PLR 16865. MO.

ARKANSAS ACADEMY OF SCIENCE

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Hendrix College, Conway, AR

Frank Hardcastle President	**Stephen Addison** President-Elect	**Andrew Sustich** Vice-President	**R. Panneer Selvam** Past President	**Collis Geren** Secretary
Mostafa Hemmati Treasurer	**Mostafa Hemmati** JAAS Editor-in-Chief	**Ivan Still** JAAS Managing Editor	**Rami Alroobi** Webmaster	**Collis Geren** Historian
	R. Panneer Selvam Newsletter Editor		**Stephen Addison** Undergraduate grants	

Secretary's Report
MINUTES OF THE 103rd MEETING

ARKANSAS ACADEMY OF SCIENCE SPRING 2019 BUSINESS MEETING MINUTES
March 30, 2019– 12:00 noon.

Hendrix College, Conway

The meeting was called to order at 12:00 pm. by President Frank Hardcastle.

1. **President's Report**

Frank welcomed all and thanked particularly Todd Tinsley and his committee, the Hendrix President, the speaker and all those who assisted with the meeting who are listed in the Academy Resolution later in these minutes for a very successful meeting. He also thanked particularly Mostafa Hemmati and the rest of the Executive Committee of the Academy.

2. **Local Arrangements Committee: Todd Tinsley**

Based on Online registrations as of 3/28/19, there were 272 registrants for the meeting. A total of 162 presentations, with 67 being oral and 95 posters. These broke down across the following subject areas: 87 (54% of total) Biology, with 38 orals (57% of orals) and 49 posters (52% of posters); 28 Chemistry and Physics presentations: 6 orals, 15 chemistry posters, 7 Physics posters; 24 Engineering presentations with 11 orals, 13 posters; 13 Computer Science and Math. Presentations with 5 Computer Science orals, 3 Math orals 5 Computer Science and Math posters; 10 Geosciences presentations, with 4 Geosciences orals and 6 posters.

In addition to the data provided above, Todd also thanked all those people mentioned in the Academy's Resolution contained later in these minutes.

A motion to approve the report was made by Mostafa and the second was provided by Panneer. Approval was unanimous.

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

Collis presented a review of the mintes of the executive committee meeting in January 2019 & April 2018 business meeting). Collis reminded members that minutes of the business meeting were already published in the Journal V72, but only after being reviewed twice by the executive committee. Membership for 2018 was 155 life, regular, and student members. The number of dues paying institutional members was 12.

4. **Treasurer's Report: Mostafa Hemmati**

Mostafa reported it was a good year for the Academy. Collis reported for the Auditing Committee (Dr. Collis Geren and Dr. Ivan Still, who verified all calculations) that all accounts balance and that the treasurer is pathologically honest (see AAS financial statement in appendix.)

The Treasurer's and Auditing committee's reports were approved unanimously.

5. **Historian's Report: Collis Geren**

The 2019 spring meeting of the Arkansas Academy of Science at Hendrix College in Conway, Arkansas is the 103rd annual meeting of the Academy. This will mark the fifth time that Hendrix will have hosted the Academy having done so previously in 2005, 1979, 1963 and 1939.

In 1876 Central Institute, which was to become Hendrix College, was established in Altus, Arkansas, by the Rev. Isham L. Burrow. Burrow was serving as a minister in the Methodist Episcopal Church, South (now a part of the United Methodist Church) at the time. The school became Central Collegiate Institute in 1881 with the addition of secondary and collegiate departments. The institute was purchased in 1884 by conferences of the Southern Methodist Church in Arkansas and was renamed Hendrix College in honor of Bishop Eugene R. Hendrix in 1889. The following year the college moved to Conway, Arkansas. By the mid-1930s Hendrix had firmly established its role as a small, coeducational, undergraduate, residential, liberal arts, church-related institution. From the mid-1950s Hendrix gradually increased student enrollment until it stabilized at just under 1,000 in the 1970s. Through the end of the 20th century, Hendrix added more than 15 buildings, developed a new master plan for growth of the campus, and revised and enhanced its curriculum and added faculty in key areas. The College's long-standing commitment to experiential education gave birth to the Hendrix Odyssey Program in 2005. This component of the curriculum, which requires three engaged learning experiences of every Hendrix graduate, is helping the College build a national reputation for leadership in engaged learning. From the foundation of more than 140 years of excellence in education, Hendrix College is moving confidently into the 21st century (from www/hendrix.edu/College History).

Today Hendrix is a highly rated private, Christian college with an enrollment of 1,228 undergraduate students. Admissions is somewhat competitive as the Hendrix acceptance rate is 80%. Popular majors include Psychology, Biology, and Economics. The total number of majors offered is 32. The graduation rate is 75% (from NICHE).

6. Journal (JAAS #72) Report: Editor-In-Chief Mostafa Hemmati

During the spring 2018 semester, 37 manuscripts were submitted for consideration for publication in volume 72 of the Journal of the Arkansas Academy of Science (JAAS); another manuscript which was rejected the previous year was requested to be considered for publication in the Journal this year again. Soon after receiving the manuscripts, all manuscripts were sent to reviewers and several Associate Editors. The reviewers sent all manuscripts and their comments back before the end of July 2018.

Reviewers' comments were sent to the authors between July 15, 2018, and July 30, 2018. That process was completed by July 30, 2018. The authors were asked to respond to the reviewers' comments and return their manuscript back to Managing Editor, Dr. Still, by August 31, 2018. 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, 2018, was also the deadline for receipt of the payment of the page charges; we had to extend the deadline up to October 15 this time.

One manuscript was rejected due to major formatting problems, another was withdrawn by the author and three other manuscripts which required major corrections were rejected by the reviewers at the end. Therefore, volume 72 of the Journal includes 33 manuscripts. In the process of manuscript submission, no manuscripts were lost.

Three Associate Editors, 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, 2018. Printing of the Journal was completed by March 2, 2019. I used the Russellville Printing Company for printing of the Journal.

The Journal Editor-in-Chief's Report was approved unanimously.

Managing Editor Ivan Still

Thirty seven manuscripts were submitted for consideration of publication in volume 72 (2018) of the JAAS. Of these 26 Articles and 11 General notes were submitted by the electronic manuscript submission process on the Journal website.

By the beginning of May, manuscripts were checked for style, grammar, format, etc, to ensure compliance with the "Instructions to Authors". One paper was rejected due to issues with formatting that the authors failed to resolve in a timely fashion. Abstracts were sent to potential reviewers by mid to late May. Dr. Hemmati handled Physical Science papers, 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. One of the manuscripts had been rejected from the previous year and although submitted late, the original reviewers were able to process it on time.

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. Five manuscripts required major corrections and three manuscripts were rejected, one was withdrawn by the author. 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 33 (down from the 36 in Volume 71), of which 23 were Articles and 10 were in General Note format. We are also publishing an In Memoriam for our departed friend and colleague, Dr. Kim Smith. Volume 72 is 236 pages long (including cover pages).

I would like to thank the reviewers and Assistant/Associate Editors and reviewers for their help in the preparation of volume 72.

Report on issues with General Data Protection Regulations (Europe).

In August 2017, our on-line manuscript handling system, bepress, was taken over by Elsevier publishing, a European company. May 25th marked the implementation of General Data Protection Regulations in Europe, an issue that was not relayed to me at the time. In an effort to be compliant with GDPR, bepress implemented policies that have now blocked my access to review email addresses to determine if they are current. While bepress have agreed to take responsibility for interrogating the database and the web for author/reviewer's current emails and determine if they are still active in the scholarship field. It is unclear if, how and/or when this will be completed. Thus, while I had hoped to be able to keep an up-to-date reviewer database that is unlikely while bepress continues to be integrated into the Elsevier family. In the meantime, I was able to recruit a student volunteer, Jackson Gray, who has worked off an excel file provided by bepress to at least provide an "off-line" expertise and current email database for us to work from. After integration, I am hoping that bepress's system will provide the benefits of Elsevier's reviewer database system.

Pursuant to recruitment of Associate Editors for biology

In the newsletter, I have pinpointed the need for two new Associate Editors for the Journal: one for Invertebrate biology and the other for Vertebrate biology. This would decrease the Managing Editor's load for the future, and might encourage a Managing Editor to come forward, who is not necessarily in the field of Biology.

I am now pleased to announce that Cristina Blanco (ATU) has volunteered her services as an Associate Editor for Vertebrate Biology.

On a professional note, I would also like to thank Dr. Hemmati for his years of service as Editor-in-Chief of the Journal, through a time of change for the publication process of the Journal and its move to the electronic handling system that we now use. Dr. Hemmati has always been supportive in these measures. On a personal note, I have enjoyed working with Mostafa, both within the Academy and also as a valued colleague and friend at Arkansas Tech. I wish him a long and happy retirement!

The Journal Managing Editor's Report was approved unanimously.

7. **Webmaster: Rami Alroobi**

Rami reported the web site is current and invites input on its content and structure. He reported he planned to make a Twitter site for the Academy.

A report of this meeting is now available on Twitter as the Arkansas Academy of Science.

8. **Newsletter: Panneer Selvam**

Panneer reminded members that the spring newsletter is what brought details of this meeting. Input from the membership is invited. Please advise Panneer if you are not receiving the newsletter.

Report was approved unanimously.

9. **Committee Reports:**

Nominations Committee: Mostafa Hemmati

Stephen Addison inherited the presidency of the Academy, with Andy Sustich as President-Elect and Frank Hardcastle becomes Past President.

The following nominations from the committee were reported: Todd Tinsley for Vice President; Andy Sustich for Treasurer; Abdul Bachri for Historian; Steve Addison for Undergraduate

Research Coordinator; Ivan Still for Editor-in-Chief, JAAS.

The nominations were unanimously approved with no new nominations from the membership. The nominees were elected by acclamation.

Undergraduate Research Awards: Stephen Addison

Three awards were approved, and presented by Dr. Addison:

1. Alicen Wilcox (Harding), Determine the effect of TDP-43 on microglial polarization, Faculty Mentor David Donley.
2. Raymond J. Weldon (Henderson State), Design of polyesters and polyamides based on the imidazole structural motif, Faculty Mentor: Martin J. Campbell.
3. Blake T. Mitchell (UCA), Exploring potential differences in habitat use and body condition of darter species, *Etheostoma fragi* and *Etheostoma caeruleum.*

Outreach Committee Report- Edmund Wilson

Ed reminded the membership that the Arkansas Academy of Science has been in existence for 103 years. It is up to the membership to make sure that its existence is not a secret. We need to make sure that local newspapers report on accomplishments of the Academy and its individual members and particularly the accomplishments of students. Please contact wilson@harding.edu for further information on how you can help.

Report was approved unanimously.

10. Business Old and New:

Item 1: Upcoming meetings

Kannan reported that planning had started and he and the three other faculty who accompanied him, Kristine Garner, Amy Skypala, and David McClellen were part of the planning group who were present at the Executive Committee Meeting to learn. April 3 & 4, 2020 are the proposed dates of the meeting.

Proposed future locations for 105th meeting in 2021 include OBU and UAPB.

Item 2: Fellows Status and Vote: Collis Geren

The following proposed addition to the Academy regulations was projected for the membership to view.

