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# Prey Composition of Barn Owl Pellets Collected in Oklahoma

**Katrina D. Hucks**

Department of Biology, University of Louisiana at Lafayette, Lafayette, LA 70504

**Christopher J. Butler**

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

**Ken J. Locey**

Department of Biology, Indiana University, Bloomington, IN 47405

**Lisa H. Locey**

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

**Paul W. Wilson**

10004 E. 156<sup>th</sup> Street North, Collinsville, OK 74021

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**Abstract:** We examined the prey composition of Barn Owl (*Tyto alba*) pellets from 26 counties in Oklahoma across five regions. A total of 49,186 pellets was collected from 1978 through 1992, representing 58,937 total prey items. The majority (98.6%) of the prey items were mammals, although birds, snakes, and invertebrates were also found. The most frequently encountered species were *Sigmodon hispidus* (n = 21,472), *Peromyscus* spp. (n = 9077), and *Chaetodipus hispidus* (n = 7381). Dietary composition by region broadly reflected published accounts of relative abundances of small mammal in each region. These results lead us to suggest that Barn Owls are generalist predators of small mammals across Oklahoma. ©2015 Oklahoma Academy of Science

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## Introduction

Barn Owls (*Tyto alba*) are widely distributed on every continent except Antarctica (Marti 1992), making this species ideal for study of regional prey preferences. They feed primarily on small mammals (Askew et al. 2007, Velarde et al. 2007, Santos-Moreno and Alfaro Espinosa 2009), although fishes were most common in a Nevada study (Bogiatto et al. 2006). Taylor (1994) mentioned that individual Barn Owls occasionally focus on bird species such as Leach's Storm-Petrel (*Oceanodroma leucorhoa*) and Red-winged Blackbird (*Agelaius phoeniceus*). Barn Owls cannot easily digest bones, feathers, or fur and eject these remains as a pellet (Glue 1974). Numerous studies

have used the remains from these pellets to evaluate the composition of the prey species community (Yom-Tov and Wool 1997, Shehab and Al Charabi 2006, Velarde et al. 2007).

However, these studies have not examined how prey composition may differ across ecological gradients. In Oklahoma, for example, many species, including birds, amphibians, reptiles, and mammals reach their eastern or western range limits (Blair and Hubbell 1938, Caire et al. 1989, Reinking 2004, Sievert and Sievert 2006). In contrast, Barn Owls are common in grasslands and some open forests in western Oklahoma, and are uncommon to rare in the northeast and southeast, probably due to lack of habitat (Reinking 2004). We

examined how Barn Owl prey diversity might vary across the state of Oklahoma.

## Methods

We grouped Oklahoma counties into five areas: the panhandle, northwest, southwest, central, and northeast. The panhandle (Cimarron County) is characterized by low precipitation and shortgrass prairie and sagebrush (Woods et al. 2005). Cimarron County receives an average of only 438.4 mm of precipitation (Oklahoma Climatological Society 2014). The northwest (Alfalfa, Blaine, Custer, Dewey, Ellis, Major, Roger Mills, Woods, and Woodward counties) consists of mixed grass prairie and cropland, with precipitation increasing eastward (Woods et al. 2005) and receives an average of 716.3 mm of precipitation. The southwest (Beckham, Caddo, Comanche, Cotton, Greer, Harmon, Jackson, Jefferson, Kiowa, Tillman, and Washita counties) consists of mixed grass prairie integrating cross-timbers in the east (Woods et al. 2005). This region receives similar amounts of precipitation as northwestern Oklahoma, an average of 770.5 mm of precipitation per year. The central (Oklahoma County) region consists mostly of cross-timbers with mixed grass prairie in the west (Woods et al. 2005). Oklahoma County receives an average of 919.7 mm of precipitation annually. The northeast (Cherokee, Ottawa, Tulsa, and Wagoner counties) is composed of the oak-hickory and tallgrass prairie regions with higher amounts of rainfall (Woods et al. 2005) and receives an average of 1137.2 mm of precipitation annually.

Paul Wilson collected pellets across Oklahoma from 1978 through 1992 (Fig. 1). A total of 49,186 Barn Owl pellets was collected in 26 counties: Alfalfa (n = 2,846); Beckham (n = 1,750); Blaine (n = 1,314); Caddo (n = 46); Cherokee (n = 126); Cimarron (n = 387); Comanche (n = 196); Cotton (n = 1,489); Custer (n = 3,582); Dewey (n = 1,179); Ellis (n = 110); Greer (n = 16,439); Harmon (n = 3,378); Jackson (n = 5,231); Jefferson (n = 3,076); Kiowa (n = 298); Major (n = 1,395); Oklahoma (n = 675); Ottawa (n = 164); Roger Mills (n = 1); Tillman (n = 3,406); Tulsa (n =

467); Wagoner (n = 346); Washita (n = 251); Woods (n = 49); and Woodward (n = 985; Fig. 1) which represent 7 of 12 level III ecoregions in Oklahoma. Level III ecoregions are divisions nested within coarser level I and II ecoregions created by the Commission of Environmental Cooperation (CEC 1997). From west to east, High Plains, Southwestern Tablelands, Central Great Plains, Cross Timbers, Central Irregular Plains, Ozark Highlands, and Boston Mountains ecoregions are represented (Woods et al. 2005).

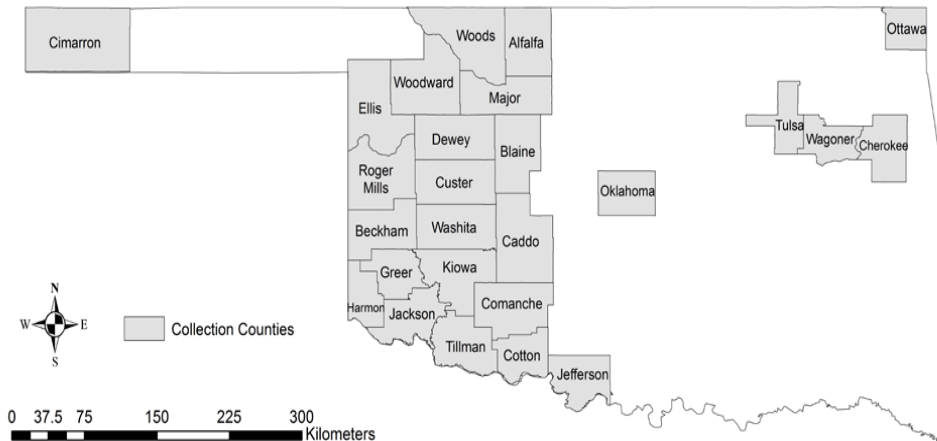
Pellets were soaked in water and dissected individually. Number of prey items were counted based on the number of similar bone structures (i.e. mandibles or parts of the cranium) that the pellet contained (Yom-Tov and Wool 1997). Complete, or mostly complete skulls were identified using Hall (1981). Prey fragments were identified to the lowest reliable taxonomic level. After identification, skulls were placed in the University of Central Oklahoma Natural History Museum. For the identified species or groups, we recorded the average body mass referencing Schmidly (2004) and Poole (2005), averaging masses for prey items identified only to genus. We did not include mass for items that we were unable to identify beyond family.

## Results

We identified 58,937 prey. The majority (98.6%) were mammals, whereas 1.4% were birds (Table 1). In addition to mammals and birds, six snakes, six crayfish, and four insects were identified, representing only 0.03% of the prey species.

Of the species identified, 84.5% (n = 49,829) were Cricetidae (New World mice), 5.9% (n = 3,497) were Soricidae (shrews), 3.0% (n = 1,769) were Geomyidae (pocket gophers), 2.7% (n = 1,590) were Heteromyidae (pocket mice and kangaroo rats), and 1.6% (n = 919) were Leporidae (rabbits). Muridae (Old World rats and mice), Talpidae (moles), Vespertilionidae (bats), and Mephitidae (skunks) represented less than 1% of the prey total. In Oklahoma, Barn Owls fed predominantly on *Sigmodon hispidus* (36.4%, n = 21,472), *Peromyscus* spp.

Fig. 1. Oklahoma counties where Barn Owl pellets were collected from 1978-1992.



(15.4%,  $n = 9,077$ ), and *Chaetodipus hispidus* (12.5%,  $n = 7,381$ ), regardless of region. Introduced rodents such as *Mus musculus* ( $n = 6$  or 0.01%), *Rattus rattus* ( $n = 206$  or 0.35%), or *R. norvegicus* ( $n = 49$  or 0.08%; Table 1) were not important components of the diet. Among avian prey, passeriforms accounted for 1.4% ( $n = 806$ ) of the prey items identified. Remains from Falconiformes (falcons), Columbiformes (pigeons and doves), Strigiformes (owls), and Piciformes (woodpeckers) accounted for only 0.1% of the prey items.

## Discussion

The percentage of mammalian prey consumed is similar (usually greater than 90%) to other studies conducted in neighboring states. *Sigmodon hispidus* was commonly consumed in studies by Parmalee (1954), Otteni et al. (1972), Goyer et al. (1981), and Baker (1991). *Microtus* voles, *Peromyscus* spp., *Reithrodontomys* spp., *Chaetodipus hispidus*, and *Cryptotis parva* were often important dietary components, likely representing commonly available species (Wooster 1936, Petitt 1951, Parmalee 1954,

Otteni et al. 1972, Goyer et al. 1981, Baker 1991, Gubanyi et al. 1992), which agree with the results of this study (Table 1). *Oryzomys palustris* was identified as a heavily predated species in Texas by Otteni et al. (1972), Goyer et al. (1981), and Baker (1991). This species was not frequently consumed in our study, which may be attributed to lack of information about Barn Owl dietary habits in southeastern Oklahoma.

We examined prey composition by region. The four most commonly encountered mammals are found throughout most of the state (Table 2). In northeastern, central, northwestern, and southwestern Oklahoma, the pellet composition was dominated by *Sigmodon hispidus* (22-68%; Table 2), which is common and abundant throughout Oklahoma (Caire et al. 1989, Clark et al. 1989). In contrast, *Peromyscus* spp. dominated the pellet composition in the panhandle (25%; Table 2). *Peromyscus* spp. also are common statewide (Caire et al. 1989). The third most consumed mammal, *Chaetodipus hispidus* (13% of the total pellets; Table 2), is present statewide except for the northeast and southeast corners of the state (Caire et al.

Table 1: Specimens from Barn Owl pellets were identified to lowest possible taxonomic level.

Class	Order	Family	Genus	Species	Number of Specimens	Average Mass (g)
Mammalia	Rodentia	Cricetidae	<i>Sigmodon</i>	<i>Sigmodon hispidus</i>	21472	115
Mammalia	Rodentia	Cricetidae	<i>Peromyscus</i>	<i>Peromyscus</i> spp.	9077	24
Mammalia	Rodentia	Heteromyidae	<i>Chaetodipus</i>	<i>Chaetodipus hispidus</i>	7381	47.5
Mammalia	Rodentia	Cricetidae	<i>Reithrodontomys</i>	<i>Reithrodontomys</i> spp.	5273	14
Mammalia	Rodentia	Cricetidae	<i>Microtus</i>	<i>Microtus ochrogaster</i>	3460	40
Mammalia	Soricomorpha	Soricidae	<i>Cryptotis</i>	<i>Cryptotis parva</i>	3450	5.8
Mammalia	Rodentia	Geomyidae	<i>Geomys</i>	<i>Geomys bursarius</i>	1768	140
Mammalia	Rodentia	Heteromyidae	<i>Dipodomys</i>	<i>Dipodomys ordii</i>	1590	65
Mammalia	Rodentia	Cricetidae	<i>Neotoma</i>	<i>Neotoma micropus</i>	1323	257
Mammalia	Rodentia	Cricetidae	<i>Onychomys</i>	<i>Onychomys leucogaster</i>	1011	36.5
Mammalia	Rodentia	Heteromyidae	<i>Perognathus</i>	<i>Perognathus flavus</i>	617	7
Mammalia	Lagomorpha	Leporidae	<i>Sylvilagus</i>	<i>Sylvilagus audubonii</i>	570	950
Mammalia	Lagomorpha	Leporidae	<i>Sylvilagus</i>	<i>Sylvilagus floridanus</i>	349	1500
Mammalia	Rodentia	Muridae	<i>Rattus</i>	<i>Rattus rattus</i>	206	200
Mammalia	Rodentia	Cricetidae	<i>Microtus</i>	<i>Microtus</i> spp.	187	25.5
Mammalia	Soricomorpha	Talpidae	<i>Scalopus</i>	<i>Scalopus aquaticus</i>	176	75
Mammalia	Rodentia	Muridae	<i>Rattus</i>	<i>Rattus norvegicus</i>	49	450
Mammalia	Soricomorpha	Soricidae	<i>Blarina</i>	<i>Blarina brevicauda</i>	47	24
Mammalia	Chiroptera	Molossidae	<i>Tadarida</i>	<i>Tadarida brasiliensis</i>	16	12.5
Mammalia	Rodentia	Heteromyidae	<i>Perognathus</i>	<i>Perognathus</i> spp.	14	12
Mammalia	Rodentia	Cricetidae	<i>Baiomys</i>	<i>Baiomys taylori</i>	9	7.8
Mammalia	Carnivora	Mephitidae	<i>Mephitis</i>	<i>Mephitis mephitis</i>	8	4000
Mammalia	Rodentia	Muridae	<i>Mus</i>	<i>Mus musculus</i>	6	38.5
Mammalia	Rodentia	Cricetidae	<i>Neotoma</i>	<i>Neotoma floridana</i>	5	275
Mammalia	Rodentia	Cricetidae	<i>Oryzomys</i>	<i>Oryzomys palustris</i>	2	51
Mammalia	Chiroptera	Vespertilionidae	<i>Myotis</i>	<i>Myotis velifer</i>	2	13.5
Mammalia	Rodentia	Cricetidae	<i>Neotoma</i>	<i>Neotoma</i> spp.	1	215
Mammalia	Rodentia	Geomyidae	<i>Geomys</i>	<i>Geomys</i> spp.	1	114
Mammalia	Soricomorpha	Soricidae	<i>Notiosorex</i>	<i>Notiosorex crawfordi</i>	1	4.3
Aves	Passeriformes	Fringillidae		Unidentified Fringillidae	408	
Aves	Passeriformes			Unidentified Passeriformes	106	
Aves	Passeriformes	Icteridae	<i>Sturnella</i>	<i>Sturnella</i> spp.	101	111
Aves	Passeriformes	Icteridae	<i>Sturnella</i>	<i>Sturnella neglecta</i>	100	102



Table 1 Continued

Class	Order	Family	Genus	Species	Number of Specimens	Average Mass (g)
Aves				Unidentified Aves	32	
Aves	Passeriformes	Emberizidae		Unidentified Emberizidae	19	
Aves	Passeriformes	Icteridae	<i>Sturnella</i>	<i>Sturnella magna</i>	7	120
Aves	Strigiformes	Tytonidae	<i>Tyto</i>	<i>Tyto alba</i>	4	525
Aves	Passeriformes	Icteridae	<i>Agelaius</i>	<i>Agelaius phoeniceus</i>	4	55
Aves	Columbiformes	Columbidae	<i>Columba</i>	<i>Columba livia</i>	3	160
Aves	Passeriformes	Cardinalidae	<i>Cardinalis</i>	<i>Cardinalis cardinalis</i>	3	45
Aves	Passeriformes	Icteridae	<i>Quiscalus</i>	<i>Quiscalus quiscula</i>	3	108
Aves	Passeriformes	Sturnidae	<i>Sturnus</i>	<i>Sturnus vulgaris</i>	3	78
Aves	Columbiformes	Columbidae	<i>Zenaida</i>	<i>Zenaida macroura</i>	2	133
Aves	Piciformes	Picidae	<i>Colaptes</i>	<i>Colaptes auratus</i>	1	135
Aves	Falconiformes	Falconidae	<i>Falco</i>	<i>Falco sparverius</i>	1	122.5
Aves	Passeriformes	Passeridae	<i>Passer</i>	<i>Passer domesticus</i>	1	28
Reptilia	Squamata			Unidentified Serpentes	6	
Decapoda				Unidentified Decapoda	10	
Insecta				Unidentified Insecta	4	

1989). *Reithrodontomys* spp. (9% of the total pellets; Table 2) are found statewide, though *R. fulvescens* and *R. montanus* are more common than *R. humulis* and *R. megalotis* (Caire et al. 1989). *Microtus ochrogaster* (6% of the total pellets; Table 2) is uncommon and found only in the northeastern to north-central part of Oklahoma (Caire et al. 1989).

Table 3 shows the relative mammal abundance reported in previous studies in five areas of Oklahoma where our data were concentrated. We contrasted their studies to the five most consumed species in our study. *Peromyscus* spp. (*P. leucopus* and *P. maniculatus*) and *Sigmodon hispidus* were the most abundant species in the northeast, northwest, and southwest regions, which mirrors the results of our study. *Sigmodon hispidus* was generally less common than *Peromyscus* spp. in the small mammal surveys but was the most common prey item in our study (excluding the Panhandle). This may be attributed to sampling biases in small mammal trapping as well as biases in pellet data. *Peromyscus* spp. may be overrepresented in small mammal trapping, while *Sigmodon*

*hispidus* may be overrepresented in the pellets. Barn Owls could also be feeding preferentially on *Sigmodon hispidus*.

We detected proportionately more *Microtus ochrogaster* than these small mammal surveys indicated, which may mean that Barn Owls disproportionately prey upon *Microtus ochrogaster*, or this species is overrepresented in the diet. Whereas *Microtus ochrogaster* accounted for only 6% of the top five Barn Owl prey items, the species was the second most frequently consumed prey in the northwest, northeast, and central Oklahoma regions (Table 2). *Microtus ochrogaster* is generally uncommon across Oklahoma and is encountered frequently only in northeastern Oklahoma (Table 3). Our data also reflected a larger percentage of *Chaetodipus hispidus* than shown in the abundance studies (13%; Table 3). This supports that *Chaetodipus hispidus* is an important dietary component of Oklahoma Barn Owls. One species of *Reithrodontomys*, *R. montanus*, was abundant only in the northeast (Table 3).

The most abundant mammal for the

**Table 2. Five most consumed mammal species in five areas of Oklahoma according to our study.**

Panhandle	Percent (%)	Northwest	Percent (%)	Southwest	Percent (%)	Northeast	Percent (%)	Central	Percent (%)	Overall	Percent (%)
<i>Peromyscus</i> spp.	25	<i>Sigmodon hispidus</i>	22	<i>Sigmodon hispidus</i>	41	<i>Sigmodon hispidus</i>	53	<i>Sigmodon hispidus</i>	68	<i>Sigmodon hispidus</i>	36
<i>Sigmodon hispidus</i>	17	<i>Microtus ochrogaster</i>	19	<i>Peromyscus</i> spp.	17	<i>Microtus ochrogaster</i>	23	<i>Microtus ochrogaster</i>	16	<i>Peromyscus</i> spp.	15
<i>Reithrodontomys montanus</i>	17	<i>Peromyscus</i> spp.	13	<i>Chaetodipus hispidus</i>	14	<i>Cryptotis parva</i>	6	<i>Cryptotis parva</i>	10	<i>Chaetodipus hispidus</i>	13
<i>Onychomys leucogaster</i>	11	<i>Chaetodipus hispidus</i>	11	<i>Reithrodontomys montanus</i>	9	<i>Peromyscus</i> spp.	5	<i>Reithrodontomys</i> spp.	2	<i>Reithrodontomys</i> spp.	9
<i>Chaetodipus hispidus</i>	11	<i>Dipodomys ordii</i>	7	<i>Cryptotis parva</i>	6	<i>Reithrodontomys montanus</i>	5	<i>Peromyscus</i> spp.	2	<i>Microtus ochrogaster</i>	6

**Table 3. Five most abundant mammal species in five areas of Oklahoma contrasted to our study.**

Panhandle (Leslie et al. 2008)	Percent (%)	Northwest (Leslie et al. 2008)	Percent (%)	Southwest (Leslie et al. 2008)	Percent (%)	Northeast (Payne and Caire 1999)	Percent (%)	Central (Mitchell and Burns 1964)	Percent (%)	Our Study	Percent (%)
<i>Dipodomys ordii</i>	26	<i>Peromyscus leucopus</i>	35	<i>Peromyscus leucopus</i>	37	<i>Peromyscus maniculatus</i>	28	<i>Sigmodon hispidus</i>	76	<i>Sigmodon hispidus</i>	36
<i>Onychomys leucogaster</i>	21	<i>Sigmodon hispidus</i>	31	<i>Sigmodon hispidus</i>	36	<i>Sigmodon hispidus</i>	26	<i>Mus musculus</i>	9.7	<i>Peromyscus</i> spp.	15
<i>Peromyscus maniculatus</i>	15	<i>Chaetodipus hispidus</i>	6	<i>Peromyscus maniculatus</i>	7	<i>Peromyscus leucopus</i>	18	<i>Peromyscus maniculatus</i>	6.5	<i>Chaetodipus hispidus</i>	13
<i>Perognathus flavus</i>	11	<i>Dipodomys ordii</i>	6	<i>Chaetodipus hispidus</i>	4	<i>Microtus ochrogaster</i>	11	<i>Peromyscus leucopus</i>	4.8	<i>Reithrodontomys</i> spp.	9
<i>Chaetodipus hispidus</i>	7	<i>Peromyscus maniculatus</i>	5	<i>Neotoma micropus</i>	4	<i>Reithrodontomys montanus</i>	4	<i>Cryptotis parva</i>	1.6	<i>Microtus ochrogaster</i>	6

panhandle (Table 3) is *Dipodomys ordii*, which is not reflected in the pellet composition for this study. We suggest that Barn Owls do not actively prey upon kangaroo rats in Oklahoma. Stangl et al. (2005) suggested that *Dipodomys* spp. are generally underrepresented in Barn Owl diets where present. Species within this genus may have well-developed predator avoidance mechanisms that allow them to escape predation by Barn Owls (Stangl et al. 2005).

Barn Owls in Oklahoma consumed 1.4% avian prey. Of this percentage, the majority of species fell in the families Fringillidae and Icteridae (0.69% and 0.56% respectively). While not an important component of the diet compared to small mammals, flocking passerines may have acted as a buffer against low mammal populations, as suggested by Otteni et al. (1972). The percentage of avian prey items present in the pellets is similar to studies from Nebraska, Kansas, and Texas (Petitt

1951, Goyer et al. 1981, Gubanyi et al. 1992).

The diet of Barn Owls in Oklahoma, although relatively diverse, was composed primarily of small mammals and is comparable to many other Barn Owl pellet studies in North America (Marti 1992), especially those of surrounding states. Important prey varied only slightly between regions, suggesting that dietary composition across Oklahoma is similar despite habitat and precipitation differences.

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## References

- Askew, NP, JB Searle, and NP Moore. 2007. Prey selection in a barn owl *Tyto alba*. *Bird Study* 54:130-132.
- Baker, RH. 1991. Mammalian prey of the common Barn-Owl (*Tyto alba*) along the Texas coast. *Southwestern Naturalist* 36:343-347.
- Blair, WF and TH Hubbell. 1938. The biotic districts of Oklahoma. *American Midland Naturalist* 20:425-454.
- Bogiatto, RJ, JM Broughton, VI Cannon, K Dalton, and S Arnold. 2006. Fish remains dominate barn owl pellets in northwestern Nevada. *Western North American Naturalist* 66:395-396.
- Caire W, JD Tyler, BP Glass, and MA Mares. 1989. *Mammals of Oklahoma*. Norman, OK: University of Oklahoma Press.
- CEC. 1997. *Ecological Regions of North America: Toward a Common Perspective*. Montreal. Commission for Environmental Conservation.
- Clark, BK, BS Clark, TR Homerding, and WE Munsterman. 1998. Communities of small mammals in six grass-dominated habitats of southeastern Oklahoma. *The American Midland Naturalist* 139:262-268.
- Glue, DE. 1974. Food of the barn owl in Britain and Ireland. *Bird Study*, 21:200-210.
- Goyer, N, AL Barr, and ARP Journet. 1981. Barn Owl pellet analysis in northwestern Harris County, Texas. *Southwestern Naturalist* 26:202-204.
- Gubanyi, JA, RM Case, and G Wingfield. 1992. Diet and nesting success of Barn Owls breeding in western Nebraska. *American Midland Naturalist* 127:224-232.
- Hall, ER. 1981. *The Mammals of North America*. 2nd Ed. John Wiley & Sons, New York, NY. v 1: 1-600+1-90 p and v 2: 601-1181+1-90 p.
- Leslie, DM, RA Van den Bussche, ZP Roehrs, BS Coyner, KN King, DL Martinez, JK Braun, and MJ Hamilton. 2008. Survey of mammals of special concern in western Oklahoma. Oklahoma Department of Wildlife Conservation.
- Marti, CD. 1992. Barn Owl. *The Birds of North America*, No. 1 (A. Poole, P. Stettenheim, and F. Gill, Eds.). Philadelphia: The Academy of Natural Sciences; Washington, DC: The American Ornithologists' Union.
- Mitchell, C and RD Burns. 1964. Mammal records for Oklahoma County. *Proceedings of the Oklahoma Academy of Science* 44:63-64. Oklahoma Climatological Society.
2014. *Oklahoma Climatological Survey: County Climate*. Norman, OK.
- Otteni, LC, EG Bolen, and C Cottam. 1972. Predator-prey relationships and reproduction of the Barn Owl in southern Texas. *Wilson Bulletin* 84:434-448.
- Parmalee, PW. 1954. Food of the Great Horned Owl and Barn Owl in east Texas. *Auk* 71:469-470.
- Payne, T and W Caire. 1999. Species diversity of small mammals in the Tallgrass Prairie Preserve, Osage County, Oklahoma. *Proceedings of the Oklahoma Academy of Science* 79:51-59.
- Petitt, RM. 1951. Vertebrate remains in Barn Owl pellets from Stanton County, Kansas. *Transactions of the Kansas Academy of Science* 54:395.
- Poole, A. (Editor). 2005. *The Birds of North America*. Cornell Laboratory of Ornithology, Ithaca, NY.
- Reinking, DL. (Editor). 2004. *Oklahoma Breeding Bird Atlas*. University of Oklahoma Press, Norman, OK.
- Santos-Moreno, A and AM Alfaro Espinosa. 2009. Mammalian prey of barn owl (*Tyto alba*) in southeastern Oaxaca, Mexico. *Acta Zoologica Mexicana* 25:143-149.
- Schmidly, DJ. 2004. *The Mammals of Texas*. Rev. ed. Austin: Texas University Press.
- Shehab, AH and SM Al Charabi. 2006. Food of the Barn Owl *Tyto alba* in the Yahmool Area, Northern Syria. *Turkish Journal of Zoology* 30:175-179.
- Sievert, G and L Sievert. 2006. *A Field Guide to Oklahoma's Amphibians and Reptiles*. Oklahoma Department of Wildlife Conservation, Oklahoma City, OK.
- Stangl, FB, Jr., MM Shipley, JR Goetze, and C Jones. 2005. Comments on the predator-prey relationship of the Texas kangaroo rat (*Dipodomys elator*) and Barn Owl (*Tyto alba*).

- American Midland Naturalist 153:135-141.
- Taylor, I. 1994. Barn owls: predator-prey relationships and conservation. Cambridge, UK: Cambridge University Press.
- Velarde, E, R Avila-Flores, and RA Medellin. 2007. Endemic and introduced vertebrates in the diet of the barn owl (*Tyto alba*) on two islands in the Gulf of California, Mexico. Southwestern Naturalist 52:284-290.
- Woods, AJ, JM Omernik, DR Butler, JG Ford, JE Henley, BW Hoagland, DS Arndt, and BC Moran. 2005. Ecoregions of Oklahoma (color poster with map, descriptive text, summary tables, and photographs): Reston, Virginia, U.S. Geological Survey (map scale 1:1,250,000).
- Wooster, LD. 1936. The contents of owl pellets as indicators of habitat preferences of small mammals. Transactions of the Kansas Academy of Science 39:395-397.
- Yom-Tov, Y, and D Wool. 1997. Do the contents of barn owl pellets accurately represent the proportion of prey species in the field? Condor 99:972-976.

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# Soil Invertebrate Community Response to Climate Patterns in a South-Central Oklahoma Grassland

Erica A. Corbett

Department of Biological Sciences, Southeastern Oklahoma State University, Durant, OK 74701

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**Abstract:** Soil invertebrates are an important, but often overlooked, component of the soil ecosystem. In temperate grassland, they are involved in nutrient cycling, breakdown of detritus, and soil fertility. Soil invertebrate populations are affected by the quality of detritus in the soil; there is evidence that both grazing and fire can increase invertebrate populations by improving the quality of litter in soil. It is also possible their population sizes are affected by climatic conditions. I surveyed a degraded grassland site over six years and quantified invertebrate populations (by order) in the top 8 cm of soil. During the years of this survey, a serious drought (2011) took place, which apparently affected invertebrate diversity. Over the years of the study, Shannon diversity decreased but number of invertebrates increased. That increase appears to be the result of increases in the Acarina, Collembola, and the ants (specifically, red imported fire ants). Management recommendations for the site include either burning or grazing to remove accumulated fuel, and removal of invading woody plants. ©2015 Oklahoma Academy of Science

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## Introduction

Soil invertebrates, primarily in Phyla Annelida, Aschelminthes, and Arthropoda, play many vital roles in soil ecosystems. They are involved in breakdown of detritus and nutrient cycling (Davis et al 2006, Seastedt 2000). They can improve soil fertility and structure and can alter primary production (Davis et al. 2006). The soil presents a complete ecosystem and a complex food web (Seastedt 1984). However, because of the small size and inconspicuous location of soil invertebrates, they are relatively little-known, and their effects on decomposition rates are not widely studied (Wall et al. 2008). Seastedt (2000) notes that soil invertebrates may control certain ecosystem processes, but this fact may be "opaque" to many researchers in ecosystems or soils, because often the contributions of the invertebrates are summarized in proxy variables

that are easier to measure. Additionally, identifying individual species of some groups is very challenging (e.g., Aschelminthes must be identified based on mouthpart anatomy).

Soil invertebrates are particularly involved in nutrient cycling in prairie communities; Tschardtke and Greiler (1995) suggest that the soil invertebrate community consumes two to ten times the amount of standing crop in the soil that aboveground herbivores do. In tallgrass prairie, the soil fauna can be particularly diverse: Seastedt et al. (1988) note that there is a great deal of root turnover in the tallgrass prairie, leading to a buildup of organic matter. This leads to a soil fauna that is "diverse and abundant," according to them. Additionally, within different types of grassland, there is considerable variation as to what group of soil invertebrates is dominant (Seastedt 2000). Just as communities vary

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by location, because locations differ in abiotic factors like temperature and moisture availability, so could the community at a single site vary over time with fluctuations in climate or other environmental factors.

Soil invertebrates can be affected by fire (Seastedt et al. 1988), seasonal changes in soil temperature profile (Dowdy 1944), grazing or mowing (Seastedt and Reddy 1991), and vegetational changes. Any factor that affects root productivity can affect soil invertebrate populations. Some soil invertebrate populations show wide short-term fluctuation without large effects on long-term population dynamics (Brand 2002). These populations are often referred to as "resilient" – that is, able to rebound following disturbance (Wall et al 2008). There are few recent studies of soil invertebrate population change, in particular as a result of drought. Because the soil invertebrate community tends to vary from season to season and year to year, single samples do not give a complete picture of the diversity of soil invertebrates at a site. There are some historical papers that address seasonal and yearly patterns of change (Dowdy 1944, Shackleford 1942), but few more recent studies. With long-term climate change patterns, including an increased risk of drought in Western states, understanding its effects on soil detritivores and soil invertebrates in general will be important.

The data reported in the current study were collected to develop a baseline of soil-invertebrate order abundance and diversity during prairie restoration of formerly grazed land. The aim was to determine if, and how, the community of invertebrates changed as vegetation changed. Initial results of these data (using a slightly different method of classifying organisms; I used a simplified classification, going only to order level, in this paper) were previously published by Corbett (2012).

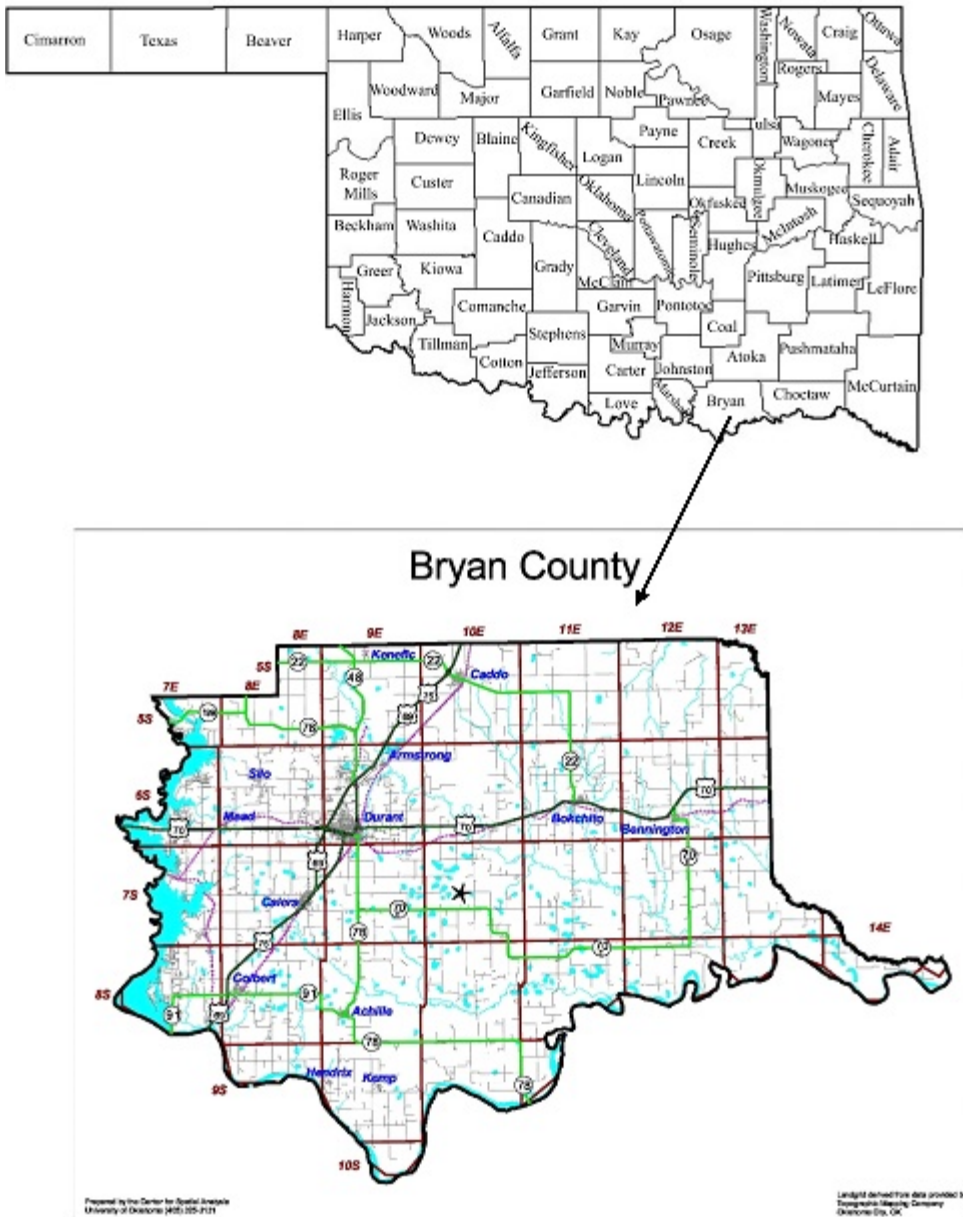
To examine changes in abundance and diversity of soil invertebrate populations over time, I tested for patterns over the seasons and years of this study, and compared these patterns to patterns in climate. Dowdy (1944) Proc. Okla. Acad. Sci. 95: pp 10 - 19(2015)

proposed that soil invertebrates move up and down in the soil column over the course of the year, as the "temperature turnover" happens in the soil from fall to spring. In addition, as site conditions like vegetation heterogeneity or moisture availability change over the longer term, soil-invertebrate community structure can change and undergo a sort of succession (Jonas et al 2002). In particular, I examined the relationship between certain community parameters (number of organisms, number of orders represented, Shannon diversity) and environmental factors (in particular, rainfall and temperature). I also examined the most abundant taxa for changes in population size over time.