A proposal to establish Fellows and Honorary Fellows in the Arkansas Academy of Science

FELLOWS

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

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

HONORARY FELLOWS

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

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

Collis reported that this document has been revised more than 3 times and comes to the membership unanimously approved by the Executive Committee so it comes with an automatic move and second. The vote to approve was unanimous.

Collis then made a motion to name Doug James as the first Fellow of the Academy and Kim Smith the second. The motion was seconded and the vote for approval was unanimous.

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.

12. <u>Special Award and Gift to Mostafa Hemmati</u>

President Frank Hardcastle presented a special plaque and gift to Mostafa Hemmati. Mostafa is past president of the Academy and long time Treasurer of the Academy. Since 2008, Mostafa has been Editor-in-Chief and has worked with Dr. Still and Cedar Middleton (UARK) to move the Journal to the current electronically handled system. He will be missed!

13. <u>Transfer of Presidency</u>

Stephen Addison was installed as the new President of the Academy. Stephen presented Frank a plaque for his Service as President of the Academy.

The meeting was adjourned 1:15 pm by Steve Addison.

Minutes prepared by Secretary Collis Geren, April 16, 2019.

Treasurer’s Report
ARKANSAS ACADEMY OF SCIENCE
2019 FINANCIAL STATEMENT
December 7, 2019

Balance – December 7, 2019	**$158,834.34**
Balance – December 3, 2018	**<u>$150,132.66</u>**
Net Gain	**$8,701.68**

DISTRIBUTION OF FUNDS

Checking Account Dec. 7, 2019 Arvest Bank	$12,328.44
PayPal Account: Available funds on Dec. 7, 2019	$87.12
Certificate of Deposit Dec. 7, 2019 Includes Phoebe and George Harp Endowment Arvest Bank	$52,786.07
Certificate of Deposit Dec. 7, 2019 Arvest Bank	$52,786.07

Certificate of Deposit Dec. 7, 2019 Arvest Bank	$40,846.64
TOTAL	**$158,834.34**

INCOME

1. GIFTS

a. Hendrix University	$1,000.00
Total	**$1,000.00**

2. INTEREST (Interest Earned Year to Date December 7, 2019)

a. Checking Account, Arvest Bank	$0
b. CD1 (Arvest Bank)	$1,215.03
c. CD2 (Arvest Bank)	$1,215.03
d. CD3 (Arvest Bank)	$846.34
All interest was added to the CDs	**$3,276.40**

3. JOURNAL

a. Page Charges	$7,699.99
b. Subscriptions, University of Arkansas	$150.00
Total	**$7,849.99**

4. MEMBERSHIP

a. Individual/Associate (reimbursements for double payments)	$320.98
b. Individual collected at the meeting	$2,505.00
c. Institutional	$1,100.00
Total	**$3,925.98**

5. MEETING INCOME	**$0**

6. MISCELLANEOUS INCOME

a. Unspent/returned UG awards	$129.84
Total	**$129.84**

TOTAL INCOME	**$16,182.21**

EXPENSES

1. STUDENT AWARDS	**$0**

2. AWARDS (Organizations)

a. Arkansas State Science Fair	$400
b. Arkansas Junior Academy of Science	$400
c. Arkansas Junior Science and Humanities Sym.	$400
Total	**$1,200.00**

3. UNDERGRADUATE RESEARCH AWARDS

a. Dr. Ginny Adams,, UCA	$1,000
b. Dr. Martin Campbell, Henderson State University	$1,000
c. Dr. David Donley, Harding University	$1,000
Total	**$3,000.00**

4. JOURNAL

a. Volume 72 Printing Cost	$2,681.40
b. Journal Mailing Cost	$97.95
Total	**$2,779.35**

5. MISCELLANOUS EXPENSES

a. Reimburse Collis for Plaques	$187.68
b. Reimburse Rami for Website registration	$143.50
c. Reimburse Andy for Quicken subscription	$29.99
d. Reimburse Andy for checks/stamps	$60.01
e. Bank fees	$60.00
f. Tax report prep	$20.00
Total	**$501.18**

6. MEETING EXPENSES	**$0**
TOTAL EXPENSES	**$7,480.53**

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
72 (2018)	180	229	$2,681.40	$2,779.35	$15.44	$12.14

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

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

APPENDIX A

AWARD WINNERS FROM THE 103rd ANNIVERSARY AKANSAS ACADEMY OF SCIENCE (awardees are underlined)

UNDERGRADUATE ORAL PRESENTATION AWARDS: Biological and Medicinal Chemistry

1st Place

Melanin Concentrating Hormone Receptor 1 (MCH1R) Antagonists for Treating Addiction, by Britny Kirkpatrick; Jim Tarrant; Jacob Hatvany; and Thomas Pencarinha (Harding University)

UNDERGRADUATE POSTER PRESENTATION AWARDS: General Biology

1st Place

The Antimicrobial Activity of *Callicarpa americana* Berry Extracts, by Kara Burchfield and Elizabeth Wess; and Antoinette Y. Odendaal (Southern Arkansas University)

UNDERGRADUATE POSTER PRESENTATION AWARDS: Medicine, Molecular & Cellular Biology

1st Place

Papillary Thyroid Cancer Cells Display Differences in Drug Sensitivity that are Dependent on Extracellular Matrix Composition, by Lauren Dwyer; Brianna Kelly; Hannah Moore; and Sesalie Satterwhite (Hendrix College)

UNDERGRADUATE ORAL PRESENTATION AWARDS: Ecology and Organismal Biology

1st Place

Biodiversity of Hymenoptera Across Sky Islands of Arkansas, by Allison Monroe[1]; Oliver J. Kuhns[1]; Sierra C. Hubbard[1]; Reynol Rodriguez[1]; Maureen R. McClung[1]; Matthew D. Moran[1]; and Michael W. Gates[2] ([1]Hendrix College; [2]Smithsonian National Museum of Natural History)

GRADUATE ORAL PRESENTATION AWARDS: Ecology and Organismal Biology

1st Place

Energy Allocation Patterns in a Girdling and a Non-Girdling Caterpillar, by Brianna Trejo; and David Dussourd; Matthew Gifford. University of Central Arkansas

UNDERGRADUATE ORAL PRESENTATION AWARDS: Geosciences

1st Place

Pointing Isn't Rude: A Proof-of-Concept HAB Stabilizer, by Russell Jeffery (University of Central Arkansas)

UNDERGRADUATE POSTER PRESENTATION AWARDS: Geosciences

1st Place

Locating Legacy Oil and Gas Wells in Arkansas: Refining Magnetic Methods, by Michael Davis; Candy Roberts, Hunter Vickers; Jason A. Patton; Jessica Buenrostro; and Allie Roach (Arkansas Tech University)

UNDERGRADUATE POSTER PRESENTATION AWARDS: Physics

1st Place

Quantitative Binding of Divalent Metal Ions to DNA Hairpin Loops, by Harrison Russell; Julie Gunderson; and William Gunderson (Hendrix College)

UNDERGRADUATE ORAL PRESENTATION AWARDS: Chemistry and Physics

1st Place

Investigation Anion Interactions with Tripodal Urea-Based Anion Transporters, by Natalie Lowry (John Brown University)

UNDERGRADUATE POSTER PRESENTATION AWARDS: Chemistry

1st Place - Tie

Microenvironment-sensitive Probes for Selective Recognition of Serum Albumin Protein in Solution, by Kallie Mendenhall; Siddhi Patel; and Rajib Choudhury (Arkansas Tech University)

Design and Optimization of a Low-Cost, Arduino-Controlled Fluorometer, by Jacob Belding; William Gunderson; and Julie Gunderson (Hendrix College)

UNDERGRADUATE POSTER PRESENTATION AWARDS: Engineering

1st Place
Developing Soft Actuators at SAU Engineering, by Kenneth Escudero; Daniel McKague; and Mahbub Ahmed (Southern Arkansas University)

GRADUATE ORAL PRESENTATION AWARDS: Engineering

1st Place
Electromagnetic Tunability of Charged Particles in Altered Dielectric Systems, by Tamal Sarkar and Brandon A. Kemp (Arkansas State University)

GRADUATE ORAL PRESENTATION AWARDS: Mathematics

1st Place
The Effects of Selection History on Perceptual and Semantic Interactions in Visual Search, by Taylor Dague; Caroline Dacus; Ken Sobel; and Amrita Puri (University of Central Arkansas)

UNDERGRADUATE ORAL PRESENTATION AWARDS: Computer Science

1st Place
Employing Ensemble Learning for the Categorization of Android Malware Types, by Brett Baker; and Rami Alroobi (Southern Arkansas University)

UNDERGRADUATE POSTER PRESENTATION AWARDS: Computer Science

1st Place
Scalability Studies for Compressible Flow Simulations, by John McGarigal; Alaina Edwards; and Tulin Kaman (University of Arkansas at Fayetteville)

GRADUATE ORAL PRESENTATION AWARDS: Computer Science

1st Place
Cyber Security Awareness Training Program for University Students, by Tanim Sardar; and Luay A. Wahsheh (Arkansas Tech University)

GRADUATE POSTER PRESENTATION AWARDS: Computer Science

1st Place
How Powerful Can Deep Learning Be Compared with Machine Learning? A Entity Resolution Case, by Xinming Li; John R. Talburt; Ting Li; and Xiangwen Liu (University of Arkansas at Little Rock)

APPENDIX B: RESOLUTIONS

Arkansas Academy of Science
103rd Annual Meeting, 2019 Resolutions

Be it resolved that we, the membership of the Arkansas Academy of Science (AAS), offer our sincere appreciation to Hendrix College for hosting the 103rd annual meeting of the Academy.

We thank the local arrangements committee: Susan Ablondi, Heidi Dahlmann, Bobby Engeler-Young, Joyce Hardin, Brenda Houck, Laura MacDonald, Maureen McClung, and Todd Tinsley (chair) who supported the program and meeting arrangements listed in the AAS proceedings.

We sincerely thank Hendrix College for providing its facilities and service during the meeting and the Arkansas Academy of Computing for sponsoring oral and poster sessions. We especially thank our keynote speaker, Dr. Luis Bettencourt, for his wonderful talk.

The Academy recognizes the important role of oursession chairs: Ben Cash (UCA), Rajib Choudhury (ATU), Taylor Dague (UCA), Frank Hanh (Hampton University), Courtney Hatch (Hendrix), Lionel Hewavitharana (SAU), Donghoon Kim (ASU), Maureen McClung (Hendrix), Matt Moran (Hendrix), Rajesh Sharma (ASU), Stan Trauth (ASU), and Brianna Trejo (UCA).