## Methods

The field site is formerly-grazed land near Roberta, Oklahoma (N335230, W 0961500 – see Figure 1). The site is considered an upland site and has a homogenous soil association throughout (Crockett-Durant complex: USDA, 1978). The site has been free of grazing since 2001. In 2004, a colleague (Tim Patton) and I began a process of prairie restoration on the site. For sampling purposes, forty 10m x 10m blocks were laid out and were marked in their northwest corner with metal poles so that the sites could be relocated (see figure 2). The blocks were arranged in five rows of eight each. Vegetation on the site was a mixture of native grassland species and introduced pasture species. Dominant species of grasses include several species of bluestems (*Andropogon spp.* and *Schizachyrium scoparius*). Three-awn grass (*Aristida oligantha*), sand lovegrass (*Eragrostis trichoides*) and Scribner's panic grass (*Panicum oligosanthos*) are also present. The dominant forbs include several species of aster (primarily heath aster, *Aster ericoides*), Iva (*Iva annua*) and goldenrod (most likely *Solidago gigantea*). Blackberry (*Rubus oklahomus*) is also present. (All nomenclature follows Noble Foundation's Plant Image Gallery, <http://www.noble.org/Apps/PlantImageGallery/index.aspx>). In 2006, some of the blocks were raked and overseeded with a prairie mix.

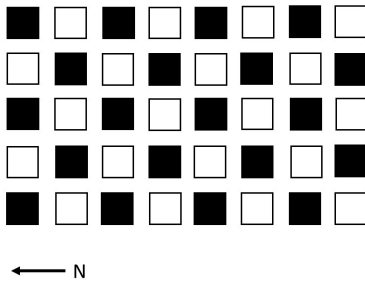
I began sampling the site for soil



**Figure 1: Location of sample site in Bryan County, Oklahoma. Approximate location of sample site is shown with a star.**

invertebrates in early spring 2009. Three samples per year were taken: early March, early June, and early October. Twenty of the forty blocks (see Figure 2) were sampled, following a "staggered" pattern (e.g., blocks 1, 3, 5, and 7 in the first row, and 10, 12, 14, and 16 in the

second row). Five soil samples were collected per block sampled, 6.5 cm in diameter by 5 cm deep. A standard bulb planter ("Garden Plus 5 inch steel bulb planter," Lowe's) was used to collect the samples. Each block's samples were placed in a zipper-top bag and were



**Figure 2: Diagram showing sample site. Filled-in blocks represent blocks sampled in this study. Block size is 10 m x 10m**

kept in a cooler at  $\sim 20^{\circ}\text{C}$  until transported to Southeastern Oklahoma State University.

Soil invertebrates were extracted from the soil using a combination of a modified Berlese funnel method and a floatation method. For the funnel extraction, heavy foil 2-quart aluminum casserole pans (Hefty EZ Foil) and plastic needlepoint mesh with 1 mm holes (e.g., JoAnn Fabric and Crafts) was used. Most of the bottom of each pan was cut out and replaced with a slightly larger circle of the needlepoint mesh. These "funnels" were then set on top of 1000 mL beakers containing about 20 mL of 70% isopropyl alcohol as a preservative. Soil samples were placed in the casserole pan and a 25 watt light bulb shone on the surface of the soil for 24 hours. (A low-wattage light bulb was used because the rooms were unattended during the time of extraction). After extraction, the alcohol and any invertebrates in it were transferred to 120-mL plastic specimen cups for storage. Additionally, the remaining soil in the funnel was searched using a "floatation" technique to find and extract invertebrates that did not travel through the holes in the mesh. In some cases, this was because they were too large (beetles and larger isopods); in other cases, they did not travel far enough down through the soil. Each soil sample was split into smaller portions (to scarcely fill the bottom of a 150 mm petri dish), wetted excessively, and examined under 20X magnification using a dissecting microscope. Any invertebrates observed were Proc. Okla. Acad. Sci. 95: pp 12 - 19(2015)

removed using a dissecting needle or forceps and placed in the appropriate sample's vial.

After completing the full extraction procedure, I examined the contents of each vial. Again, I examined the organisms using a dissecting microscope on 20X magnification. A red filter (a sheet of translucent plastic placed over the baseplate) was used to make the organisms easier to see (and to reduce eyestrain). Samples were broken down into 4-5 mL aliquots to make examination easier. Each aliquot was examined and individual invertebrates were identified to order and counted. The "Kwik-key to soil invertebrates" (Meyer, 1994) was used to assist with identification. In most cases, organisms were separated to order or class; in a few cases (like Aschelminthes) where that type of separation was difficult, identification was left at the phylum level. However, the identification scheme used was consistent across all samples, so relative comparisons of numbers and diversity should be valid. In general, the identification scheme was similar to that used by Wall et al. (2008).

Data were analyzed using Shannon diversity indexes (Magurran, 1988). Base-ten logarithms were used in the calculation of the Shannon index. Evenness was also calculated for each sample. In addition, I analyzed samples based on total number of organisms present and on number of orders represented in the sample (order richness). Statistical analyses were performed using IBM SPSS version 20 (IBM, 2013). Initially, data were analyzed using a non-parametric analogue of Analysis of Variance (Kruskal-Wallis). Two sets of analyses were performed: using season (spring, summer, fall) as the grouping variable or using year (2009-2014) as the grouping variable. The dependent variables included Shannon diversity, evenness, number of soil invertebrates, and number of orders represented in the sample. There were insufficient degrees of freedom to run a two-way GLM analysis to examine the interaction term, so simple one-way analysis was used.

Because the samples were small and generally did not fulfill the assumption of



**Table 1: Temperature data for South-Central Oklahoma during the period of the soil-invertebrate study. "Average temperature" is the average temperature for the entire year. "Previous month" is the average temperature for the month prior to the sample. "Current Month" is the temperature for the month of the sample.**

Sample period	Average temperature	Previous month	Current Month
March 2009	16.6° C	10.6° C	13.2° C
June 2009	16.6° C	19.8° C	26.9° C
October 2009	16.6° C	22.2° C	14.2° C
March 2010	16.9° C	3.5° C	10.72° C
June 2010	16.9° C	21.5° C	28.0° C
October 2010	16.9° C	24.7° C	17.3° C
March 2011	17.8° C	6.2° C	13.6° C
June 2011	17.8° C	20.4° C	29.3° C
October 2011	17.8° C	23.1° C	17.7° C
March 2012	18.3° C	8.5° C	16.5° C
June 2012	18.3° C	23.1° C	26.3° C
October 2012	18.3° C	24.6° C	16.3° C
March 2013	16.2° C	7.7° C	10.3° C
June 2013	16.2° C	20.2° C	26.1° C
October 2013	16.2° C	25.7° C	17.7° C
March 2014	16.2° C	4.0° C	9.3° C
June 2014	16.2° C	21.1° C	26.0° C
October 2014	16.2° C	24.0° C	19.5° C

**Table 2: Precipitation data for South-Central Oklahoma during the period of the soil-invertebrate study. "Annual precipitation" is the total precipitation for the entire year. "Previous month" is the total precipitation for the month prior to the sample. "Current Month" is the total precipitation for the month of the sample.**

Sample period	Annual precipitation	Previous month	Current Month
March 2009	119.5 cm	3.5 cm	5.5 cm
June 2009	119.5 cm	20.2 cm	8.2 cm
October 2009	119.5 cm	13.0 cm	24.8 cm
March 2010	87.8 cm	7.5 cm	6.4 cm
June 2010	87.8 cm	11.9 cm	9.3 cm
October 2010	87.8 cm	16.2 cm	4.9 cm
March 2011	61.5 cm	4.3 cm	0.7 cm
June 2011	61.5 cm	14.5 cm	0.9 cm
October 2011	61.5 cm	3.7 cm	9.8 cm
March 2012	74.3 cm	3.7 cm	13.7 cm
June 2012	74.3 cm	6.9 cm	7.8 cm
October 2012	74.3 cm	8.5 cm	9.8 cm
March 2013	96.9 cm	7.7 cm	4.6 cm
June 2013	96.9 cm	18.1 cm	11.8 cm
October 2013	96.9 cm	4.8 cm	9.9 cm
March 2014	81.8 cm	1.5 cm	6.2 cm
June 2014	81.8 cm	6.4 cm	14.6 cm
October 2014	81.8 cm	5.9 cm	7.9 cm

**Table 3: Most abundant taxa by sampling period, with number of individuals observed.**

Year	March		June		October	
2009	Protura	68	Diplura	130	Protura	47
	Acarina	35	Hymenoptera	76	Acarina	43
	Collembola	23	Acarina	56	Hymenoptera	21
				Annelida	31	
2010	Protura	49	Acarina	213	Acarina	103
	Annelida	36	Collembola	96	Hymenoptera	84
	Acarina	22	Hymenoptera	48	Collembola	56
2011	Hymenoptera	203	Hymenoptera	192	Acarina	59
	Acarina	99	Acarina	117	Hymenoptera	26
	Collembola	59	Collembola	34	Coleoptera	19
	Protura	53	Coleoptera	32		
2012	Collembola	212	Acarina	316	Acarina	292
	Hymenoptera	63	Collembola	222	Collembola	123
	Acarina	55	Hymenoptera	115	Hymenoptera	101
2013	Acarina	269	Acarina	172	Acarina	188
	Collembola	155	Collembola	126	Hymenoptera	97
	Diplura	25	Hymenoptera	110	Collembola	65
2014	Hymenoptera	181	Hymenoptera	533	Hymenoptera	292
	Acarina	159	Acarina	250	Acarina	204
	Collembola	29	Collembola	50	Collembola	103

equal variances, they were analyzed using non-parametric methods. However, because of a lack of follow-up tests for the nonparametric analyses, the significant analyses were tested parametrically using REGWQ: the Ryan-Einot-Gabriel-Welch  $q$  test: a modification of the  $t$  test used as a follow up after analyses of variance (IBM, 2013) to determine which groups differed.

Additionally, Pearson correlations were run on pairs of variables to look for trends. I compared year, season, and number of the sample (1 through 18: eighteen samples were collected over the course of this study) against diversity and abundance to determine if there were any temporal or seasonal patterns.

To analyze the data in greater detail, I obtained climate data for the South Central Oklahoma region (Climate Region 8 on <http://www.ncdc.noaa.gov/cag/time-series/us>). First, I obtained annual rainfall totals for the years 2009 through 2014, and average annual temperatures (see table 1). In addition, I compiled data on monthly precipitation (table 2) and monthly temperatures (table 1) for the month of the sampling and the month immediately previous to it. Samples were typically collected early in the month, so it is assumed the previous month's conditions could have an effect. Again, Pearson correlations were run to determine possible relationships between variables.

## Results

Over the course of the study, the "majority" taxa (those with the greatest number of individuals) were fairly consistent (see table 3). Generally, mites and springtails showed high abundance throughout the study. Numbers seemed to increase post-2011, perhaps as a result of the drought that year. Over time, diversity decreased but number of individuals in the samples increased; that increase may be linked to these three taxa increasing over time.

Analysis for year effect and for season effect using Kruskal-Wallis analysis revealed only one significant result: that of year on diversity. The  $p$  value of this test (SPSS does not provide the Proc. Okla. Acad. Sci. 95: pp 16 - 19(2015)

value of the Kruskal-Wallis test statistic) was 0.038. None of the other comparisons against year (of evenness, of order richness, of total number of invertebrates) showed significance, and none of the comparisons against season was significant. There was considerable variation from year-to-year within a single season.

Comparing individual years more closely, years 2009, 2010, and 2011 were not significantly different in their diversity. Years 2010-2014 were also not significantly different in their diversity. However, year 2009 was significantly more diverse than years 2012-2014. There is a trend of a decline in diversity following 2011. In southern Oklahoma, 2011 was a severe drought year, with 34.8 cm less rainfall than average. (National Climate Data Center).

Correlation analysis revealed additional patterns. There were significant correlations between year of sampling and diversity ( $p < 0.001$ ,  $n = 15$ , correlation coefficient  $-0.796$ ). Diversity declined with year of sampling, which agrees with the Kruskal-Wallis analysis. There was also a significant correlation between year and number of organisms ( $p = 0.035$ ,  $n = 15$ , correlation coefficient  $= 0.546$ ). As time passed, there was a trend toward decreasing diversity but increasing number of organisms. Season showed no significant correlation with any of the other variables.

Comparing the most abundant taxa against year, season, and "sample order" (where the first sample in spring 2009 was 1 and the sample in fall 2014 was number 18) revealed a few patterns. Three groups showed a significant increase over the years of the study (Collembola:  $p = 0.004$ , correlation coefficient  $= 0.643$ ; Isopoda:  $p = 0.021$  correlation coefficient  $= 0.541$ ; Acarina:  $p = 0.004$ , correlation coefficient  $= 0.643$ ). Only one group, Isopoda, showed a relationship with season ( $p = 0.026$ , correlation coefficient  $= 0.522$ ), demonstrating that the number of isopods tended to increase later in the year. For "sample number," again Collembolans, Isopoda, and Acarina showed significant correlations, but ants also showed a marginally significant

( $p=0.50$ , correlation coefficient = 0.468) correlation. These groups apparently increased over the period of sampling of this site.

Climatological patterns, beyond mere seasonal differences, can have an effect on soil invertebrates (McLean et al, 1977. Jonas et al 2002). There was variation in precipitation (see table 2) across the years of the study. There was also variation in temperature, both in terms of average annual temperature and in temperature of the months surrounding the sampling times (see table 1 for temperature data). Monthly temperatures showed no significant correlations with any of the community variables, but there was a significant correlation between average annual temperature and number of orders present (coefficient = -0.489,  $p=0.039$ ). In warmer years, the number of orders appearing in samples declined. Similarly, there were no patterns related to monthly rainfall amounts, but total annual precipitation was correlated with diversity (coefficient = 0.524,  $p=0.026$ ). As total precipitation in a year increased, diversity also increased.

Individual taxa showed relatively few patterns in response to environmental variables. Phylum Annelida decreased in abundance with increasing annual temperature ( $p=0.037$ , correlation coefficient = -0.494) and decreased with increasing previous month's temperature ( $p=0.025$ , coefficient = -0.520). Springtails (Order Collembola, Phylum Arthropoda) decreased with increasing annual precipitation ( $p=0.044$ , correlation coefficient = -0.480). Isopods (Order Isopoda, Phylum Arthropoda) showed an increase in abundance with increasing temperatures in the previous month ( $p=0.014$ , correlation coefficient = 0.568).

## Discussion

Kruskal-Wallis analysis of year and season effects on diversity, evenness, order richness, and total number of organisms revealed a significant effect of year on diversity. Diversity declined over the course of the study, with the first year showing high diversity, and years 2012-2014 being significantly lower in diversity. It is

notable that 2011 was an extreme drought year for southern Oklahoma, with almost 35 cm of precipitation less than average (roughly 96 cm). The year 2012 was also droughty, with 22 cm less precipitation than the average (National Climate Data Center). Seasons showed no pattern as far as diversity, evenness, or numbers of taxa/numbers of organisms were concerned.

The year 2011 was a serious drought year that apparently had an effect on species diversity (2012 had the lowest average diversity). Different taxa are affected to different degrees by drought; in a study in wet meadows, Davis et al (2006) noted a decline in earthworm and scarab beetle populations in drought years, but an increase in Isopoda. Whether the declines observed are actual declines, or merely evidence of the invertebrates moving deeper into the soil column is unclear, but perhaps the effect on upper-horizon decomposition and nutrient cycling would be similar in either case.

The two main patterns over the sampling period are a decrease in diversity after the drought of 2011, and an increase in total number of organisms over time. The trend of increasing numbers of organisms over time is more difficult to explain; few manipulations have been done to the site since it was abandoned from grazing in 1980. The increase may be the result of gains in numbers, at least for some sampling periods, in ants, mites, and collembolans. While overall diversity tended to decline, a few taxonomic groups became more abundant, which would, itself, have reduced diversity. Dowdy (1965) states that "moisture does not appear to be a factor" in the abundance and diversity of mites and collembolans; perhaps they are less affected by these kinds of environmental fluctuations than some other groups, and they were able to take advantage of reduced competition following the dry period of 2011-2012.

Examining individual orders of invertebrates and their abundance, in general the same few groups showed highest abundance in each sample. Mites (order Acarina) were in the top three groups (in terms of abundance) in every sampling period. (see table 3). Springtails

(Order Collembola) were the most abundant order in 13 of the 16 samples. Other highly abundant groups included beetles (Coleoptera), earthworms (Phylum Annelida), and ants (order Hymenoptera). Maclean et al. (1977), in their study in Alaska, determined that mites and springtails were the most abundant groups present in their soils. The abundance of mites and collembolans should not be surprising; Seastedt (1984) noted that they comprise roughly 95% of arthropods found in grassland ecosystems.

Some types of habitat loss or fragmentation may affect invertebrates strongly without showing large effects on vertebrate animals. This is especially true of small-scale or short-duration disturbances (Jonas et al., 2002). Even though soil organisms are often considered to have population "resiliency" (where they can rebound rapidly after a disturbance), prolonged or multiple disturbances could alter community make-up over time. Disturbances can alter soil moisture, organic matter, root biomass, and soil chemistry, all factors that could affect invertebrate populations (Davis et al 2006). Wall et al (2008) suggest that actual taxonomic diversity of soil organisms may be as important to decomposition rates as sheer number of organisms – and therefore any long-term loss of diversity could alter decomposition and nutrient-cycling patterns at a site. Chemical pollution, overuse of pesticides, extended drought, or other climate changes could alter the soil community and as a result alter decomposition rates, litter accumulation, and nutrient dynamics at a site.

## References

Brand RH. 2002. The effect of prescribed burning on epigeic springtails (Insecta: Collembola) of woodland litter. *Am Midl Nat* 148: 383-393.

Corbett. 2012. Initial survey of soil invertebrates in a disturbed Oklahoma grassland. In: Williams D, Butler B, Smith D, editors. *Proceedings of the 22<sup>nd</sup> North American Prairie Conference* p. 101-105.

Davis CE, Austin JF, Buhl DA. 2006. Factors Influencing Soil Invertebrate Communities

in Riparian Grasslands of the Central Platte River Floodplain. *Wetlands* 26:438-454.

Dowdy WW. 1944. The Influence of Temperature on Vertical Migration of Invertebrates Inhabiting Different Soil Types. *Ecology* 25: 449-460.

Dowdy, WW. 1965. Studies on the Ecology of Mites and Collembola. *Am Midl Nat* 74:196-210.

IBM. 2013. IBM SPSS Statistics version 20.0.0.

Jonas JL, Whiles MR, Charlton RE. 2002. Aboveground Invertebrate Responses to Land Management Differences in a Central Kansas Grassland. *Environ Entomol* 31: 1142-1152.

MacLean SF, Douce GK, Morgan EA, Skeel MA. 1977. Community Organization in the Soil Invertebrates of Alaskan Arctic Tundra. *Ecol Bull* 25: 90-101.

Magurran AE. 1988. *Ecological Diversity and Its Measurement*. Princeton (NJ): Princeton University Press. 179 p.

Meyer JR. 1994. *Kwik-Key to Soil-Dwelling Invertebrates*. Raleigh (NC): Vision Press. 43 p.

National Climate Data Center 2015. Available from: <http://www.ncdc.noaa.gov/cag/time-series/us> (Accessed 28 February 2015).

Noble Foundation's Plant Image Gallery. 2015. Available from: <http://www.noble.org/Apps/PlantImageGallery/index.aspx> (Accessed March 2, 2015).

Seastedt TR. 2000. Soil Fauna and controls of carbon dynamics: Comparisons of rangelands and forests across latitudinal gradients. In: Coleman DC, Hendrix P, editors. *Soil Arthropods as Webmasters of Ecosystems*. Athens (GA): CAB International. p. 293-313.

Seastedt TR. 1984. The role of Microarthropods in Decomposition and Mineralization Processes. *Annu Rev Entomol.* 29: 25-46.

Seastedt TR, Reddy MV. 1991. Mowing and Insecticide Effects on Sternorrhyncha (Homoptera) Densities in Tallgrass Prairie. *J Kansas Entomol Soc* 64: 238-242.

Seastedt TR, James SW, Todd TC. 1988. Interactions Among Soil Invertebrates, Microbes, and Plant Growth in the Tallgrass

- Prairie. *Agr Ecosyst Environ* 24: 219-228.
- Shackleford M. 1942. The Invertebrate Population of a Central Oklahoma Prairie. *Am Midl Nat* 28: 408-415.
- Tscharntke T, Greiler H-J. 1995. Insect communities, grasses, and grasslands. *Annu Rev Entomol* 40: 535-558.
- USDA. 1978. Soil Survey of Bryan County, Oklahoma. United States Department of Agriculture Soil Conservation Service, in cooperation with Oklahoma Agricultural Experiment Station.
- Wall DH, Bradford BA, St. John MF, Trofymow JA, Behan-Pelletier V, Bignell DE, Dangerfield JM, Gardel HZ, Ayuke FO, Bashford R, Beljakova OI, Bohlen PJ, Brauman A, Flemming S, Henschel R, Johnson DL, Kutny L, Lin K-C, Maryati M, Masee D, Pokarzhecskii A, Rahman H, Sabara MG, Salamon J-A, Swift MA, Varela A, Vasconcelos HL, White D, and Zou, X. 2008. Global decomposition experiment shows soil animal impacts on decomposition are climate-dependant. *Glob Change Biol* 14: 2661-2677.

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# Notes on the Distribution of the Ozark Logperch (*Percina fulvitaenia*) in the Lower Cimarron River

**Dustin T. Lynch**

Arkansas Cooperative Fish and Wildlife Research Unit, Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701

**Loren W. Stearman**

Pacific States Marine Fisheries Commission, Marysville, CA 95901

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**Abstract:** We document three new localities for Ozark logperch (*Percina fulvitaenia*) in the Lower Cimarron River of Oklahoma, including one population 166 river km upstream from any previously known collection of this species. We hypothesize that *P. fulvitaenia* may be more widely distributed in the Cimarron River than previously thought, but that it utilizes habitat that could make it difficult to detect during the non-breeding season. ©2015 Oklahoma Academy of Science

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## Introduction

The currently understood distribution of the Ozark logperch, *Percina fulvitaenia* (Morris and Page 1983) includes much of the Ozark Highlands in the Missouri, White, and Arkansas River systems and tributaries to the Arkansas River in the periphery of the Ozark Highlands, as well as isolated populations in Red River tributaries in the Arbuckle Mountains of south-central Oklahoma (Miller and Robson 2004, Page and Burr 2011). In Oklahoma, historical records have indicated that within the Arkansas River basin, the species is restricted to tributaries east of the main stem; however, Luttrell et al. (1994) and Luttrell (1996) documented its occurrence west of the main stem Arkansas River at a handful of scattered localities in the lower Cimarron River drainage. We document three new collection localities for this species, and develop a hypothesis addressing the apparently sporadic occurrence of Ozark logperch in the lower Cimarron River system.

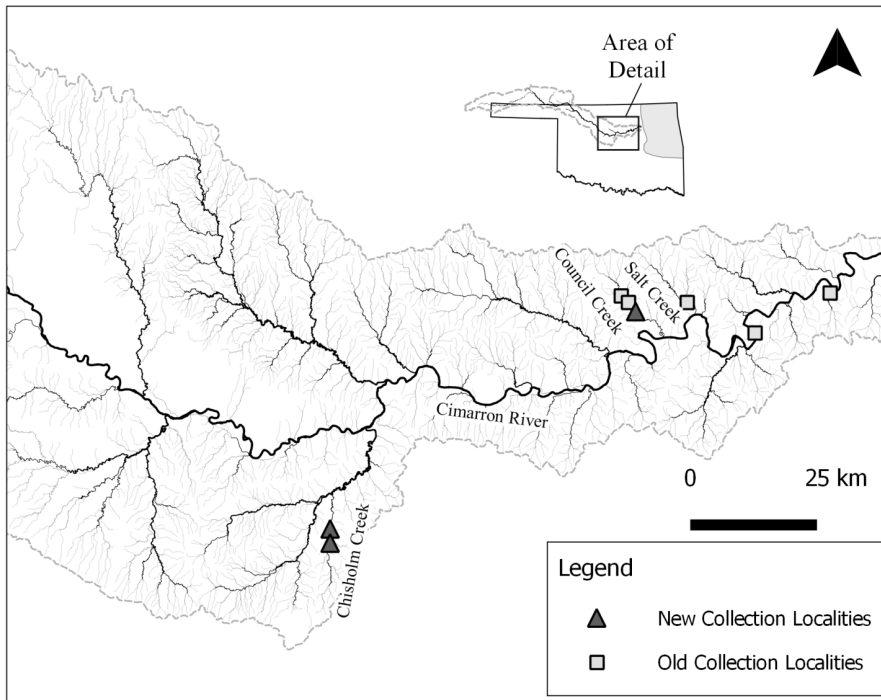
## Methods and Results

We collected Ozark logperch at three new localities, all 3rd order streams, during field collections from streams in the lower Cimarron

River drainage in April of 2009 (Figure 1). On 2 April, we collected ten specimens (OSUS 27838, SL 63 to 81 mm) from a site on Council Creek, Payne Co., Oklahoma, downstream from previously reported localities (36.101119 N, -96.852122 W). On 4 April, we collected a single specimen (OSUS 27824, SL 92 mm) near the outflow of the Deer Creek water treatment facility on Chisholm Creek, north of the city of Edmond, Oklahoma Co., Oklahoma (35.696451 N, -97.527083 W). On 5 April, we collected three more individuals (OSUS 27835, SL 83 to 104 mm) at a locality approximately 3 km downstream from the Deer Creek water treatment facility on Chisholm Creek (35.725624 N, -97.527512 W).

All individuals were captured during kicksets in riffles 0.1 to 0.5 m deep with 3.2 mm mesh seines. We found logperch in Council Creek over substrates of sand, gravel, and cobble. In Chisholm Creek, logperch utilized riffles created by collapsed bank-stabilizing rip-rap. Females appeared gravid, and laboratory examinations of gonads confirmed mature oocytes were present. Females in both populations ranged from mature (MA) to ripe (RE), and all males examined were ripe (RE), following Heins and Machado's (1993) index of visual gonad status. Larger individuals in all three collections had the orange band in the





**Figure 1. Map of the lower Cimarron River system showing previous collection localities from Luttrell et al. (1994) (squares) and localities from this report (triangles). Map inset: Lower Cimarron River system (dashed line) and historically accepted distribution of Ozark logperch within Oklahoma (grey).**

first dorsal fin that distinguishes this species from logperch, *Percina caprodes* (Rafinesque 1818), which is found in Red River tributaries in the southeast portion of the state and has historically been treated as conspecific (Miller and Robison 2004). As part of a wider study examining the phylogeography of *P. fulvitaenia* in Oklahoma, fin-clips were taken from all individuals and used to sequence the mitochondrial gene ND2 (NADH dehydrogenase subunit 2; 1047 base pairs). The Council Creek population had moderately high haplotype diversity ( $h=0.742 \pm 0.084$ ) while the Chisolm Creek collections consisted of four individuals sharing a single haplotype (Lynch 2010). Field samplings at the same localities on Council Creek in August 2008 and Chisholm Creek during August 2009 failed to yield additional individuals.

## Discussion

The previous westernmost locality for Ozark

logperch in the Cimarron River system has been reported as Lake Optima, Beaver County, Oklahoma (Pigg 1987). However, Luttrell et al. (1994) suggest that the lone individual collected at this locality, nearly 500 river km upstream from any previous locality, probably represented a transient introduction. If the record from Lake Optima is indeed a transient introduction, then our record from Chisholm Creek may represent the westernmost known distribution of Ozark logperch in the Cimarron River system, 166 river km upstream from the next nearest collection locality on Council Creek.

Whether the distribution of Ozark logperch is actually highly sporadic in the lower Cimarron River, or such apparently sporadic occurrence is driven by seasonal vulnerability to sampling is an interesting question. Many of the pools in these streams are excessively deep ( $> 2\text{m}$ ) for effective seining and excessively turbid for effective snorkel surveys (OK DEQ 2010).

Logperch may occupy deeper water following spawning during the spring in many areas (Winn 1958, Page 1983), and residence in deep, turbid pools would make them difficult to detect. Our own success of capturing gravid females during April in both Chisholm and Council Creek, and subsequent lack of success capturing individuals during August at these same sites suggests that individuals may have left shallower riffle habitat following reproduction. Similarly, the records reported by Luttrell et al. (1994) occurred from March to July. The only previous record of fish collections from Chisholm Creek that we were able to locate occurred during November and contained no logperch (Margraf and Plitt 1982). Unfortunately, Luttrell (1996) does not comment on the temporal variability in logperch abundance in Council Creek. While logperch are probably not abundant in the lower Cimarron River system, we hypothesize that sampling riffle habitat in larger tributary streams during the spring may reveal this species to be more widespread than previously thought.

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## References

- Heins DC, Machado MD. 1993. Spawning season, clutch characteristics, sexual dimorphism and sex ratio in the redbfin darter (*Etheostoma whipplei*). *Am Midl Nat* 129:161-171.
- Luttrell GR, Haraughty SJ, Tompkins JD, Corley BA. 1994. Occurrence of the logperch (*Percina caprodes*) in the Cimarron River drainage of Oklahoma. *Proc Okla Acad Sci* 74:1-2.
- Luttrell GR. 1996. The fishes of Council Creek, Payne County, Oklahoma. *Proc Okla Acad Sci* 76:39-42.
- Lynch DT. 2010. Phylogeography of the Ozark logperch (*Percina caprodes fulvitaenia*) in the Red and Arkansas River basins [MSc thesis]. Stillwater (OK): Oklahoma State University. 45 p. Available from: OSU Library.
- Margraf FJ, Plitt DW. 1982. The aquatic macrofauna and water quality of Cottonwood Creek. Oklahoma. *Proc Okla Acad Sci* 62:1-6.
- Miller RJ, Robison HW. 2004. The Fishes of Oklahoma. 2nd ed. Norman (OK): University of Oklahoma Press. 450 p.
- Morris MA, Page M. 1981. Variation in western logperches (Pisces: Percidae), with description of a new subspecies from the Ozarks. *Copeia* 1981: 95-108.
- OK DEQ (Oklahoma Department of Environmental Quality). 2010. Turbidity total maximum daily loads for the Lower Cimarron River-Skeleton Creek Area [online]. Available from: [http://www.deq.state.ok.us/wqdnew/tmdl/skeleton\\_cimarron\\_turbidity\\_tmdl\\_2010-04.pdf](http://www.deq.state.ok.us/wqdnew/tmdl/skeleton_cimarron_turbidity_tmdl_2010-04.pdf)
- Page LM, Burr BM. 2011. Peterson Field Guide to Freshwater Fishes of North America North of Mexico. 2nd ed. New York (NY): Houghton Mifflin Harcourt. 663 p.
- Page LM. 1983. Handbook of Darters. Neptune (NJ): THF Publications. 271 p.
- Pigg J. 1987. Survey of fishes in the Oklahoma panhandle and Harper County, northwestern Oklahoma. *Proc Okla Acad Sci* 67:45-59
- Winn HE. 1958. Comparative reproductive behavior and ecology of fourteen species of darters (Pisces: Percidae). *Ecol Monogr* 28: 155-191.

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***Oochoristica whitentoni* Steelman, 1939 (Cestoda: Cyclophyllidea: Linstowiidae) and *Cruzia testudinis* (Nematoda: Ascaridida: Kathlaniidae) from a Three-toed Box Turtle, *Terrapene carolina triunguis* (Testudines: Emydidae) from Oklahoma: Second Report from Type Host Species and New State Record for *C. testudinis***

**Chris T. McAllister**

Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

**Charles R. Bursey**

Department of Biology, Pennsylvania State University-Shenango Campus, Sharon, PA 16146

The tapeworm genus *Oochoristica* Lühe is a large, unwieldy complex of parasitic worms that infect a variety of reptiles (primarily lizards) and mammals, and currently includes 93 species (Schuster 2012). One, *Oochoristica whitentoni* Steelman, was described from a three-toed box turtle, *Terrapene carolina triunguis* from Stillwater, Payne County, Oklahoma (Steelman 1939). It has since also been reported from the false iguana, *Ctenosaura pectinata* from Guerrero, Mexico (Flores-Barroeta 1955) and reticulate Gila monster, *Heloderma suspectum suspectum* from Arizona (Goldberg and Bursey 1991). The validity of the former record has been questioned; its possible inclusion in *O. acapulcoensis* Brooks, Pérez-Ponce de León and García-Prieto has been suggested (Brooks et al. 1999) and we concur. Therefore, only two valid hosts of *O. whitentoni* may occur. Here, we provide only the second report of *O. whitentoni* from the type host species since the original description over 75 yr ago. In addition, we document only the second

report of a nematode, *Cruzia testudinis* Harwood in *T. c. triunguis* and, most importantly, the first report of this nematode from Oklahoma.

On 8 May 2015 a single adult *T. c. triunguis* was found dead on road off St. Hwy 82, Le Flore County (34.814878°N, 95.04472°W). It was placed on ice, taken to the laboratory and, since its shell was already cracked, the gastrointestinal tract was split longitudinally and the contents placed in a Petri dish containing 0.85% saline. A single live tapeworm was found in the small intestine, fixed in near boiling water, transferred to 70% ethanol, stained with acetocarmine, and mounted in Canada balsam. Numerous nematodes were found in the intestinal tract and fixed in near boiling water, transferred to 70% ethanol and examined as temporary mounts after placement on a glass slide in a drop of glycerol. A host voucher specimen was deposited in the Henderson State University Collection (HSU), Arkadelphia, Arkansas; parasite vouchers were deposited in the Harold W. Manter Laboratory

**Table 1. Comparison of measurements between original and current specimens.**

Character	Steelman 1939	Current specimen
Length	12.3 (1.5–27.5) cm	17.1 cm
Suckers	0.16 (0.15–0.18) mm	0.17 mm
Scolex	0.40 (0.36–0.44) mm	0.41 mm
Segmentation begins	3.3 (2.9–3.6) mm from anterior	2.3 mm from anterior
*Immature segments	0.26 (0.09–0.39) mm long × 0.57 (0.33–0.73) mm wide	0.26–0.39 mm long × 0.58–1.08 mm wide
*Mature segments	0.95 (0.56–1.36) mm long × 1.04 (0.79–1.13) mm wide	0.64–1.47 mm long × 1.34–2.51 mm wide
Gravid segments	absent	absent
Cirrus sac	0.19 (0.13–0.23) mm long × 0.09 (0.07–0.12) mm wide	0.22–0.31 mm long × 0.05–0.08 mm wide
Ovary	0.29 (0.13–0.36) mm wide	0.47–0.51 mm wide
Testes	0.04 (0.02–0.06) mm diameter	0.02–0.05 mm diameter
Number	100–150	105–110

\*Strobilus width is significantly larger in the current specimen.

of Parasitology (HWML), Lincoln, Nebraska.

Closer examination revealed the tapeworm (HWML 101644) fit the description of *O. whitentoni* provided by Steelman (1939): i.e., scolex rounded anteriorly, four unarmed suckers, narrowed neck; segmentation as light transverse lines separating bands of deeper-staining tissue; genital primordia first apparent as an ovoid mass of deeply staining tissue centrally placed in segment which eventually becomes medially elongate; genital pores marginal, irregularly alternate, located in anterior third of segment; ovary median comprising two lateral lobed wings connected by a transverse median portion. Vitellarium ovoid just posterior to ovary. Testes located behind and posterolateral to ovary, spheroid in shape, forming crescentic band with concavity directed forward. Steelman (1939) reported one strobila, measuring 9.5 cm in length to contain 134 immature and 77 mature proglottids; our specimen possessed 153 immature and 101 mature proglottids; in both specimens, gravid proglottids were absent. Morphological features between Steelman's specimen and the current specimen are given in Table 1. However, with the exception of

strobilus width (our specimen is larger than original description), all of our measurements are within the ranges provided by Steelman (1939).

In the original description, Steelman (1939) mentioned the low prevalence of infection of *O. whitentoni* as he observed it from only one of 11 (9%) *T. c. triunguis*, while no *O. whitentoni* were found in 35 ornate box turtles, *Terrapene ornata* from Oklahoma. In an unpublished thesis, Mays (1960) examined 77 *T. c. triunguis* and 89 *T. ornata* from Oklahoma and did not report *O. whitentoni*. Indeed, we have also examined 45 *T. c. triunguis* for coccidian parasites from Arkansas and Oklahoma via fecal flotation technique (McAllister et al. 2015), and while coccidia and nematode ova were often observed, none of the box turtles were found to be passing linstowiid tapeworm ova. Therefore, prevalence of this tapeworm in box turtles is likely very low which may account for its absence in helminth surveys of *Terrapene* spp. (see Mays 1960; Ernst and Ernst 1977; Baker 1987; Dodd 2001) in the United States.

In addition to *O. whitentoni*, numerous nematodes (HWML92074) found in the intestinal

tract fit the description of *Cruzia testudinis* that Harwood (1932) described previously from *T. c. triunguis* collected in Houston, Harris County, Texas. Since that time, we are unaware of additional reports of this nematode in three-toed box turtles or any other host.

In summary, we provide only the second report of *O. whitentoni* and *C. testudinis* from the type host species since the original description over 75 and 83 years ago, respectively. In addition, we document the first report of *C. testudinis* in Oklahoma. Further sampling of box turtles may eventually allow description of gravid segments of *O. whitentoni* and additional reports of *C. testudinis* in three-toed and other box turtles from other parts of their range.

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### References

Baker MR. 1987. Synopsis of the Nematoda parasitic in amphibians and reptiles. Mem. Univ. Newfoundland Occas. Pap. Biol. 11:1-325.

Brooks DR, Pérez-Ponce de León G., García-Prieto L. 1999. Two new species of *Oochoristica* Lühe, 1898 (Eucestoda: Cyclophyllidae: Anoplocephalidae: Linstowiinae) parasitic in *Ctenosaura* spp. (Iguanidae) from Costa Rica and Mexico. J. Parasitol. 85:893–897.

Dodd CK Jr. 2001. North American box turtles: A natural history. Norman (OK): University of Oklahoma Press. 231 p.

Ernst EM, Ernst CH. 1977. Synopsis of helminths endoparasitic in native turtles of the United States. Bull. Maryland Herp. Soc. 13:1–75.

Flores-Barroeta L. 1955. Céstodos de Vertebrados II. Rev. Ibérica Parasitol. 15:115–134.

Goldberg SR, Bursey CR. 1991. Gastrointestinal helminths of the reticulate Gila monster, *Heloderma suspectum suspectum* (Sauria: Helodermatidae). J. Helminthol. Soc. Wash. 58:146–149.

Harwood PD. 1932. The helminths parasitic in the Amphibia and Reptilia of Houston, Texas and vicinity. Proc. U.S. Natl. Mus. 81:1–71.

Mays ML. 1960. A survey of helminth parasites of box turtles in central Oklahoma [MSc Thesis]. Norman (OK): University of Oklahoma. (Available from Univ. of Oklahoma Library).

McAllister CT, Seville RS, Motriuk-Smith D, Hudson C, Connior MB, Robison HW. 2015. Coccidia (Apicomplexa: Eimeriidae) of three-toed box turtles, *Terrapene carolina triunguis* (Reptilia: Testudines), from Arkansas and Oklahoma. J. Arkansas Acad. Sci. 69:(in press).

Schuster RK. 2012. A new species of *Oochoristica* (Cestoda, Linstowiidae) from the Arabian toad-headed agama, *Phrynocephalus arabicus* (Sauria, Agamidae) from the United Arab Emirates. Vest. Zool. 46:29–32.

Steelman GM. 1939. *Oochoristica whitentoni*, a new anoplocephalid cestode from a land tortoise. J. Parasitol. 25:479–482.

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# New Host and Geographic Distributional Records for *Eustrongylides* sp. (Nematoda: Dioctophymatoidea: Dioctophymatidae) from Eight Vertebrates (Osteichthyes, Amphibia, Reptilia) from Arkansas, Oklahoma and Texas

**Chris T. McAllister**

Division of Science and Mathematics, Eastern Oklahoma State College, Idabel, OK 74745

**Charles R. Bursey**

Department of Biology, Pennsylvania State University-Shenango Campus, Sharon, PA 16146

**Thomas J. Fayton**

Gulf Coast Research Laboratory, University of Southern Mississippi, 703 E. Beach Drive, Ocean Springs, MS 39564

**Henry W. Robison**

Department of Biology, Southern Arkansas University, Magnolia, AR 71754

**Stanley E. Trauth**

Department of Biological Sciences, Arkansas State University, State University, AR 72467

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**Abstract:** During May 1989 and again between February 2012 and July 2015, the following eight vertebrates (five fishes, one amphibian, two reptiles) from Arkansas, Oklahoma or Texas were collected and found to be infected with larval nematodes, *Eustrongylides* sp. as follows: Ozark Bass (*Ambloplites constellatus*, Arkansas), Grass Carp (*Ctenopharyngodon idella*, Arkansas), Grass Pickerel (*Esox americanus*, Oklahoma), Golden Topminnow (*Fundulus chrysotus*, Arkansas), Green Sunfish (*Lepomis cyanellus*, Oklahoma), western lesser siren (*Siren intermedia nettingi*, Arkansas), Midland water snake (*Nerodia sipedon pleuralis*, Arkansas) and Gulf Coast ribbon snake (*Thamnophis proximus orarius*, Texas). In the life cycle of this nematode, these new hosts represent paratenic or transport hosts. This is the first time *Eustrongylides* sp. has been reported from Oklahoma, and we document seven new host records for the parasite. ©2015 Oklahoma Academy of Science

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## Introduction

Nematodes of the genus *Eustrongylides* Jägerskiöld, 1909 are pathogenic parasites found as adults in the proventriculus of piscivorous Proc. Okla. Acad. Sci. 95: pp 26 - 32 (2015)

wading birds (Spalding and Forrester 1993) with larvae encysted in the body cavity and musculature of fishes (Hoffman 1999). This parasite causes pathology in piscivorous fish hosts (Mitchell et al. 2009) and renders smaller forage fish more susceptible to predation

(Coyner et al. 2001). There are two valid species of *Eustrongylides* that occur in the New World, *E. ignotus* Jägerskiöld, 1909 and *E. tubifex* (Nitzsch in Rudolphi, 1819) (Measures 1988a).

In the life cycle, early larval development occurs in blood vessels of first intermediate host freshwater tubificine oligochaetes after they ingest infective eggs (Measures 1988b; Anderson 2000), planktivorous and benthivorous fishes are second intermediate hosts (Measures 1988c) and piscivorous birds are generally considered to be the definitive host (Spalding and Forrester 1993; Franson and Custer 1994). However, predatory fish and reptiles and amphibians that ingest infected fish serve as paratenic, or transport, hosts (Xiong et al. 2013). Rarely mammals, including humans have become infected with larval *Eustrongylides* sp. (Abram and Lichtenfels 1974; Guerin et al. 1982; Deardorff and Overstreet 1991; Cole 1999).

To our knowledge, nothing has been published previously on *Eustrongylides* sp. in any host from Oklahoma (Hoffman 1999) and, as far as we know, there are three recent reports (McAllister et al. 2014, 2015, 2016) from Arkansas fishes. Here, we provide new host records for this nematode as well as the first report of the genus from Oklahoma.

## Methods

During May 1989 and again between February 2012 and July 2015, the following eight vertebrate species were collected and examined for helminth parasites, including one Ozark Bass (*Ambloplites constellatus*) from Marion County, Arkansas, five Golden Topminnows (*Fundulus chrysotus*) from Lincoln County, Arkansas, two Grass Carp (*Ctenopharyngodon idella*) from Pulaski County, Arkansas, five Green Sunfishes (*Lepomis cyanellus*) from McCurtain County, Oklahoma, five Grass Pickerel (*Esox americanus*) from McCurtain County, Oklahoma, three western lesser sirens (*Siren intermedia nettingi*) from Craighead ( $n = 1$ ) and Sevier ( $n = 2$ ) counties, Arkansas, nine Midland water snakes, (*Nerodia sipedon pleuralis*) from Franklin ( $n = 1$ ), Fulton ( $n =$

1), Independence ( $n = 5$ ), Marion ( $n = 1$ ) and Montgomery ( $n = 1$ ) counties, Arkansas, and one Gulf Coast ribbon snake, *Thamnophis proximus orarius* from Harris County, Texas. Fishes and sirens were collected with a dipnet or 3.7 m (1.6 mm mesh) seine and snakes by hand or tong. Fish and sirens were placed in containers with cool aerated habitat water and snakes in cloth bags on ice; all were necropsied within 24 hr. We followed accepted guidelines for the use of fish (AFS 2004) and amphibians and reptiles in research (HACC 2004); specimens were overdosed with a concentrated Chloretone solution and a mid-ventral incision was made to expose the gastrointestinal tract and internal viscera. Nematodes were studied as temporary mounts in glycerol. Voucher specimens of *Eustrongylides* sp. were deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, and Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. Host voucher specimens were deposited in the Arkansas State University Museum of Zoology Herpetology Collection (ASUMZ), State University, Arkansas or Henderson State University Museum (HSU), Arkadelphia, Arkansas. Prevalence, mean intensity, and range of infection are provided in accordance with terminology given in Bush et al. (1997).

## Results

Overall, eight of 31 (26%) vertebrate specimens collected, including one each of *A. constellatus*, *C. idella*, *E. americanus*, *F. chrysotus*, *L. cyanellus*, *S. i. nettingi*, *N. s. pleuralis* and *T. p. orarius* harbored nematodes fitting the description of *Eustrongylides* sp. Watersheds herein where *Eustrongylides* were found in hosts include the Arkansas, Ouachita, St. Francis, and White river systems in Arkansas, the Red River drainage in Oklahoma and the Trinity/San Jacinto river basin in Texas. A summary of host data is as follows:

### **Nematoda: Dioctophymatoidea: Dioctophymatidae *Eustrongylides* sp. (larvae) (Fig. 1)**

*Hosts and localities:* *A. constellatus*  
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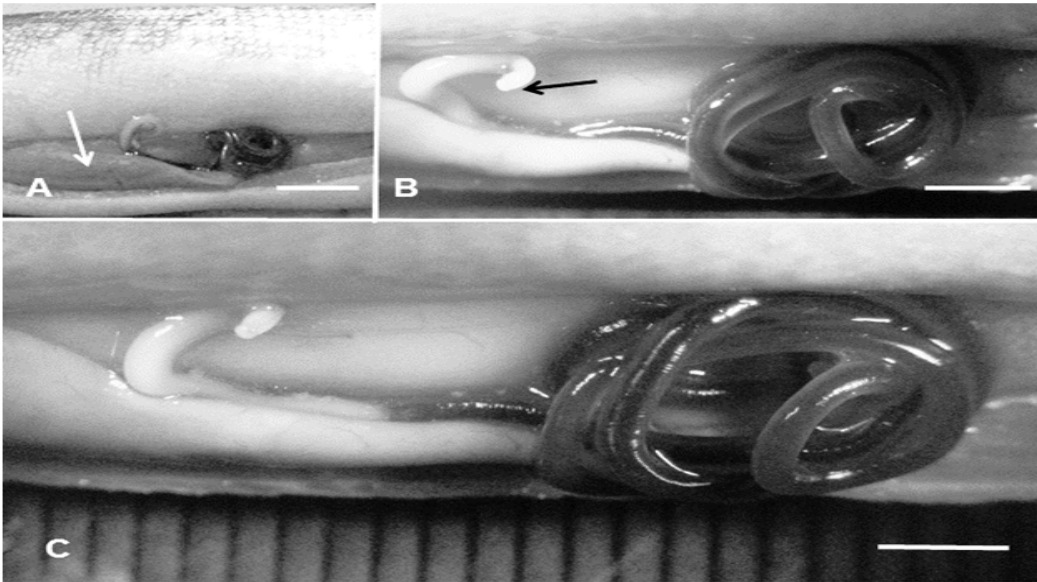
collected on 23 July 2014 from Crooked Creek at Kelly's Slab, Marion County, Arkansas (36°15'09.9"N, 94°26'25.8"W); *C. idella* collected on 17 October 2014 from Freeze Fish Farm at Keo, Pulaski County, Arkansas (34° 37' 11.6688"N, 92° 01' 28.1922"W); *E. americanus* collected on 29 July 2015 from Yashau Creek, McCurtain County, Oklahoma (34° 00' 41.115"N, 94° 44' 59.7258"W); *F. chrysotus* collected on 29 June 2014 from Cane Creek Lake, Lincoln County, Arkansas (33° 55' 0.3102"N, 91° 45' 56.9592"W); *L. cyanellus* collected on 23 November 2014 from Yashau Creek, McCurtain County, Oklahoma (34° 00' 41.115"N, 94° 44' 59.7258"W); *S. i. nettingi* (ASUMZ 31985) collected on 23 February 2012 from 0.4 km E of South Culberhouse Road off county road 412, Craighead County, Arkansas (35° 45' 46.8648"N, 90° 42' 27.1614"W); *N. s. pleuralis* collected on 24 July 2014 from Crooked Creek at Kelly's Slab, Marion County, Arkansas (36°15'09.9"N, 94°26'25.8"W); *T. p. orarius* collected on 11 May 1989 from San Jacinto Street, Harris County, Texas (29° 44' 35.0262"N, 95° 22' 24.114"W).

*Prevalence:* 8/31 (26%) overall; 1/1 (100%) *A. constellatus*; 1/5 (20%) *E. americanus*; 1/5 (20%) *F. chrysotus*; 1/2 (50%) *C. idella*; 1/5 (20%) *L. cyanellus*; 1/3 (33%) *S. i. nettingi*; 1/9 (11%) *N. s. pleuralis*; 1/1 (100%) *T. p. orarius*.

*Intensity:* Most hosts harbored a single larval worm except *S. i. nettingi* which was infected with 12 advanced fourth-stage larval *Eustrongylides* sp.

*Site of infection:* Encapsulated (all alive) in fat body and mesentery. From fishes, these conspicuous red-colored coiled nematodes when teased from their capsules measured  $130.0 \pm 23.3$ , range 90–143 mm in total length, similar to lengths of third-stage larval *Eustrongylides* (see Lichtenfels and Piliitt 1986).

*Other reported North American hosts:* Larval *Eustrongylides* spp. have been reported from a number of fish within 14 orders (see Xiong et al. 2013); in amphibians, including three-toed amphiuma, *Amphiuma tridactylum* ([experimental infection] von Brand 1944;



**Figure 1.** *Eustrongylides* sp. infection in *Esox americanus* from Yashau Creek, McCurtain County, Oklahoma. **A.** Free worm in coelomic cavity near liver (arrow); scale bar = 10 mm. **B.** Closer view of infection showing anterior end of worm (arrow); scale bar = 5 mm. **C.** Higher magnification showing infection; scale bar = 2 mm.



Paneser and Beaver 1979), mudpuppy, *Necturus maculosus* ([experimental infection, von Brand 1944), Blanchard's cricket frog, *Acris blanchardi* (Muzzell and Sonntag 2012), leopard frog, *Lithobates pipiens* ([experimental infection], von Brand 1944; Walton 1949), African clawed frog, *Xenopus laevis* (introduced population, Kuperman et al. 2004), American bullfrog, *Lithobates catesbeianus* (von Brand 1944; [experimental infection], Modzelewski and Culley 1974); in reptiles, including common snapping turtle, *Chelydra serpentina serpentina* (Rausch 1947), painted turtle, *Chrysemys picta* ([experimental infection], von Brand 1944), American alligator, *Alligator mississippiensis* ([experimental infection], von Brand 1944), copperhead, *Agkistrodon contortrix*, eastern racer, *Coluber constrictor*, coachwhip, *Coluber flagellum*, indigo snake, *Drymarchon corais*, northern water snake, *Nerodia sipedon sipedon*, gopher snake, *Pituophis catenifer*; eastern pine snake, *Pituophis melanoleucus*, Mexican garter snake, *Thamnophis eques*, eastern garter snake, *Thamnophis sirtalis sirtalis* and red-sided garter snake, *Thamnophis sirtalis parietalis* ([experimental infection], Lichtenfels and Lavies 1976) (see Winsor 1948; Ernst and Ernst 2006).

*Other Western Hemisphere hosts:* Cuban treefrog, *Hyla septentrionalis*, Cuba (Walton 1940); Paraguayan caiman, *Caiman yacare*, Paraguay (Goldberg et al. 1991).

*Geographic range of genus outside of North America:* North and South America, Caribbean, Europe, eastern Africa, China, and Turkey (Xiong et al. 2013).

*Geographic range of genus in North America:* **U.S.A.:** Arkansas (McAllister et al. 2014, 2015, 2016), California, Florida, Louisiana, Maine, Maryland, Massachusetts, Mississippi, Montana, Nevada, New York, Ohio, Oklahoma (this report), Oregon, Pennsylvania (Philadelphia Zoo captives), Tennessee, Texas (also this report), Utah, Virginia, Washington (DC), Wyoming (Hoffman 1999). **Canada:** British Columbia, Manitoba, Ontario (Hoffman 1999). **Mexico:** Campeche, Guanajuato, Guerrero, Hidalgo, Jalisco, Morelos, Oaxaca, San Luis

Potosí, Veracruz, Yucatán (Salgado-Maldonado 2006), Michoacán (Hoffman 1999; Ernst and Ernst 2006; Salgado-Maldonado 2006).

*Additional Arkansas records:* Pirate Perch, *Aphredoderus sayanus* (McAllister et al. 2014) collected on 24 October 2013 from the Rolling Fork River, Sevier County (34.064667N, 94.380023W); Blackspotted Topminnow, *Fundulus olivaceus* (McAllister et al. 2015) collected on 23 July 2014 from Crooked Creek at Harmon, Boone County (36° 14' 02.0328"N, 92° 55' 20.2224"W); Northern Studfish, *Fundulus catenatus* (McAllister et al. 2016) collected on 23 July 2014 from Crooked Creek at Kelly's Slab in Yellville, Marion County (36°15'9.9"N, 94°26'25.8"W).

*Additional Oklahoma records:* None.

*Additional Texas records:* Rock Bass, *Ambloplites rupestris*, Warmouth, *Lepomis gulosus*, Largemouth Bass, *Micropterus salmoides*, upper San Marcos River, Hays County (Underwood and Dronen 1984).

*Specimens deposited:* USNPC 105449, 107686, HWML 64707-09 (in EtOH).

## Discussion

Specific identification of *Eustrongylides* requires rearing larvae in an avian host and our study did not include this experimental transmission. We did not observe any noticeable pathological effect on these hosts; however, Kaur et al. (2013) reported that fecundity of fish could be reduced with a decline in fish populations, while Mir et al. (2012) noted that fish had a profound decrease in luteinizing hormone with accompanying abnormal histology of their ovaries and testes. When these larvae migrate under the skin and musculature of fishes, it can cause extensive inflammation and necrosis with severe pathologic changes in adjacent tissues (Paperna 1974; Xiong et al. 2009). In one of the largest surveys to date (Weisberg et al. 1986), over 11,000 fishes (20 species) were examined for *Eustrongylides* infection from the Chesapeake Bay region of

Maryland, and only three species of *Fundulus* were found to be harboring these worms. In addition, epizootics are known in various piscivorous birds which can lead to large-scale mortality, particularly among nestlings (Wiese et al. 1977; Roffe 1988; Xiong et al. 2009).

Interestingly, a previous helminth survey on *S. i. nettingi* in Arkansas (McAllister et al. 1994) did not report *Eustrongylides* sp. from this amphibian. And, to our knowledge, this nematode has also not been previously reported from *A. constellatus*, *C. idella*, *E. americanus*, *F. chrysotus*, *L. cyanellus*, *N. s. pleuralis* or *T. p. orarius* (Hoffman 1999; Ernst and Ernst 2006). No *Eustrongylides* sp. were found in 31 and 20 northern water snakes, *N. s. sipedon* from North Carolina and Ohio by Collins (1969) and Gibson and Rabalais (1973), respectively; however, Winsor (1948) was the first to find *Eustrongylides* sp. in *N. s. sipedon* from captive specimens at the Fairmount Park Aquarium in Pennsylvania, and Bursey (1986) later reported *Eustrongylides* sp. from a natural population of *N. s. sipedon* from Virginia. In addition, no *Eustrongylides* sp. was reported from a comprehensive survey on 46 and over 200 water snakes from Alabama and Louisiana by Dettlerline et al. (1984) and Fontenot and Font (1996), respectively. As more and more surveys on helminth parasites of vertebrates of Arkansas, Oklahoma and Texas are conducted, we expect additional hosts of *Eustrongylides* sp. to be reported, particularly new fish hosts.

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## References

- Abram JB, Lichtenfels JR. 1974. Larval *Eustrongylides* sp. (Nematoda: Dioctophymatoidea) from otter, *Lutra canadensis*, in Maryland. Proc. Helminthol. Soc. Wash. 41:253.
- American Fisheries Society (AFS). 2004. Guidelines for the use of fishes in research [web application]. J. G. Nickum (Chair). American Fisheries Society, Bethesda, Maryland. [http://fisheries.org/docs/policy\\_useoffishes.pdf](http://fisheries.org/docs/policy_useoffishes.pdf). Accessed 2015 Aug. 1.
- Anderson RM. 2000. Nematode parasites of vertebrates: Their development and transmission. Second Ed. Wallingford, UK: CABI Publishing. 650 p.
- Bursey CR. 1986. Histological aspects of natural eustrongyloid infections in the northern water snake, *Nerodia sipedon*. J. Wildl. Dis. 22:527–532.
- Bush AO, Lafferty KD, Lotz JM, Shostak A. W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 83:575–583.
- Cole RA. 1999. Eustrongylidosis. Pages 223–228 in M. Friend and J. C. Franson, eds. Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds. Biological Resources Division, Information and Technology Report 1999–2001, U. S. Geological Survey, Washington, D.C.
- Collins RF. 1969. The helminths of *Natrix* spp. and *Agkistrodon piscivorus piscivorus* (Reptilia: Ophidia) in eastern North Carolina. J. Elisha Mitchell Sci. Soc. 85:141–144.
- Coyner DF, Schaack SR, Spalding MG, Forrester DJ. 2001. Altered predation susceptibility of Mosquitofish infected with *Eustrongylides ignotus*. J. Wild. Dis. 37:556–560.
- Deardorff TL, Overstreet RM. 1991. Seafood-transmitted zoonoses in the United States: The fishes, the dishes, and the worms. Pages 211–265 in D. R. Ward and C. R. Hackney, eds. Microbiology of Marine Food Products. Van Nostrand Reinhold, New York.
- Dettlerline JL, Jacob JS, Wilhelm WE. 1984. A comparison of helminth endoparasites in the cottonmouth (*Agkistrodon piscivorus*) and three species of water snakes (*Nerodia*). Trans.

- Amer. Micros. Soc. 103:137–143.
- Ernst CH, Ernst EM. 2006. Synopsis of the helminths endoparasitic in snakes of the United States and Canada. *SSAR Herpetol. Circ.* 34:1–86.
- Fontenot LW, Font WF. 1996. Helminth parasites of four species of aquatic snakes from two habitats in southeastern Louisiana. *J. Helminthol. Soc. Wash.* 63:66–75.
- Franson JC, Custer TW. 1994. Prevalence of eustrongylidosis in wading birds from colonies in California, Texas, and Rhode Island, USA. *Col. Waterb.* 17:168–172.
- Gibson JB, Rabalais FC. 1973. Helminth parasites of *Natrix sipedon sipedon* and *Thamnophis sirtalis sirtalis* from the Ottawa National Wildlife Refuge, Ohio. *Amer. Midl. Nat.* 89:239–241.
- Goldberg SR, Bursley CR, Aquino-Shuster AL. 1991. Gastric nematodes of the Paraguayan caiman, *Caiman yacare* (Alligatoridae). *J. Parasitol.* 77:1009–1011.
- Guerin PF, Marapudi S, McGrail L, Moravec, CL, Schiller E, Hopf EW, Thompson R, Lin FYC, Israel E, Bier JW, Jackson GJ. 1982. Epidemiological notes and reports of intestinal perforation caused by larval *Eustrongylides*-Maryland. *Morb. Mortal. Week. Rept.* 31:383–384.
- Herpetological Animal Care Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists. 2004. [Internet]. Guidelines for use of live amphibians and reptiles in field and laboratory research. 2nd Edition. Electronic database [http://research.fsu.edu/acuc/policies\\_Guidelines/ASIH\\_HACC\\_GuidelinesAmphibians.pdf](http://research.fsu.edu/acuc/policies_Guidelines/ASIH_HACC_GuidelinesAmphibians.pdf). Accessed 2015 Aug. 1.
- Hoffman GL. 1999. Parasites of North American freshwater fishes. 2nd ed. Ithaca, New York: Comstock Publishing Associates. 539 p.
- Kaur P, Shrivastav R, Qureshi TA. 2013. Physiological effects of *Eustrongylides* sp. larvae (Dioctophymatidae) infection in freshwater fish, *Glossogobius giurus* (Ham.) with special reference to ovaries. *J. Par. Dis.* 37:245–250.
- Kuperman BI, Matey VE, Fisher RN, Ervin EL, Warburton ML, Bakhireva L, Lehman CA. 2004. Parasites of the African clawed frog, *Xenopus laevis*, in southern California, USA. *Comp. Parasitol.* 71:229–232.
- Lichtenfels JR, Lavies B. 1976. Mortality in red-sided garter snakes, *Thamnophis sirtalis parietalis*, due to the larval nematode, *Eustrongylides* sp. *Lab. Anim. Sci.* 26:465–467.
- Lichtenfels JR, Pilitt PA. 1986. *Eustrongylides* sp. (Nematoda: Dioctophymatoidea): Differentiation of third- and fourth-stage larvae from Killifish, *Fundulus* sp., collected in Chesapeake Bay Area, U.S.A. *Proc. Helminthol. Soc. Wash.* 53:144–148.
- McAllister CT, Bursley CR, Robison HW, Neely DA, Connior MB, Barger MA. 2014. Miscellaneous fish helminth parasite (Trematoda, Cestoidea, Nematoda, Acanthocephala) records from Arkansas. *J. Ark. Acad. Sci.* 68:78–86.
- McAllister CT, Bursley CR, Fayton TJ, Font WF, Robison HW, Connior MB, Cloutman DG. 2015. Helminth Parasites of the Blackspotted Topminnow, *Fundulus olivaceus* (Cyprinodontiformes: Fundulidae), from the Interior Highlands of Arkansas. *J. Ark. Acad. Sci.* 69:(in press).
- McAllister CT, Bursley CR, Font WF, Robison HW, Trauth SE, Cloutman DG, Fayton TJ. 2016. Helminth parasites of the Northern Studfish, *Fundulus catenatus* (Cypriniformes: Fundulidae) from the Ouachitas and Ozarks of Arkansas, U.S.A. *Comp. Parasitol.* 83:(in press).
- McAllister CT, Goldberg SR, Trauth SE, Bursley CR, Holshuh HJ, Cochran BG. 1994. Helminths of the western lesser siren, *Siren intermedia nettingi* (Caudata: Sirenidae), from Arkansas. *J. Helminthol. Soc. Wash.* 61:234–238.
- Measures LN. 1988a. Revision of the genus *Eustrongylides* Jägerskiöld, 1909 (Nematoda: Dioctophymatoidea) of piscivorous birds. *Can. J. Zool.* 66:885–895.
- Measures LN. 1988b. The development of *Eustrongylides tubifex* (Nematoda: Dioctophymatoidea) in oligochaetes. *J. Parasitol.* 74:294–304.
- Measures LN. 1988c. The development and pathogenesis of *Eustrongylides tubifex* (Nematoda: Dioctophymatoidea) in

- piscivorous birds. *Can. J. Zool.* 66:2223–2232.
- Mir TA, Kaur P, Manohar S. 2012. Pathogenic effects of nematode parasite *Eustrongylides* sp. larvae on serum LH level and histology of gonads of freshwater fish, *Clarias gariepinus*. *Rec. Res. Sci. Tech.* 4:24–26.
- Mitchell AJ, Overstreet RM, Goodwin AE. 2009. *Eustrongylides ignotus* infecting commercial bass, *Morone chrysops* female × *Morone saxatilis* male, and other fish in the southeastern USA. *J. Fish Dis.* 32:795–799.
- Modzelewski EH Jr, Culley DD Jr. 1974. Growth responses of the bullfrog, *Rana catesbeiana* fed various live foods. *Herpetologica* 30:396–405.
- Muzzall PM, Sonntag E. 2012. Helminths and symbiotic Protozoa of Blanchard's cricket frog, *Acris blanchardi* Harper, 1947 (Hylidae), from Michigan and Ohio, U.S.A. *Comp. Parasitol.* 79:340–343.
- Panaser TS, Beaver PC. 1979. Morphology of the advanced-stage larva of *Eustrongylides wenrichi* Canavan, 1929, occurring encapsulated in the tissues of *Amphiuma* in Louisiana. *J. Parasitol.* 65:96–104.
- Paperna I. 1974. Hosts distribution and pathology of infections with larvae of *Eustrongylides* (Dioctophymidae, Nematoda) in fish from East African lakes. *J. Fish Biol.* 6:67–76.
- Rausch R. 1947. Observations on some helminths parasitic in Ohio turtles. *Amer. Midl. Nat.* 38:434–442.
- Roffe TJ. 1988. *Eustrongylides* sp. epizootic in young common egrets (*Casmerodius albus*). *Avian Dis.* 32:143–147.
- Salgado-Maldonado G. 2006. Checklist of the helminth parasites of freshwater fishes from Mexico. *Zootaxa* 1324:1–357.
- Spalding MG, Forrester DJ. 1993. Pathogenesis of *Eustrongylides ignotus* (Nematoda: Dioctophymatoidea) in Ciconiiformes. *J. Wildl. Dis.* 29:250–260.
- Underwood HT, Dronen NO Jr. 1984. Endohelminths of fishes from the upper San Marcos River, Texas. *Southwest. Nat.* 29:377–385.
- von Brand T. 1944. Physiological observations upon a larval *Eustrongylides*. VI. Transmission to various coldblooded intermediate hosts. *Proc. Helminthol. Soc. Wash.* 11:23–27.
- Walton AC. 1940. Notes on amphibian parasites. *Proc. Helminthol. Soc. Wash.* 7:87–91.
- Walton AC. 1949. Parasites of the Ranidae (Amphibia). XIV. *Trans. Ill. Acad. Sci.* 42:161–164.
- Weisberg SB, Morin RP, Ross EA, Hirshfield MF. 1986. *Eustrongylides* (Nematoda) infection in Mummichogs and other fishes of the Chesapeake Bay region. *Trans. Amer. Fish. Soc.* 115:776–783.
- Wiese JH, Davidson WR, Nettles VF. 1977. Large scale mortality of nesting ardeids caused by nematode infection. *J. Wildl. Dis.* 13:376–382.
- Winsor H. 1948. Hosts of eustrongyloid worms from Fairmount Park Aquarium and Philadelphia Zoo. *Proc. Pennsylv. Acad. Sci.* 22:68–72.
- Xiong F, Li WX, Wu SG, Zou H, Wang GT. 2013. Molecular phylogeny and host specificity of the larval *Eustrongylides* (Nematoda: Dioctophymidae) from freshwater fish in China. *J. Parasitol.* 99:137–144.
- Xiong F, Wang GT, Wu SG, Nie P. 2009. Development of *Eustrongylides ignotus* (Nematoda: Dioctophymida) in domestic ducks (*Anas platyrhynchos domestica* (L.)). *J. Parasitol.* 95:1035–1039.

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# New County Record for the Least Shrew, *Cryptotis parva*, and Notes on an Ectoparasite, *Haemogamasus longitarsus*.

**Matthew B. Connior**

Life Sciences, Northwest Arkansas Community College, Bentonville, AR 72712

**Lance A. Durden**

Department of Biology, Georgia Southern University, Statesboro, GA 30458

**Chris T. McAllister**

Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

The least shrew (*Cryptotis parva*) is a small insectivore that ranges from the northern United States west to the Midwest and south to Texas and Florida; it is also found in Central America (Whitaker 1974). In Oklahoma, *C. parva* occurs over most of the state, except the western panhandle (Caire et al. 1989); however, there are apparently no records for Delaware Co. in northeastern Oklahoma. Although the least shrew has a wide distribution, little is known of its ecology, especially its ectoparasite fauna, in Oklahoma. Here, we report a new county record for the least shrew from Oklahoma and also report on a laelapid mite, *Haemogamasus longitarsus*, collected from it.

On 9 May 2015, an adult female *C. parva* was collected as a salvage specimen from a county road just west of Maysville, AR, on Co. Rd. E0430 in Delaware Co. (36.394695°N, 94.610809°W). The surrounding habitat consisted of a matrix of pastureland and agricultural cropland. The shrew was searched for ectoparasites following previous methods described in Connior et. al. (2014). Two mites were collected and placed in vials containing 70% ethanol. Mites were cleared in lactophenol and slide-mounted in Hoyer's medium (Walters and Krantz 2009). Voucher specimens of mites were deposited in the General Ectoparasite Collection in the Department of Biology at Georgia Southern University (accession no.

L3720). The voucher host was deposited in the Henderson State University (HSU 878) collection, Arkadelphia, Arkansas.

Since Caire et al. (1989), several additional county records have been reported for *Cryptotis parva* in Oklahoma (Braun and Revelez 2005); however, these all occur in western Oklahoma. This is the first report of an additional distribution record from northeastern Oklahoma for *C. parva* to our knowledge. This record (Delaware Co.) fills a distributional hiatus from surrounding Ottawa and Adair cos. In addition, *C. parva* has been collected from adjacent Benton Co. in Arkansas, as well as Washington and Madison cos. in extreme northwestern Arkansas (Pfau et al. 2011). This shrew species commonly inhabits grassy or brushy areas, but may go undetected due to its small size and habits, unless specifically targeted. Thus, this shrew is probably more prevalent than museum records represent.