Even greater appreciation and sincere gratitude is extended to our dedicated judges for the student presentations including: Stephen Addison (UCA), Abdel Bachri (SAU), Doug Barron (ATU), David Bowles (National Park Service), Andres Caro (Hendrix), Rajib Choudhury (ATU), Shannon Clardy (Henderson), SteveCooper (Harding), Carl Frank (Arkansas Academy of Computing), Carl Frederickson (UCA), Mariusz Gajewski (ATU), Jorista Garrie (ATU), Gija Geme (SAU), Julie Gunderson (Hendrix), Franklin Hardcastle (ATU), Joyce Hardin (Hendrix), Courtney Hatch (Hendrix), Mostafa Hemmati (ATU), Lionel Hewavitharana (SAU), Md Islam (SAU), Cindy Jacobs (ATU), Kailash Jajam (UALR), Eugene Jones (Arkansas Academy of Computing), Tulin Kaman (UA), Chris Kellner (ATU), Peter Kett (Hendrix), David McClellan (UAFS), Aboozar Mosleh (ATU),Kari Naylor (UCA), Henry North (Harding), Antoinette Odendaal (SAU), Dennis Province (Harding), Andrew Schurko (Hendrix), Caitlin Scott (Hendrix), R. Panneer Selvam (UAF), Ashokkumar Sharma (UALR), Rajesh Sharma (ASU), Andrew Sustich (ASU), Todd Tinsley (Hendrix), Susanne Wache (SACC), Scott Weston (Harding), Jessica Young (ATU), and Kaiman Zeng (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 Frank Hardcastle (President), Stephen Addison (President Elect), Panneer Selvam (Past President and Newsletter Editor), Andrew Sustich (Vice President), Mostafa Hemmati (Treasurer and Journal Editor-in-Chief), Ivan Still (Journal Managing Editor), Rami Alroobi (Webmaster), and Collis Geren (Secretary and Historian).

Respectfully submitted on this 30th day of March, 2019. Resolutions Committee: Frank Hardcastle (President), Andrew Sustich (Vice President), and Mostafa Hemmati (Treasurer).

2019 ARKANSAS ACADEMY OF SCIENCE MEMBERSHIP

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Calvin	Cotton	Geographics Silk Screening Co.
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Brenda	Houck	Hendrix College
John	Hunt	University of Arkansas-Monticello
James	Hyde	Southern Arkansas University-Magnolia
Md	Islam	Southern Arkansas University-Magnolia
Kailash	Jajam	University of Arkansas-Little Rock
David	Jamieson	Crowder College
Tulin	Kaman	University of Arkansas-Fort Smith
Chris	Kellner	Arkansas Tech University
Peter	Kett	Hendrix College
Donghoon	Kim	Arkansas State University
Laura	MacDonald	Hendrix College
Chris	McAllister	Eastern Oklahoma State College-Idabel
David A.	McClellan	University of Arkansas-Fort Smith
Maureen	McClung	Hendrix College
Rahul	Mehta	University of Central Arkansas
Matthew	Moran	Hendrix University
Luis	Murilla Cortes	University of Arkansas-Fort Smith
Dever	Norman	Henderson State University
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Mike	Plummer	Harding University
Dennis	Province	Harding University
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Christie	Slay	The Sustainability Consortium
Mike	Slay	The Nature Conservancy
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Grady	Weston	Harding College
Tsunemi	Yamashita	Arkansas Tech University
Jessica	Young	Arkansas Tech University

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KEYNOTE ADDRESS

The Emerging Science of Complex Systems: From Physics to Cities and Back Again.

By Professor Luis M. Bettencourt, Pritzker Director of the Mansueto Institute for Urban Innovation, Professor of Ecology and Evolution, and Associate Faculty and Special Friend of Sociology at the University of Chicago. He is also External Professor of Complex Systems at the Santa Fe Institute

Prof. Bettencourt received a doctorate in theoretical physics from Imperial College (University of London) and held postdoctoral positions at Los Alamos National Laboratory, the Massachusetts Institute of Technology, and the University of Heidelberg. His recent accolades include an invitation to the White House Frontiers Conference (2016), service on the President's Council of Advisors on Science and Technology working group on "Technology and the Future of Cities" (2015) and the World Cities Summit Young Leaders network (2015), and induction as Kavli Fellow of the National Academy of Sciences at the Frontiers of Science Japanese-American Symposium (2014).

Prof. Bettencourt will deliver our Keynote Address, "The Emerging Science of Complex Systems: From Physics to Cities and Back Again."

Abstract: Many of the most important and difficult challenges to science and policy today ask that we conceive of phenomena in nature and in human societies as complex systems.
This approach emphasizes processes and interconnections in the way phenomena in living systems take place, using quantitative methods from physics, population biology and economics to make sense of new big data.

I will discuss my own path as a physicist, faculty of ecology and evolution and scientific director of an institute for urban research to illustrate how connections between these disciplines can be a particularly fertile ground for education and research, and how a synthesis of ideas is emerging around processes in nature that create and sustain complexity and adaptation. I will make a case for a set of wonderful new opportunities ahead for those pursuing a broad and rigorous scientific culture that is able to transcend traditional silos and engage with big questions in research and in practice.

SECTION PROGRAMS

ORAL PRESENTATIONS

(Presenter is underlined; * - Undergraduate ** - Graduate)

ORAL SESSIONS: FRIDAY 1:00-4:45

GEOSCIENCE
ROOM: DW Reynolds 8

1:00
EVALUATION OF A MICRO SPECTROMETER FOR SATELLITE MISSIONS
Michael Cruz*, Harding University
Co-Authors: E. W. Wilson, Jr. (Harding University); Y. X. Chan, (University of Arkansas Little Rock); Adam Po-Hao Huang (University of Arkansas Fayetteville)

1:15
WATER ADSORPTION ON ATMOSPHERIC CLAY MINERALS: EXPERIMENTAL AND THEORETICAL STUDIES OF INDIRECT EFFECTS ON CLIMATE
Courtney Hatch, Hendrix College
Co-Author: Paul R. Tumminello; Megan A. Cassingham; Annie L. Greenaway; Kenneth J. Harris; Matthew Christie; Rebecca Parham; and Karen Morris

1:30
GEOTHERMAL ENERGY USE AND POTENTIAL FOR THE CARIBBEAN NATION OF GRENADA
Khalil Buckmire*, University of Arkansas
Co-Authors: Christopher L. Liner

1:45
POINTING ISN'T RUDE: A PROOF-OF-CONCEPT HAB STABILIZER
Russell Jeffery*, University of Central Arkansas

ECOLOGY AND ORGANISMAL BIOLOGY I
ROOM DW Reynolds 10

1:00
THE REPRODUCTIVE SEASON OF THE HIGHLAND STONEROLLER, CAMPOSTOMA SPADICEUM, EVIDENCED BY MUSEUM SPECIMENS
Grace Wills*, Henderson State University
Co-Authors: Renn Tumlison, Henderson State University; Henry Robison (Arkansas State University, retired)

1:15
ENERGY ALLOCATION PATTERNS IN A GIRDLING AND A NON-GIRDLING CATERPILLAR
Brianna Trejo**, University of Central Arkansas
Co-Authors: David Dussourd; Matthew Gifford (University of Central Arkansas

1:30
THE SYNERGISTIC RELATIONSHIP OF BISON GRAZING AND ARTHROPOD HERBIVORY IN STRUCTURING A TALLGRASS PRAIRIE PLANT COMMUNITY
Sofie Varriano*, Hendrix College
Co-Authors: Luke H. Lefler; Krishna Patel; Carolina Kirksey; Adam Turner; Matthew D. Moran (Hendrix College)

1:45
NEW STATE RECORDS FOR THE TEXAS FROSTED ELFIN (*CALLOPHRYS IRUS HADROS*) AND ITS HOST PLANTS (BAPTISIA SPP.)
Caralee Shepard*, Hendrix College
Co-Author: Leah C. Crenshaw (Hendrix College); Natalie E. Phelan (Hendrix College); Melissa Lombardi (US Fish & Wildlife Service); William H. Baltosser (University of Arkansas at Little Rock); Maureen R. McClung (Hendrix College); Matthew D. Moran (Hendrix College)

2:00
BIODIVERSITY OF HYMENOPTERA ACROSS SKY ISLANDS OF ARKANSAS
Allison Monroe*, Hendrix College
Co-Authors: Oliver J. Kuhns (Hendrix College); Sierra C. Hubbard (Hendrix College); Reynol Rodriguez (Hendrix College); Maureen R. McClung (Hendrix College); Matthew D. Moran (Hendrix College); Michael W. Gates (Smithsonian National Museum of Natural History)

2:15
EXPLAINING THE DIVERSITY AND EVOLUTION OF COLOR IN ANTS USING THE ANTWEB IMAGE DATABASE
Jacob Idec*, Hendrix College
Co-Author: Brian Fisher (California Academy of Sciences)

CHEMISTRY AND PHYSICS
ROOM DW Reynolds 11

1:00
LASER TRAPPING OF POLYSTERENE BEADS USING OPTICAL TWEEZERS
Ashley Cotnam**, University of Central Arkansas

1:15
A COMPACT RAMAN SPECTROMETER USING COMMERCIAL OFF THE SHELF (COTS) COMPONENTS
Drake Jackson*, Harding University
Co-Authors: Brandun Barnett (Harding University); Edmond W. Wilson, Jr. (Harding University)

1:30
IMPLICIT AND EXPLICIT SOLVATION STUDIES OF SMALL MOLECULES AND IONS IN WATER
Eliza Hanson*, John Brown University
Co-Author: Jill Ellenbarger (John Brown University)

1:45
BOND VALENCE / BOND LENGTH CORRELATIONS FOR PHOSPHORUS-OXYGEN AND URANIUM-OXYGEN BONDS
Blake Ludwig**, Arkansas Tech University
Co-Authors: Kallie Mendenhall (Arkansas Tech University); Franklin D. Hardcastle (Arkansas Tech University)

2:00
INVESTIGATION ANION INTERACTIONS WITH TRIPODAL UREA-BASED ANION TRANSPORTERS
Natalie Lowry**, John Brown University

2:15
EXPLORING THE POTENTIAL OF PHENOL DERIVATIVES: CHARGE TRANSFER FLUOROPHORES AND DETECTION OF PROTEIN IN SOLUTION
Rajib Choudhury, Arkansas Tech University
Co-Authors: Siddhi Patel (Arkansas Tech University); Kallie Mendenhall (Arkansas Tech University)

MATHEMATICS
ROOM DW Reynolds 13

1:00
THE EFFECTS OF SELECTION HISTORY ON PERCEPTUAL AND SEMANTIC INTERACTIONS IN VISUAL SEARCH
Taylor Dague**, University of Central Arkansas
Co-Authors: Caroline Dacus (University of Central Arkansas); Ken Sobel (University of Central Arkansas); Amrita Puri (University of Central Arkansas)

1:15
INITIAL CONDITIONS FOR NUMERICAL SIMULATIONS OF RICHTMYER-MESHKOV INSTABILITY
Logan Sublett**, University of Arkansas
Co-Authors: Tulin Kaman; Alaina Edwards; John McGarigal

1:30
PERFORMANCE OPTIMIZATION OF THE CFD CODE FOR FLOW SIMULATIONS
Alaina Edwards*, University of Arkansas - Fayetteville
Co-Authors: John McGarigal, Tulin Kaman (University of Arkansas)

BIOLOGICAL AND MEDICINAL CHEMISTRY
ROOM DW Reynolds 8

3:00
COMPARATIVE LIPIDOMICS OF PHOSPHOLIPIDS IN GROUND BEEF EXTRACTS BY GC-MS AND MALDI-TOF MS
Frank Hahn, Hampton University

3:15
PROTEOMICS OF CARBON FIXATION IN HALOTHIOBACILLUS NEAPOLITANUS
Newton Hilliard, Arkansas Tech University

3:30
SCORPION TOXIN PROTEOMICS: SODIUM TOXIN GENE IDENTIFICATION, ISOLATION, AND PROTEIN OVEREXPRESSION
T Yamashita, Arkansas Tech University

3:45
ROLE OF THE DJ-1 PROTEIN IN MITOCHONDRIAL DYSFUNCTION AND PARKINSON'S DISEASE
Courtney Holloway*, University of Central Arkansas
Co-Author: Kari Naylor, Ph.D. (University of Central Arkansas)

4:00
GENE EXPRESSION STUDY OF SODIUM 13 TOXINS ON CENTRUROIDES VITTATUS
John Sisco*, Arkansas Tech University
Co-Authors: Chloe Fitzgerald; Grace Rice (Arkansas Tech University)

4:15
MELANIN CONCENTRATING HORMONE RECEPTOR 1 (MCH1R) ANTAGONISTS FOR TREATING ADDICTION
Britny Kirkpatrick*, Harding University
Co-Authors: Jim Tarrant, Jacob Hatvany, Thomas Pencarinha

4:30
THE ESSENTIALITY OF THE VINYL PROTON IN ANTICONVULSANT ENAMINONES
Henry North, Harding University
Co-Authors: Mariano S. Alexander (Howard University), Kenneth R. Scott (Howard University)

ECOLOGY AND ORGANISMAL BIOLOGY II
ROOM: DW Reynolds 10

3:00
OBSERVATIONS OF AN ALLIGATOR (*ALLIGATOR MISSISSIPPIENSIS*) NEST AND HATCHLINGS IN CLARK, COUNTY, WITH ANECDOTAL OBSERVATIONS OF OTHER ALLIGATOR NESTS IN ARKANSAS
Kevin Nordengren, Henderson State University
Co-Authors: Allison Surf (Henderson State University, Arkadelphia); Renn Tumlison (Henderson State University, Arkadelphia); Kelly Irwin (Arkansas Game and Fish Commission)

3:15
BILATERAL DIAPHYSEAL CHONDRODYSPLASIA AND POLYMORPHIC OSTEODYSPLASIA OF THE TIBIOFIBULAS IN A SOUTHERN LEOPARD FROG, LITHOBATES SPHENOCEPHALUS (AMPHIBIA: ANURA: RANIDAE)
Stan Trauth, Arkansas State University (Emeritus)
Co-Author: M.L. McCallum (Langston University)

3:30
RESPONSE TO RAPID HABITAT PERTURBATION IN THE SLIDER TURTLE (TRACHEMYS SCRIPTA): BEHAVIORAL AND HORMONAL EFFECTS.
Ben Cash, University of Central Arkansas

3:45
AGE ESTIMATION USING PHALANGEAL SKELETOCHRONOLOGY IN NORTHERN CRAWFISH FROGS, *LITHOBATES AREOLATUS CIRCULOSUS* (AMPHIBIA: ANURA: RANIDAE), FROM ARKANSAS
Stan Trauth, Arkansas State University (Emeritus)
Co-Author: Christopher S. Thigpen (ASU)

4:00
PHYLOGENY AND CHARACTERIZATION OF *PARAEUSTIGMATOS COLUMELLIFERUM*, AN ARKANSAS ALGA THAT REPRESENTS A NEW LINAGE OF THE EUSTIGMATALES (EUSTIGMATOPHYCEAE)
Karen Fawley, University of the Ozarks
Co-Authors: Yvonne Nemcova (Charles University, Prague, Czech Republic); Marvin Fawley (University of the Ozarks)

4:15
BLACKROLL CREEK RUNS NORTH: A FAMILY FARM HISTORY AND ITS CHANGES FROM THE 1800S TO THE PRESENT
Anthony Holt**, University of Arkansas Community College, Morrilton

ENGINEERING
ROOM: DW Reynolds 11

3:00
FLOW DEPENDENCY UPON THE CONVECTION REGION HEIGHT OF A CFD TORNADO SIMULATOR
Sumit Verma**, University of Arkansas, Fayetteville
Co-Author: R. Panneer Selvam (University of Arkansas)

3:15
EXAMINING MOLECULAR LEVEL OF PROPERTIES OF ASPHALT BINDERS TO PREDICT THEIR MOISTURE-INDUCED DAMAGE
Sumon Roy**, Arkansas State University
Co-Author: Zahid Hossain (Arkansas State University)

3:30
EVALUATION OF RICE HUSK ASH (RHA) AS AN ASPHALT MODIFIER
Kazi Islam**, Arkansas State University
Co-Author: Zahid Hossain (Arkansas State University)

3:45
ANALYSIS OF ASPHALT BINDERS FROM CHEMICAL PROSPECTIVE
Mohammad Nazmul Hassan**, Arkansas State University
Co-Author: Zahid Hossain (Arkansas State University)

4:00
EXPLORATION OF ALTERNATIVE(S) OF THE EMPIRICAL AND EXPENSIVE TESTS TO CHARACTERIZE BITUMEN
MM Tariq Morshed**, Arkansas State University
Co-Author: Zahid Hossain (Arkansas State University)

4:15
CORROSION RISKS OF METAL CULVERT PIPES IN NORTHEAST ARKANSAS
Md Ariful Hasan**, Arkansas State University
Co-Author: Zahid Hossain (Arkansas State University)

ECOLOGY AND ORGANISMAL BIOLOGY III
ROOM: DW Reynolds 13

3:00
DEVIATION OF ANTIOXIDANT CAPABILITY OF COLORED SWEETPOTATOES AND ITS UPPERS IN RELATION TO POLYPHENOLIC CONTENTS
Mosammat Briti Rabbani**, University of Arkansas at Pine Bluff
Co-Author: Shahidul Islam

3:15
WILD GAME CONSUMPTION AND GREENHOUSE GAS EMISSION SAVINGS IN THE U.S.
Jamie Johnson, Hendrix College
Co-Authors: Nathan Taylor; Benjamin Zamzow; Matthew Moran

3:30
DETERMINATION OF BAT SPECIES' USE OF ARTIFICIAL BARK ENHANCED HABITAT IN NORTHERN ARKANSAS
Sarah Martin**, University of Central Arkansas

3:45
SPATIAL ECOLOGY OF MUD SNAKES (*FARANCIA ABACURA*) IN A SMALL ISOLATED POND WITHIN A BOTTOMLAND HARDWOOD FOREST
Caleb O'Neal*, Harding University
Co-Authors: Michael V. Plummer; Ryan Stork; Steven M. Cooper

4:00
EFFECT OF SELECTION AND GENETIC DRIFT ON PHENOTYPIC DIVERSIFICATION IN THE EASTERN COLLARED LIZARD.
Andrew Feltmann**, University of Central Arkansas
Co-Authors: Matthew Gifford (University of Central Arkansas); Emily Field (University of Central Arkansas)

4:15
INTEGRATION OF MIXED METHODS INTO COMMUNITY-BASED PARTICIPATORY RESEARCH (CBPR): A METHODOLOGICAL APPROACH AND HEALTH-CENTERED CASE STUDY
Mason Rostollan**, University of Central Arkansas
Co-Authors Leah Horton (University of Central Arkansas)

4:30
ESTIMATING CULTURAL ECOSYSTEM SERVICES PROVIDED BY THE BUFFALO NATIONAL RIVER USING A CONTINGENT VALUATION METHOD
Margaret Young*, Hendrix College
(Co-Authors: Maureen R. McClung (Hendrix College); Matthew D. Moran (Hendrix College); William Haden Chomphosy (Hendrix College)

ORAL SESSIONS: SATURDAY 8:00-10:15

KIM SMITH MEMORIAL SESSION
ROOM: DW Reynolds 8

8:30
INTRODUCTION TO THE SESSION: DR KIMBERLY G. SMITH'S LEGACY OF SCIENCE AND CONSERVATION
Maureen McClung, Hendrix College

8:45
WINTER OCCURRENCE AND HABITAT USE OF NORTHERN SAW-WHET OWLS (*AEGOLIUS ACADICUS*) IN NORTHWESTERN ARKANSAS
Mitchell Pruitt**, University of Arkansa
Co-Author: Kimberly G. Smith (University of Arkansas)

9:00
HISTORY AND CURRENT STATUS OF THE INCA DOVE (COLUMBINA INCA) IN ARKANSAS
Ragupathy Kannan, University of Arkansas--Fort Smith

9:15
THERMAL ECOLOGY OF PRAIRIE LIZARDS IN THE ARKANSAS RIVER VALLEY
Chris Kellner, Arkansas Tech University

9:30
DRIVERS OF GLOBAL FOREST LOSS AND WHAT IT MEANS FOR ARKANSAS
Christy Slay, The Sustainability Consortium
Co-Author: F.E. Follett

9:45
RECENT STUDIES ON THE BEHAVIORAL RESPONSES OF ARKANSAS WILDLIFE TO ANTHROPOGENIC NOISE
Maureen McClung, Hendrix College

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 Job;, 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

ECOLOGY AND ORGANISMAL BIOLOGY IV
ROOM: DW Reynolds 10

8:30
LONG-TAILED WEASEL (MUSTELA FRENATA) STATUS AND DISTRIBUTION SURVEY IN ARKANSAS
Blake Sasse, Arkansas Game and Fish Commission
Co-Authors: SD Johnson (Tennessee Tech University), and RE Kissell Jr. (Tennessee Tech University)

8:45
NEW HOST AND DISTRIBUTIONAL RECORDS FOR HELMINTH PARASITES (TREMATODA, CESTODA, NEMATODA) OF ARKANSAS REPTILES (TESTUDINES, OPHIDIA)
Chris McAllister, Eastern OK St. College
Co-Authors: C.R. Bursey (Pennsylvania State University, Shenango Campus, Sharon); T.J. Fayton (Lamar Fish Health Center, U.S. Fish and Wildlife Service, Lamar, PA); H.W. Robison (Arkansas State University, retired); V. V. Tkach (University of North Dakota)

9:00
PARASITES (APICOMPLEXA, TREMATODA, NEMATODA, PHTHIRAPTERA) OF TWO ARKANSAS RAPTORS (FALCONIFORMES, STRIGIFORMES: STRIGIDAE)
Chris McAllister, Eastern OK St. College
Co-Authors: L.A. Durden (Georgia Southern University, Statesboro, GA); C.R. Bursey (Pennsylvania State University, Shenango Campus, Sharon, PA); J.A. Hnida (Midwestern University, Glendale, AZ); V.V. Tkach and T.J. Ackatz (University of North Dakota)

9:15
OCCURRENCE OF THE SINUS NEMATODE SKRJABINGYLUS SP. (NEMATODA: METASTRONGYLOIDEA) INFERRED FROM NASAL LESIONS IN ARKANSAS MUSTELIDAE AND MEPHITIDAE
Renn Tumlison, Henderson State University
Co-Author: T. L. Tumlison (Arkadelphia, AR)

9:30
VERTEBRATE NATURAL HISTORY NOTES FROM ARKANSAS, 2019
Renn Tumlison, Henderson State University
Co-Authors: C.T. McAllister (Eastern Oklahoma State College, Idabel, OK); H.W. Robison (Arkansas State University, retired); M.B. Connior (Northwest Arkansas Community College, Bentonville, AR); D.B. Sasse (Arkansas Game and Fish Commission, Mayflower, AR); P.R. Port (Arkansas Game and Fish Commission, Mountain Home, AR)

9:45
NEW ANGIOSPERM RECORDS FROM ARKANSAS
Jonathan Hardage*, Henderson State University
Co-Authors: Keenan Serviss (Arkadelphia, AR); Brook Olsen (Henderson State University); Brett Serviss (Henderson State University); and James Peck (Cedar Key, FL)