Two mites (1 female, 1 nymph) identified as *Haemogamasus longitarsus* were recovered from *C. parva*. This laelapid mite has been previously reported from the shrew of the genus *Blarina* (McCay 2001; Whitaker et al. 2007) and rodents from Delaware, Florida, Georgia, Idaho, Illinois, Indiana, Maryland, New Hampshire, New York, North Dakota, Pennsylvania, Virginia, and West Virginia. (Whitaker et al. 2007). It has not been, to our knowledge, previously reported from any

mammalian host in Oklahoma. We therefore document a new state record for *H. longitarsus* in Oklahoma. Furthermore, this is only the second report of a mite from an insectivore from Oklahoma (McAllister and Durden 2014).

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### References

- Braun JK, Revelez MA. 2005. Distributional records and comments on Oklahoma mammals. *Texas J. Sci.* 57:3-24.
- Caire W, Tyler JD, Glass BP, Mares MA. 1989. *Mammals of Oklahoma*. Norman (OK): University of Oklahoma Press. 567 p.
- Connior MB, Durden LA, McAllister CT. 2014. New records of ectoparasites and other epifaunistic arthropods from *Scalopus aquaticus* and *Blarina carolinensis* in Arkansas. *J. Ark. Acad. Sci.* 68:137-139.
- McAllister CT, Durden L. 2014. *Haemogamasus harperi* Keegan (Arthropoda: Acari: Laelapidae): New to the Mite Fauna of Oklahoma. *Proc. Okla. Acad. Sci.* 94:70-72.
- McCay TS. 2001. *Blarina carolinensis*. *Mammal. Species* 673:1-7.
- Pfau RS, Sasse DB, Connior MB, Guenther IF. 2011. Occurrence of *Blarina brevicauda* in Arkansas and notes on the distribution of *Blarina carolinensis* and *Cryptotis parva*. *J. Ark. Acad. Sci.* 65:61-66.
- Walters DE, Krantz GW. 2009. Collection, rearing and preparing specimens. In: Krantz GW, Walter DE, editors. *A manual of acarology*, 3<sup>rd</sup> edition. Lubbock (TX): Texas Tech University Press. p. 83-96.
- Whitaker JO Jr. 1974. *Cryptotis parva*. *Mammal. Species* 43:1-8.
- Whitaker JO Jr., Walters BL, Castor LK, Ritzi CM, Wilson N. 2007. Host and distribution lists of mites (Acari), parasitic and phoretic, in the hair or on the skin of North American wild mammals north of Mexico: records since 1974. *Fac. Publ. H. W. Mantor Lab. of Parasitol. Paper* 1. 173 p.

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# First Report of the Asian Fish Tapeworm, *Bothriocephalus acheilognathi* (Cestoda: Bothriocephalidea:Bothriocephalidae) from Oklahoma with New Host Records in Non- Hatchery Fishes in Arkansas

**Chris T. McAllister**

Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

**Charles R. Bursey**

Department of Biology, Pennsylvania State University-Shenango Campus, Sharon, PA 16146

**Thomas J. Fayton**

Gulf Coast Research Laboratory, University of Southern Mississippi, Ocean Springs, MS 39564

**Matthew B. Connior**

Life Sciences, Northwest Arkansas Community College, Bentonville, AR 72712

**Henry W. Robison**

Department of Biology, Southern Arkansas University, Magnolia, AR 71754

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**Abstract:** The Asian fish tapeworm, *Bothriocephalus acheilognathi* is an invasive pathogen in North America and beyond. It first appeared in North America in the mid-1970s likely arriving in shipments of introduced Grass Carp, *Ctenopharyngodon idella* from China to control aquatic vegetation. This tapeworm now can be found in many parts of the world (except Antarctica) where it infects over 200 species of fishes, including several raised in commercial hatcheries. In examining 67 fishes, including 11 Bluntnose Minnow (*Pimephales notatus*), one Spotted Sucker (*Minytrema melanops*), 17 Western Mosquitofish (*Gambusia affinis*) and 22 Creek Chub (*Semotilus atromaculatus*) from four watersheds in Arkansas, and 16 *G. affinis* from a watershed in Oklahoma, we found *B. acheilognathi* in 15 (22%) of them. In addition, examination of 256 fishes from the same watersheds where other fishes were infected with this tapeworm yielded no *B. acheilognathi*. Here, we report, for the first time, *B. acheilognathi* in an Oklahoma fish, document two new host records, and confirm the parasite in Arkansas in non-hatchery fishes. ©2015 Oklahoma Academy of Science

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## Introduction

*Bothriocephalus acheilognathi* Yamaguti, 1934, the Asian fish tapeworm, is a potentially pathogenic cestode species native to eastern Asia in Japan, China, and Russia (Scholz 1997); the first report of *B. acheilognathi* in North America was in 1975, likely in shipments from China of introduced Grass Carp (*Ctenopharyngodon idella*) to control aquatic vegetation (Hoffman 1999). Since then, this parasite has spread in the Western Hemisphere and been reliably reported in numerous fishes from at least 16 U.S. states (including Hawaii, and four states in hatcheries only), Manitoba, Canada, Honduras, Panama, Puerto Rico, Mexico, Argentina and Brazil (Bunkley-Williams and Williams 1994; Font and Tate 1994; Salgado-Maldonado and Pineda-López 2003; Choudhury et al. 2006; Salgado-Maldonado 2006; Salgado-Maldonado et al. 2015). This tapeworm is established on all continents except Antarctica and currently infects about 235 species (19 families) of freshwater fish, primarily cyprinids and poeciliids (Salgado-Maldonado and Pineda-López 2003; Scholz et al. 2012; Choudhury and Cole 2015).

The life cycle of *B. acheilognathi* includes four stages: (1) eggs which are shed in feces from a definitive host fish, (2) a free-swimming coracidium that is swallowed by an intermediate host, usually a cyclopoid copepod (of at least five genera), (3) a proceroid that develops in the intermediate host, and (4) an adult worm that develops from a proceroid in the definitive host (either smaller cyprinid fishes that complete the life cycle or piscivorous fishes that eat smaller fish and complete the life cycle) (Marcogliese and Esch 1989; Kline et al. 2009).

In Arkansas, *B. acheilognathi* has been documented in fish hatcheries/commercial ponds (Scott and Grizzle 1979; Choudhury et al. 2006) but not yet in native fish populations. Rogers (1976) reported transfer of this tapeworm from Arkansas to Florida in *C. idella* and Golden Shiners (*Notemigonus crysoleucus*). In addition, bait minnows (*N. crysoleucus*) said to have originated from Arkansas were reported to be infected with *B. acheilognathi*

obtained from bait shops in Nevada (Heckmann et al. 1993). However, we are not aware of any previous reports of *B. acheilognathi* reported from non-hatchery fishes from Arkansas, nor are there published reports of this tapeworm in any fish from Oklahoma. Here, we report, for the first time, *B. acheilognathi* in an Oklahoma fish and confirm the parasite in Arkansas but in non-hatchery raised (native) fishes.

## Methods

Between July 2014 and August 2015, 67 fishes were collected with seine (3.7 m, 1.6 mm mesh), dipnet or electroshocker as follows: 11 Bluntnose Minnow (*Pimephales notatus*) from East Branch of Gulpha Creek, Garland County, Arkansas (Ouachita River drainage, 34.470655°N, 92.986162°W), 22 Creek Chub (*Semotilus atromaculatus*) from East Flint Creek at Springtown, Benton County, Arkansas (Arkansas River drainage, 36.261867°N, 94.421601°W), one Spotted Sucker (*Minytrema melanops*) from the Arkansas River at Pendleton, Desha County, Arkansas (Arkansas River drainage, 33.987451°N, 91.362222°W), 17 Western Mosquitofish (*Gambusia affinis*) from the Rolling Fork River, Sevier County, Arkansas (Red River drainage, 34.064701°N, 94.38003°W) and 16 *G. affinis* from Yashau Creek at Memorial Drive, McCurtain County, Oklahoma (Red River drainage, 34.011507°N, 94.749615°W). An additional 256 fishes from the same watersheds (Appendix I) were also examined. Fish were placed in containers with cool aerated habitat water; all were necropsied within 24 hr following accepted guidelines for the use of fish in research (AFS 2004). Specimens were overdosed with a concentrated Chloretone solution and a mid-ventral incision was made to expose the gastrointestinal tract, which was split lengthwise, and internal viscera. Tapeworms were rinsed in saline, fixed in nearly boiling distilled water, preserved in DNA grade ethanol (70-95%), stained with acetocarmine, and mounted in Canada balsam. Voucher specimens of parasites were deposited in the Harold W. Manter Laboratory



Table 1. Fishes infected with *Bothriocephalus acheilognathi* from four watersheds of Arkansas and one watershed of Oklahoma.

Species	County Location	Watershed	Intensity <sup>1</sup>	Prevalence <sup>2</sup>
<i>Semotilus atromaculatus</i> <sup>3,4</sup>	Benton Co., AR	E. Flint Creek	1.5 ± 0.6 (1-2)	4/22 (18%)
<i>Minytrema melanops</i> <sup>3,4</sup>	Desha Co., AR	Arkansas River	2.0 ± 0.0 (2)	1/1 (100%)
<i>Pimephales notatus</i> <sup>4</sup>	Garland Co., AR	Gulpha Creek	1.3 ± 0.5 (1-2)	6/17 (35%)
<i>Gambusia affinis</i> <sup>4</sup>	Sevier Co., AR	Rolling Fork River	1.0 ± 0.0 (1)	3/11 (27%)
<i>G. affinis</i> <sup>4</sup>	McCurtain Co., OK	Yashau Creek	6.0 ± 0.0 (6)	1/16 (6%)
			<b>overall = 15/67 (22%)</b>	

<sup>1</sup>mean ± 1SD (range).<sup>2</sup>infected/examined × 100 = percent.<sup>3</sup>New host record.<sup>4</sup>New distributional record.

of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. Host voucher specimens were deposited in the Henderson State University Museum (HSU), Arkadelphia, Arkansas. Prevalence, mean intensity, and range of infection are provided in accordance with terminology given in Bush et al. (1997).

## Results and Discussion

Of the 67 individual fishes originally examined (Table 1), 15 (22%) were found to harbor tapeworms fitting the description of *B. acheilognathi*. This species is easily distinguished from other *Bothriocephalus* spp. reported from fishes based on the scolex and strobilar morphology; it possesses an arrowhead-shaped or heart-shaped, fleshy scolex with anterolaterally directed narrow slit like openings and a medial position of the genital opening, which is unique among *Bothriocephalus* spp. (Scholz 1997). A total of 12 *B. acheilognathi* (overall mean  $\pm$  1SD intensity =  $1.3 \pm 0.5$ , range 1–2) were removed from the intestinal tract of these four hosts.

Of the four fish species found to be infected, *G. affinis* and *P. notatus* have previously been reported as hosts of *B. acheilognathi*. The Asian fish tapeworm is known from *G. affinis* in California (Choudhury et al. 2006), Louisiana (W. Font pers. comm.), North Carolina (Granath and Esch 1983; Scholz 1997), Texas (Bean and Bonner 2010) and China (syn. *Coelobothrium gambusiense* Yang, Wang, Peng, Zhou and Liu, 2005 per Kuchta et al. 2008). Marcogliese (2008) reported *B. acheilognathi* from *P. notatus* from the Great Lakes, Michigan. However, we document *B. acheilognathi* in *M. melanops* and *S. atromaculatus* for the first time.

We examined other fishes ( $n = 256$ ) from the watersheds from which a species was reported positive for *B. acheilognathi*, but none of them were found to be harboring this tapeworm (Appendix I). Those examined included 72 cyprinids which are usually not suitable hosts for other North American *Bothriocephalus* spp. (Choudhury et al. 2006) and some have been previously reported as hosts of this helminth

(Hoffman 1999). Therefore, although prevalence and intensity of infection is relatively low for the fishes we examined from four sites (three river drainages) in two states, this tapeworm should still be considered a potential pathogenic species that could spread into new drainages (most recently the Rio Grande of North America, see Bean and Bonner 2010) and contribute to the global spread of this invasive parasite. Indeed, *B. acheilognathi* was found infecting two IUCN-listed endangered fishes, including the Humpback Chub (*Gila cypha*) in Arizona (Stone et al. 2007) and the Woundfin (*Plagopterus argentissimus*) in Utah (Heckmann et al., 1986; Heckman 2009); further research indicated that *G. cypha* could maintain this parasite in the Little Colorado River without the presence of carp or other non-native fish species (Hoffnagle et al. 2006; Stone et al. 2007). Ultimately, certain populations of fish could be negatively affected and this tapeworm could inhibit their recovery.

In summary, we provide the first record of *B. acheilognathi* from Oklahoma and the first report of this tapeworm in non-hatchery fishes in Arkansas, including two new host records (*M. melanops*, *S. atromaculatus*). It is now documented herein that the Asian tapeworm occurs in fish hosts in three major river systems in Arkansas, including the lower Arkansas River that drains directly into the Mississippi River. Additional research in both states, particularly Oklahoma, will surely increase the host list and watersheds affected by this invasive parasite.

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## References

- American Fisheries Society (AFS). 2004. Guidelines for the use of fishes in research [web application]. J. G. Nickum (Chair). American Fisheries Society, Bethesda, Maryland. [http://fisheries.org/docs/policy\\_useoffishes.pdf](http://fisheries.org/docs/policy_useoffishes.pdf). Accessed 2015 July 31.
- Bean MG, Bonner TH. 2010. Spatial and temporal distribution of the Asian fish tapeworm *Bothriocephalus acheilognathi* (Cestoda: Bothriocephalidea) in the Rio Grande (Río Bravo del Norte). *J. Aquat. Anim. Health* 22:182–189.
- Bunkley-Williams L, Williams EH. 1994. Parasites of Puerto Rican freshwater sport fishes. Puerto Rico Department of Natural and Environmental Resources, San Juan, PR, and Department of Marine Sciences, University of Puerto Rico, Mayaguez, PR. 164 p.
- Bush AO, Lafferty KD, Lotz JM, Shostak A. W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.* 83:575–583.
- Choudhury A, Charipar E, Nelson P, Hodgson JR, Bonar S, Cole RA. 2006. Update on the distribution of the invasive Asian fish tapeworm, *Bothriocephalus acheilognathi*, in the U.S. and Canada. *Comp. Parasitol.* 73:269–273.
- Choudhury A, Cole RA. 2015. Invasive species compendium: *Bothriocephalus acheilognathi* <http://www.cabi.org/isc/datasheet01669>. Accessed 2015 August 11.
- Font WF, Tate DC. 1994. Helminth parasites of native Hawaiian fishes: An example of extreme ecological isolation. *J. Parasitol.* 80:682–688.
- Granath WO Jr, Esch GW. 1983. Seasonal dynamics of *Bothriocephalus acheilognathi* in ambient and thermally altered areas of a North Carolina cooling reservoir. *Proc. Helminthol. Soc. Wash.* 50:205–218.
- Heckmann RA. 2009. The fate of an endangered fish species (*Plagiopterus argentissimus*) due to an invasive fish introduction (*Cyprinella lutrensis*) infected with Asian tapeworm (*Bothriocephalus acheilognathi*): Recovery methods. *Proc. Parasitol.* 47:43–52.
- Heckmann RA, Deacon JE, Greger PD. 1986. Parasites of the Woundfin Minnow, *Plagiopterus argentissimus*, and other endemic fishes from the Virgin River, Utah. *Great Basin Nat.* 46:662–676.
- Heckmann RA, Greger PD, Furtek RC. 1993. The Asian fish tapeworm, *Bothriocephalus acheilognathi*, in fishes in Nevada. *J. Helminthol. Soc. Wash.* 60:127–128.
- Hoffman GL. 1999. Parasites of North American freshwater fishes. 2<sup>nd</sup> ed. Ithaca, New York: Comstock Publishing Associates. 539 p.
- Hoffnagle TL, Choudhury A, Cole RA. 2006. Parasitism and body condition in Humpback Chub from the Colorado and Little Colorado rivers, Grand Canyon, Arizona. *J. Aquat. Anim. Health* 18:184–193.
- Kline SJ, Archdeacon TP, Bonar SA. 2009. Effects of praziquantel on eggs of the Asian tapeworm *Bothriocephalus acheilognathi*. *N. Amer. J. Aquacul.* 71:380–383.
- Kuchta R, Scholz T, Bray RA. 2008. Revision of the order Bothriocephalidea Kuchta, Scholz, Brabec & Bray, 2008 (Eucestoda) with amended generic diagnoses and keys to families and genera. *Syst. Parasitol.* 71:81–136.
- Marcogliese DJ. 2008. First report of the Asian fish tapeworm in the Great Lakes. *J. Great Lakes Res.* 34:566–569.
- Marcogliese DJ, Esch GW. 1989. Experimental and natural infection of planktonic and benthic copepods by the Asian tapeworm, *Bothriocephalus acheilognathi*. *Proc. Helminthol. Soc. Wash.* 56:151–155.
- Rogers WA. 1976. Grass Carp hosts Asian tapeworm. *Sports Fish. Inst. Bull.* 276:2–3.
- Salgado-Maldonado G. 2006. Checklist of helminth parasites of freshwater fishes from Mexico. *Zootaxa* 1324:1–357.
- Salgado-Maldonado G, Matamoros WA, Krieser BR, Caspeta-Mandujano JM, Mendoza-Franco EF. 2015. First record of the invasive Asian fish tapeworm *Bothriocephalus acheilognathi* in Honduras, Central America. *Parasite* 22:1–6.
- Salgado-Maldonado G, Pineda-López R. 2003. The Asian fish tapeworm *Bothriocephalus acheilognathi*: A potential threat to native freshwater fish species in Mexico. *Biol. Inv.* 5:261–268.
- Scholz T. 1997. A revision of the species of

- Bothriocephalus* Rudolphi, 1808 (Cestoda: Pseudophyllidea) parasitic in American freshwater fishes. Syst. Parasitol. 36:85–107.
- Scholz T, Kuchta R, Williams C. 2012. *Bothriocephalus acheilognathi*. In: Woo PTK, Buchmann K, editors. Fish parasites: Pathobiology and protection. Wallingford (UK): CAB International. p. 282–297.
- Scott AL, Grizzle JM. 1979. Pathology of cyprinid fishes caused by *Bothriocephalus gowkongiensis* Yeh, 1955 (Cestoda: Pseudophyllidea). J. Fish. Dis. 2:69–73.
- Stone DM, Haverbeke DR van, Ward DL, Hunt TA. 2007. Dispersal of nonnative fishes and parasites in the intermittent Little Colorado River, Arizona. Southwest. Nat. 52:130–137.

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**Appendix I. Other fishes ( $n = 256$ ) from same watersheds examined for *Bothriocephalus acheilognathi* and found to be negative.**

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**E. Flint Creek, Benton Co., AR ( $n = 76$ )**

- 1 *Ambloplites rupestris*
- 12 *Campostoma anomalum*
- 6 *Chrosomus erythrogaster*
- 4 *Cottus carolinae*
- 17 *Etheostoma spectabile*
- 17 *Luxilus cardinalis*
- 7 *Notropis boops*
- 4 *N. nubilis*
- 8 *Noturus exilis*

**Arkansas River, Desha Co., AR ( $n = 11$ )**

- 1 *Carpionodes velifer*
- 8 *Cyprinella venusta*
- 2 *Moxostoma erythrurum*

**Gulpha Creek, Garland Co., AR ( $n = 5$ )**

- 2 *Campostoma spadiceum*
- 3 *Fundulus catenatus*

**Rolling Fork River, Sevier Co., AR ( $n = 9$ )**

- 1 *Lepomis megalotis*
- 1 *Lythrurus umbratilis*
- 7 *Noturus gyrinus*

**Yashau Creek, McCurtain Co., OK ( $n = 155$ )**

- 17 *Ameiurus melas*
- 5 *A. natalis*
- 4 *Aphredoderus sayanus*
- 1 *C. spadiceum*
- 6 *Esox americanus*
- 2 *Etheostoma radiosum*
- 101 *Fundulus notatus*
- 2 *Lepomis cyanellus*
- 2 *L. gulosus*
- 1 *L. megalotis*
- 1 *L. umbratilis*
- 5 *Luxilus chrysocephalus*
- 8 *Notemigonus crysoleucus*

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***Salsuginus seculus* (Monogenoidea: Dactylogyrida: Ancyrocephalidae) from the Western Mosquitofish, *Gambusia affinis* (Cyprinodontiformes: Poeciliidae): New distributional records for Arkansas, Kansas and Oklahoma**

**Chris T. McAllister**

Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

**Donald G. Cloutman**

P. O. Box 197, Burdett, KS 67523

**Henry W. Robison**

Department of Biology, Southern Arkansas University, Magnolia, AR 71754

Studies on fish monogeneans in Oklahoma are relatively uncommon (Seamster 1937, 1938, 1960; Mizelle 1938; Monaco and Mizelle 1955; McDaniel 1963; McDaniel and Bailey 1966; Wheeler and Beverley-Burton 1989) with little or no published work in the past two decades or more. Members of the ancyrocephalid genus *Salsuginus* (Beverley-Burton) Murith and Beverley-Burton have been reported from various fundulid fishes including those from Alabama, Arkansas, Illinois, Kentucky, Nebraska, New York, Tennessee, and Texas, and Newfoundland and Ontario, Canada, and the Bahama Islands; additionally, two species have been reported from the Western Mosquitofish, *Gambusia affinis* (Poeciliidae) from California, Louisiana, and Texas, and the Bahama Islands (see Hoffman 1999).

To our knowledge, nothing has been published on any species of *Salsuginus* in Oklahoma. In Arkansas, two species were recently reported in fundulid fishes, including *S. umbraensis* (Mizelle) Murith and Beverley-Burton in Blackspotted Topminnow, *Fundulus olivaceus*,

and *S. fundulus* (Mizelle) Murith and Beverley-Burton in Northern Studfish, *Fundulus catenatus* (McAllister et al. 2015, 2016). In Kansas, a single species, *S. thalkei* Janovy, Ruhnke, and Wheeler (syn. *S. fundulus*) has been reported from Northern Plains Killifish, *Fundulus kansae* (see Janovy et al. 1989). Here, we report new distributional records for a species of *Salsuginus* in Arkansas, Kansas and Oklahoma.

During June 1983 (Kansas only) and again between April 2014 and September 2015, 36 Western Mosquitofish, *Gambusia affinis* were collected by dipnet, seine (3.7 m, 1.6 mm mesh) or backpack electrofisher from Big Spring at Spring Mill, Independence County, Arkansas ( $n = 4$ ; 35.828152°N, 91.724273°W), Rolling Fork River, Sevier County, Arkansas ( $n = 10$ ; 34.064701°N, 94.38003°W), Yashau Creek in Broken Bow, McCurtain County, Oklahoma ( $n = 21$ ; 34.011507°N, 94.749615°W), and Rattlesnake Creek, 10 km S of Macksville, Stafford County, Kansas ( $n = 1$ , 37.845555°N, 98.967777°W). Fish from Arkansas and Oklahoma were placed in

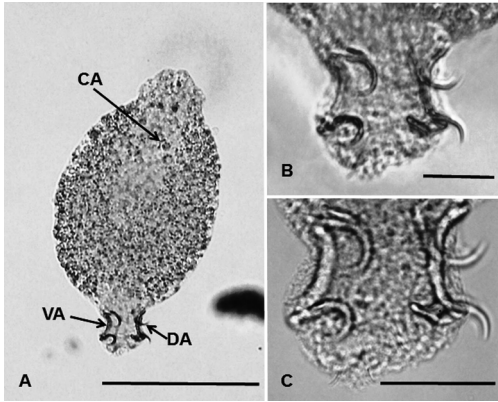


Figure 1. *Salsuginus seculus* from *Gambusia affinis* (HWML 101873). A. Entire specimen showing male copulatory apparatus (CA); dorsal anchor and dorsal bar (DA); ventral anchors and ventral bar (VA). Scale bar = 100  $\mu$ m. B. View showing marginal hooks on haptor. Scale bar = 20  $\mu$ m. C. Closer view showing marginal hooks. Scale bar = 20  $\mu$ m.

overdosed by immersion in a concentrated chloreto solution for approximately one hr and preserved in 10% formalin. The fish from Kansas was placed on ice for approximately one hr and preserved in 10% formalin. The gills of all the fish were examined under a stereomicroscope for monogeneans, and when found, they were picked with minuten nadeln directly from the gills. The parasites from Kansas were mounted in glycerin jelly and those from Arkansas and Oklahoma in Gray and Wess medium stained with Gomori's trichrome. Voucher specimens were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska as HWML 101873-101876. Host voucher specimens were deposited in the Henderson State University Collection (HSU), Arkadelphia, Arkansas.

containers with cool aerated water from their collection site and necropsied within 24 hr. We followed accepted guidelines for the use of fish in research (AFS, 2004); specimens were

Twenty-eight of 36 (78%) of the Western Mosquitofish were found to be infected with a monogenean matching the description of *S. seculus* (Mizelle and Arcadi, 1945) Murith and Beverley-Burton, 1985 (Fig. 1). These included two of four (50%, mean  $\pm$  1SD intensity = 4.0

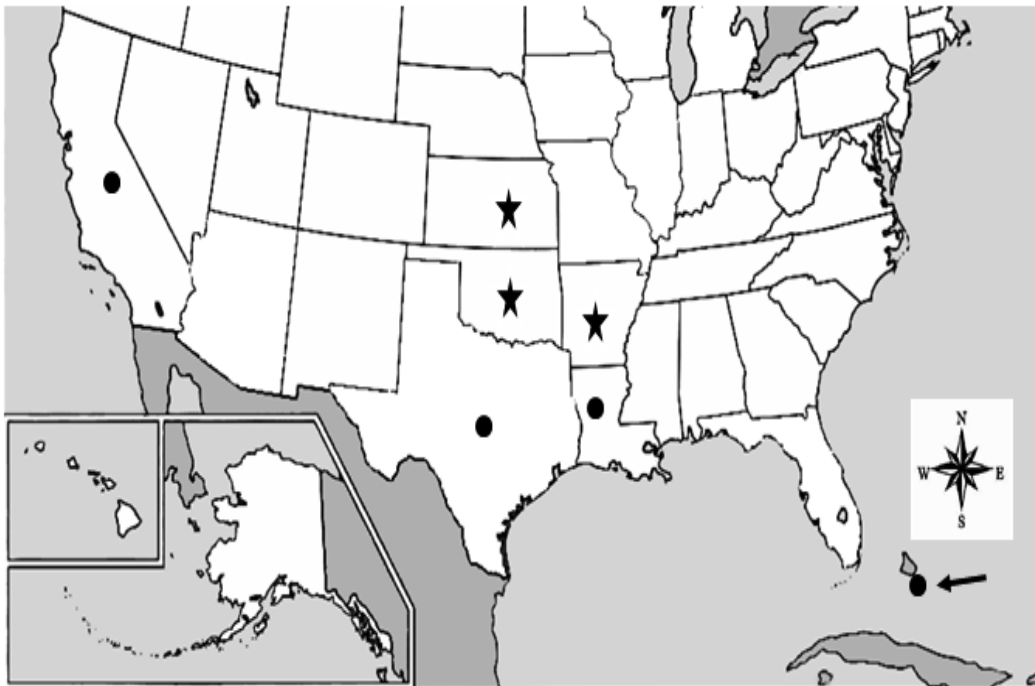


Figure 2. Records of *Salsuginus seculus* in the USA and the Bahamas (arrow). Dots = previous records; stars = new records.

± 1.4, range 3–5) from Big Spring at Spring Mill, Arkansas, eight of 10 (80%, 2.5 ± 0.8, 2–4) from the Rolling Fork River, Arkansas, 17 of 21 (81%, 5.3 ± 2.5, 1–14) from Yashau Creek, Oklahoma, and one of one (100%, 4 worms) from Rattlesnake Creek, Kansas.

This monogenean has previously been reported on *G. affinis* from California, Louisiana, Texas and the Bahama Islands (Mizelle and Arcadi 1945; Seamster 1948; Nowlin et al. 1967; Hanek and Fernando 1972; Meade and Bedinger 1972; Duobinis-Gray and Corkum 1985) (Fig. 2). In their revision of the monogenean genus *Salsuginus*, Murith and Beverley-Burton (1985) reported on previously unrecognized diversity and high host specificity within the genus due to difficulty in observing the weakly developed male copulatory apparatus. *Salsuginus seculus* appears to be monoxenous and widely distributed on *G. affinis*, including populations introduced outside its native range (Page and Burr 2011) in the Bahamas, California, and Kansas.

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We thank the Arkansas Game and Fish Commission and Oklahoma Department of Wildlife Conservation for Scientific Collecting Permits issued to CTM. We also thank Drs. Scott L. Gardner (HWML) and Renn Tumilson (HSU) for expert curatorial assistance and Nikolas H. McAllister (Lukfata Elementary, Broken Bow, OK) for assistance in collecting.

## References

- American Fisheries Society (AFS). 2004. Guidelines for the use of fishes in research [web application]. J. G. Nickum (Chair). American Fisheries Society, Bethesda, Maryland. [http://fisheries.org/docs/policy\\_useoffishes.pdf](http://fisheries.org/docs/policy_useoffishes.pdf). Accessed 2015 Aug. 1.
- Duobinis-Gray LF, Corkum KC. 1985. Monogenea (Platyhelminthes) of various freshwater fishes of Louisiana. Proc. Helminthol. Soc. Wash. 52:133–135.
- Hanek G, Fernando C. 1972. Monogenetic trematodes from New Providence Island, Bahamas. J. Parasitol. 58:1117–1118. Proc. Okla. Acad. Sci. 95: pp 44 - 45 (2015)
- Hoffman GL. 1999. Parasites of North American freshwater fishes. Second edition. Ithaca (NY): Comstock Publishing Associates. 539 p.
- Janovy J Jr, Ruhnke TR, Wheeler TA. 1989. *Salsuginus thalkeni* n. sp. (Monogenea: Ancyrocephalidae) from *Fundulus zebrinus* in the South Platte River of Nebraska. J. Parasitol. 75:344–347.
- McAllister CT, Bursey CR, Fayton TJ, Font WF, Robison HW, Connior MB, Cloutman DG. 2015. Helminth parasites of the Blackspotted Topminnow, *Fundulus olivaceus* (Cyprinodontiformes: Fundulidae), from the Interior Highlands of Arkansas. Journal of the Arkansas Academy of Science 69:(in press).
- McAllister CT, Bursey CR, Font WF, Robison HW, Trauth SE, Cloutman DG, Fayton TJ. 2016. Helminth parasites of the Northern Studfish, *Fundulus catenatus* (Cyprinodontiformes: Fundulidae) from the Ouachitas and Ozarks of Arkansas, U.S.A. Comparative Parasitology 83:(in press).
- McDaniel JS. 1963. Parasites from the genus *Lepomis* (Centrarchidae) in Lake Texoma, Oklahoma. Trans. Amer. Microsc. Soc. 82:423–425.
- McDaniel JS, Bailey HH. 1966. Parasites of Centrarchidae from Little River, Oklahoma. Trans. Kans. Acad. Sci. 69:45–47.
- Meade TG, Bedinger CA. 1972. Helminth parasitism in some species of fresh water fishes of eastern Texas. Southwest. Nat. 16:281–295.
- Mizelle JD. 1938. Comparative studies on trematodes (Gyrodactyloidea) from the gills of North American fresh-water fishes. Ill. Biol. Monogr. 17:1–81.
- Mizelle JD, Arcadi JA. 1945. Studies on monogenetic trematodes. XIII. *Urocleidus seculus*, a new species of Tetraonchinae from the viviparous top minnow, *Gambusia affinis affinis* (Baird and Girard). Trans. Amer. Microsc. Soc. 65:293–296.
- Monaco LH, Mizelle JD. 1955. Studies on monogenetic trematodes. XVII. The genus *Dactylogyrus*. Amer. Midl. Nat. 52:455–477.
- Murith D, Beverley-Burton M. 1985. *Salsuginus* Beverley-Burton, 1984 (Monogenea: Ancyrocephalidae) from Cyprinodontoides (Atheriniformes) in North America with



- descriptions of *Salsuginus angularis* (Mueller, 1934) Beverley-Burton, 1984 from *Fundulus diaphanus* and *Salsuginus heteroclitii* n. sp. from *F. heteroclitus*. Can. J. Zool. 63:703–714.
- Nowlin WJ, Price CE, Schlueter EA. 1967. First report of freshwater monogenetic trematodes of Texas. Tex. J. Sci. 19:110–111.
- Page LM, Burr BM. 2011. Peterson field guide to freshwater fishes of North America north of Mexico. Second edition. New York (NY): Houghton Mifflin Harcourt Publishing Company. 663 p.
- Seamster A. 1937. Gill trematodes of Oklahoma fishes. Proc. Okla. Acad. Sci. 18:13–15.
- Seamster A. 1938. Studies on gill trematodes from Oklahoma fishes. Amer. Midl. Nat. 20: 603–612.
- Seamster A. 1948. Gill parasites from Louisiana fishes, with a description of *Urocleidus wadei* n. sp. Amer. Midl. Nat. 39:165–168.
- Seamster A. 1960. A new species of *Dactylogyrus* from the silver chub. Sobr. Libro. Homen. Dr. Eduardo Caballero Caballero. Caballero Jub. vol, p. 269–270.
- Wheeler TA, Beverley-Burton M. 1989. Systematics of *Onchocleidus* Mueller, 1936 (Monogenea: Ancyrocephalidae): Generic revision. Can. J. Zool. 67:136–157.

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# Estimating Spawning Times of Alligator Gar (*Atractosteus spatula*) in Lake Texoma, Oklahoma

**Richard A. Snow**

Oklahoma Department of Wildlife Conservation, Oklahoma Fishery Research Laboratory, Norman, OK 73072

**James M. Long**

U.S. Geological Survey Oklahoma Cooperative Fish and Wildlife Research Unit, Department of Natural Resource Ecology and Management, Oklahoma State University, Stillwater, OK 74078

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**Abstract:** In 2013, juvenile Alligator Gar were sampled in the reservoir-river interface of the Red River arm of Lake Texoma. The Red River, which flows 860 km along Oklahoma's border with Texas, is the primary in-flow source of Lake Texoma, and is impounded by Denison Dam. Miniflyke nets were deployed using an adaptive random cluster sampling design, which has been used to effectively sample rare species. Lapilli otoliths (one of the three pair of ear stones found within the inner ear of fish) were removed from juvenile Alligator Gar collected in July of 2013. Daily ages were estimated by counting the number of rings present, and spawn dates were back-calculated from date of capture and subtracting 8 days (3 days from spawn to hatch and 5 days from hatch to swim-up when the first ring forms). Alligator Gar daily age estimation ranged from 50 to 63 days old since swim-up. Spawn dates corresponded to rising pool elevations of Lake Texoma and water pulses of tributaries. ©2015 Oklahoma Academy of Science

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## Introduction

The Alligator Gar (*Atractosteus spatula*) is the largest freshwater fish species in Oklahoma and the third largest in North America (Page and Burr 1991). Alligator Gar inhabit sluggish backwater areas in large rivers from Mexico to Florida (Miller and Robison 2004) and populations are declining thru much of their range (Robinson and Buchanan 1988, Etnier and Starnes 1993, Ferrara 2001). Alligator Gar is considered vulnerable in several states and has been extirpated from Ohio and Illinois (NatureServe 2012). Only Texas and Louisiana populations are considered stable (NatureServe 2012). Until recently, this species has received little attention from fisheries biologists who are just now beginning to understand their life history and develop management plans for conservation.

In its native range, Alligator Gar spawns from early April through the middle of June in conjunction with seasonal flooding events (Etnier and Starnes 1993, Ferrara 2001, Inebnit 2009). In Oklahoma, Alligator Gar are thought to spawn in early May (Miller and Robison 2004). Documented reports of Alligator Gar spawning are limited, but Oklahoma Department of Wildlife Conservation (ODWC) personnel videotaped a spawning event in Lake Texoma on 11 May, 2007. Spawning habitat is thought to consist mainly of flooded backwater areas and floodplains (Brinkman 2008, Inebnit 2009). On 16 June 1993, 21 age-0 Alligator Gar were collected from a large, shallow floodwater area below Robert S. Kerr Lock and Dam, and was thought to be the first evidence of reproduction of this species in Oklahoma since the early 1980s (Pigg and Gibbs 1996).

Because spawning seems to be related to seasonal flooding, spawning and recruitment may be inconsistent, accounting for the paucity of young Alligator Gar reported in the literature (May and Echelle 1968, Brinkman 2008).

In 2007 and 2008, multiple year classes of juvenile Alligator Gar were successfully collected from Lake Texoma during April through November using mini-fyke nets in back water areas and coves with woody vegetation and debris (Brinkman 2008). Intensive sampling in these habitats with mini-fyke nets may yield capture of significant numbers of juvenile Alligator Gar to provide insights into the role of spawning and early life history to recruitment. The ability to capture young-of-year gar would enable one to remove otoliths and estimate age (in days), calculate growth, and estimate spawning date. With this information, one can then look at environmental conditions and factors that may influence spawning.

## Methods

**Sampling site.**— Alligator Gar were sampled in the river-reservoir interface section of the Red River arm of Lake Texoma (Figure 1). The Red River flows 860 km along Oklahoma's border with Texas and is impounded by Denison Dam forming Lake Texoma. The Red River is a typical braided prairie river composed of sand and silt substrate with woody debris deposited from flooding events and occasional rock outcroppings. During high water events, the Red River reconnects to adjacent flood plains and cut-off oxbow lakes where Alligator Gar spawn.

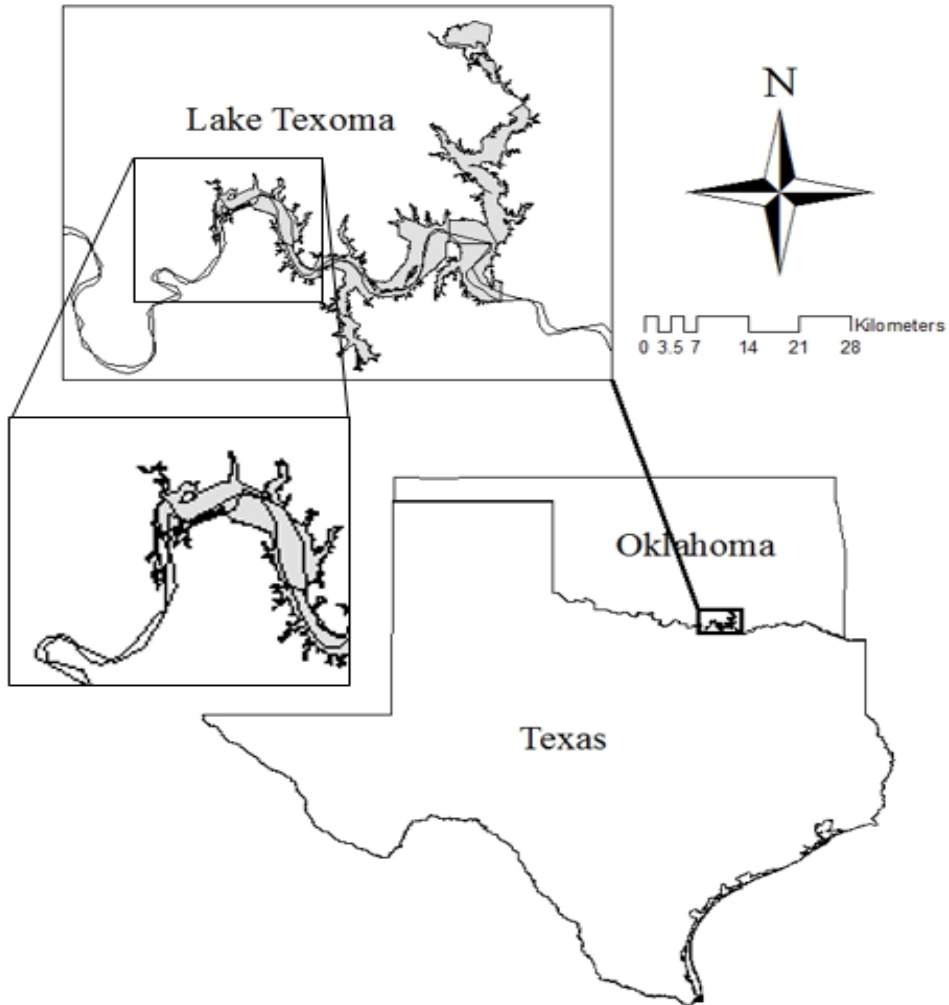
In the summer of 2013 mini-fyke nets with two different lead lengths ("short lead" 0.6 m x 6.35 m; with 3.18 mm mesh, 4.57 m lead, 0.6 m x 1.92 m rectangular cod, and "long lead" 510 mm metal throat and 0.6 m x 6.35 m; with 3.18 mm mesh, 9.14 m lead, 0.6 m x 1.92 m rectangular cod, and 510 mm metal throat) were used to sample age-0 Alligator Gar. Nets were set randomly in backwater areas and coves where herbaceous vegetation and woody debris are abundant (Brinkman 2008). Nets were deployed using an adaptive random cluster sampling

design (Tompson 1990). A 100-m gridded map of all backwaters and shallow-water coves in the river-reservoir interface was used to randomly select initial sample sites. When a juvenile Alligator Gar was collected, additional neighboring grid sites were subsequently sampled until no additional Alligator Gar were collected. All age-0 Alligator Gar collected were measured to the nearest mm, weighed to the nearest gm and the lapilli otoliths were removed for daily age estimation because they provided the most precise and accurate estimates of age compared to the other otolith pairs (Snow 2014).

Otoliths are calcium carbonate accretions associated with the inner ear of teleost fishes and are used for orientation and hearing (Mathiesen and Popper 1987). There are three pair of otoliths (lapillus, sagitta, and asteriscus) (Popper and Lu 2000) and they grow in relation to fish metabolism creating a permanent record of growth throughout the life of the fish. The ability to use otoliths as a technique to estimate fish age gives fisheries managers insight into population dynamics.

Otoliths were removed as described by Snow (2014), positioning the specimen dorsal side down under a dissecting scope and removing the head by making a cut just in front the pectoral girdle. Dissection pins were used to secure the head, allowing the lower jaw and gill structures were removed with forceps while the ventral side of the braincase was exposed. The parasphenoid was then detached to expose the inner ear structures, located just under the large bulbous portion of the parasphenoid. After removing the parasphenoid, brain matter was then removed from around the utricle structures to remove the lapilli otoliths.

Otoliths were cleaned and stored dry, then browned at 104°C on a hot plate to increase contrast between accretion and discontinuous zones (Secor et al.1992, Snow 2014). After browning, otoliths were embedded in Loctite 349 (Mauck and Boxrucker 2004, Snow 2014) for sectioning with a low speed IsoMet® saw (127 mm x 0.4 mm). Lapilli were sectioned in a transverse plane, mounted to glass microscope



**Figure 1.** Location of Lake Texoma in south central Oklahoma where age-0 Alligator Gar were sampled.

slides (3" x 1" x 1mm) with thermoplastic cement, and polished wet with 600-grit sandpaper. Immersion oil was applied to the otolith to enhance visibility of daily rings. Daily rings were counted independently by two readers (Hoff et al. 1997) using a high resolution monitor connected to an optic-mount digital camera attached to an Olympus BH-2 microscope. Back calculating to date of spawn was done by adding 8 days to the age estimated (3 days for hatching, 5 days to swim-up) and subtracting from capture date (Mendoza et al. 2002).

Linear regression analysis was used to

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determine relationships for Alligator Gar: 1) daily age and total length, 2) daily age and weight, and 3) total length and weight. Length and weight were log<sub>10</sub> transformed to correct for non-linearity. Pool elevation data for Lake Texoma at Denison Dam (U.S. Army Corps of Engineers) and river discharge for the Red River upstream of Lake Texoma (U.S. Geological Survey gage #07316000) were compared with back-calculated spawning dates for Alligator Gar.

## Results

Nine age-0 Alligator Gar (102 to 176 mm

TL) were collected during June and July of 2013 and were estimated to be between 50–63 days old since swim-up. Spawn dates were estimated to have occurred between May 18 to June 1, 2013. Lake Texoma pool elevation rose steadily during the estimated time of spawning, from 187 m on May 15 to 188 m on June 10, 2013 (Figure 2). Flow data from upstream in the

Red River recorded two pulse events coincident with the estimated time of spawning (Figure 3).

Based on the slope of the regression equation relating size to age, Alligator Gar captured in summer of 2013 grew an average of 5.49 mm ( $r^2 = 0.85, P < 0.01$ ; Figure 4) and 1.15g ( $r^2 = 0.82, P < 0.01$ ; Figure 5) per day. The

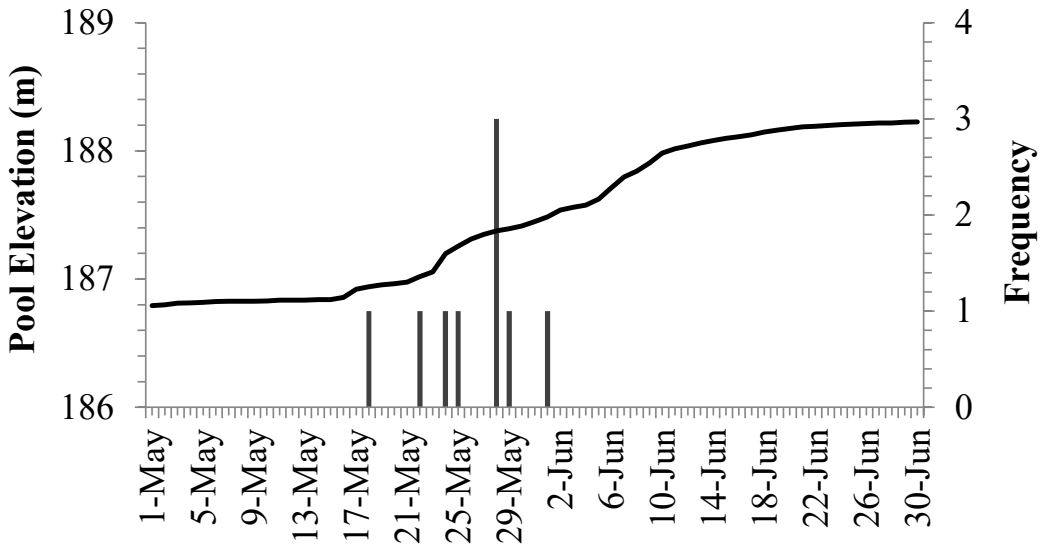


Figure 2. Frequency of back-calculated spawn dates in 2013 estimated from daily rings in lapilli otoliths of Alligator Gar in relation to pool elevation of Lake Texoma at Denison Dam, Oklahoma.

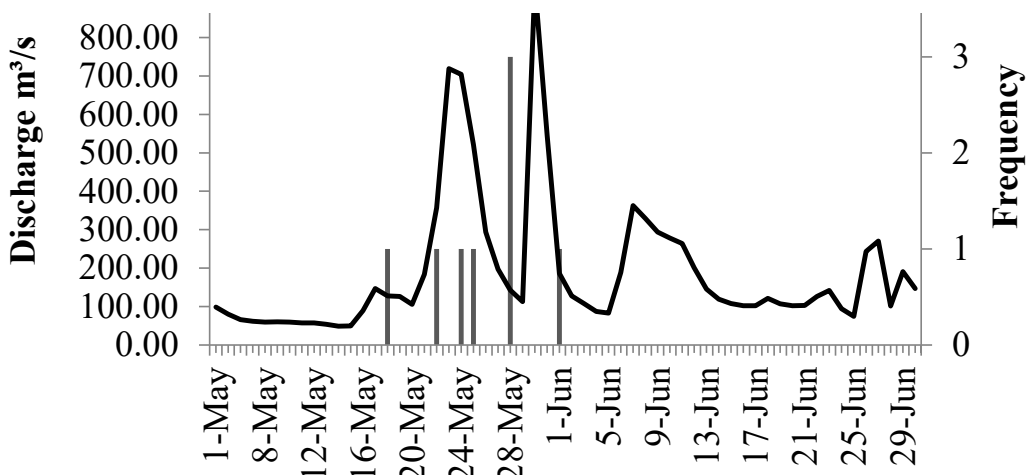


Figure 3. Frequency of back-calculated spawn dates in 2013 estimated from daily rings in lapilli otoliths of Alligator Gar in relation to discharge (USGS gage #07316000) of the Red River into Lake Texoma, Oklahoma.

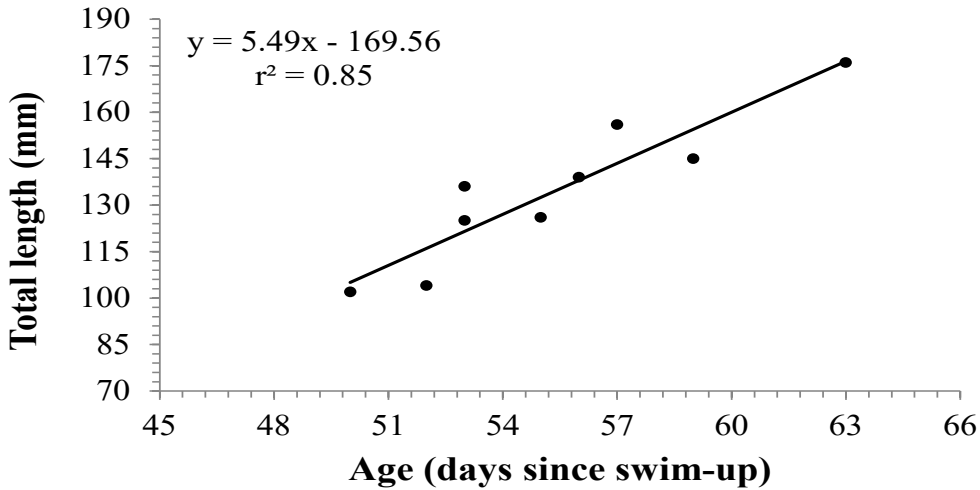


Figure 4. Relationship between total length and age of age-0 Alligator Gar captured from Lake Texoma, Oklahoma in July of 2013. Solid line is fit from linear regression.

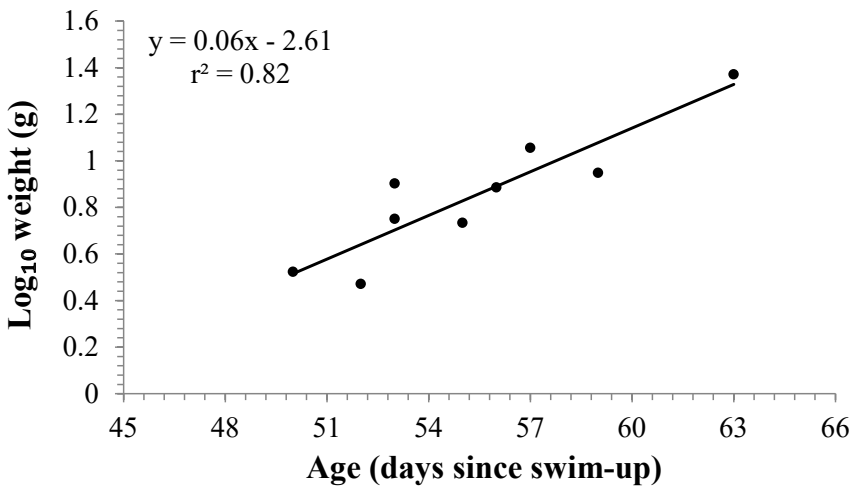


Figure 5. Relationship between  $\log_{10}$  weight and age for age-0 Alligator Gar captured from Lake Texoma, Oklahoma in July of 2013. Solid line is fit from linear regression.

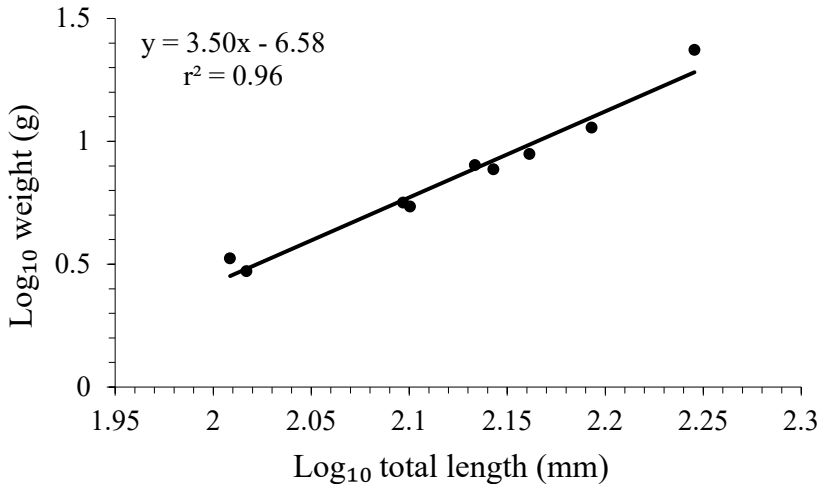
length-weight regression indicated a cubed relationship ( $\log_{10}$  weight (g) =  $3.50 (\log_{10}$  TL (mm) - 6.58;  $r^2 = 0.96$ ,  $P < 0.01$ ; Figure 6).

## Discussion

Collection of age-0 Alligator Gar proved difficult, with only 9 individuals captured out of 70 nights of netting. Developing a better sampling strategy would be beneficial. Anecdotally, we did catch more juvenile Alligator Gar in long-lead nets ( $N = 5$ ) compared to short-lead

nets ( $N = 2$ ) and two others were captured haphazardly, but we did not test for statistical differences. Additional studies on the utility of long-lead nets could prove beneficial. Longer leads allow for increased sampling range while still allowing the cod end to remain in shallow water where juvenile Alligator Gar occur.

The back-calculated spawn dates for wild-caught Alligator Gar corresponded to an increase in pool elevation and two pulses of water from the Red River, which has been generally



**Figure 6. Relationship between  $\log_{10}$  weight and  $\log_{10}$  length for age-0 Alligator Gar captured from Lake Texoma, Oklahoma in July of 2013. Solid line is fit from linear regression.**

accepted as their mode of reproduction. However, few observations of Alligator Gar spawning behavior and collections of age-0 fish exist in Lake Texoma, providing limited location-specific insight into reproduction of this species (May and Echelle 1968; Echelle and Riggs 1972; Brinkman 2008; Inebnit 2009). But, because Alligator Gar deposit eggs on vegetation, where they adhere and juvenile gar develop over a period of days (Aguilera et al. 2002; Balfour and Parker 1882), water level history in Lake Texoma provides some insight. Lake Texoma water storage in general has been reduced through sedimentation (Patton and Lyday 2008) and persistent drought since 2011 kept water levels low in Lake Texoma until early 2013, allowing herbaceous vegetation to establish in back-water areas. Subsequent inundation of these areas created attachment substrate for Alligator Gar eggs and larvae. The cycle of drying, encroachment of terrestrial vegetation, and subsequent flooding should be examined in future research related to spawning and recruitment of Alligator Gar in reservoirs.

Fish spawning activity may be limited in some ageing reservoirs due to sedimentation or poor quality of availability sites (Summerfelt, 1993); this is particularly important to nest building species. However, siltation and fragmentation caused by sedimentation in an

ageing reservoir create habitats in the river-reservoir interface that function as a floodplain (Buckmeier et al. 2014; Patton and Lyday 2008) as an alternative for spawning habitat that was lost due to original impoundment (Buckmeier et al. 2014). For some native species, such as Alligator Gar, this habitat is critical for completing their life cycle (Graebs et al. 2009). The ageing of reservoirs may actually benefit Alligator Gar because the floodplains that were originally lost through impoundment of the watershed may now be coming back through a prolonged process of sedimentation. However, future research on the potential influence of these artificial habitats on spawning success of Alligator Gar in the river-reservoir interface would be required to test this hypothesis.

Understanding that Alligator Gar spawning coincides with rises in reservoir pool elevation and pulses of water from inflowing rivers can be used for management purposes. Because Alligator Gar spawning is linked to seasonal flooding of riparian vegetation, successful recruitment may be infrequent. Alligator Gar has the potential to overcome year-class failures because of late maturity, high fecundity, and long life span (Ferrara 2001; Buckmeier 2008; Inebnit 2009). Reservoir water levels could, in theory, be manipulated to allow re-vegetation of backwaters. Subsequent flooding during the

Alligator Gar spawning season might enhance recruitment. Water levels would need to be maintained for at least 8 days after spawning to allow Alligator Gar to reach the lecithoexthrophic stage (i.e., swim-up) when larvae are no longer attached to vegetation (Inebnit 2009). How this strategy would fit with management of other species (e.g., Largemouth Bass [*Micropterus salmoides*] or Striped Bass [*Morone saxatilis*]) is unknown, but it appears that this level of management could be one aspect to consider within the fisheries management plans developed for impoundments such as Lake Texoma.

## Acknowledgments

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## References

- Aguilera, C., R. Mendoza, G. Rodriguez, and G. Marquez. 2002. Morphological description of Alligator Gar and Tropical Gar larvae, with an emphasis on growth indicators. *Tran Am Fish Soc* 131:899-909.
- Balfour F. M. and W. N. Parker. 1882. On the structure and development of *Lepisosteus*. *Phil Tran Royal Soc London* 178:359-442.
- Brinkman, E.L. 2008. Contributions to the life history of Alligator Gar, *Atractosteus spatula* (Lacepede), in Oklahoma [MSc thesis]. Stillwater (OK): Oklahoma State University
- Buckmeier, D. L. 2008. Life history and status of alligator gar *Atractosteus spatula*, with recommendations for management. TPWD Inland Fisheries Report, Heart Hills Fish Sci Cen (2008).
- Buckmeier, D.L., N.G. Smith, B. P. Fleming and K. A. Bodine. 2014. Intra-annual variation in river-reservoir interface fish assemblages: implications for fish conservation and management in regulated rivers. *River Res Applic* 30:780-790.
- Echelle, A. A. and C. D. Riggs. 1972. Aspects of the early life history of gar (*Lepisosteus*) in Lake Texoma. *Tran Am Fish Soc* 101:106-112.
- Etnier, D. A. and Starnes, W.C. 1993. Alligator Gar. In: *The fishes of Tennessee*. Knoxville, (TN): University of Tennessee Press 109-110 p.
- Ferrara, A.M. 2001. Life-history strategy of Lepisosteidae: implications for the conservation and management of Alligator Gar [DRc Dissertation]: Auburn (AL): Auburn University.
- Greab, B.D.S, D.W Willis, and B.D. Spindler. 2009. Shift in sauger spawning locations after 40 years of reservoir ageing: influence of a novel delta ecosystem in the Missouri River, USA. *River Res Appl* 25:153-159
- Hoff, G. R., D. J. Logen, and M. F. Douglas. 1997. Otolith morphology and increment validation in young Lost River and Shortnose suckers. *Tran Am Fish Soc* 126:488-494
- Inebnit III, T. E. 2009. Aspects of the reproductive and juvenile ecology of Alligator Gar in the Fourche LaFave River, Arkansas [MSc thesis]. Conway (AR): University of Central Arkansas
- Mathiesen, C and A.N. Popper. 1987. The ultrastructure and innervation of the ear of the Gar, *Lepisosteus osseus*. *J Morphol* 194:129-142.
- Mauck, P. and J. Boxrucker. 2004. Abundance, growth, and mortality of the Lake Texoma Blue Catfish population: implications for management. *Proc of the Sou Ass of Fish and Wild Ag* 58: 57-65.
- May, E. B. and A. A. Echelle. 1968. Young-of-year Alligator Gar in Lake Texoma, Oklahoma. *Copeia* 629-630 p.
- Mendoza, R., C. Aguilera, G. Rodriguez, M. Gonzalez, and R. Castro. 2002. Morphophysiological studies on Alligator Gar (*Atractosteus spatula*) larval development as a basis for their culture and repopulation of their natural habitats. *Fish Bio and Fish* 12:133-



142.

- Miller, R.J. and Robison, H.W. 2004. Alligator Gar. In: *Fishes of Oklahoma*. Norman (OK): University of Oklahoma Press 52-53 p.
- NatureServe. 2012. NatureServe Explorer: An online encyclopedia of life [web application]. Version 7.1. NatureServe, Arlington, Virginia. Available <http://www.natureserve.org/explorer>. (Accessed: Oct 13, 2012).
- Page, L.M. and Burr, B.M. 1991. A field guide to freshwater fishes. Boston, Massachusetts: Houghton Mifflin Company 432 p.
- Patton, T. and C. Lyday. 2008. Ecological succession and fragmentation in a reservoir: effects of sedimentation and habitats on fish communities. Pg 147-167. In M.S. Allen, S. Sammons, and M.J. Maceina [ed.] *Balancing fisheries management and water uses for impounded river systems*. Am Fish Soc, Sym 62, Bethesda, Maryland.
- Pigg, J. and Gibbs, R. 1996. Observations on the propagation of two rare fish species in Oklahoma. *Proc Okla Acad of Sci* 76: 89.
- Popper, A.N. and Z. Lu. 2000. Structure-function relationships in fish otolith organs. *Fisheries Research* 46:15-25.
- Robison, H.W. and Buchanan, T.M. 1988. *Fishes of Arkansas*. Fayetteville (AR): University of Arkansas Press 536 p.
- Secor, D. H., J. M. Dean, and E. H. Laban. 1992. Otolith removal and preparation for microstructural examination. Pg 19-57. In D. K. Stevenson and S. E. Campana [ed.] *Otolith microstructure examination and analysis*. Can Sp Pub Fish Aqua Sci 117 p.
- Snow, R.A. 2014. Documenting utility of otoliths for estimating age and spawning times of Alligator Gar (*Atractosteus spatula*) [MSc thesis] Stillwater, OK Oklahoma State University.
- Summerfelt, R.C., 1993. Lake and Reservoir Habitat Management. In: *Inland Fisheries Management in North America* (eds C.C. Kohler and W.A. Hubert), Bethesda, Maryland, USA: Am Fish Soc.
- Tompson, S. K. 1990. Adaptive cluster sampling. *Jou Am Stat Ass* 85:1050-1059.

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# Microhabitat Preferences of a Small Mammal Assemblage in Canadian County, Oklahoma

**Sophia Hackney**

Oklahoma City University, Department of Biology, Oklahoma City, OK 73106

**Anthony J Stancampiano**

Oklahoma City University, Department of Biology, Oklahoma City, OK 73106

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**Abstract:** We conducted a small mammal ecological study at John Nichols Scout Ranch in Southeastern Canadian County, Oklahoma, during the summers of 2013 and 2014. Microhabitat preferences of *Peromyscus leucopus*, *P. maniculatus*, *Neotoma floridana*, and *Sigmodon hispidus* were determined using species' presence/absence and 60 habitat variables at 10 randomly selected plots. These data were subjected to principle components analysis and niche overlap. *Peromyscus leucopus* and *N. floridana* occupy similar woodland areas, featuring high degrees of litter and overhang canopy, as well as rocky outcrops. *Peromyscus maniculatus* was associated with areas of annual, barren, shrub, and tree coverage, with adjacent habitat heterogeneity—an edge. *Sigmodon hispidus* was found in open grasslands with homogeneous adjacent habitat. *Peromyscus leucopus* and *N. floridana* had the highest degree of niche overlap, while *P. maniculatus* had a smaller overlap with these species. *Sigmodon hispidus* had the lowest degree of niche overlap among all studied species, indicating high habitat specificity. ©2015 Oklahoma Academy of Science

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## Introduction

Rodentia comprises approximately one-third of all known mammalian species. These highly successful organisms have evolved to fill a variety of niches in most terrestrial habitats (Witmer 2004). The activities of rodent populations heavily influence both the biotic and abiotic components of their ecosystem, such as vegetation composition/dispersal, and the populations of higher-level consumers; as well as nutrient cycling and soil weathering (Beard et al. 2013, Galiano et al. 2014; Ims et al. 2013; Pavey et al. 2008). Rodents are vital to the health of terrestrial ecosystems. Like plants, rodents serve as a base that sustains the complex web of ecosystem interactions (Avenant 2011; Jones 2010). The characterization and comparison of rodent species' habitat affinities is essential to understand fundamental ecosystem interactions. Establishing rodent microhabitat preferences can yield information about these interactions, which may be useful to urban developers and wildlife managers as a tool for conservation.

*Peromyscus maniculatus* (deer mouse) is the most widely distributed species of the genus *Peromyscus*, and has been documented living in both grassland and forested areas (Clark et al. 2000; Hall 1981; Whitaker 1980). Lack of habitat correlation has been reported in many studies (Gore 1988; Marinelli and Millar 1989; Maser et al. 1981); indicating that *P. maniculatus* is successful in multiple habitat types. Local studies in Oklahoma have found populations of *P. maniculatus* primarily in tall grass prairies (Drabek 1977; Stancampiano and Schnell 2004). A related species, *P. leucopus* (white-footed mouse), may also be found in a variety of habitat types (Clark et al. 2000; Stancampiano and Schnell 2004), but is positively correlated with vertical vegetation complexity, overhang cover, snags and other forms of litter, and rocky outcrops (Kaufman et al. 1983; M'Closkey 1975).

*Sigmodon hispidus* (cotton rat) is a dominant species in tall grass prairies across the southern United States and Mexico (Bradley et al. 2008; Drabek 1977). Regional studies in Oklahoma and Texas concluded that *S. hispidus* prefers

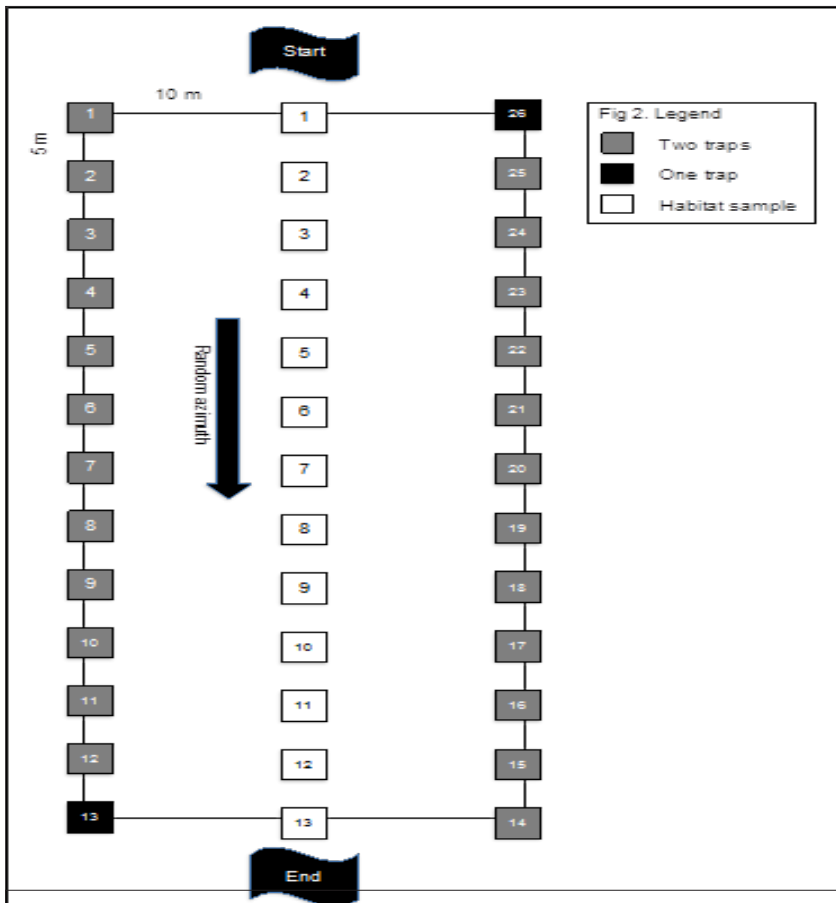
homogeneous grassy habitats (Cameron and Spencer 2008; Stancampiano and Schnell 2004). *Neotoma floridana* (eastern woodrat) is traditionally classified as a woodland species (Clark et al. 2000; Stancampiano and Schnell 2004). Many subspecies populations are rapidly decreasing due to high habitat specificity coupled with habitat disturbance (McCleery et al. 2006).

**Methods**

*Study area*— John Nichols Scout Ranch (97-ha) is located in extreme southeastern Canadian County in Central Oklahoma (35.349987 N, -97.672389 W). The Canadian River traverses the southern border of the property. The site features a diversity of habitats—including riparian, prairie, seasonally flooded creeks, rocky bluffs, and temperate forests—with

various levels of human influence. Based on satellite imagery, we determined the study site to consist of 70% forested and 30% grassland areas. This proportion was replicated in 10 randomly selected study plots (*Appendix A*). To avoid bias, the coordinates for the trap sites were selected via random.org.