COMPUTER SCIENCE
ROOM: DW Reynolds 11

8:30
ANALYZING THE ADOPTION RATE OF LOCAL VARIABLE TYPE INFERENCE IN OPEN SOURCE JAVA 10 PROJECTS
Clayton Liddell*, Arkansas State University
Co-Author: Donghoon Kim (Arkansas State University)

8:45
HOW CAN YOU BECOME A SOFTWARE ENGINEER IN PRESTIGIOUS COMPANIES?
Donghoon Kim, Arkansas State University

9:00
AUTOMATIC CUSTOMIZATION OF WEB PAGES TO ENHANCE USER EXPERIENCE
Zezhang Lin*, UALR
Co-Author: Dr. Chia-Chu Chiang (UALR)

9:15
CYBER SECURITY AWARENESS TRAINING PROGRAM FOR UNIVERSITY STUDENTS
Tanim Sardar**, Luay A. Wahsheh, Arkansas Tech University

9:30
EMPLOYING ENSEMBLE LEARNING FOR THE CATEGORIZATION OF ANDROID MALWARE TYPES
Brett Baker*, Southern Arkansas University
Co-Author: Rami Alroobi (Southern Arkansas University

ENGINEERING
ROOM: DW Reynolds 13

9:30
CADMIUM SULFIDE-BUFFERED PV SYSTEMS: ASSESSING THE ENVIRONMENTAL, HEALTH, AND ECONOMIC IMPACTS
Rajesh Sharma, Arkansas State University
Co-Author: Maqsood Ali Mughal (Worcester Polytechnic Institute, Worcester, MA)

9:45
ELECTROMAGNETIC TUNABILITY OF CHARGED PARTICLES IN ALTERED DIELECTRIC SYSTEMS
Tamal Sarkar**, Arkansas State University
Co-Author: Brandon A. Kemp (Arkansas State University)

10:00
EXPERIMENTAL INVESTIGATION OF COUNTER-FLOW HEAT EXCHANGERS EXPOSED TO AMBIENT HEAT LEAKS
Lionel Hewavitharana, Southern Arkansas University
Co-Author: Kenneth Escudero (Southern Arkansas University)

10:15
DESIGN AND DEVELOPMENT OF A VISION BASED LANE KEEPING SYSTEM
Kaiman Zeng, Arkansas Tech University
Co-Authors: Michael Langley; Nasser Alshahrani

10:30
MOORE'S LAW AND SPACE EXPLORATION: NEW INSIGHTS AND NEXT STEPS
Michael Howell**, University of Arkansas at Little Rock
Co-Authors: Venkat Kodali (CARTI); Richard Segall (Arkansas State University); Hyacinthe Aboudja (Oklahoma City University); Daniel Berleant (University of Arkansas at Little Rock)

POSTER PRESENTATIONS

*** Undergraduate student ** Graduate student**

GENERAL BIOLOGY AND ECOLOGY

1 **LIKE MOTHS TO A FLAME**
Erin Guerra*

2 **NOTES ON THE NATURAL HISTORY OF SELECTED INVERTEBRATES**
Matt Connior

3 **A DOBSONFLY (MEGALOPTERA: CORYDALIDAE, *CORYDALUS CORNUTUS*) FROM ARKANSAS WITH ABERRANT MANDIBLES**
David Bowles

4 **THE ROLES FOR SPECIFIC CHROMATIN ENVIRONMENTS IN PROMOTING DISSOCIATION OF THE FACT COMPLEX FROM 3' ENDS OF GENES FOLLOWING TRANSCRIPTION.**
JunHyeuk Shin*

5 **HISTORY OF SPRING RIVER CRAYFISH (*FAXONIUS ROBERTI*) COLLECTIONS IN THE STRAWBERRY RIVER, ARKANSAS**
Brian Wagner

6 **DE NOVO DEVELOPMENT OF MICROSATELLITE MARKERS FOR GENETIC CHARACTERIZATION OF HOUSE FINCHES**
Edgar Sanchez*

7 **EFFECT OF SEX ON OSMOREGULATION OF THE OHIO SHRIMP, *MACROBRACHIUM OHIONE***
Drew Castleberry*

8 **A TALE OF TWO SYLAMORES: UNDERSTANDING RELATIONSHIPS AMONG LANDUSE, NUTRIENTS, AND AQUATIC COMMUNITY ASSEMBLAGES ACROSS A SUBSIDY-STRESS GRADIENT**
Brianna Trejo**

9 **EFFECTS OF RECREATIONAL ACTIVITIES ON MUSSELS AND THE ASIATIC CLAM IN THE LOWER SALINE RIVER**
Makayla Nguyen* and Katie Pike*

10 **PLAINS SPOTTED SKUNK PELT PURCHASE TRENDS IN THE OZARKS AND OUACHITAS, 1943-1990**
Blake Sasse

11 **PHOTOGRAPHIC RECORD OF A GREATER ROADRUNNER (*GEOCOCCYX CALIFORNIANUS*) FROM DREW COUNTY, ARKANSAS.**
John Hunt

12 **BEHAVIORAL RESPONSE OF CAROLINA WRENS (*THRYOTHORUS LUDOVICIANUS*) TO SONGS ALTERED TO ESCAPE MASKING EFFECTS OF ANTHROPOGENIC NOISE**
Tristian Wiles*

13 **SONGBIRDS ALTER THEIR USE OF BIRD FEEDERS IN RESPONSE TO VOCALIZATION PLAYBACK**
Shelby Sarna*

14 **USING RADIO FREQUENCY IDENTIFICATION TO TEST THE ASSUMPTIONS OF OPTIMAL FORAGING THEORY ON WINTERING BIRDS**
Hannah Adams* and Sarah Roddy*

15 **DO PHENOTYPIC TRAITS PREDICT FEEDER USE BY WILD BIRDS?**
Colton Barrett*

16 **COMPARISON OF SUGAR-BASED CLEARING TECHNIQUES IN AVIAN EMBRYOS**
Kira Gibbs*

17 **DISTRIBUTION, HABITAT, AND LIFE HISTORY ASPECTS OF THE DWARF CRAYFISHES OF THE GENUS CAMBARELLUS (DECAPODA: CAMBARIDAE) IN ARKANSAS**
Chris McAllister

18 **DUMORTIER'S LIVERWORT, *DUMORTIERA HIRSUTA (SW.) NEES* (HEPATICOPHYTA: MARCHANTIALES: DUMORTIERACEAE) IN ARKANSAS**
Chris McAllister

19 ***COTYLOGASTER OCCIDENTALIS* (ASPIDOGASTREA: ASPIDOGASTRIDAE) FROM FRESHWATER DRUM, *APLODINOTUS GRUNNIENS* (PERCIFORMES: SCIAENIDAE), FROM NORTHEASTERN OKLAHOMA**
Chris McAllister

20 **HAEMOGREGARINA SP. (APICOMPLEXA: EUCOCCIDIORIDA: ADELEORINA) FROM EASTERN SPINY SOFTSHELL, *APALONE SPINFERA SPINIFERA* (TESTUDINES: TRIONYCHIDAE), FROM ARKANSAS**
Chris McAllister

21 **MORE DISTRIBUTIONAL AND HOST RECORDS FOR TWO ACANTHOCEPHALAN PARASITES FROM ARKANSAS FISHES (APHREDODERIDAE, CATOSTOMIDAE, CENTRARCHIDAE, CYPRINIDAE)**
Chris McAllister

22 **SURVEY OF AEDES ALBOPICTUS OVIPOSITION IN SOUTHEAST ARKANSAS**
Kameron Skinner*

23 **ENERGY CONTENT OF SEEDS OF TEXAS DOVEWEEN (*CROTON TEXENSIS*) FROM THE DIET OF MOURNING DOVES (*ZENAIDA MACROURA*) FROM SOUTHEASTERN NEW MEXICO**
Emily Neilson*

24 **CREATING A NEW MOLECULAR PHYLOGENY OF ARCEUTHOBIUM**
Kate Sanders*

25 **SURVEYING VARIABILITY IN CYANIDE PRODUCTION OF WHITE CLOVER (*TRILLIUM REPENS*) ACROSS AN URBANIZATION GRADIENT IN LITTLE ROCK, AR AND MEMPHIS, TN**
Sierra Hubbard*

26 **SOIL ALGAL COMMUNITIES OF WARREN PRAIRIE NATURAL AREA**
Marvin Fawley

27 **SOIL CRUST ALGAL COMMUNITIES OF WARREN PRAIRIE NATURAL AREA**
Karen Fawley

28 **THE ANTIMICROBIAL ACTIVITY OF *CALLICARPA AMERICANA* BERRY EXTRACTS**
Kara Burchfield* and Elizabeth Wess*

29 **THE BACTERIAL MICROBIOME OF THE SOCIAL AMOEBAE**
Eleni Sallinger**

30 **THE EFFECTS OF CAFFEINE AND CHLOROGENIC ACID ON *DAPHNIA MAGNA***
Ashley Stewart*

31 **EFFECT OF MULTI-COURSE PRENATAL STEROIDS ON FIBER-TYPE PROFILE AND ENZYME ACTIVITY IN THE GUINEA PIG RECTUS THORACIS**
Tel Johnson*

32 **BIODIVERSITY AND COMMUNITY STRUCTURE OF AQUATIC INSECTS IN THE LITTLE MISSOURI RIVER**
Dustin Booth* and Logan Pearson*

33 **SUPERNUMERARY SOURCES OF HUMAN DIET AND BIOENERGY FOR THE TWENTY-FIRST CENTURY; SOYBEAN (*GLYCINE MAX L.*)**
Ishrar Islam*

34 **CHEMICAL ANALYSIS OF POND DEVELOPMENT**
Audrey Lawrence*

35 **CHARACTERIZATION OF SMOKE PARTICLE EMISSIONS FROM ROCKET STOVES VERSUS THREE-STONE FIRES**
Mackenzie Hoogshagen* and Elizabeth Versluis*

36 **VARIATION IN HABITAT USE AND BODY CONDITION OF *ETHEOSTOMA CAERULEUM* AND *ETHEOSTOMA FRAGI* IN THE STRAWBERRY RIVER, ARKANSAS**
Blake Mitchell*

MEDICINE, MOLECULAR AND CELLULAR BIOLOGY

37 **ANALYSIS OF DE NOVO PEPTIDES FOR POTENTIAL ANTIMICROBIAL ACTIVITY**
Hannah Smith*

38 **ANALYZING THE ROLE OF FSZA-GFP IN MITOCHONDRIAL DYNAMICS OF *DICTYOSTELIUM DISCOIDEUM***
Jacie Cooper* and Kennedy Kuykendall*

39 **CHRONIC ETHANOL ADMINISTRATION TO RODENTS INDUCES MITOCHONDRIAL BIOGENESIS**
Grishma Patel*

40 **DISSEMINATION OF OXALATE AND VITAMIN C AMONG ASSORTED GENOTYPES OF SWEETPOTATO (*IPOMOEA BATATAS L.*) LEAVES**
Jessica Harston*

41 **INTEGRIN SIGNALING IS REQUIRED FOR COLLAGEN-MEDIATED TUMORIGENICITY OF PAPILLARY THYROID CANCER CELLS**
Sarah Gilmour*, Jonathan Jenkins*, Moira Moore*, Cole Stanton*, andKeith Taylor*