*Sampling*—Trapping took place each summer of 2013 and 2014. Each 650-m<sup>2</sup> trapping plot featured two parallel Sherman trap lines, spaced 10 m from the longitudinal axis of the plot, following a random azimuth (random.org). Trap stations in each line were spaced 5 m apart. Trap sets were laid at stations 1-12 and 14-25; individual traps were laid at stations 13 and 26, for a total of 50 traps per site (*Fig. 1*). Each trap was baited with rolled oats and peanut butter. The traps were checked for two consecutive



**Figure 1. Orientation of the trap and habitat sampling lines in each plot.**

**Table 1. Description of the 60 microhabitat variables, and their associated eigenvalues (as determined by the Principle Components Analysis). Vectors with absolute values of  $\geq 0.7$  considered significant. Some habitat variables are described as the percentage of the total ground cover within the quadrat. Other habitat variables are the measured distance of vertical vegetation within the sample, and also horizontal distances relative to the quadrat.**

Variable	Description	Eigenvectors	
		Component I	Component II
CN00-20	0-20% no cover	<b>0.7005</b>	<b>0.7129</b>
CN21-40	21-40% no cover	0.2374	<b>-0.9672</b>
CN41-60	41-60% no cover	<b>-0.7953</b>	0.6050
CN61-80	61-80% no cover	0.3868	<b>-0.9206</b>
CN81-100	81-100% no cover	<b>0.8870</b>	0.2413
CA00-20	0-20% annual cover	<b>0.9532</b>	0.3023
CA21-40	21-40% annual cover	<b>0.9749</b>	-0.1973
CA41-60	21-60% annual cover	<b>-0.7418</b>	0.6691
CA61-80	61-80% annual cover	-0.4723	<b>-0.8783</b>
CA81-100	81-100% annual cover	<b>-0.7980</b>	-0.5868
CL00-20	0-20% litter cover	<b>-0.9879</b>	-0.1541
CL21-40	21-40% litter cover	<b>-0.8452</b>	0.5320
CL41-60	41-60% litter cover	<b>0.9480</b>	0.2857
CL61-80	61-80% litter cover	<b>0.9954</b>	-0.0596
CL81-100	81-100% litter cover	<b>0.9511</b>	0.2900
CR00-20	0-20% rock cover	<b>0.9500</b>	0.3062
CR21-40	21-40% rock cover	<b>0.9535</b>	0.3014
CR41-60	41-60% rock cover	<b>0.9401</b>	0.3109
CR61-80	61-80% rock cover	<b>0.9403</b>	0.3109
CR81-100	81-100% rock cover	<b>0.9401</b>	0.3109
CS00-20	0-20% shrub cover	<b>0.9510</b>	0.2899
CS21-40	21-40% shrub cover	<b>0.9401</b>	0.3109
CT00-20	0-20% tree cover	<b>0.9935</b>	-0.0905
CT21-40	21-40% tree cover	<b>0.9525</b>	0.2927
CT41-60	41-60% tree cover	<b>0.8870</b>	0.2413
COC00-20	0-20% overhang canopy cover	-0.0015	<b>-0.9993</b>
COC21-40	21-40% overhang canopy cover	<b>0.9515</b>	0.3048
COC41-60	41-60% overhang canopy cover	<b>0.8966</b>	-0.4289
COC61-80	61-80% overhang canopy cover	<b>0.9927</b>	-0.1011
COC81-100	81-100% overhang canopy cover	<b>0.9495</b>	0.3066
COC00-20	0-20% overhang canopy cover	<b>0.9401</b>	0.3109
CM00-20	0-20% moss cover	<b>0.9401</b>	0.3109
CM21-40	21-40% moss cover	-0.1744	<b>-0.9831</b>
MHA00-02	0-2 dm mean annual height	-0.7223	0.6912

MHA02-04	2-4 dm mean annual height	<b>0.9744</b>	-0.2220
MHA04-06	4-6 dm mean annual height	<b>0.9844</b>	-0.1760
MHA06-08	6-8 dm mean annual height	<b>0.7708</b>	-0.6271
MHA08-10	8-10 dm mean annual height	<b>0.9401</b>	0.3109
MHS00-05	0-5 dm mean shrub height	0.3427	<b>-0.9281</b>
MHS05-10	6-10 dm mean shrub height	<b>0.9537</b>	0.3009
MHS11-20	11-20 dm mean shrub height	<b>0.9401</b>	0.3109
MHT00-15	0-15 dm mean tree height	<b>0.8729</b>	-0.4608
MHT16-40	16-40 dm mean tree height	<b>0.9535</b>	0.3014
MHT41-85	41-85 dm mean tree height	<b>0.9359</b>	0.3120
VegA	Annuals are major vegetation	<b>-0.9226</b>	0.3761
VegT	Trees are major vegetation	<b>0.9163</b>	-0.3884
VegS	Shrubs are major vegetation	<b>0.9198</b>	-0.3923
VegN	No major vegetation	<b>0.8870</b>	0.2413
DHA00-10	0-10 m distance to human activity	<b>0.9474</b>	0.3080
DHA11-20	11-20 m distance to human activity	<b>0.9435</b>	0.3098
DHA21-30	21-30 m distance to human activity	<b>0.9439</b>	0.3097
DHAgt30	>30 m distance to human activity	<b>-0.9808</b>	-0.1471
DDH00-10	0-10 m distance to different habitat	0.3669	<b>-0.9295</b>
DDH11-20	11-20 m distance to different habitat	<b>-0.7052</b>	<b>0.7089</b>
DDH21-30	21-30 m distance to different habitat	-0.1201	<b>0.9926</b>
DDHgt30	>30 m distance to different habitat	0.2867	<b>0.9503</b>
DT00-05	0-5 m distance to closest tree	<b>0.7666</b>	-0.6415
DT06-10	6-10 m distance to closest tree	-0.6362	<b>0.7714</b>
DT11-15	11-15 m distance to closest tree	<b>-0.7509</b>	0.6600
DT16-20	16-20 m distance to closest tree	<b>-0.7953</b>	0.6050
DT26-30	26-30 m distance to closest tree	<b>-0.7953</b>	0.6050

**Table 2. Small mammal niche overlap using Horn's and Pianka's indexes of niche overlap.**

Horn's Index	<i>P. l</i>	<i>N. f</i>	<i>S. h</i>	<i>P. m</i>
<i>P. leucopus</i>	1.0			
<i>N. floridana</i>	0.995	1.0		
<i>S. hispidus</i>	0.622	0.627	1.0	
<i>P. maniculatus</i>	0.852	0.828	0.703	1.0

Pianca's Index	<i>P. l</i>	<i>N. f</i>	<i>S. h</i>	<i>P. m</i>
<i>P. leucopus</i>	1.0			
<i>N. floridana</i>	0.994	1.0		
<i>S. hispidus</i>	0.618	0.629	1.0	
<i>P. maniculatus</i>	0.890	0.869	0.729	1.0

mornings, for a total of 2,000 trap nights. We identified, determined the sex of, weighed, and then released collected individuals. The majority of habitat sampling took place each summer from 2013-2014 (site 10 was sampled in the fall of 2013). Habitat samples were collected every 5 m using 1-m<sup>2</sup> quadrats, for a total of 13 samples per site. Each habitat sampling line ran directly down the middle of each plot, paralleling the trap lines. A total of 105 habitat variables, variations of biotic and abiotic components within and adjacent to the 1-m<sup>2</sup> quadrat, such as percentage of ground rock cover or distance to nearest tree, were measured in each sample (only 60 variables were considered for analysis) (Table 1).

## Results

We collected a total of 88 small mammals, representing 4 different species: *P. leucopus* (60), *P. maniculatus* (1), *N. floridana* (23), *S. hispidus* (2), and unknown (2). The two unknown mammals were assumed to be *P. maniculatus*, however the handling time with these organisms was insufficient to make a positive identification.

*Principle Components Analysis*— We calculated the mean value of each microhabitat variable at each plot to represent habitat structure. These data were then aligned with species' presence within each given plot. This data matrix was then subjected to a principle components analysis (PCA). The PCA produced 60 variables with eigenvectors (Table 1). Only vectors with absolute values of  $\geq 0.7$  were used in the subsequent analysis.

Components I and II accounted for 98.45% of the data variation. Therefore, the third component was not included in further analysis. Component I accounted for 70.63% of microhabitat preference variation. This component represents an overall gradient of open, grassy habitats without larger vegetation, to denser forests with a high degree of litter cover. Component II accounted for 27.82% of the variation, and represented a gradient from heterogeneous adjacent habitat with shrub presence to homogeneous adjacent habitat without shrubs.

*Niche Overlap*—The data matrix was subjected to Pianka's and Horn's indexes of niche overlap (Horn 1966; Pianka 1974). *Peromyscus leucopus* and *N. floridana* had a high degree of niche overlap in both models. The niche overlap between *P. leucopus* and *P. maniculatus* was found to be lower than the overlap between *N. floridana* and *P. leucopus* in both models. Overall, *S. hispidus* had the lowest overlap with other species (Table 2).

## Discussion

The projections of *P. leucopus* and *N. floridana* were closely clustered in quadrant I, indicating that these species have similar habitat requirements (Fig. 2). The other two species showed very different habitat preferences, and were located in quadrant III (*P. maniculatus*), and quadrant II (*S. hispidus*).

In this study, *P. leucopus* and *N. floridana* were found in areas with high litter and overhang canopy cover; low annual and shrub cover; some exposed rock, moss, and/or barren ground coverage; and dense tree coverage. These habitat characteristics are typical of a shaded forest. The species' presence with low annual cover, shrubs, moss, and rock may indicate that *P. leucopus* and *N. floridana* prefer aged forests— with sufficient levels of moisture and complex vertical structure. *Peromyscus leucopus* and *N. floridana* were trapped in plots featuring steep inclines and sandstone bluffs, which may also explain the species' association with barren ground and rock coverage. This habitat characterization of *N. floridana* is consistent with many studies (Clark et al. 2000; Kaufman et al. 1983; Stancampiano and Schnell 2004). *Peromyscus leucopus* has been documented in a variety of habitats, including prairies, swamps, and rocky areas; however, when studies were conducted over multiple habitat types, *P. leucopus* was more prevalent in wooded areas (Clark et al. 2000; Stancampiano and Schnell 2004). In his 2007 thesis, Sato calls *P. leucopus* a habitat generalist in woodlands, but a habitat specialist in other areas (Sato 2007). The niche overlap of 0.994 and 0.995 between *P. leucopus* and *N. floridana* indicate similar

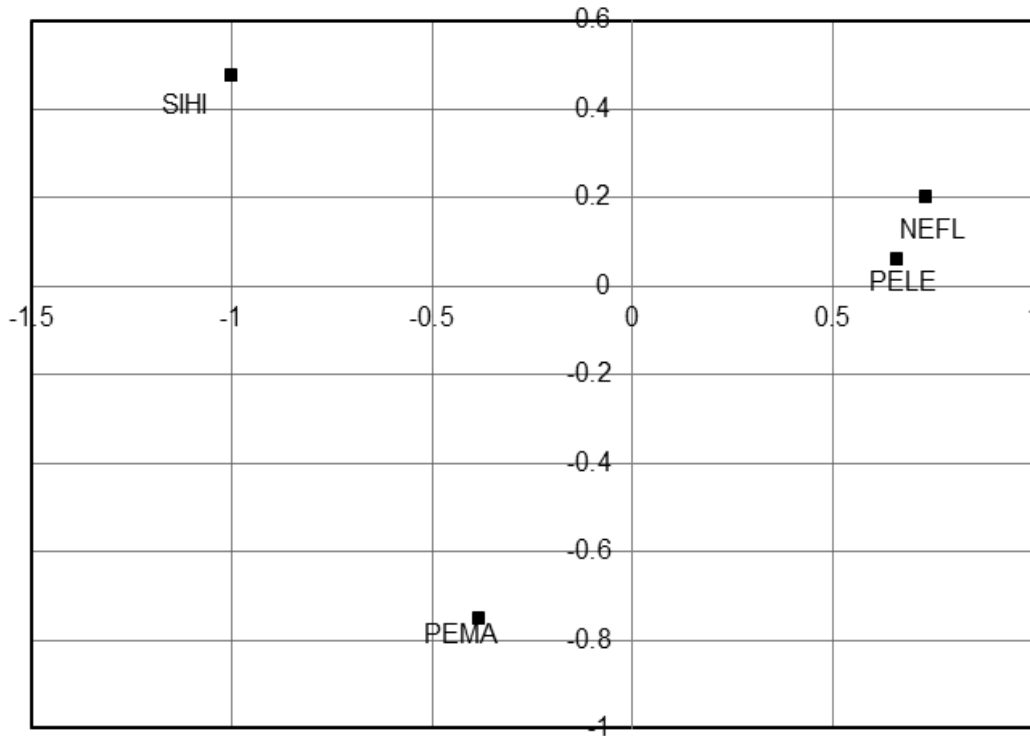


Figure 2. Projections of *P. leucopus*, *P. maniculatus*, *N. floridana*, and *S. hispidus* onto the principle components of microhabitat variable variation.

Fig. 2 Legend

NEFL= *N. floridana* *P. maniculatus*= PEMA

PELE= *P. leucopus* *S. hispidus*= SIHI

microhabitat preferences, and therefore support the PCA results. It is also interesting to note that every successful trapping plot featured both of these species; in other words, one species was never observed without the other in this study. This degree of association and niche overlap may suggest limited competitive interactions between the two species (Kaufman et al. 1983).

*Peromyscus maniculatus* was associated with an edge habitat, featuring some shrubs and barren ground; many annuals; and mid-distant trees with little overhang canopy. This is in contrast to other Oklahoma studies, which characterized *P. maniculatus* as a grassland species. However, a lack of habitat correlation has been described for this species in several studies, indicating that *P. maniculatus* is successful in many habitat

types (Gore 1988; Maser et al. 1981). This species was only trapped at site 8; therefore the habitat affinities attributed to this species may be particular to the trapping location. Although no other trapping plot, for this analysis, featured a gradual edge. Interestingly, the niche overlaps between *P. maniculatus* and *P. leucopus* (sympatric species) was lower than the niche overlaps between *P. leucopus* and *N. floridana* (Table 2). For our current trapping season, three new trapping locations were added to validate habitat affinities. One of the three new locations includes a similar grassland-to-forest gradient that *P. maniculatus* was previously documented in. Despite being similar in horizontal and vertical structure, the sites differ in humidity and vegetation profile. Trap site 8 is extremely arid, with its dried grasslands featuring cacti, as compared to the new location, which has lush annual coverage. The second new site is a light forest with pockets of shaded clearings dominated by small shrubs and annuals.

The other new location is described below.

*Sigmodon hispidus* was associated with open grasslands featuring high annual coverage, little litter coverage, and some degree of barren ground. This species was also associated with the presence of homogeneous landscape and mid-distant trees. Only two *S. hispidus* were trapped at one location; the relatively low trapping rate of this species was most likely due to the fact that all grassland plots had moderate-heavy levels of human activity (*Appendix A*). During the current trapping season, we trapped two *S. hispidus* at this location again. Our low trapping success of the species may indicate a low population at the study area. Because *S. hispidus* was only captured at site 8, our attribution of the species' habitat specialty may be due to the landscape features of the trapping site alone. Despite this, *S. hispidus* was not trapped in any non-clearing areas, and has been well documented as a grassland species (Bradley et al. 2008; Cameron and Spencer 2008; Drabek 1977; Stancampiano and Schnell 2004). In both models of niche overlap, *S. hispidus* had the lowest degree of similarity to all species of this study, indicating entirely different habitat affinities. To confirm the habitat affinities of *S. hispidus*, a new grassland site was added to the current trapping season. This new site has an adjacent harsh forest edge to the west and about 20-30 m of habitat homogeneity to the east; this site also includes a relatively unused path for vehicles.

The characterization of small mammal microhabitat preferences is important to understanding fundamental ecosystem interactions. As human influence continues to expand into natural habitats, it is important to have preservation as a goal. The goal of ecological studies, such as this one, is to understand how organisms interact with their environment; then to use this knowledge to maintain, restore, and prevent harm to ecosystems.

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## References

- Avenant N. 2011. The potential utility of rodents and other small mammals as indicators of ecosystem "integrity" of South African Grasslands. *Wildlife Res.* 38(7): 626-639.
- Beard K, Faulhaber CA, Howe FP, Edwards TC. 2013. Rodent-mediated interactions among seed species of differing quality in a shrubsteppe ecosystem. *West N Am Naturalist.* 73(4):426-441.
- Bradley RD, Hensen DD, Durish ND. 2008. Re-evaluation of the geographic distribution and phylogeography of the *Sigmodon hispidus* complex based on mitochondrial DNA sequences. *Southwestern Naturalist.* Available from: <http://www.biomedsearch.com/article/Re-evaluation-geographic-distribution-phylogeography/186998757.html> (Accessed February 2, 2014).
- Cameron GN, Spencer SR. 2008. Mechanisms of habitat selection by the hispid cotton rat (*Sigmodon hispidus*). *J Mammal.* 89(1):126-131.
- Clark BK, Kaufman DW, Kaufman GA. 2000. Small mammals in native and anthropogenic habitats in the Lake Wilson area of north-central Kansas. *Southwest Nat.* 45(1):45-60.
- Drabek CM. 1977. Some population aspects of cricetid rodents in a central Oklahoma tall grass prairie. *Proc Okla Acad Sci* 57:25-28.
- Galiano D, Kubiak BB, Overbeck GE, Thales, RO. 2014. Effects of rodents on plant cover, soil hardness, and soil nutrient content: a case study on tucos-tucos (*Ctenomys minutus*). *Acta Theriol.* 59(4):583-587.
- Gore JA. 1988. Habitat structure and the distribution of small mammals in a northern hardwoods forest. *Management of amphibians, reptiles, and small mammals in North America: Proceedings of the symposium; 1988 July 19-21; Flagstaff, AZ. Gen. Tech. Rep. RM-166.* Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment. Available from: [http://www.fs.fed.us/rm/pubs\\_rm/rm\\_gtr166/rm\\_gtr166\\_319\\_327.pdf](http://www.fs.fed.us/rm/pubs_rm/rm_gtr166/rm_gtr166_319_327.pdf) (Accessed February



- 7, 2015).
- Hall RE. 1981. The mammals of North America. 2nd ed. Vol. 2. New York (NY): John Wiley and Sons. 127 p.
- Horn HS. 1966. Measurement of "overlap" in comparative ecological studies. *Am Nat.* 100(1):419-424.
- ImsRA, HendenJA, ThingnesAV, KillengreenST. 2013. Indirect food web interactions mediated by predator-rodent dynamics: relative roles of lemmings and voles. *Biol Lettters*. Available from: <http://rsbl.royalsocietypublishing.org/content/9/6/20130802> (Accessed February 12, 2015).
- Jones H. 2010. Prognosis for ecosystem recovery following rodent eradication and seabird restoration in an island archipelago. *Ecol Appl.* 20(5):1204-1216.
- Kaufman DW, Peterson SK, Pristik R, Kaufman GA. 1983. Effect of microhabitat features on habitat use by *Peromyscus leucopus*. *Am Midl Nat.* 110(1):177-185.
- Marinelli L, Millar JS. 1989. The ecology of beach-dwelling *Peromyscus maniculatus* on the Pacific coast. *Can J Zool.* 67:412-417.
- Maser C, Mate BR, Franklin JF, Dyrness CT. *Natural history of Oregon Coast mammals*. U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station. (1981) 496 p.
- McCleery RA, Lopex RR, Silvy NJ, Frank PA, Klett SB. 2006. Population status and habitat selection of the endangered key largo woodrat. *Am Midl Nat.* 155(1):197-209.
- M'Closkey RT. 1975. Habitat dimensions of white-footed mice, *Peromyscus leucopus*. *Am Midl Nat.* 93(1):158-167.
- Pavey C, Eldridge SR, Heywood M. 2008. Population dynamics and prey selection of native and introduced predators during a rodent outbreak in arid Australia. *J Mammal.* 89(3):674-683.
- Pianka ER. 1974. Niche overlap and diffuse competition. *P Natl Acad Sci USA.* 71(5):2141-2145.
- Sato T. 2007. Habitat Preferences of *Peromyscus leucopus*, *Blarina brevicauda*, and *Glaucomys volans*. [MSc thesis]. Ann Arbor (MI): University of Michigan. Available from: <http://deepblue.lib.umich.edu/handle/2027.42/55334> (Accessed February 2, 2015).
- Stancampiano AJ, Schnell GD. 2004. Microhabitat Affinities of Small Mammals in Southwestern Oklahoma. *J Mammal.* 85(5):948-958.
- Whitaker JO. 1980. *National Audubon Society field guide to North American mammals*. New York (NY): Alfred A. Knopf, Inc. 745 p.
- Witmer GW. 2004. Rodent Ecology and Plague in North America. *Proceedings of the 19<sup>th</sup> International Congress of Zoology.* 19:154-156. Available from: [http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1395&context=icwdm\\_usdanwrc](http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1395&context=icwdm_usdanwrc) (Accessed February 1, 2015).

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## Appendix A

### A description of the study sites.

#### Site 1: Forest

This area is a thin forest, with taller, mature trees and a flat forest floor. This area is within 30 m of a road and an abandoned wooden outhouse. The forest has little overhang canopy, and has plenty of barren ground. Site 1 was a temporarily closed site—no human activity allowed—for the first year of study. The site was reopened the second year of study, although there was not an obvious change to the area. There were no small mammals captured here.

#### Site 2: Grassland

Site 2 is a grassy clearing, surrounded by a dense forest. This area is seasonally mowed, although the area appeared overgrown during the second year of study. The ground is flat, but inclined slightly toward the west. Deer frequent the area. There is also a deer blind on the perimeter of the site. This was the only location where *S. hispidus* was found.

#### Site 3: Grassland

This site is primarily grassland, with occasional clusters of trees. Site 3 is at the pinnacle of human activity at the ranch. The site is next to a gravel road, the ranch house, and a recreation hall. There is a large bathroom facility on the site itself, as well as inactive gopher mounds. There were no small mammals captured here.

#### Site 4: Forest

This is an extremely dense forest, with lots of overhang canopy. There were occasional clearings, all of which were completely surrounded by *Smilax*. There are also many snags and other forms of litter. The density of the forest and overhang canopy created an extremely humid environment. Many *P. leucopus* and *N. floridana* were found here.

#### Site 5: Forest

The site progressed from areas thick with dead Junipers, to a dense lush forest. There was a distinct 3m drop-off, at which point the density of the forest decreased, and the amount of rock cover increased dramatically. There was a standing stream at the end of the site. *Peromyscus leucopus* and *N. floridana* were captured here.

#### Site 6: Grassland

This site is narrow, and runs between the edge of a forest and County Line Road. This area appeared mowed in some areas, but unkempt in others. Part of the trap lines ran parallel to a horse pen. No small mammals were captured here.

**Site 7: Forest**

The trap lines began at the edge of the forest. Overall, the area is a U-shaped depression, although there are many elevation changes within the site. There are many rocky bluffs and pockets of thick vegetation, making the site very difficult to navigate in. Both *P. leucopus* and *N. floridana* were captured here, although there were many more *N. floridana*.

**Site 8: Forest**

This area is a moderately dense forest with a shallow winding creek. The trapping site was not too far from a hiking trail. This area was also U-shaped, although the elevation changes were much more gradual than in site 7. The overhang canopy was lighter, which allowed more sunlight and airflow. *Peromyscus leucopus* and *N. floridana* were found here.

**Site 9: Forest**

The trap lines of this site began at the edge of a light forest. Annuals are dominant vegetation of the area, although there were occasional shrubs and trees—increasing as the trap lines progressed into the forest. This site was on the high hills of a steep valley, and was much higher in elevation than the other locations. Cacti are present in the open, grassy areas. This was the only site where *P. maniculatus* was found.

**Site 10: Forest**

The trap lines began at the edge of a 20 m rocky cliff. The entire trap site is extremely steep, sloping upward toward a proximal, manmade clearing. Overall, this was a moderately-dense forest with few pockets of clearing. This site had frequent rock exposure, and more barren ground than other sites. Both *P. leucopus* and *N. floridana* were captured here.

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# A Second Ornithischian Dinosaur from the Antlers Formation (Lower Cretaceous) of Southeastern Oklahoma

**Joseph A Frederickson**

University of Oklahoma, Department of Biology, Norman, OK 73019

**Joshua E Cohen**

University of Oklahoma, Department of Biology, Norman, OK 73019

**Richard L Cifelli**

Sam Noble Oklahoma Museum of Natural History, 2401 Chautauqua Ave, Norman, OK 73072

## Introduction

The Lower Cretaceous (Upper Aptian) Antlers Formation of Oklahoma is the terrigenous, non-marine extension of the Trinity Group of Texas. Decades of fossil collecting by the Sam Noble Oklahoma Museum of Natural History ([SN]OMNH) has yielded a relatively diverse vertebrate fauna from this formation, including freshwater sharks, fishes, amphibians, lizards, mammals, crocodylians, and dinosaurs (Cifelli et al. 1997). Although most groups are represented by multiple species, the only ornithischian dinosaur recognized hitherto is the relatively common, large-bodied iguanodontian *Tenontosaurus tilletti*. When compared with other contemporaneous rock units from North America, the ornithischian assemblage of the Antlers Formation is a depauperate anomaly. Most of these formations preserve multiple ornithischian dinosaurs, ranging from the large, armored ankylosaurs to cat-sized basal neoceratopsians (Oreska et al. 2013; Farke et al. 2014). Here, we describe a nearly complete tooth, OMNH 34881, representing only the second ornithischian known from the Antlers Formation of Oklahoma.

The largest and most diverse collection from

the Antlers Formation of Oklahoma comes from a locality in Atoka County (V706; Locality data are on file at OMNH and are available upon request from qualified investigators). This site includes two separate bone-bearing horizons. The stratigraphically higher bed has yielded multiple articulated skeletons of the herbivorous *Tenontosaurus tilletti*, along with associated material from the predatory *Deinonychus antirrhopus* (Theropoda, Dromaeosauridae; Brinkman et al. 1998). Approximately 1.5 m below this unit is a highly fossiliferous microsite that yielded OMNH 34881, together with a wide array of other vertebrate taxa. These deposits are interpreted as representing overbank lags in a fluvial environment with close approximation to the paleo-Gulf of Mexico (Cifelli et al. 1997).

## Results and Discussion

OMNH 34881 is a single, relatively complete tooth that is short (total height: 4.05 mm; crown height: 1.25 mm) when contrasted with other dinosaurian teeth from the Antlers Formation. The crown is low, possessing five incomplete marginal denticles. A straight central cusp forms the largest denticle, while the two most lateral denticles are outgrowths of the basal cingulum

(A<sub>1</sub> Fig.1). The other two accessory denticles are small and formed on the same plane as the lateral-most denticles. The root is wide near the crown and gently tapers to a rounded point; a root 'neck' is only moderately developed below the crown base. Enamel is deposited on both sides of the cusp, but may be thinner on the side that bears a large wear facet (A<sub>2</sub> Fig 1).

OMNH 34881 is unlikely to belong to *Tenontosaurus tilletti*, which is by far the most commonly encountered dinosaur in the Antlers Formation. Teeth of *Tenontosaurus*, including juveniles, are thicker, more inflated, and bear a greater number of denticles on the margin of the crown. One of the most striking differences is that the root in *T. tilletti* is angled lingually as compared to OMNH 34881, which is relatively straight. Additionally, affinities with Thyreophora (armored dinosaurs) can be excluded because teeth from this group are often broadly triangular in lateral view bearing a sinusoidal ridge, which is often adorned with more denticles than observed in OMNH 34881 (Oreska et al. 2013). Hence, OMNH 34881 represents a non-*Tenontosaurus* cerapodan, likely either a basal ornithopod or a marginocephalian. Ornithopod maxillary and

dentary teeth are mesiodistally broad and bear multiple denticles on the margins. Although many species have more denticles than OMNH 34881, some species bear as few as five (Norman et al. 2004). In addition, it is not uncommon for posterior dentary or maxillary teeth of basal euornithopods to have a limited number of marginal denticles (Oreska et al. 2013). Ornithopods are known from North America during the late Aptian, including at least one species from the roughly concurrent Trinity Group of Texas (Langston 1974). Specifically, a substantial sample of ornithopod material is known from the Proctor Lake locality in the basal Trinity Group. This ornithopod material awaits comprehensive study, but from what has been described it does not match OMNH 34881. Teeth from this undescribed species are leaf-shaped, bear many marginal denticles, and lack the large central cusp seen in the Oklahoma specimen (Winkler et al. 1988). OMNH 34881 also resembles neoceratopsian teeth; however, this specimen does not compare well with material from contemporaneous deposits in North America. For example, *Aquilops americanus* from the Cloverly Formation of Montana has teeth similar in size and appearance to OMNH 34881, except that placement of the

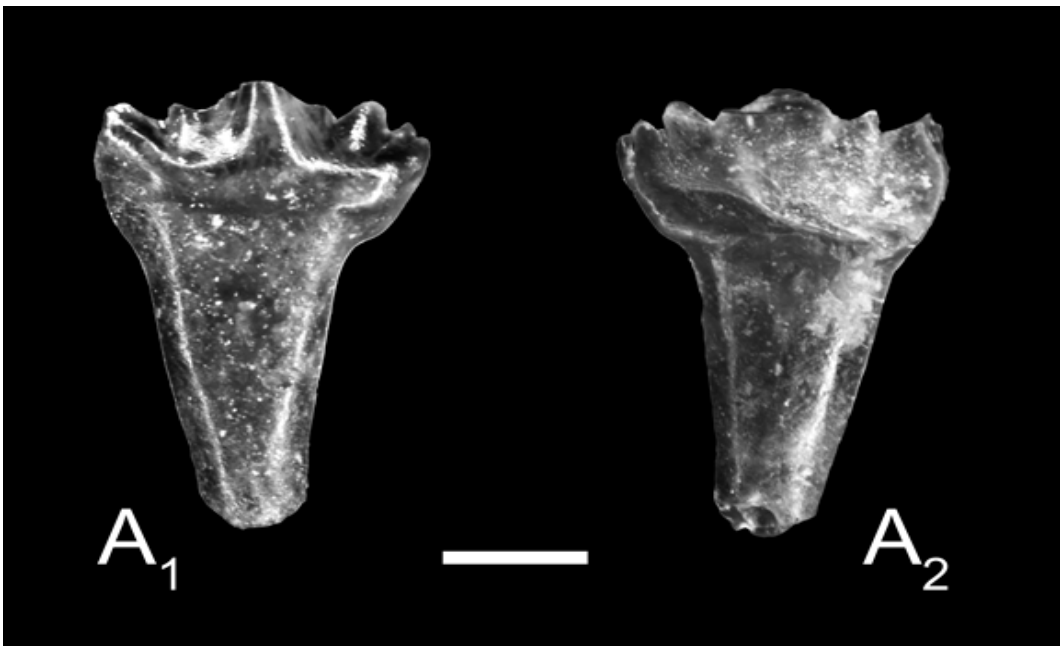


Figure 1. OMNH 34881 in two views (A<sub>1</sub> and A<sub>2</sub>). Scale is equal to 1 mm.

central cusp in this species is asymmetric (Farke et al. 2014); a feature commonly observed in basal neoceratopsians (You and Dodson, 2004). A third possibility is that this tooth belongs to an early pachycephalosaur. Pachycephalosaur teeth tend to be small, triangular, transversely compressed, and bear relatively large denticles (Maryńska et al. 2004). The geologically oldest pachycephalosaur previously reported from North America is of earliest Cenomanian age (Cifelli et al. 1999), and hence is slightly younger than OMNH 34881.

Although a definitive identification for OMNH 34881 cannot be made at this time, it is apparent that the specimen represents a previously unrecognized ornithischian from the Antlers Formation of Oklahoma. Additional specimens are needed to determine whether OMNH 34881 is an exemplar for a species with a unique dentition, or an abnormal tooth from a better-known North American species. In either scenario, this discovery significantly adds to the known ornithischian diversity from the Early Cretaceous of Oklahoma.

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### References

- Brinkman DL, Cifelli RL, Czaplewski, NJ. 1998. First Occurrence of *Deinonychus Antirrhopus* (Dinosauria: Theropoda) from the Antlers Formation (Lower Cretaceous, Aptian-Albian) of Oklahoma. Oklahoma Geologic Survey Bulletin 146: 1–27.
- Cifelli RL, Gardner JD, Nydam RL, Brinkman DL. 1997. Additions to the vertebrate fauna of the Antlers Formation (Lower Cretaceous), southeastern Oklahoma. Oklahoma Geology Notes 57(4): 124–131.
- Cifelli RL, Nydam RL, Gardner JD, Weil A, Eaton JG, Kirkland JI, Madsen SK. 1999. Medial Cretaceous vertebrates from the Cedar Mountain Formation, Emery County, Utah: the Mussentuchit local fauna. Vertebrate paleontology in Utah 99(1): 219–242.
- Farke AA, Maxwell WD, Cifelli RL, Wedel MJ. 2014. A ceratopsian dinosaur from the Lower Cretaceous of Western North America, and the biogeography of Neoceratopsia. PLoS one 9(12).
- Langston W. Jr. 1974. Comanchean vertebrates from Texas. Geoscience and Man 3:77–102.
- Maryńska T, Chapman RE, Weishampel DB. 2004. Pachycephalosauria. In: Weishampel DB, Dodson P, Osmólska H, eds. The Dinosauria. Berkeley: University of California Press. pp 464–478.
- Norman DB, Sues H-D, Wimer LM, Coria RA. 2004. Basal Ornithopoda. In: Weishampel DB, Dodson P, Osmólska H, eds. The Dinosauria. Berkeley: University of California Press. pp 393–412.
- Oreska MP, Carrano MT, Dzikiewicz KM. 2013. Vertebrate paleontology of the Cloverly Formation (Lower Cretaceous), I: faunal composition, biogeographic relationships, and sampling. Journal of Vertebrate Paleontology 33(2): 264–292.
- Winkler DA, Jacobs LL, Branch JR, Murray PA, Downs WR, Trudel P. 1988. The Proctor Lake Dinosaur locality, Lower Cretaceous of Texas. Hunteria 2(5): 1–8.
- You HL, Dodson P. 2004. Basal Ceratopsia. In: Weishampel DB, Dodson P, Osmólska H, eds. The Dinosauria. Berkeley: University of California Press. pp 478–493.

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# ***Bashkirovitrema canadense* (Trematoda: Digenea: Echinostomatidae) from Northern River Otter, *Lontra canadensis* (Carnivora: Mustelidae), from Southeastern Oklahoma**

**Chris T. McAllister**

Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

**Thomas J. Fayton**

Gulf Coast Research Laboratory, University of Southern Mississippi, Ocean Springs, MS 39564

The northern river otter, *Lontra canadensis* (Schreber) is a large semi-aquatic mustelid that ranges from Alaska and most of Canada south to northern California and northern Utah in the west and from Newfoundland southward to Florida in the east (Reid 2006). In Oklahoma, *L. canadensis* is found sporadically in 15 counties of the state, primarily in the eastern portion (Hatcher 1984; Caire et al. 1989; Barrett and Leslie 2010). Unfortunately, *L. canadensis* populations have been severely affected because of habitat destruction, human settlement, unregulated harvest and water pollution (Larivière and Walton 1998). The river otter primarily feeds on fish, but crayfish, amphibians, reptiles, birds, and other mammals are also taken (Tumlison et al. 1986; Melquist et al. 2003; Ligon and Reasor 2007; Reed-Smith 2012).

Concerning helminth parasites, *L. canadensis* has been the subject of several surveys conducted on populations in 14 US states, including Alabama, Alaska, Arkansas, Florida, Georgia, Louisiana, Maryland, Massachusetts, Michigan, Montana, New York, North Carolina, Oregon and Tennessee, and Newfoundland and Ontario, Canada (summarized by Fleming et al. 1977; Shoop and Corkum 1981; Tumlison et al. 1984; Addison et al. 1988; Snyder et al. 1989; Forrester 1992; Hoberg et al. 1997;

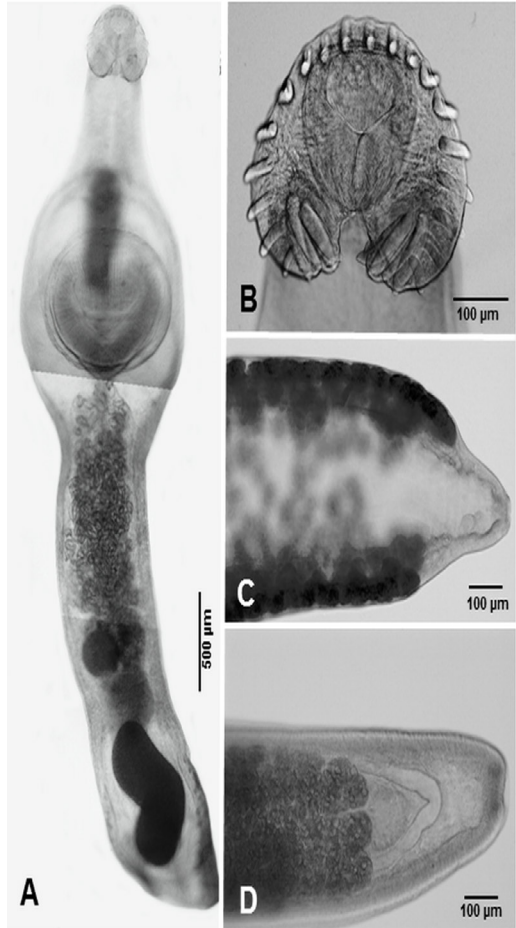
Kollars et al. 1997; Kimber and Kollias 2000; Dronen 2009; Crait et al. 2015). Interestingly, Kimber and Kollias (2000) reported that during an Oklahoma reintroduction program of river otters originally from Louisiana, 40% of the *L. canadensis* were infected with nematodes, *Capillaria* sp. and one infection was a concurrent *Ancylostoma* spp. infection. To our knowledge, however, nothing has been published on any parasite from river otters originating from Oklahoma. Here, we report on a digenean found in a single *L. canadensis* from the southeastern corner of the state.