42 **USING A YEAST TWO-HYBRID APPROACH TO INVESTIGATE DNA REPAIR IN BDELLOID ROTIFERS** Kevin Bombinski*

43 **CHARACTERIZATION OF THE 1957-1958 INFLUENZA PANDEMIC IN ARKANSAS AND ARIZONA**
Kaitlyn Kemp*

44 **CHEMOKINE SECRETION VARIES SIGNIFICANTLY IN PAPILLARY AND FOLLICULAR THYROID CANCER TUMOR CELLS**
Nathan Andress*, Michaela Edwards*, Amber Melcher*, and Brock Sullivan*

45 **COMPARING THE EFFECTIVENESS OF ANTIBIOTICS AND ESSENTIAL OILS ON LABORATORY AND ENVIRONMENTAL BACTERIA STRAINS**
Vi Le*

46 **EFFECTS OF SIMULATED MICROGRAVITY AND RADIATION ON SERCA EXPRESSION IN ARTERIES**
Claudy Sarpong*

47 **OBJECT DISCRIMINATION ABILITIES IN BLIND INDIVIDUALS USING ECHOLOCATION**
Michael Ezeana*

48 **PAPILLARY THYROID CANCER CELLS DISPLAY DIFFERENCES IN DRUG SENSITIVITY THAT ARE DEPENDENT ON EXTRACELLULAR MATRIX COMPOSITION**
Lauren Dwyer*, Brianna Kelly*, Hannah Moore* andSesalie Satterwhite*

49 **ANALYSIS OF DE NOVO PEPTIDE 3337 FROM THE VENOM OF *R. RABIDA***
Landon Wolfe*

CHEMISTRY

50 **ANALYSIS OF BETX MIXTURES USING RAMAN SPECTROMETRY**
Drake Jackson*

51 **ANALYSIS OF OXIDATIVE STRESS OF MEMBRANE LIPIDS**
Sydnye Shuttleworth*

52 **DESIGN AND OPTIMIZATION OF A LOW-COST, ARDUINO-CONTROLLED FLUOROMETER**
Jacob Belding*

53 **DEVELOPMENT OF A MODIFIED MICHAELIS-MENTEN LANGMUIR KINETIC MODEL FOR SUPPORTED LIPID BILAYER FORMATION**
Kirstyn Baker*

54 **INVESTIGATING THE ROLE OF A CRITICAL VESICLE CONCENTRATION IN THE FORMATION OF SUPPORTED LIPID BILAYERS**
Jackson Bridges*

55 **IRON CONTENT IN DRIED FRUIT CHIPS VERSUS PUREED BABY FOOD USING TWO DIFFERENT METHODS**
Whitney Austin*

56 **DETERMINATION OF CAFFEINE CONTENT IN POPULAR ENERGY DRINKS WITH HIGH PERFORMANCE LIQUID CHROMATOGRAPHY**
Jada Fricks*

57 **ANALYSIS OF COPPER IN LOCAL ARKANSAS WINES**
Tanner Parrott*

58 **LEAD CONCENTRATIONS IN SOIL AT THE LOCAL SHOOTING RANGE**
Zach Hazeslip*

59 **MICROENVIRONMENT-SENSITIVE PROBES FOR SELECTIVE RECOGNITION OF SERUM ALBUMIN PROTEIN IN SOLUTION**
Kallie Mendenhall*

60 **ORGANOCATALYTIC PERICYCLIC REACTIONS: CATALYST REACTIVITY AND SUBSTRATE SCOPE**
Hayden Criswell*, Reece Mitchell*, and Shawqi Musallam*

61 **PHOTOCATALYSIS AS A MEANS OF DISINFECTING WATER DURING SPACE FLIGHT**
Elizabeth Reed*

62 **PHOTOCATALYTIC ACTIVITY OF TIO2 IN A CLOSED CIRCUIT: ELIMINATING ORGANIC CONTAMINANTS IN WATER USING METHYL ORANGE AS A MODEL COMPOUND.**
Rebecca Sain*

63 **UNDERSTANDING THE ROLE OF FLOW RATE AND LIPID CONCENTRATION IN THE KINETICS OF SUPPORTED LIPID BILAYER FORMATION**

Robbie Kiss*

64 **USE OF UV/VIS SPECTROSCOPY TO MEASURE ASA IN ASPIRIN**
Preston Eubanks*

COMPUTER SCIENCE & MATHEMATICS

65 **SKIN CANCER SPATIAL SURVIVAL MODELS USING R/SAS**
Thy Dao**

66 **HOW POWERFUL CAN DEEP LEARNING BE COMPARED WITH MACHINE LEARNING? A ENTITY RESOLUTION CASE**
Xinming Li**

67 **COMPUTER VISION SYSTEM FOR IDENTIFYING AND QUANTIFYING WASTE**
Mariofanna Milanova

68 **HUMAN INTERACTION WITH MULTIVARIATE SENTIMENT DISTRIBUTIONS OF STOCKS INTRADAY**
Mariofanna Milanova

69 **SCALABILITY STUDIES FOR COMPRESSIBLE FLOW SIMULATIONS**
John McGarigal*

PHYSICS

70 **A REAL TIME AUTOMATED MICROCLIMATE ECOSYSTEM**
Kayce Conville*

71 **DESIGN AND IMPLEMENTATION OF 3D-PRINTABLE OPTOMECHANICAL COMPONENTS**
Dylan Mitchell*

72 **DEVELOPMENT OF AN ACOUSTIC FIELD SCANNER**
Nick Scoles*

73 **INVESTIGATING RELATIONSHIP BETWEEN STRAIN APPLIED TO RAT'S LEG BONE AND BONE'S MECHANICAL STRENGTH**
Yelaman Zhenis*

74 **PREPARATION OF POLYCRYSTALLINE TIN SELENIDE TO INVESTIGATE THERMOELECTRIC PROPERTIES**
Alex Golden*

75 **PROTOSTELLAR OUTFLOWS IN L1448**
Jordan Rhoades*

76 **QUANTITATIVE BINDING OF DIVALENT METAL IONS TO DNA HAIRPIN LOOPS**
Harrison Russell*

ENGINEERING

77 **AN INVESTIGATION OF THERMOELECTRIC ELEMENT POWER GENERATION AND HEAT PUMPING ABILITY**
Isaac Raphael*

78 **DEVELOPING A FLUID MECHANICS EXPERIMENT USING A 3D PRINTED VENTURIMETER**
Joel Howell*, Tristan Nivens* Jerry Ramsey* and Austen Wood*

79 **EVALUATION OF THE STRESS-STRAIN STATE FOR THE 3D PRINTED AIRFOIL USING FINITE ELEMENT METHOD**
Tristan Nivens*

80 **EXPERIMENTAL INVESTIGATION OF STRESS CONCENTRATION IN PLASTIC MATERIALS USED FOR 3D PRINTING**
Kenneth Escudero*

81 **DEVELOPING SOFT ACTUATORS AT SAU ENGINEERING**
Kenneth Escudero* and Daniel McKague*

82 **EFFECTIVE THERMAL CONDUCTIVITY OF OPEN CELLED COPPER FOAM METALS**
Elias Perez Reyes*

83 **EXPERIMENTATION AND MODIFICATION OF VARIOUS 3-D PRINTED WATER PUMP DESIGNS**
Becka Wilson* and Moriah York*

84 **IMPACT ENERGY ABSORPTION BEHAVIOR OF LIGHTWEIGHT INTERPENETRATING PHASE COMPOSITE FOAM**
Kailash Jajam and Sherif Selim*

85 **MIXING OF FLUIDS IN MACRO-CHANNELS**
Derrick Fuell*

86 **NUMERICAL SIMULATION OF OXY-FUEL COMBUSTION**
Lucas Blake* and Matthew Gustafson*

87 **TROUT FISHING TACKLE BOX**
Li Morrow*

88 **WATER RECYCLING SYSTEM FOR A HYDRAULIC RAM PUMP**
Jacob Jackson*

89 **WEARABLE TACKLE/UTILITY BOX**
James Smith*

GEOSCIENCE

90 **BIOLOGICAL AND ECOSYSTEM LEVEL CHANGES FROM THE ADDITION OF RESERVOIRS TO HEADWATER STREAMS**
Krishna Patel*

91 CLOUD CONDENSATION NUCLEI (CCN) MEASUREMENTS: DESIGN AND CALIBRATION OF A CCN ANALYSIS SYSTEM
Karen Morris* and Rebecca Parham

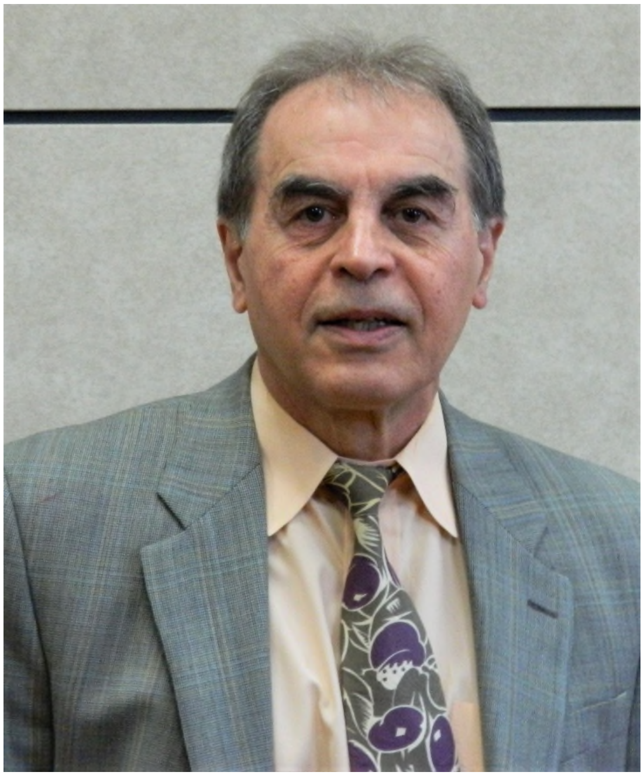
92 LOCATING LEGACY OIL AND GAS WELLS IN ARKANSAS: REFINING MAGNETIC METHODS
Michael Davis, Candy Roberts* and Hunter Vickers*

93 RESTORATION POTENTIAL OF ABANDONED WELLS IN THE FAYETTEVILLE SHALE
Varenya Nallur*

94 WATER ADSORPTION ON POLYHYDROXYLATE MICROSPHERES AS A FUNCTION OF RELATIVE HUMIDITY USING A QUARTZ CRYSTAL MICROBALANCE
Hailey Hayes*

95 WATER ADSORPTION ON POLYHYDROXYLATE MICROSPHERES AS A FUNCTION OF RELATIVE HUMIDITY USING AN FTIR SPECTROMETER EQUIPPED WITH A FLOW CELL
Henry Dana*

Tribute to Dr. Mostafa Hemmati



1983 was a very good year for Arkansas Tech University (ATU) and subsequently Arkansas. During that year a young man named Mostafa Hemmati joined the Physical Science faculty of ATU as an associate professor. He had received his B.S. in Physics from Meshad University in Iran in 1972, an M.S. in Physics from the University of Oklahoma (OU) in 1980 and then a Ph.D. in Physics in 1983 from the same institution. He had been both a teaching assistant and a research assistant while at OU. While at OU he also furthered his teaching skills at South Oklahoma City Junior College where he taught applied physics, introduction to physical science and introduction to astronomy. Mostafa received the Nielson Award for Outstanding Dissertation, from the University of Oklahoma in 1983.

From the very beginning of his academic career Mostafa was focused on his students and it shows. The following are comments about Mostafa on Rate your Professor.com from his students:

Dr. Hemmati is a fantastic lecturer, he is very passionate about the subject matter. At first his handwriting is tough to read so listening is key. Every day it's important to be in class as he constantly covers material, there is no such thing as a light day in his course.

He is simply awesome. His lectures are interesting. He wishes goodwill to everyone during class, lab, quiz and test. I will highly recommend him for all the physics classes and labs.

All around great professor. Challenging but not hard, plenty of opportunity for grades, goes into meticulous detail in his lectures. Be sure to get a seat front-row center, it can be difficult to see his work at the far end of the board.

Dr. Hemmati is a great physic professor. Learn what he teaches and study what he tells you to study, and you're good. You have to be able to understand the work instead of just doing the problems to do well on the tests. He teaches Modern Physics, and he teaches at your speed. He's humorous and his labs are fun.

Great Professor!!! TAKE HIM! His tests are difficult, but very fair. Lectures are anything but boring and provide more than sufficient understanding of the material. He is enthusiastic about his classes and demands respect of his students. Also a wonderful lab instructor!

If you prefer student TAGS on Mostafa are as follows: Skip class you will not pass; participation matters; amazing lectures; tough grader; clear grading criteria; and, extra credit available.

Thus, while Physics professors are not known for their popularity with students. Mostafa is a very conspicuous exception.

From 1996 to 2001 Mostafa was Head of the Department of Physical Science at ATU. In 2001 he was promoted to full Professor. From 2001 until his retirement he has also been Director of Undergraduate Research at ATU. In this role, he promoted and supported the necessary research aspirations of junior faculty as they sought promotion and tenure, and thereby enhancing the training and development of undergraduate students, so improving their ability to compete post-graduation. During this time, he has been very active in outreach in the support of science to a variety of entities. One of the more surprising of such entities are grade schools. It takes a very special physics professor who can talk to elementary students and be invited back!

Mostafa has taught almost every conceivable physics class at ATU including both majors and nonmajors physics, optics, atomic and nuclear physics both basic and advanced, thermodynamics and statistical mechanics, theory of electricity and magnetism, mechanics, quantum mechanics, as well as special problems in physics, colloquium, and introduction to physical science.

He has also received the following awards at ATU:

1996 Faculty Award of Excellence in Scholarship;
1996 Blue Key – Cardinal Key Outstanding Faculty Member Award;
2004 Faculty Award for Excellence for Scholarship; and the
2005 Faculty Award for Excellence for Service.

During the last 10 years Mostafa has mentored more than 40 students many of whom have gone on to graduate school. He has more than 30 significant publications, and has been awarded more than $300,000 in grant money to support his undergraduate researchers. He has managed to attend on the average one major research conference each year for the last two decades and presented many papers. His research emphasis is in the area of electrical breakdown waves.

Mostafa is very service-oriented. From 1998 to 2002 he served two terms as President of the Arkansas-Oklahoma-Kansas Section of the American Association of Physics Teachers. From 2003 to 2006 he served as Councilor to the Council on Undergraduate Research of the American Physical Society (APS). From 2001 to present he has been the ATU representative to the Arkansas Space Grant Consortium. From 2005 to present Mostafa has been President of the Iranian American Association of Arkansas. From 2008 to 2010 he was President of IrAP (Iranian-American Physicists Network Group). Mostafa was cited extensively in the article entitled *Iranian Scientists Battle Numerous Restrictions* (APS NEWS, Volume 20, Number 7).

Mostafa has been most active in the Arkansas Academy of Science. He was President of the AAS 1999-2000. Since 2002 he has been Chair of the Nominations Committee and Treasurer since 2005. When he took over as Treasurer, the AAS was barely breaking even each year, but as noted below that was going to change significantly. From 2004 to 2009 Mostafa was the AAS Representative to the American Association for the Advancement of Science annual meeting. In 2008 Mostafa accepted his most daunting assignment for the Academy: that of Editor-in-Chief of the peer-reviewed Journal of the Arkansas Academy of Science during its transformation from predominantly paper format to an increasingly more web-based presence.

By serving the intense roles of Treasurer and Journal Editor-in-Chief, Mostafa has provided a unique, powerful and long-lasting impact on the Academy. In 2008, Mostafa recruited Ivan Still to work on the Journal as Interim and then duly elected Managing Editor. Mostafa promoted a dramatic change to the publication mechanics, with authors needing to format their submitted articles in a "near-finished" format; Ivan subsequently would handle any "final edits" after peer-review to bring all manuscripts to publication quality and then assembling meeting reports etc. to form the completed *JOURNAL* for publication. Mostafa skillfully handled resistance to these controversial moves, but this innovation in the publication process was successful as evidenced by the quality of the finished product. Significantly, the new process removed the need for an external copy-editing and publishing service, previously a massive drain on the finances of the Academy. Indeed, as of 2018, the Academy now has sufficient resources to fund research grants for undergraduate students as well as other support activities of AAS. However, in the last couple of years, further changes to the Journal were coming. Since 1950, the Proceedings, and then, with the name change in 1997, the Journal of the Arkansas Academy of Science has been published each year in hardcopy, and then archived electronically by the University of Arkansas Libraries. When UA adopted the Scholarworks Digital Commons platform through bepress^{TM}, Mostafa supported the move of the Journal to the new system. In collaboration with Cedar Middleton (UA), Melody Herr (UA), Ivan and the staff of bepress, the Journal transferred to the Digital Commons system, with all manuscript review and processing now handled electronically. The overriding and long-lasting impact of this final change to the Journal during Mostafa's tenure as Editor-in-Chief returns to major objectives of the Academy. The current form of the Journal further aids "the promotion and diffusion of knowledge in the fields of Science", and in particular promotes the research efforts of Faculty and students in Arkansas by being easily and freely available throughout the world through the journal homepage at https://scholarworks.uark.edu/jaas/.

We of the Academy wish Professor Mostafa Hemmati a very happy retirement in New York City!

Collis Geren
Secretary and Historian, Arkansas Academy of Science

Ivan H. Still
Managing Editor, JAAS

In Memoriam: Douglas A. James, 1925-2018

On December 17, 2018, Arkansas lost a giant in ornithology and conservation. Douglas A. James, a faculty member at the University of Arkansas (Fayetteville) from 1953 to 2016, was the authority on the birds of Arkansas and was one of the state's leading conservationists in the second half of the last century. Widely considered as "The Bird Man of Arkansas", he co-authored *Arkansas Birds: Their Distribution and Abundance* (University of Arkansas Press) with Joseph C. Neal in 1986. He arranged the first meeting of what would become the Ozark Society, which was responsible for saving the Buffalo River from damming. Starting with studies of scrubland birds in northwestern Arkansas, Doug expanded to studying scrubland birds in Africa, Nepal, and Belize as one of the few people to have received three Fulbright Scholar awards. He also spent spring of 1995 as a Visiting Scholar at Cambridge University in England.

Douglas Arthur James was born on 25 July 1925 in Detroit, Michigan, to Arthur Collins James and Sigrid Elizabeth Hovey. He began his career in ornithology by drawing and coloring bird pictures in the 4th grade. Later in elementary school, his teacher asked him to lead class field trips and continued to do so in junior high. He attended the University of Michigan, receiving a Bachelor of Science degree in 1946 and a Master of Science degree in 1947. He then went to the University of Illinois to study under the famous avian physiologist and ecologist, S. C. Kendeigh, where he completed a doctoral degree in 1957 on the ecology of roosting blackbirds.

In 1953, he was offered a position as the first ornithologist at the University of Arkansas. Prior to that, ornithology was first taught at the University by William Baerg, a professor in the Department of Entomology. Doug last taught ornithology in the spring of 2015, meaning that the course was taught for nearly 90 years by just two professors. His ornithology course was known for decades for its annual South Texas field trip in the spring. Doug was the first faculty member in his department to receive the special appointment University Professor of Biological Sciences in 2004. When he retired in 2016, he had been at the University of Arkansas for 64 years.


Douglas A. James at Holla Bend National Wildlife Refuge with trumpeter swan, February 2010. Photo by Sue Pekel.

In his decades-long illustrious career, Doug published over a hundred scientific papers and received 77 research grants (totaling over $1.5 million) from local and national agencies. He cherished the opportunity to attend ornithological meetings and gave over 300 presentations of which 70 were invited. He mentored more than 80 graduate students, including 30 doctoral students. Notable subjects of his international studies included the endangered Great Hornbill in India, Jabiru in Belize, Pallas's Fishing Eagle in Mongolia, Musk Deer in Nepal, Scrubland bird communities in India, and avifauna of Malaysian rubber plantations. More locally, he authored several papers on the endangered Red-cockaded Woodpecker in Arkansas. He played a significant role in the development of the field of multivariate statistics to describe bird habitats. A historical plaque on the university campus notes the accomplishments in statistical ecology of James' lab and students.

Doug received several life-time achievement awards, including the W. Frank Blair Eminent Naturalist Award (2006) from the Southwestern Association of Naturalists, which recognizes excellence in a lifetime of commitment to outstanding study or conservation of the flora or fauna of the southwestern United States, Mexico, and Central America, and William and Nancy Klamm Service Award (2014) from the Wilson Ornithological Society (WOS), which honors the history of service and dedication to the society. Altogether, he received 14 awards for his excellence in teaching and research from various entities including an international teaching award. In 2002, he received the prestigious Charles and Nadine Baum Teaching Award, the highest teaching award given by the University of Arkansas. Doug served as President of the WOS (1977-79); and was elected Fellow to the American Association for Advancement of Science (1968) and American Ornithologists' Union (AOU; 1988). As President of WOS, he pushed for encouraging amateur involvement in ornithology.

Doug James was the quintessential versatile naturalist, almost equally adept with reptiles and mammals, as he was with birds. His non-avian publications covered the gamut from box turtles and bats, to salamanders and small mammals. With a clever mixture of field and lab investigations, his sharp and interdisciplinary mind enabled him address questions not answered by any one discipline. He was as comfortable with the binoculars as he was with museum skins and specimens, a quality that is becoming increasingly rare among academics. Using these attributes, he determined, for example, that Great Hornbills do not use mud to seal nest cavities (Wilson J. Ornithology, 2007). Among his notable Arkansas projects were the successful translocation of wild turkeys to northwest Arkansas; discerning perplexing chickadee specimens using skeletal measurements; identifying Colibri hummingbirds using indirect measurements from photographs; and interpreting the cargos of birds blown into Arkansas by hurricanes (J. Ark. Acad. Sci. 1983-2010).

Doug was a great and inspiring teacher. He trained his graduate students first by an excellent regimen of his own courses. His courses were strongly supported by intensive 4-hour lab sessions, in which he hammered the importance of accuracy and attention for detail while dealing with complex data. His superior grasp of statistics and ability to incorporate statistical methods into data actually collected by students was invaluable. His courses were also heavily field-oriented, and this excellent combination of lab and field exposure shaped many a biologist's career, as is evident from the enormous success of his former students. He hated jargon and obfuscation in scientific writing and demanded simplicity and clarity. He imposed tough but fair standards in his classes, and decried the rise of grade inflation, often telling his classes that he finished his B.S. degree with a 2.9 G.P.A., yet led the graduation march for topping his class.