On 13 July 2015, an adult male *L. canadensis* was found dead on the road north of Broken Bow off US 259, McCurtain County (34.054366°N, 94.73956°W) and salvaged under an Oklahoma Department of Wildlife Conservation Scientific Collecting Permit. The nearest watershed is Yanubbee Creek, approximately 500m due east of the collection site. The river otter was immediately taken to the laboratory and necropsied for helminth parasites. A midventral incision was made and the entire gastrointestinal tract from the esophagus to anus was removed and split lengthwise and several 15 cm segments were cut and placed in Petri dishes and their contents rinsed in 0.9% saline. The lungs, heart, kidneys, liver and gonads were also similarly examined

under a stereomicroscope. Six trematodes (Fig. 1A) were removed from the stomach and upper small intestine, fixed in near boiling distilled water without coverslip pressure and placed in 70–95% DNA grade ethanol. They were stained in acetocarmine or Ehrlich's hematoxylin, cleared in methyl salicylate and mounted in Canada balsam. A host voucher specimen (skull only) was deposited in the Henderson State University Collection (HSU), Arkadelphia, Arkansas, with the skin retained in the Eastern Oklahoma State College-Idabel collection; parasite vouchers were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska as HWML 101026–101027.

All trematodes found possessed 27 collar spines (Fig. 1B), and with their arrangement, a bipartite internal seminal vesicle, and vitelline fields confluent in the post-testicular region our species belongs in *Bashkirovitrema* Skrjabin, 1944 (Digenea: Echinostomatidae). Measurements of five adult specimens (in  $\mu\text{m}$ , long  $\times$  wide) are as follows: body 14,560–19,382  $\times$  1,033–1,305; forebody 895–1,527 long, representing 5–8% body length (BL); oral sucker 266–308  $\times$  261–308; ventral sucker 583–1,037  $\times$  840–963; head collar 425–537  $\times$  485–625; lateral collar spines 93–150  $\times$  27–33; corner collar spines 140–165  $\times$  41–50; postcecal space 105–292, representing 0.6–2.0% BL; pharynx 202–285  $\times$  147–192; esophagus 612–874 long; anterior testis 845–1,527  $\times$  315–369; posterior testis 878–1,240  $\times$  278–368, separated from anterior testis by distance of 50–223, representing 0.4–1.0% BL; cirrus sac 612–874  $\times$  284–383, representing 3.4–6.0% BL; ovary 268–298  $\times$  273–293, separated from anterior testis by 150–283, representing 0.7–1.6% BL; posterior extent of vitelline field 108–425, representing 0.6–2.8% BL; eggs 97–107  $\times$  55–66. These measurements and the relative position of morphological features falls within the range of those reported for *B. canadense* Dronen, 2009 from *L. canadensis* reported from Georgia, Louisiana, New York, North Carolina, and Ontario, Canada by Dronen (2009). However, our specimens are unique in the posterior extent of the ceca and vitelline field and in the condition of the excretory vesicle. Dronen

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**Figure 1.** *Bashkirovitrema canadense* from *Lontra canadensis*. **A.** Ventral mount. **B.** Anterior end showing oral sucker and collar spines. **C.** Posterior end showing posterior extent of vitelline field. **D.** Posterior end showing relative positions of looping diverticula and posterior extent of ceca and vitelline field.

(2009) describes the ceca and vitelline field as terminating "...some distance from the posterior extremity..." and, although no measurements for the position of termination of these features are provided, measurements from his illustration of *B. canadense* (see his Fig. 8) indicate that the posterior extents of the ceca and vitelline field are approximately 95.8% and 94.8% of BL, respectively, whereas those of our specimens are 98–99.4% and 97.2–99.4%, respectively, with posterior extents nearly reaching the end of the body (Fig. 1C). As to whether the posterior



extent of the ceca and vitelline fields in our specimens represents intraspecific variation for *B. canadense* requires examination of type material, and this would be well supplemented through DNA sequencing of *Baskirovitrema* from Oklahoma and elsewhere in the Nearctic. Our specimens also possessed prominent lateral diverticula extending from the excretory vesicle that distinctly loop near the posterior end of the body (Fig. 1D), a feature that has not been previously described for *B. canadense*.

In summary, we document a new distributional record (Oklahoma) for *B. canadense* and add comparative morphological data on the parasite. Further research needs to include molecular sequencing to differentiate between interspecific and intraspecific morphological variation in the genus *Baskirovitrema*.

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## References

- Abram JB, Lichtenfels JR. 1974. Larval *Eustrongylides* sp. (Nematoda: Dioctophymatoidea) from otter, *Lutra canadensis*, in Maryland. Proc. Helminthol. Soc. Wash. 41:253.
- Addison EM, Strickland MA, Stephenson AB, Hoeve J. 1988. Cranial lesions possibly associated with *Skrjabingylus* (Nematoda: Metastrongyloidea) infections in martens, fishers, and otters. Can. J. Zool. 66:2155–2159.
- Barrett DA, Leslie DM Jr. 2010. Current distribution of North American river otters in central and eastern Oklahoma, with seven new county records. Occ. Pap. Mus. Texas Tech Univ. 294:1–13.
- Caire W, Tyler JD, Glass BP, Mares MA. 1989. Mammals of Oklahoma. Norman (OK): University of Oklahoma Press. 567 p.
- Crait JR, McIntosh AD, Greiner EC, Ben-David M. 2015. The influence of changing prey availability on the prevalence of *Diphyllobothrium* in river otters from Yellowstone National Park. J. Parasitol. 101:240–243.
- Dronen ND. 2009. Re-evaluation of the species composition of *Baskirovitrema* Skrjabin, 1944 (Digenea: Echinostomatidae), with the description of two new species of this genus and the proposal of *Kostadinovatrema novaeguiniense* n. g., n. sp. Syst. Parasitol. 74:169–185.
- Fleming WJ, Dixon CF, Lovett JW. 1977. Helminth parasites of river otters (*Lutra canadensis*) from southeastern Alabama. Proc. Helminthol. Soc. Wash. 44:131–135.
- Forrester DJ. 1992. Parasites and diseases of wild mammals in Florida. Gainesville (FL):Univ. Press Florida. 459 p.
- Hatcher RT. 1984. River otters in Oklahoma. Proc. Okla. Acad. Sci. 64:17–19.
- Hoberg EP, Henry CJ, Hedstrom OR, Grove RA. 1997. Intestinal helminths of river otters (*Lutra canadensis*) from the Pacific Northwest. J. Parasitol. 83:105–110.
- Kimber KR, Kollias GV. 2000. Infectious and parasitic diseases and contaminant-related problems of North American river otters (*Lontra canadensis*): A review. J. Zoo Wildl. Med. 31:452–472.
- Kollars TM, Lizotte, RE, Wilhelm WE. 1997. Gastrointestinal helminths in the river otter (*Lutra canadensis*) in Tennessee. J. Parasitol. 83:158–160.
- Larivière S, Walton LR. 1998. *Lontra canadensis*. Mamm. Spec. 587:1–8.
- Ligon DB, Reasor J. 2007. Predation on alligator snapping turtles (*Macrochelys temminckii*) by northern river otters (*Lontra canadensis*). Southwest. Nat. 52:608–610.
- Melquist WE, Polechla PJ Jr, Toweill D. 2003. River otter: *Lontra canadensis*. In: Feldhamer GA, Thompson BC, Chapman JA, editors. Wild mammals of North America: Biology, management, and conservation. Baltimore (MD): Johns Hopkins University Press. p 708–734.
- Reed-Smith J. (ed.) 2012. North American (Nearctic) river otter husbandry notebook 4th Ed., Section 2, Chapters 7–10. Grand Rapids

- (MI): John Ball Zoo. 229 p.
- Reid FA. 2006. Mammals of North America. Boston (MA): Houghton Mifflin Company. 579 p.
- Shoop WL, Corkum KC. 1981. Some trematodes of mammals in Louisiana, USA. Tulane Univ. Stud. Zool. Bot. 22:109–121.
- Snyder DE, Hamir AN, Nettles VF, Rupprecht CE. 1989. *Diriofilaria immitis* in a river otter (*Lutra canadensis*) from Louisiana. J. Wildl. Dis. 25:629.
- Tumlison R, King AW, McDaniel VR. 1986. The river otter in Arkansas. IV. Winter food habits in eastern Arkansas. Proc. Ark. Acad. Sci. 40:76–77.
- Tumlison R, Smith R, Hudspeth J, Polechia PJ, King AW. 1984. Dracunculiasis in some Arkansas carnivores. J. Parasitol. 70:440.

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# Possible Alteration of Circadian Rhythms in Bats at a Heavy Metal Contaminated Site

**Jimmy J. Lovett**

Department of Integrative Biology and Collection of Vertebrates, Oklahoma State University, Stillwater, OK 74078

**Karen McBee**

Department of Integrative Biology and Collection of Vertebrates, Oklahoma State University, Stillwater, OK 74078

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**Abstract:** The ability of organisms to exhibit daily patterns of behavior and hormone regulation is deeply connected to the changing light levels over any 24 hour solar cycle. Both morphological and physiological mechanisms allow for proper timing of key behaviors such as emergence and sleep so as to maximize benefits and minimize risks to survival. We were interested in how heavy metal contamination may affect circadian activity levels in wild bat populations. Specifically we hoped to determine if the emergence time of bats within the Tar Creek Superfund Site (TC) in northeastern Oklahoma differed from that at two uncontaminated locations within the Oologah Wildlife Management Area (OWMA, Oklahoma). We recorded emergence times visually and by using an acoustical bat detector and compared them to sunset times at each location. We found emergence times of bats at TC occurred significantly later ( $p = 0.022$ ,  $df = 3$ ) than at the combined locations within OWMA. ©2015 Oklahoma Academy of Science

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## Introduction

The existence of circadian rhythmicity in regulation of hormonal and behavioral activity of organisms ranges from single-celled forms to higher vertebrates, which points to the importance of this daily phenomenon to survivability and persistence (Young and Kay 2001). This circadian rhythmicity is controlled in mammals by a region of the brain, inside the hypothalamus called the suprachiasmatic nucleus (SCN), that regulates the rhythmic expression of genes by a series of complex feedback mechanisms (Zelinski et al. 2014). Information about time of day projects from the retina through the retinohypothalamic tract (RHT), via glutamate, directly into the SCN (Gompf et al. 2015; Schmoll et al. 2011). In this way the circadian regulation of hormonal activity important to metabolism, sleep-wake cycles, and health follows or entrains to changing light and

dark cycles of any given 24 hour period (Schmoll et al. 2011). Research implicates alterations to these circadian regulations or abilities in many human diseases and disorders such as diabetes and cancer (Zelinski et al. 2014). Likewise, circadian regulation of hormones is important to reproduction and health of developing offspring (Boden et al. 2013; Zelinski et al. 2014).

Wild animals are exposed to many different environmental toxicants. Heavy metals such as lead (Pb), cadmium (Cd), and zinc (Zn), when found in the environment, are particularly dangerous because of the risk they pose to development and proper function of the central nervous system of animals (Tokar et al. 2013). Pb is a neurotoxin that, among other effects, inhibits growth of synapses in the brain (Neal et al. 2011). Cd is both a neurotoxin and endocrine disrupting chemical and affects many aspects of behavior and central nervous system function (Huang et al. 2015; Leret et al. 2003).

These metals may also pose a risk to important mechanisms involved in entrainment of circadian processes in organisms experiencing chronic or acute environmental exposure.

Experiments have shown effects by these metals on various components of the circadian system in animals. For example, Rojas-Castañeda et al. (2007, cited in Rojas-Castañeda et al. 2011) found chronic Pb exposure changes circadian activity levels of Wistar rats (*Rattus norvegicus*). This effect may be due to alterations to photoentrainment mechanisms that might occur as Pb accumulates in the retina (Fox and Boyes 2013) and, at chronically low exposure levels, induces rod photoreceptor death (He et al. 2000, 2003). Increases in the time it takes to adjust to changes in light levels may result from this environmental Pb exposure (Fox and Boyes 2013). Furthermore, both Pb and Cd impair glutamate transport (Borisova et al. 2011) which is the main neurotransmitter released from the RHT into the SCN (Gompf et al. 2015). Pb also increases DNA methylation of genes relevant to behavior in animals (Nye et al. 2015). Finally, early Pb exposure affects development and physical properties of the SCN itself (Rojas-Castaneda et al. 2011). Cd may also affect circadian regulatory mechanisms because in Wistar rats it decreased the expression of two major clock genes, *Per 1* and *Per 2*, involved in regulation of circadian cycles (Cano et al. 2007).

During spring and summer 2013 we conducted both visual and acoustical observations of bat activity along Tar Creek (TC; N 36°57.495, W 094°50.731) within the Tar Creek Superfund Site, which is contaminated with toxic levels of Pb, Cd, and Zn (USEPA 2010), and at two uncontaminated locations, Plum Creek (PL; N 36°35.5063, W 095°32.4197) and Panther Creek (PA; N 36°37.747, W 095°31.372), located within the Oologah Wildlife Management Area (OWMA). We compared emergence time, relative to sunset, of bats at TC, PL, and PA. We hypothesized that emergence times would differ at TC when compared to the two uncontaminated sites. Emergence of bats, like all mammals, is in part determined by the phase of endogenous feedback mechanisms controlled by the SCN

(Zelinski et al. 2014). Because Pb and Cd affect these mechanisms, as outlined above, and impair the reception of light information by the SCN (Borisova et al. 2011), thus reducing the capacity of animals to properly entrain circadian cycles to changing photoperiods, it is plausible that sleep-wake cycles in exposed animals could be temporally shifted compared to unexposed animals. Because of this potentially reduced ability to photoentrain their sleep-wake cycles to daily changes in photoperiod, we predicted that emergence time, an indicator of the sleep-wake cycle, would occur later in the evening for bats within TC compared to emergence times at the two uncontaminated sites.

## Methods

This study was conducted simultaneously with a companion study (Eguren 2014) in which bats were captured with mist nets set across streams at the same locations and dates. Bats captured in mist nets were removed and identified to species in hand. Total number of each captured bat species for each location was recorded. Straight-line distances between sites were determined using the ruler tool in Google Earth Pro. The contaminated site (TC) is separated from the uncontaminated Plum Creek (PL) by 72.8 km and from Panther Creek (PA) by 70.1 km. The two uncontaminated sites are approximately 3.2 km apart. Data collection occurred on separate nights May-August 2013. Twelve nights were spent afield (TC = 8, PL = 2, PA = 2). Mist nets were opened and observation began 30 minutes before sunset and continued for 5 hours or until 1:00 am. On each night of observation we sat about 2 m from each creek adjacent to mist nets set across the creek while holding a bat detector (Convergence Tech Inc., Belfry Bat Detector) pointed directly upward toward the sky. This detector is rated as capable of detecting echolocation calls at distances of 75 to 200 ft (Convergence Tech, Inc. 2015). The same bat detector was used for each sampling night. An observation of a bat was defined as visual observation of a bat around the nets or a clear repeating auditory signal from the bat detector. The time of the first observation (visual or acoustic) of a bat was recorded for each night.

We defined emergence time as the number of minutes after sunset time for each sampling location (USNO 2014) of each recorded first bat observation. The mean emergence time (min) of all sampling nights was determined for each location. To compare mean emergence time of bats at the two uncontaminated sites a two-tailed Student's t-test was used. A one-tailed Student's t-test was conducted in statistical calculations of mean emergence time between contaminated and combined uncontaminated locations.

To estimate local habitat composition at each site, satellite images, dated 23 April 2013, of each sampling location was obtained using Google Earth Pro (GE). Using the ruler tool in GE a circle of radius 250 m was drawn centered on each observation location for each site. Three habitat types were defined: forest, water, and open. Forest was defined as unbroken tracts of tree canopy and isolated clumps of trees. Isolated individual trees were not included. Water was defined as streams or ponds containing water. Open was considered as everything (including roads) that did not fit into forest or water. There were no residential areas within or near any of the sampling sites. In GE, individual polygons were fitted around forest and water habitat within each circle at each location. The surface area (m<sup>2</sup>) within each polygon denoting habitat types within each circle was determined. The proportion of each habitat type contained within each circle was then determined and compared across sites.

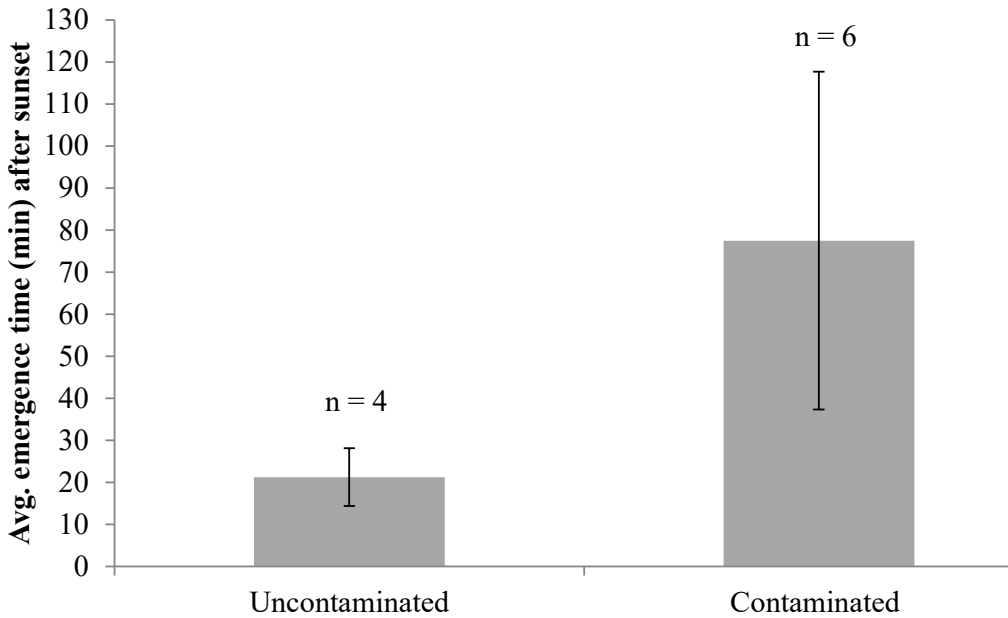
## Results and Discussion

There was only one observation period in which bats were neither seen nor heard. In order to account for any seasonal variation in emergence and activity times (Hayes 1997) only data from months in which the contaminated and uncontaminated sites were both sampled were included in analysis (July and August). This resulted in one observation being discarded for May from TC. Due to flooding at two of the three sites, there were no observations made during June. We did not attempt to identify bats to species based on calls picked up on the acoustical detector; however, the most

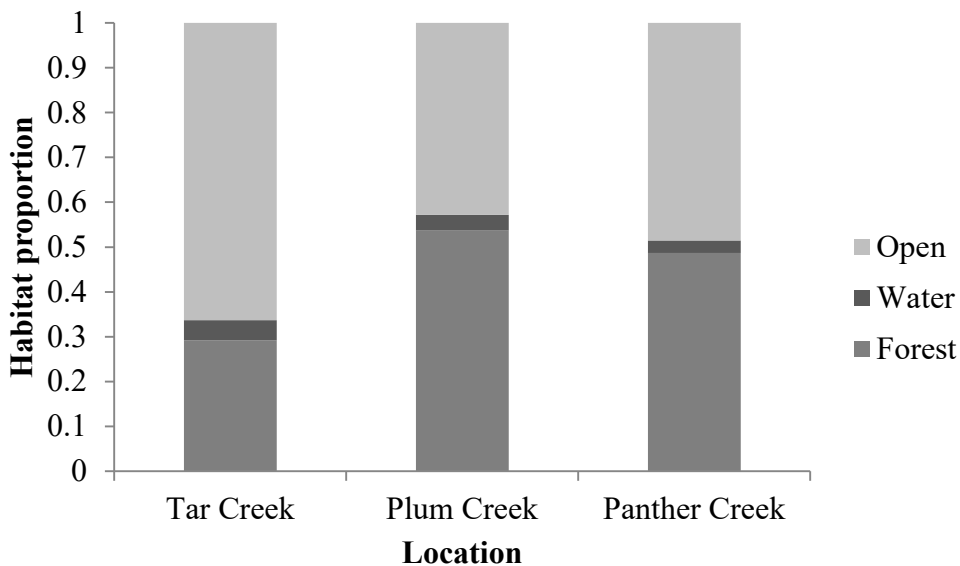
commonly collected species at each location in the companion study (Eguren 2014) was the Eastern Red Bat (*Lasiurus borealis*). On the nights and locations in which we recorded observations, 12 total bats were captured across all three sites. Eleven of them (91.7%) were *L. borealis*. The average number of minutes after sunset until the first bat was observed for TC, PL, and PA was  $77.50 \pm 40.17$  minutes ( $n = 6$ ),  $26.00 \pm 7.07$  minutes ( $n = 2$ ), and  $16.50 \pm 0.71$  minutes ( $n = 2$ ), respectively. Emergence times at PL and PA were not statistically different ( $p = 0.31$ ,  $df = 1$ ) so observations from these locations were combined. The mean time of emergence for combined uncontaminated locations was  $21.25 \pm 6.85$  min ( $n = 4$ ). Bat emergence time at TC was significantly later ( $p = 0.022$ ,  $df = 3$ ) than emergence time of bats at the combined uncontaminated sites (Fig. 1).

Forest habitat comprised 29.2% (TC), 53.7% (PL), and 48.7% (PA) of total surface area contained within the local landscape of each location. Water habitat comprised 4.5% (TC), 3.5% (PL), and 2.8% (PA). Open habitat comprised 66.3% (TC), 42.8% (PL), and 48.5% (PA) (Fig. 2).

We found that bats at a site contaminated with the heavy metals Pb, Cd, and Zn did exhibit a different pattern of emergence compared to bats at uncontaminated locations as hypothesized. Bats from the contaminated site had a mean emergence time significantly later than bats from the combined uncontaminated reference sites ( $p = 0.022$ ,  $df = 3$ ). However, variability in emergence times was nearly 6 times higher (5.86x) at TC compared to the uncontaminated sites. This may be because individual bats at TC experience differential exposures to the contaminants thus adding to variability in behavioral outcomes. Eguren (2014) found that *L. borealis* collected from TC had liver Pb levels that ranged from 0.002-0.026  $\mu\text{g/g}$  but liver Pb in bats from PA and PL ranged from below detection limits to 0.014  $\mu\text{g/g}$ . Levels of Pb in water at TC ( $0.935 \pm 0.658$  mg/L) were elevated compared to PL ( $0.219 \pm 0.153$  mg/L) but not PA ( $1.579 \pm 1.356$  mg/L—Eguren 2014). Also, Cd levels in water were much higher at TC (6.546



**Figure 1.** The average number of minutes after sunset that bats emerged at the contaminated site was significantly different ( $p = 0.022$ ,  $df = 3$ ) from that at the combined uncontaminated sites. Standard deviations are indicated by the bars.



**Figure 2.** The proportion of habitat types (forest, water, open) found within a 250 m radius at each location. Tar Creek had less forest habitat and more water and open habitats when compared to each of the reference sites.

$\pm 0.952 \mu\text{g/L}$ ) compared to PA ( $0.353 \pm 0.148 \mu\text{g/L}$ ) and PL ( $0.038 \pm 0.004 \mu\text{g/L}$ —Eguren 2014). Furthermore, Eguren (2014) found Cd levels in insects were 34% higher at TC and another contaminated location compared to the two uncontaminated sites in this study.

One of the outcomes of low level chronic Pb exposure is apoptosis of the rod photoreceptors (He et al. 2000, 2003). Of the three types of photoreceptive cells in the retina of mammals, rods contribute the most to sensitivity of photoentrainment of circadian cycles (Altimus et al. 2010; Lucas et al. 2012). It is possible bats experiencing retinal damage due to rod photoreceptor cell death, induced by chronic Pb exposure, would exhibit reduced sensitivity to changes in light levels and therefore require more light in order to properly photoentrain their circadian cycles. Behavioral effects could include later emergence or more variable emergence times in the evening than unexposed bats. Future studies could determine if bats at Tar Creek are in fact experiencing retinal deficits due to photoreceptor apoptosis. Also Gompf et al. (2015) found that mice without glutamate transmission from intrinsically photosensitive retinal ganglionic cells (ipRGC) had greatly reduced abilities of photoentrainment. Therefore, it is possible bats experiencing Pb and Cd exposures may exhibit poor photoentrainment abilities due to reduced or impaired glutamate transmission induced by these toxicants (Borisova et al. 2011).

Although our hypothesis is supported by this study, the low sample size at each location and the fact that this study was conducted during only one brief sampling period means that we don't know if this pattern of behavior is consistent from year to year or if it is a consistent representation of bat behavior at each site. Future studies should greatly increase sample sizes at each location and compare results for longer sampling periods from multiple years. Bat activity levels vary greatly through time (Hayes 1997). While we only included results of observations conducted during the same months, and we tried to visit each site in sequence so that there was little change in weather or moon phase during

each observation period, a future study could improve upon this design by simultaneous data gathering at each location in order to compare results of observations at each site on the same evening (Hayes 1997). This would allow greater control of other factors that may contribute to timing of bat emergence such as moon phase (Thomas and Jacobs 2013), vegetation structure (Russo et al. 2007), presence of predators (Lima and O'Keefe 2013), and insect prey abundance (Thomas and Jacobs 2013).

Bat activity patterns also typically exhibit some species dependence (Kunz 1973). Possibly our results are simply due to differing community structure of bats at each site. However, Eguren (2014) did not find differences in diversity or number of bats captured at the same locations during her study and *L. borealis* was the most commonly encountered species (92.5%,  $n = 37$ ) at all three sites over a period of two years. This indicates the population structure of bats at each site is similar. Future studies could focus on passive acoustic sampling surveys and using bat call identification software to better assess community structure at each site (Coleman et al. 2014).

TC contained different proportions of forest, water, and open habitat within a 250 m radius of our sampling location compared to the two uncontaminated sampling sites. TC generally contained less forest and more water and open habitat than either of the uncontaminated locations. It is unclear if this difference influenced emergence times. Russo et al. (2007) showed that emergence of bats occurred later in more loose canopy structure compared to dense canopy structure due to increased light levels remaining later in the evening in central Italy. Therefore, the later emergence time detected in bats at TC may be due to the occurrence of more open habitat compared to the two uncontaminated sites. However, all sampling locations in this study were in similar immediate local habitat and adjacent to areas of dense forest.

Because the community structure of bats at each of our locations appears to be dominated by *L. borealis*, it is important to understand

how habitat structure and roosting ecology of this tree-roosting species affects its detection and emergence time (O'Keefe et al. 2009). A consistent finding in studies containing analysis of *L. borealis* activity patterns is that they are frequently among the first bats active after sunset (Caire et al. 1988; Kunz 1973). Also, open areas and streams adjacent to hardwood forest edges appear to be important foraging habitats for Eastern Red Bats (Limpert et al. 2007; O'Keefe et al. 2009). These habitat conditions are present at each of our sampling locations and include forests dominated by hardwood trees such as oaks (Phelps and McBee 2009). Therefore, each of our sampling locations represent quality foraging habitat for the most frequently detected bat species that consistently exhibits among the earliest emergence times of bat species in the area.

In conclusion, this study serves as an intriguing observation suggesting possible behavioral consequences of exposure to environmental heavy metals. While it is possible emergence times differed as a result of detrimental effects of heavy metal exposure there are also other possible causal agents involved that were not controlled for in this study. Our results should encourage future studies that take into account these agents as well as possible physiological, functional, and morphological changes in bats exposed to environmental heavy metal contamination.

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## References

- Altimus CM, Güler AD, Alam NM, Arman AC, Prusky GT, Sampath AP, Hattar S. 2010. Rod photoreceptors drive circadian photoentrainment across a wide range of light intensities. *Nat Neurosci* 13:1107-1113.
- Boden MJ, Varcoe TJ, Kennaway DJ. 2013. Circadian regulation of reproduction: from gamete to offspring. *Prog Biophys Mol Biol* 113:387-397.
- Borisova T, Krisanova N, Sivko R, Kasatkina L, Borysov A, Griffin S, Wireman M. 2011. Presynaptic malfunction: the neurotoxic effects of cadmium and lead on the proton gradient of synaptic vesicles and glutamate transport. *Neurochem Int* 59:272-279.
- Caire W, Hardesty RM, Lacy KE. 1988. Capture heights and times of *Lasiurus borealis* (Chiroptera: Vespertilionidae) in Southeastern Oklahoma. *Proc Okla Acad Sci* 68:51-53.
- Cano P, Poliandri AHB, Jiménez V, Cardinali DP, Espuifino AI. 2007. Cadmium-induced changes in Per 1 and Per 2 gene expression in rat hypothalamus and anterior pituitary: effect of melatonin. *Toxicol Lett* 172:131-136.
- Coleman LS, Ford WM, Dobony CA, Britzke ER. 2014. A comparison of passive and active acoustic sampling for a bat community impacted by white-nose syndrome. *J Fish Wildlife Manage* 5:217-226.
- Convergence Tech, Inc. 2015. The Belfry Bat Detector [online]. Available from: <http://www.econvergence.net/The-Belfry-Bat-Detector-s/1830.htm> (Accessed October 28, 2015).
- Eguren RE. 2014. Community composition, population demographics, and tissue metal concentrations in bats (Chiroptera) at Tar Creek Superfund Site: with focus on use of a behavioral flight cage assay. [MSc thesis], Stillwater (OK): Oklahoma State University. 105 p. Available from: OSU Library.
- Fox DA, Boyes WK. 2013. Toxic responses of the ocular and visual system. In: Klaassen CD, editor. *Casarett and Doull's Toxicology: the basic science of poisons*. 8<sup>th</sup> ed. McGraw-Hill Education. p. 767-798.



- Gompf HS, Fuller PM, Hattar S, Saper CB, Lu J. 2015. Impaired circadian photosensitivity in mice lacking glutamate transmission from retinal melanopsin cells. *J Biol Rhythm* 30:35-41.
- Hayes JP. 1997. Temporal variation in activity of bats and the design of echolocation-monitoring studies. *J Mammal* 78:514-524.
- He L, Poblenz AT, Medrano CJ, Fox DA. 2000. Lead and calcium produce rod photoreceptor apoptosis by opening the mitochondrial permeability transition pore. *J Biol Chem* 275:12175-12184.
- He L, Perkins GA, Poblenz AT, Harris JB, Hung M, Ellisman MH, Fox DA. 2003. Bcl-x<sub>L</sub> overexpression blocks Bax-mediated mitochondrial contact site formation and apoptosis in rod photoreceptors of lead-exposed mice. *P Natl Acad Sci USA* 100:1022-1027.
- Huang MY, Duan RY, Ji X. 2015. The influence of long-term cadmium exposure on phototaxis in male *Pelophylax nigromaculata*. *Chemosphere* 119:763-768.
- Kunz TH. 1973. Resource utilization: temporal and spatial components of bat activity in central Iowa. *J Mammal* 54:14-32.
- Leret ML, San Millán JA, Antonio MT. 2003. Perinatal exposure to lead and cadmium affects anxiety-like behavior. *Toxicol* 186:125-130.
- Lima SL, O'Keefe JM. 2013. Do predators influence the behavior of bats? *Biol Rev* 88:626-644.
- Limpert DL, Birch DL, Scott MS, Andre M, Gillam E. 2007. Tree selection and landscape analysis of eastern red bat day roosts. *J Wildlife Manag* 71:478-486.
- Lucas RJ, Lall GS, Allen AE, Brown TM. 2012. How rod, cone, and melanopsin photoreceptors come together to enlighten the mammalian circadian clock. *Prog Brain Res* 199:1-18.
- Neal AP, Worley PF, Guilarte TR. 2011. Lead exposure during synaptogenesis alters NMDA receptor targeting via NMDA receptor inhibition. *NeuroToxicology* 32:281-289.
- Nye MD, Hoyo C, Murphy SK. 2015. In vitro lead exposure changes DNA methylation and expression of IGF2 and PEG1/MEST. *Toxicol in Vitro* 29:544-550.
- O'Keefe JM, Loeb SC, Lanham JD, Hill Jr. HS. 2009. Macrohabitat factors affect day roost election by eastern red bats and eastern pipistrelles in the southern Appalachian Mountains, USA. *Forest Ecol Manag* 257:1757-1763.
- Phelps KL, McBees K. 2009. Ecological characteristics of small mammal communities at a superfund site. *Am Midl Nat* 161:57-68.
- Rojas-Castañeda JC, Viguera-Villaseñor RM, Escobar C, Rios C. 2007. Alteraciones inducidas por la exposición crónica a plomo sobre la morfología y función del núcleo supraquiasmático de la rata. 1ª. Reunión bial de investigación pediátrica 37. Cited in Rojas-Castañeda et al. 2011.
- Rojas-Castañeda JC, Viguera-Villaseñor RM, Rojas P, Chávez-Saldaña M, Gutiérrez-Pérez O, Montes S, Rios C. 2011. Alterations induced by chronic lead exposure on the cells of circadian pacemaker of developing rats. *Int J Exp Pathol* 92:243-250.
- Russo D, Cistrone L, Jones G. 2007. Emergence time in forest bats: the influence of canopy closure. *Acta Oecol* 31:119-126.
- Schmoll C, Lascaratos G, Dhillon B, Skene D, Riha RL. 2011. The role of retinal regulation of sleep in health and disease. *Sleep Med Rev* 15:107-113.
- Thomas AJ, Jacobs DS. 2013. Factors influencing the emergence times of sympatric insectivorous bat species. *Acta Chiropterol* 15:121-132.
- Tokar EJ, Boyd WA, Freedman JH, Waalkes MP. 2013. Toxic effects of metals. In: Klaasen CD, editor. *Casarett and Doull's Toxicology: The basic science of poisons*. 8<sup>th</sup> ed. McGraw-Hill Education. p. 981-1030.
- USEPA (United States Environmental Protection Agency), Region 6. 2010. Fourth Five-year Review, Tar Creek Superfund Site, Ottawa County, Oklahoma. United States Environmental Protection Agency, Washington, D.C.
- USNO. 2014. Complete sun and moon data for one day [online]. Available from: [http://aa.usno.navy.mil/data/docs/RS\\_OneDay.php](http://aa.usno.navy.mil/data/docs/RS_OneDay.php) (Accessed August 10, 2015).
- Young MW, Kay SA. 2001. Time zones: A comparative genetics of circadian clocks. *Nat Rev Genet* 2:702-715.

Zelinski EL, Deibel SH, McDonald RJ. 2014.  
The trouble with circadian clock dysfunction:  
multiple deleterious effects on the brain and  
body. *Neurosci Biobehav R* 40:80-101.