In 1955, Doug and his then wife Frances James spear-headed the formation of the Arkansas Audubon Society, a vibrant organization that continues today. In 1972, Doug helped form the Arkansas Audubon Society Trust (AAST) with the intention of funding avian research and conservation projects within Arkansas. The AAST has continued to grow and its endowment is approaching $200,000 as it nears its 50th anniversary in 2022. Due to his vision, hundreds of graduate and undergraduate students have been funded by the Trust. One AAST award was named the Douglas James Award, given annually to a project involving birds. Doug was the first Curator of bird records for the Arkansas Audubon Society. He helped with writing the bylaws of the society, organized the first fall meeting, became the first newsletter editor, and initiated several of the annual awards bestowed by the society. While he was curator, he helped accumulated over 30,000 bird records for Arkansas, which became the foundation for his Arkansas Birds book. He used a punch card system that foresaw the coming of computers. All those cards have now been digitized and are available on line.

During his career, Doug filled nearly 150 field notebooks based on his research and travels: 106 from North America (mostly Arkansas), 24 from Central and South America, 8 from Asia, 7 from Africa, and 4 from Europe. He always told his students "if you didn't write it down, it didn't happen." Doug remained active in publishing till the end, although some of his long-term studies (like plant succession and bird communities in the forests of northern Michigan, spanning several decades) unfortunately remain unpublished. His last paper appeared the year he died. It documented changes in small mammal abundances in Lake Fayetteville over 32 years, the data having been collected as part of his Mammalogy class field trips from 1983 (J. Arkansas Acad. Sci. 72).

Doug loved to tell stories of his rich life-experiences. Notable among the anecdotes he shared was one of being bitten by a skunk that later tested positive for rabies; walking into a Michigan gas station with a live freshly caught rattlesnake at hand ("it emptied the place in a hurry"); and chasing away and being chased by elephants and bison while running in India. One of his college classmates was Nobel Laureate James Watson of DNA-structure fame. When Watson visited Fayetteville in May 2005, Doug joked to a packed audience: "We both aspired to be ornithologists.

I made it. He didn't." Once while setting off on a hike through an Indian rainforest searching for hornbill nest sites, he applied leech repellant to one of his feet. When asked why he left the other unsprayed, he quipped "That's my control!" He loved gags. He wore a hog hat to drive long-winded speakers off the stage at the AOU meeting he hosted in Fayetteville. His frequent and much sought-after talks on his global studies were colorful, literally and figuratively. He proudly wore a traditional attire of the country he was speaking about, like a Nepali hat or an Indonesian batik. After a short stint in India, he walked into his classroom wearing a garland he was given by an Indian family. Students rarely fell asleep in his classes!

Doug was an avid runner most of life, having run 10 marathons. He was indefatigable in the field, often challenging his much younger graduate students to keep up with him. After a long day of field work, he often ran to refresh and rejuvenate. His immense knowledge of natural history of the localities, his ability to design field projects tailor-made to local situations, and his stamina and endurance in the field made him an invaluable mentor. Doug lived a simple life and shunned luxuries. His intellectual curiosity was insatiable, and he waxed eloquent on all matters. His interests were eclectic, and included history, ballet, opera, and art galleries. He loved all kinds of dancing.

Apart from his own contributions to natural history, Doug James's ultimate legacy will be the generations of competent field biologists and teachers his mentorship spawned. The first three former students who were recognized as Distinguished Alums of the department were Doug's graduate students. He is survived by his wife of over 30 years, Elizabeth Adam, three daughters from his first marriage with Frances James, two grandchildren, Travis and Sydney Olson, and one great granddaughter, Linnea Olson. Two of his daughters, Helen and Avis, followed their parents' footsteps to fruitful careers in biology. The third, Sigrid, a lawyer, is a bird artist who helped illustrate his bird book.

At the 103rd Arkansas Academy of Science annual meeting on March 30th 2019, Doug James was posthumously named as the First Honorary Fellow of the Arkansas Academy of Science. Doug bequeathed much of his life-time earnings to the University of Arkansas to establish a future Douglas A. James Endowed Chair in Ornithology.

Ragupathy Kannan, Department of Biology, University of Arkansas—Fort Smith, Arkansas 72913

Joseph C. Neal, Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas 72701

The following song was sung during the memorial celebration of Doug James' life on March 31st 2019:

ODE TO DOUG JAMES
Still On the Hill
By Kelly & Donna Mulhollan

He's gone to a place, just past the rainbow
He's gone to a place, where the birding's always good
Where birds of all feathers, fly on forever
And back here on earth, we'll always remember

He's gone to a place, where he won't need no raincoat
No hat and no jacket, no long underwear
No spotting scope or tripod, no glasses or field guide
No duct tape or insecticide
He won't need those things up there

That cold December morning, when the birds were all counted
An event that he founded, so many years ago
And little did we know, that eve he had departed
With wings of his own, Doug James had flown

Journal Acknowledgements

The Arkansas Academy of Science gratefully acknowledges the Editorial board for volume 73 of the Journal during 2019.

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

Instructions to Authors

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

A. General Policies

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

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

B. General Requirements

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

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

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

C: Review Procedure

Evaluation of a paper submitted to the *JOURNAL* begins with critical reading by the Managing Editor. The manuscript is then submitted to referees for critical review for scientific content, originality and clarity of presentation. To expedite review, authors should provide, in a cover letter, the names and current e-mail address of at least three reviewers within the appropriate field, with whom they have not had a collaboration in the past two years. Potential reviewers that the authors wish to avoid due to other conflicts of interest can also be provided. Attention to the preceding paragraphs will also facilitate the review process. Reviews will be returned to the author together with a judgement regarding the acceptability of the manuscript for publication in the *JOURNAL*. The authors will be requested to revise the manuscript where necessary. Time limits for submission of the manuscript and publication charges will be finalized in the accompanying letter from the Managing Editor (see "Proposed timetable for manuscript processing"). The authors will then be asked to return the revised manuscript, together with a cover letter detailing their responses to the reviewers' comments and changes made as a result. The corresponding author will be responsible for submitting the total publication cost of the paper to the Treasurer of the Academy, when the revised manuscript is returned to the Editor assigned to your manuscript. Failure to pay the publication charges in a timely manner will prevent processing of the manuscript. If the time limits are not met, the paper will be considered withdrawn by the author. Please note that this revised manuscript will be the manuscript that will enter into the bound journal. Thus, authors should carefully read for errors and omissions so ensure accurate publication. A page charge will be billed to the author of printed errata; however, no charge is made for errata that are only "printed" in the on-line journal (contact the Editor-in-Chief for more details). All final decisions concerning acceptance or rejection of a manuscript are made by the Managing Editor and/or the Editor-in-Chief.

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

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

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

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

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F: Proposed Timetable for Manuscript Processing

It is the policy of the Arkansas Academy of Science that 1) at least one of the authors of a paper submitted for publication in the *JOURNAL* must be a member of Arkansas Academy of Science, 2) only papers presented at the annual meeting are eligible for publication, and 3) manuscript submission is due at the annual meeting. Thus, manuscripts should be submitted to the *JOURNAL* website: https://scholarworks.uark.edu/jaas/, two days before the meeting. Authors who have submitted manuscripts via the system previously, should use the contact/email and password that was used previously. New authors should follow instructions on the site to establish their profile. Authors can subsequently update their profile with any changes to their contact and account information as necessary

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

Timetable

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

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

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

End of May: Manuscripts sent to reviewers.

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

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

The prompt return of revised manuscripts 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.
5. Set words in italics that are to be printed in italics (e.g., scientific names).
6. In scientific text, **Arabic numerals** should be used in preference to words when the number designates anything that can be counted or measured: 3 hypotheses, 7 samples, 20 milligrams. However, numerals are not used to begin a sentence; spell out the number, reword the sentence, or join it to a previous sentence. Also, 2 numeric expressions should not be placed next to each other in a sentence. The pronoun “one” is always spelled out.
7. Use of footnotes is not permitted
8. A **feature article** is 2 or more pages in length. Most **feature articles** should include the following sections: Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgments, and Literature Cited.
9. A **general note** is generally shorter, usually 1 to 2 pages and rarely utilizes subheadings. A note should have the title at the top of the first page with the body of the paper following. Abstracts are not used for general notes.
10. A **review article** should contain a short abstract followed by the body of the paper. The article may be divided into sections if appropriate, and a final summary or concluding paragraph should be included.

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

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

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

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

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

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

B. Specific considerations

1. Title section

(see Fig. 1 above for layout).

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

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

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

iv. Insert a single 10 point blank line after the "Running Title" and add a Continuous section break.

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

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

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

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

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

ii. **Figures**: A short caption should be written under each figure in 9 point, normal. Figure 2 shows an example for the format of a figure inserted into the manuscript. All figures should be created with applications that are capable of preparing high- resolution PhotoShop compatible files. The figure should be appropriately sized and cropped to fit into either one or two columns. Figures should be inserted as JPEG, TIFF images or PhotoShop compatible files. Arrows, scale bars etc., must be integral to the figure: i.e. not "added over" the figure once place in the word document: "independent arrows, etc., will be lost in manuscript formatting. While the *JOURNAL* is printed in black and white, we encourage the inclusion of color figures and photographs that can be viewed in the online version. Please note that the figures directly imported from PowerPoint frequently show poor color, font and resolution issues. Figures generated in PowerPoint should be converted to a high resolution TIFF or JPEG file (see your software user's manual for details). If a

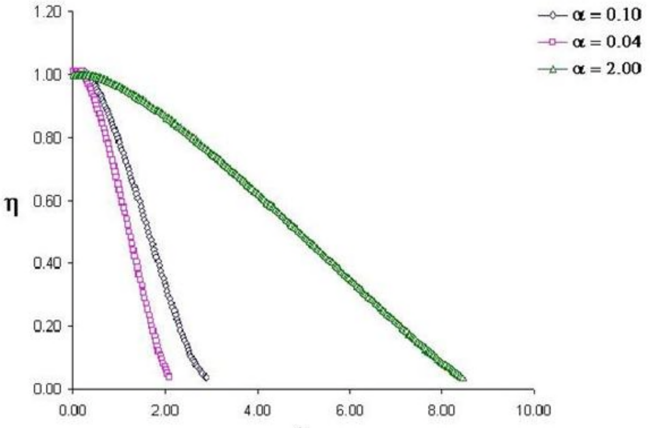

Figure 2. Electric field, η, as a function of position ξ, within the sheath region for three different wave speeds, α.

figure/table is taken from a powerpoint slide, the figure title/legend from that slide should be removed: the only title and legend that should be associated with the figure should be the caption as described at the start of this section, and as shown in the example Figure 2.

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

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

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

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

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

General form:

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

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

Please note below, that we have included "cited in text as" to show you the form of citation in the text, only, i.e. the "cited in text" part is not placed in the Literature cited section.

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.
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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.
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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.

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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

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

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

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

Online resources

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

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

Cited in text as: (USGS 1979)

Multiple Citations are Cited in text as:

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

8. Submission of Obituaries and *In Memoria*

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

BUSINESS & SUBSCRIPTION INFORMATION

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

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