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# ***Henneguya* sp. (Myxosporea: Bivalvulida: Myxobolidae) from the Black Bullhead, *Ameiurus melas* (Siluriformes: Ictaluridae), from Southeastern Oklahoma**

**Chris T. McAllister**

Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

**Stanley E. Trauth**

Department of Biological Sciences, Arkansas State University, State University, AR 72467

Myxosporeans of the genus *Henneguya* Thélohan are one of the most speciose of myxozoans and includes at least 204 described species infecting freshwater, estuarine and marine fishes (Lom and Dyková 2006). In the catfish family Ictaluridae, 20 species have been reported from commercially-raised Blue Catfish (*Ictalurus furcatus*) and Channel Catfish (*Ictalurus punctatus*), hybrid crosses of *I. punctatus* and *I. furcatus*, and wild Black Bullhead (*Ameiurus melas*) and Brown Bullhead (*Ameiurus nebulosus*) (Bosworth et al. 2003; Iwanowicz et al. 2008; Pote et al. 2012). Of the species from ictalurids, five (25%), including *H. adiposa*, *H. exilis*, *H. gurleyi*, *H. ictaluri*, and *H. sutherlandi* have been identified using molecular techniques via 18S rDNA sequence (see Griffin et al. 2009). In addition, only three species (*H. ameiuensis*, *H. exilis*, *H. gurleyi*) are known to infect bullheads (*Ameiurus* spp.) and, despite frequent examination of these fishes, especially of their skin abnormalities, cutaneous species of *Henneguya* are not commonly observed in ameiuroids (Iwanowicz et al. 2008; Griffin et al. 2009).

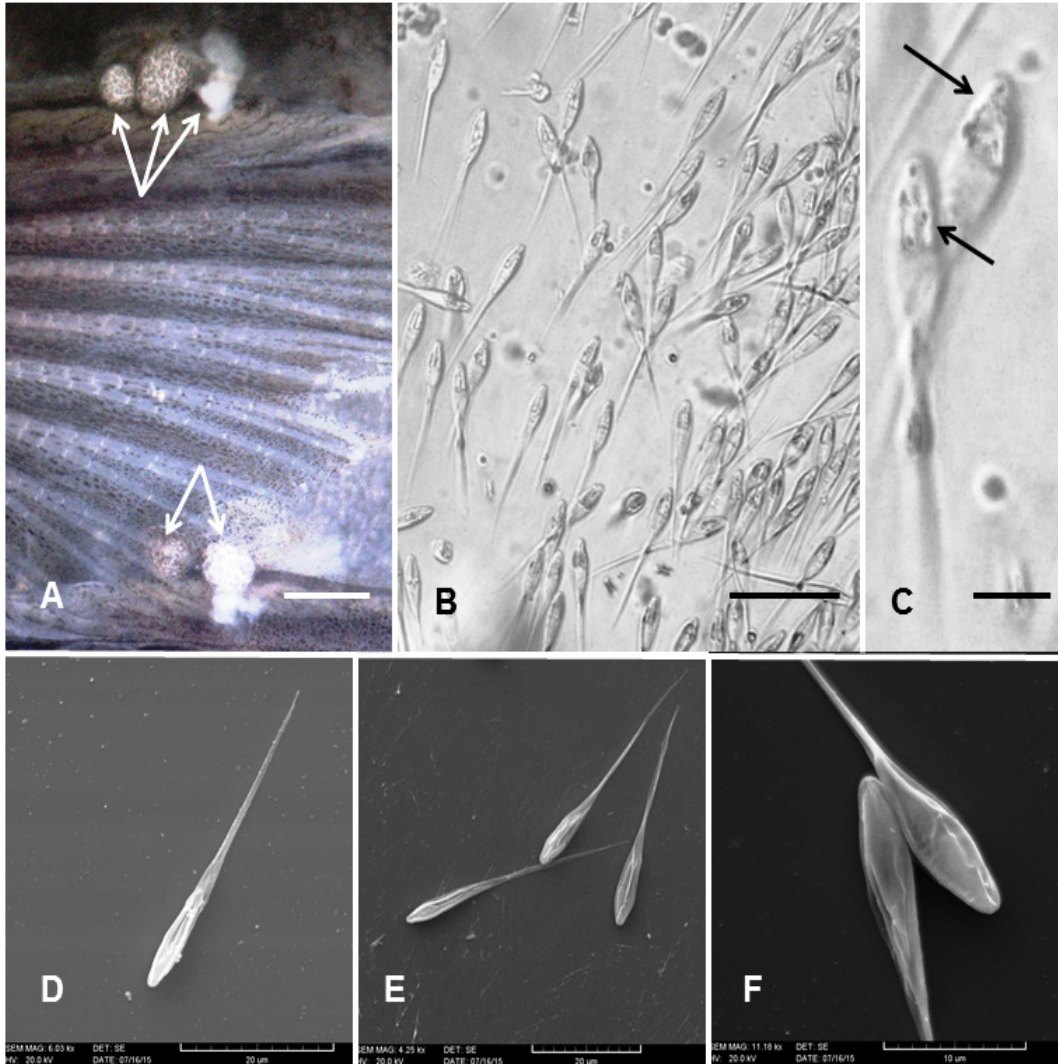
As far as we know, there are only two reports of *Henneguya* in Oklahoma fishes as follows: (1) the report by Parker et al. (1971) of *H. gambusi* from skin cysts of 5 of 143 (3%)

Western Mosquitofish, *Gambusia affinis* from Payne County, and (2) a “mandibular form” of a *Henneguya* sp. from a wild *I. punctatus*, also from Payne County (McCraren et al. 1975). Nothing else has been published about any other *Henneguya* infecting fish in the state, particularly those in the genus *Ameiurus*. Here, we report, for the first time, a species of *Henneguya* from *A. melas* from southeastern Oklahoma, and include light and scanning electron microscopy of the plasmodia and myxospores.

Between May 2013 and July 2015, 17 juvenile and adult *A. melas* (mean  $\pm$  1SD total length [TL] = 143.0  $\pm$  43.8, range 92–200 mm) were collected by seine or backpack electroshocker from Yashau Creek, McCurtain County (34.018852°N, 94.756761°W). Fish were placed in aerated creek water and taken to the laboratory for necropsy. They were killed by immersion in a concentrated chloretone solution, measured for TL, and their integument, gills, fins, peritoneal cavity, liver and kidney were examined for myxozoan plasmodia using a stereomicroscope. When plasmodia were seen, they were photographed *in situ*, excised by sharp dissection and their contents placed on a microscopic slide with a drop of 0.85% saline for microscopic examination. Additional samples were placed directly into 10% neutral buffered

formalin (NBF) for examination via scanning electron microscopy (SEM) or in 95% DNA grade ethanol for future molecular sequencing. For SEM, we transferred liquid suspensions in 10% NBF containing free myxospores through a graded series of increasing ethanol solutions (70–100%). Specimens were then extracted from vials with a pipette and placed

onto segments of glass coverslips (18 × 4 mm) previously coated with Poly-L-Lysine. An Autosamdri®–815 critical point drier (Tousimis Research Corporation, Rockville, MD; 31°C, 1072 psi, ventilation rate ~100 psi/min) was used to remove excess ethanol from cells. Dehydrated specimens on coverslips were then adhered to rectangular copper transfer boats (25



**Figure 1.** *Henneguya* sp. from *Ameiurus melas* from Oklahoma. **A.** In situ view showing plasmodia (arrows) on the pectoral fin. Scale bar = 2 mm. **B.** Numerous myxospores from wet mount preparation. Scale bar = 50  $\mu$ m. **C.** Wet mount preparation of unstained *Henneguya* sp. myxospores in sutural view showing the coiled polar filaments (arrows) within the polar capsule. Scale bar = 10  $\mu$ m. **D.** Scanning electron micrograph (SEM) of single myxospore. **E.** SEM of group of three myxospores showing variation in morphology. **F.** Higher magnification SEM showing morphology of spore body of myxospore.

× 5 mm) with double-sided tape and mounted onto sticky-tapped 12 mm aluminum pin stub mounts. Copper boats and stubs were then coated with gold using a Cressington 108 sputter coater (Cressington Scientific Instruments Ltd, Watford, UK). Specimens were examined with a Vega TS 5136XM digital scanning electron microscope (Tescan USA Inc., Cranberry Township, PA) at 19.5 kV. A host voucher specimen was deposited in the Henderson State University Collection (HSU), Arkadelphia, Arkansas; a parasite photovoucher was deposited in the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska.

One of 17 (6%) *A. melas* (juvenile, 102 mm TL) collected on 25 June 2015 was found to possess multiple white, ovoidal plasmodial pseudocysts measuring 1–2 mm in diameter on its dorsal and both pectoral fins (Fig. 1A). There were no pseudocysts on the gills or any other part of the body (barbels), including the peritoneal cavity, liver and kidney. Rupture and subsequent removal of the exudate for wet mount preparation revealed numerous lanceolate myxospores containing two polar granules with an elongate caudate process (Figs. 1B–C), fitting the description of *Henneguya* (see Hoffman 1999). Examination of wet mount material revealed myxospores with an elongate, lanceolate spore body, flattened parallel to the suture line and measuring 16.3 (range 15–19) long × 3.8 (3.7–4.0) μm wide in sutural view; total spore length (TSL) was 45.5 (range 42–50) μm. There were two valves and two polar capsules which were pyriform and of unequal size, oriented in plane with the sutural ridge.

In addition, 230 other fishes from Yashau Creek, including four *Aphredoderus sayanus*, four *Ameiurus natalis*, 14 *Campostoma spadiceum*, 10 *Cyprinella venusta*, 25 *C. whipplei*, seven *Esox americanus*, 101 *Fundulus notatus*, 10 *Gambusia affinis*, 13 *Labidesthes sicculus*, two *Lepomis cyanellus*, three *L. gulosus*, one *L. megalotis*, 26 *Luxilus chrysocephalus*, six *Lythrurus umbratilis*, and eight *Notemigonus crysoleucas*, were not found to be infected with *Henneguya* plasmodia.

The Black Bullhead has been previously reported to harbor three *Henneguya* spp. Kudo (1920) reported *H. gurleyi* from the dorsal fin of bullheads from Iowa, Kudo (1929) documented *H. exilis* from the gills of *A. melas* from Illinois, Guilford (1965) reported *H. limatula* from the gall bladder of *A. melas* from Wisconsin, and Iwanowicz et al. (2008) found *H. gurleyi* on fish from North Carolina. The measurements of myxospores of our specimens of *Henneguya* sp. from dermal plasmodia on fins most closely matches that of *H. diversis* Minchew from pectoral fins of *I. punctatus* from Mississippi (Minchew 1977); all *Henneguya* spp. previously reported from *A. melas* are larger in TSL compared to our specimens. However, we understand that myxospores can be indistinguishable from others based solely on morphological comparisons. Host- and tissue-specificity is also necessary for accurate identification in the absence of molecular analyses. Indeed, the most sensitive approach at defining species of myxozoans is utilization of small-subunit ribosomal RNA (SSU rDNA) gene sequences (Iwanowicz et al. 2008). Future studies are designed to obtain these sequences from our material (C. Whipps, *pers. comm.*) and examine additional ictalurids for this parasite.

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## References

Bosworth BG, Wise DJ, Terhune JS, Wolters WR. 2003. Family and genetic group effects for resistance to proliferative gill disease in Channel Catfish, Blue Catfish and Channel Catfish x Blue Catfish backcross hybrids.

- Aquacult. Res. 34:569–573.
- Griffin MJ, Khoo LH, Torrains L, Bosworth BG, Quiniou SM, Gaunt PS, Pote LM. 2009. New data on *Henneguya pellis* (Myxozoa: Myxobolidae), a parasite of Blue Catfish *Ictalurus furcatus*. J. Parasitol. 95:1455–1467.
- Guilford HG. 1965. New species of Myxosporidia from Green Bay (Lake Michigan). Trans. Amer. Microsc. Soc. 84:566–573.
- Hoffman GL. 1999. Parasites of North American freshwater fishes. 2<sup>nd</sup> ed. Ithaca, New York: Comstock Publishing Associates. 539 p.
- Iwanowicz LR, Iwanowicz DD, Pote LM, Blazer VS, Schill WB. 2008. Morphology and 18S rDNA of *Henneguya gurliei* (Myxosporea) from *Ameiurus nebulosus* (Siluriformes) in North Carolina. J. Parasitol. 94:46–57.
- Kudo RR. 1920. Studies on Myxosporidia. A synopsis of genera and species of Myxosporidia. Ill. Biol. Monogr. 5:1–265.
- Kudo RR. 1929. Histoic Myxosporidia found in fresh water fishes of Illinois. Archiv Protisten. 65:364–378.
- Lom J, Dyková I. 2006. Myxozoan genera: Definition and notes on taxonomy, life-cycle terminology and pathogenic species. Folia Parasitol. 53:1–36.
- McCrary JP, Landolt ML, Hoffman GL, Meyer FP. 1975. Variation in response of Channel Catfish to *Henneguya* sp. infections (Protozoa: Myxosporidea). J. Wild. Dis. 11:2–7.
- Minchew CD. 1977. Five new species of *Henneguya* (Protozoa: Myxosporida) from ictalurid fishes. J. Protozool. 23:467–477.
- Parker JD, Spall RD, Warner MC. 1971. Two new myxosporidia, *Henneguya gambusi* sp. n. and *Myxosoma pharyngeus* sp. n., in the Mosquitofish, *Gambusia affinis* (Baird and Girard). J. Parasitol. 57:1297–1301.
- Pote LMW, Khoo L, Griffin M. 2012. *Henneguya ictaluri*. In: Woo PTK, Buchmann K, editors. Fish parasites: Pathobiology and protection. Wallingford (UK): CAB International. p. 177–192.

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# Commensal Protista, Cnidaria and Helminth Parasites of the Cajun Chorus Frog, *Pseudacris fouquettei* (Anura: Hylidae), from Oklahoma

**Chris T. McAllister**

Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

**Charles R. Bursey**

Department of Biology, Pennsylvania State University-Shenango Campus, Sharon, PA 16146

**Dana M. Calhoun**

Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309

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**Abstract:** Twenty adult Cajun chorus frogs (*Pseudacris fouquettei*) were collected in McCurtain County, Oklahoma, and examined for commensal protozoans and helminth parasites. All 20 frogs harbored with one or more species, including 13 each (65%) with *Opalina* sp., and *Nyctotherus cordiformis*, 10 (50%) with *Cystodiscus melleni*, three (15%) with unknown reniferid metacercaria, three (15%) with *Mesocoelium* sp., one (5%) with *Cylindrotaenia americana*, four (20%) with *Oswaldocruzia leidy*, five (25%) with *Cosmocercoides variabilis*, and three (15%) with unidentified acuariid larva. All (100%) harbored two or more protists, a cnidarian and/or helminths each. The *Mesocoelium* sp. appears to be a new species and new host records are reported for it as well as reniferid metacercaria; new distributional records in the state are documented for *N. cordiformis*, *C. melleni*, *Mesocoelium* sp. and *O. leidy*. ©2015 Oklahoma Academy of Science

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## Introduction

The Cajun chorus frog (*Pseudacris fouquettei*) ranges from extreme southern Missouri south to western Mississippi, through all of Louisiana and Arkansas, and west to eastern Texas and Oklahoma (Lemmon et al. 2008). In Oklahoma, *P. fouquettei* is found in the central and eastern part of the state in partly wooded areas and prairies (Sievert and Sievert 2011). It is one of the first frogs to call in the winter and breeds during and after heavy rains in early spring; an adult frog is rarely found except during the breeding season.

McAllister et al. (2008) reported the cnidarian (myxozoan), *Cystodiscus* (= *Myxidium*) *melleni* from *P. fouquettei* (as *P. triseriata feriarum*) from Texas, and more recently, McAllister et al. (2013a) reported on protozoan and helminth parasites of *P. fouquettei* from Arkansas and Texas. Several helminths, including three digeneans (*Brachycoelium salamandrae*, *Glyphelmis quieta*, *Mesocoelium monas*), a tapeworm (*Cylindrotaenia americana*), and five nematodes (*Cosmocercoides variabilis*, *Oswaldocruzia leidy*, *O. pipiens*, *Physaloptera* sp., and acuariid larvae) have been previously reported from *P. fouquettei* from Arkansas (McAllister et al. 2013), Oklahoma (as *P. nigrita*

*triseriata*, *P. triseriata* or *P. feriarum*, Kuntz 1941) and Texas (as *P. triseriata*, Harwood 1930, 1932; McAllister et al. 2013a). Here, for the first time for a moderately-sized population from Oklahoma, we report new information on commensal protists, a cnidarian and helminth parasites of *P. fouquettei* in a survey of individuals from the southeastern part of the state.

## Methods

During 11–13 March 2015, 20 adult (18 male, 2 female) *P. fouquettei* (mean  $\pm$  1SD snout–vent length [SVL] =  $32.2 \pm 2.7$ , range 27–39 mm) were collected by hand from temporary wetland in Hochatown off US 259 in McCurtain County (34.162096°N, 94.755017°W). Specimens were placed on ice in individual bags and taken to the laboratory within 24 hr for necropsy. Frogs were overdosed by immersion in a concentrated chlorethone solution and a mid-ventral incision from mouth to cloaca was made to expose the gastrointestinal (GI) tract. The entire GI tract from the mouth to cloaca was split lengthwise and along with gall bladder, kidneys, liver, lungs, and gonads were placed in Petri dishes and examined using a stereomicroscope. The eustachian tubes were not examined. Frogs were also examined for select protists, including the gall bladder for cnidarians, the rectum for opalinids and ciliates, and the feces for coccidia following methods of Upton and McAllister (1988), McAllister et al. (1989), and McAllister and Trauth (1995). Trematodes and cestodes were fixed in nearly boiling tap water without coverslip pressure, transferred to 70–95% DNA grade ethanol, stained with acetocarmine and mounted in Canada balsam. Nematodes were fixed in hot 70% ethanol and placed on a glass slide in a drop of undiluted glycerol for identification. Photovoucher and regular voucher specimens of parasites were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. Host voucher specimens were deposited in the Arkansas State University Herpetological Collection (ASUMZ), State University, Arkansas. Prevalence, mean intensity, and range of infection are provided in accordance with terminology given in Bush et al. (1997).

## Results and Discussion

All 20 of the *P. fouquettei* (Table 1) were found to harbor at least one of two commensal protists, a cnidarian and/or six helminths, including 13 (65%) with *Opalina* sp., and *Nyctotherus cordiformis* (Fig. 1A), 10 (50%) with *Cystodiscus melleni* (Figs. 1B–C), three (15%) with numerous reniferid metacercaria (Figs. 1D–F), three (15%) with *Mesocoelium* sp. (Fig. 1G), one (5%) with a single *Cylindrotaenia americana*, four (20%) with five female *Oswaldocruzia leidyi*, five (25%) with 10 (2 male, 3 female, 5 immature) *Cosmocercoides variabilis*, three (15%) with numerous acuariid larvae (Fig. 1H). All (100%) harbored multiple infections of two or more protists, cnidarians and/or helminths each. None of the *P. fouquettei* was passing coccidian oocysts in feces at the time they were sampled.

The commensal protist, *Nyctotherus cordiformis* (Ehrenberg, 1838) Stein, 1867 as well as commensal *Opalina* sp. have been reported previously from *P. fouquettei* from Arkansas and Texas (McAllister et al. 2013a). Trowbridge and Hefley (1934) reported an *Opalina* sp. in several Oklahoma anurans. A “very light” infection of an unidentified *Nyctotherus* sp. was reported in a Texas horned lizard (*Phrynosoma cornutum*) from Norman, Oklahoma, by Zimmerman and Brown (1952). However, we are unaware of any published report of *N. cordiformis* in Oklahoma anurans. Although this protist is cosmopolitan in distribution, we document it (HWML photovoucher 101835) in an Oklahoma frog for the first time.

*Cystodiscus* (syn. *Myxidium*) *melleni* Jirků, Bolek, Whipps, Janovy, Kent, and Modrý, 2006 was reported originally from western chorus frog (*Pseudacris triseriata*) and Blanchard’s cricket frog (*Acris blanchardi*) from Nebraska (Jirků et al. 2006). Since then, this cnidarian was found in *P. fouquettei* in Arkansas (McAllister et al. 2013a) and Texas (McAllister et al. 2008). Ribosomal DNA sequencing of trophozoites and free spores (HWML photovoucher 101836) from our Oklahoma *P. fouquettei* confirmed the identity as *C. melleni* (C. Whipps, pers. comm.). We add

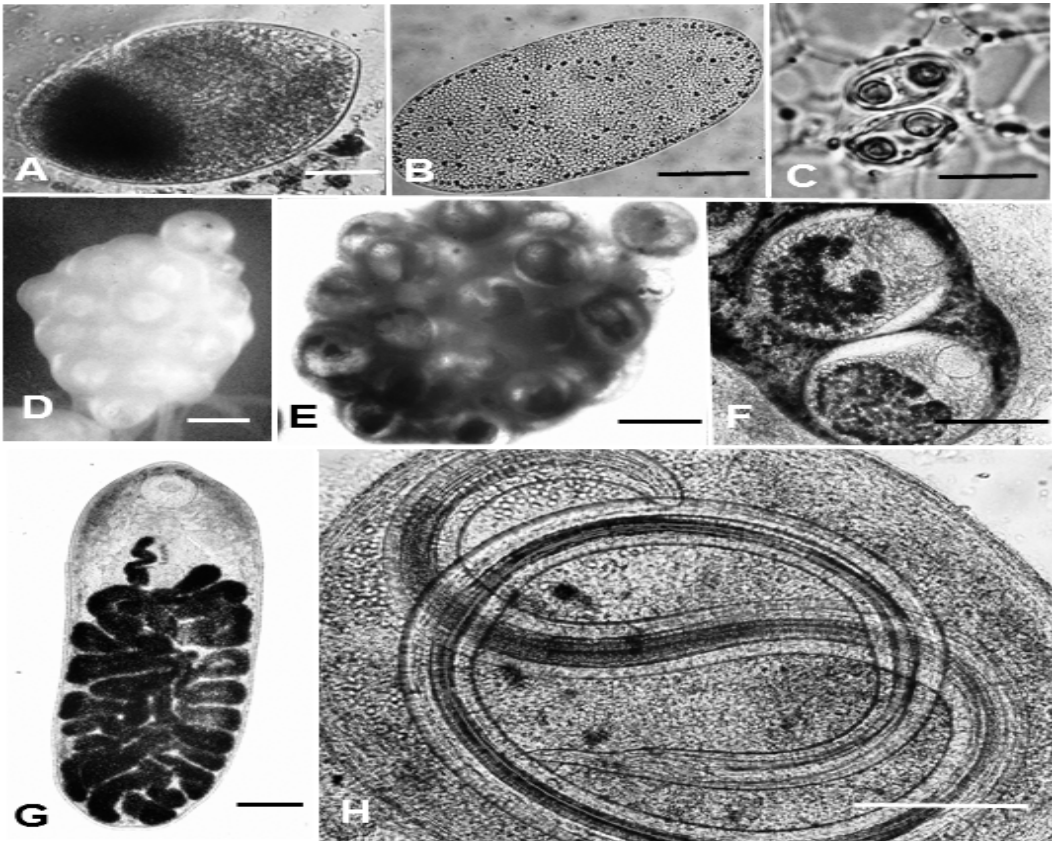


Table 1. Presence (+) or absence (-) of each parasite/commensal from 20 individual *Pseudacris fouquettei* in Oklahoma.

Parasite/Commensal	Frog Number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<b>Protista</b>																				
<i>Nyctotherus cordiformis</i> *	-	-	+	+	+	+	+	-	+	+	+	-	+	+	+	-	+	-	+	+
<i>Opalina</i> sp.	+	-	+	+	-	-	+	+	+	-	-	+	-	+	+	-	-	+	+	+
<b>Cnidaria</b>																				
<i>Cleidodiscus mellini</i> *	-	+	-	+	+	+	-	+	-	-	-	-	+	-	-	+	-	+	-	+
<b>Trematoda</b>																				
<i>Mesocoelium</i> sp. *, †	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+
<i>Renijer metacercaria</i> †	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-
<b>Cestoda</b>																				
<i>Cylindrotaenia americana</i>	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Nematoda</b>																				
Acutariid larvae	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
<i>Cosmoecoides variabilis</i>	+	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Oswaldocruzia leidyi</i> *	-	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	+	-

\*New distributional record.

†New host record.



**Figure 1.** Commensal protists, a cnidarian, and helminths from *Pseudacris fouquettei* from Oklahoma (all unstained). **A.** *Nyctotherus cordiformis*. Scale bar = 50  $\mu\text{m}$ . **B.** *Cystodiscus melleni* trophozoite (plasmodium). Scale bar = 100  $\mu\text{m}$ . **C.** *Cystodiscus melleni* spores. Scale bar = 10  $\mu\text{m}$ . **D.** Macroscopic view of reniferid metacercariae in cysts. Scale bar = 1 mm. **E.** Another view of same cysts. Scale bar = 1 mm. **F.** Two reniferid metacercariae in cysts. Scale bar = 500  $\mu\text{m}$ . **G.** *Mesocoelium* sp. Scale bar = 200  $\mu\text{m}$ . **H.** Acuariid larvae in cyst. Scale bar = 200  $\mu\text{m}$ .

Oklahoma to the geographic range of *C. melleni*.

Unidentified reniferid metacercariae (HWML 91942, photovoucher 101837) were found encapsulated in the coelomic cavity of *P. fouquettei* (Figs. 1D-F). Numerous (> 100) metacercariae appeared grouped together similar to the appearance of a “cluster of berries” (see Fig. 1D). *Renifer* (syn. *Ochetosoma*) and *Pneumatophilus* spp., as adults, occur in the oral cavity, esophagus and lungs of mostly natricine and other snakes (Tkach 2008). This is the first time, to our knowledge, these kind of metacercariae have been reported from this anuran host.

McAllister et al. (2013a) previously reported *Mesocoelium monas* (Rudolphi, 1819) Frietas, Proc. Okla. Acad. Sci. 95: pp 86 - 92 (2015)

1958 from one of 14 (7%) *P. fouquettei* from Arkansas. Recently, Calhoun and Dronen (2012) reevaluated specimens previously identified as *M. monas* and proposed keys to some 43 species. Our species of *Mesocoelium*, however, differs from *M. monas* by having a genital pore bifurcal compared with prebifurcal (Calhoun and Dronen 2012; Dronen et al. 2012). Dronen et al. (2012) divided the genus of *Mesocoelium* into six body types based primarily on cecal length, genital pore location compared to cecal bifurcation, and finally whether the genital pore is median or submedian on the body. Using these key characters developed by Dronen et al. (2012) our specimens of *Mesocoelium* (HWML photovoucher 101838) places them in the *Mesocoelium sociale* (Lühe, 1901) Odhner,

Table 2. Summary of commensals and parasites reported from *Pseudis fouquettei* from Arkansas, Oklahoma and Texas.

Commensal/Parasite	State	Prevalence*	Intensity†	Reference
<b>Protista</b>				
<i>Nyctotherus cordiformis</i>	AR	13/14 (93%)	–	McAllister et al. (2013)
	OK	13/20 (65%)	–	This study
	TX	2/5 (40%)	–	McAllister et al. (2013)
<i>Opalina</i> sp.	AR	13/14 (93%)	–	McAllister et al. (2013)
	OK	13/20 (65%)	–	This study
	TX	5/5 (100%)	–	McAllister et al. (2013)
<b>Cnidaria</b>				
<i>Cleidodiscus melini</i>	AR	6/14 (43%)	–	McAllister et al. (2013)
	OK	10/20 (50%)	–	This study
	TX	1/6 (17%)	–	McAllister et al. (2008)
<b>Trematoda</b>				
<i>Brachycoelium salamandrae</i> ‡	TX	2/26 (8%)	not given	Harwood (1932)
	OK	1/3 (33%)	not given	Kuntz (1940) §
	TX	2/26 (8%)	not given	Harwood (1932)
<i>Megalodiscus temperatus</i>	OK	3/20 (15%)	2.6 ± 2.1 (1–5)	This study
<i>M. monas</i>	AR	1/14 (7%)	1	McAllister et al. (2013)
<i>Renifer metacercaria</i>	OK	3/20 (15%)	–	This study

Unknown metacercaria	OK	3/25 (12%)	–	Bouchard (1953)#
<b>Cestoda</b>				
<i>Cylindrotaenia americana</i>	OK	1/25 (4%) 1/20 (5%)	1 1	Bouchard (1953)# This study
<b>Nematoda</b>				
Acuariid larvae	OK TX	3/20 (15%) 1/5 (20%)	– 1	This study McAllister et al. (2013)
<i>Cosmoceroides variabilis</i>	AR OK	2/14 (14%) 5/20 (25%)	1, 1 2.5 ± 1.0, 2–4	McAllister et al. (2013) This study
<i>Physaloptera</i> sp.	TX	16/26 (62%)	not given	Harwood (1930,1932)¶
<i>Oswaldocruzia leidyi</i>	AR OK	2/14 (14%) 4/20 (20%)	1, 1 1.3 ± 0.5, 1–2	McAllister et al. (2013) This study
<i>O. pipiens</i>	TX OK	1/5 (20%) 5/25 (20%)	3 4?	McAllister et al. (2013) Bouchard (1953)#

\*Prevalence = number infected/number examined (%).

†mean ± 1SD (range), where applicable or (–) too numerous to count.

‡Parasite originally reported as *Brachycoelium daviesi* (= *B. salamandrae*).

¶Host reported as *Pseudacris triseriata* (= *P. fouquettei*) per Lemmon et al. (2008).

§Unpublished thesis; Kuntz (1941) did not specify what host species were infected.

#Unpublished dissertation.

1910 body type by having ceca that surpass the ovary and a genital pore that is bifurcal and submedian. *Mesocoelium sociale* has previously been described from Asian black-spotted toad (*Duttaphrynus melanostictus*) in northern India and giant Asian toad (*Phrynooidus asper*) in Malaysia (Dronen et al. 2012). To date, *M. sociale* has not been reported from the United States. Our specimens differ from *M. sociale* by having a smaller oral sucker width (175–195 vs. 200–225  $\mu\text{m}$ ), slightly smaller oral sucker to pharynx ratio (1:2.0 vs. 1:2.1–1:2.3), larger oral sucker to ventral sucker ratio (1:1.4–1:1.6 vs. 1:1.2–1:1.3), and longer eggs (42.5–45.0 vs. 38–40  $\mu\text{m}$ ). Other amphibian hosts of *M. sociale* include *Fejervarya* (= *Rana*) *cancrivora*, *Hylarana erythraea*, *Kaloula baleata*, *K. pulchra*, and *Polypedates* (= *Rhacophorus*) *leucomystax* from Malaysia and Thailand (Fischthal and Kuntz 1965; Wongsawad et al. 1998).

*Mesocoelium sociale* was also reported from the GI tract of several lizards, including *Anolis sagrei*, *Bronchocela* (= *Calotes*) *crystalinus*, *Eutropis longicaudata*, *Japalura swinhonsis*, *Plestiodon elegans*, and *Sphenomorphus indicus* from Taiwan (Fischthal and Kuntz 1975; Norval et al. 2011, 2014; Goldberg et al. 2014), and *Hemidactylus frenatus*, *Cosymbotus platyurus*, *C. versicolor*, *Gecko gecko*, *Mabuya multifasciata* from Indonesia and Malaysia (Killick and Beverley-Burton 1982; Kennedy et al. 1987).

In the end, there are significant zoogeographical and anuran host family differences as our specimens of *Mesocoelium* were discovered in hylid frogs from the southwestern United States compared with *M. sociale* from bufonid toads from India and Malaysia. Therefore, we believe our specimens of *Mesocoelium* represent a new species and future study will include molecular analyses (V. Tkach, pers. comm.).

The tapeworm, *Cylindrotaenia americana* Jewell, 1916 is a commonly-encountered parasite of the small intestine of various amphibians, particularly anurans (McAllister et al. 2013b). It has been previously reported from Great Plains toad (*Anaxyrus cognatus*), Blanchard's cricket

frog (*Acris blanchardi*), American bullfrog (*Lithobates catesbeianus*), southern leopard frog (*Lithobates sphenoccephalus utricularius*) and dwarf American toad (*Anaxyrus americanus charlesmithi*) in Oklahoma (Trowbridge and Hefley 1934; McAllister et al. 2014; Vhora and Bolek 2015). Harwood (1932) reported *C. americana* in *P. fouquettei* (as *P. triseriata*) from Houston, Texas. However, we document *C. americana* (HWML 91943) for the first time in Oklahoma *P. fouquettei*.

*Cosmocercoides variabilis* (Harwood, 1930) Travassos, 1931 has previously been reported from the state in *A. a. americanus*, *L. catesbeianus*, Sequoyah slimy salamanders, *Plethodon sequoyah* and Hurter's spadefoot, *Scaphiopus hurterii* (Trowbridge and Hefley 1934; McAllister and Bursey 2004; McAllister et al. 2005, 2014). This nematode also has been previously reported from *P. fouquettei* in Arkansas (McAllister et al. 2013a). It is reported here (HWML 91944) from an Oklahoma population of *P. fouquettei* for the first time.

The strongylid nematode, *Oswaldocruzia leidy* Steiner, 1924 was reported from *P. fouquettei* from Arkansas and Texas (McAllister et al. 2013a). Trowbridge and Hefley (1934) were the first to report a similar species, *Oswaldocruzia pipiens* Walton, 1929 from Oklahoma in *Lithobates* spp. and Woodhouse's toad, *Anaxyrus woodhousii*. Kuntz and Self (1944) reported an *Oswaldocruzia* sp. from an unspecified anuran host. In an unpublished dissertation, Bouchard (1953) reported *O. pipiens* from *P. fouquettei* (as *P. triseriata*) from Oklahoma. Although *O. pipiens* was earlier reported from *P. fouquettei* (as *P. nigrita triseriata*, *P. triseriata* or *P. feriarum*) in Oklahoma by Kuntz (1941) and numerous other anurans from Arkansas, Florida, Georgia, Louisiana, Maine, Ohio, Texas, and Virginia, and Alberta and Ontario, Canada (see references in McAllister et al. 2013a), we document *O. leidy* (HWML 91945) in an Oklahoma host for the first time.

Unidentified acuariid larvae (Spirurida) were previously reported in *P. fouquettei* from

Texas (McAllister et al. 2013a). Species within the Acuariidae are primarily parasites of birds, although several species have been reported from mammals and frogs, like *P. fouquettei*, may serve as paratenic hosts (Anderson 2000). This is the second time these nematodes (HWML 91946, photovoucher 101839) have been reported from Oklahoma (see McAllister et al. 2014).

The Cajun chorus frog has now been the subject of helminth surveys from specimens collected in Arkansas, Oklahoma and Texas. To date, the helminth list of *P. fouquettei* includes seven trematode species, one tapeworm species, and five nematode species (Table 2). When compared to previous surveys on this host, all of the helminths, except *Mesocoelium* sp. and the reniferid metacercaria have been reported from *P. fouquettei*. However, new distributional records are documented in the state for *N. cordiformis*, *C. melleni*, *Mesocoelium* sp. and *O. leidyi*. As the host range also includes Missouri, and, more importantly, sites east of the Mississippi River in western Mississippi, surveys should include *P. fouquettei* from those states in order to fully compare their endoparasites with previous surveys from other states. Furthermore, future research needs to include molecular sequencing to differentiate between interspecific and intraspecific morphological variation in the genus *Mesocoelium*.

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The Oklahoma Department of Wildlife Conservation issued a Scientific Collecting Permit to CTM. We also thank Drs. Scott L. Gardner (HWML) and Stanley E. Trauth (ASUMZ) for expert curatorial assistance, Vasyl V. Tkach (Univ. of North Dakota) for information on the metacercaria, Christopher M. Whipps (SUNY-ESF) for sequencing the cnidarian, and Mr. Nikolas H. McAllister (Lukfata Elementary, Broken Bow, OK) for assistance in collecting.

## References

Anderson RC. 2000. Nematode parasites of vertebrates: Their development and transmission. Second Edition. Wallingford,

Oxon (UK): CAB International. 650 p.

Bouchard JL. 1953. An ecological and taxonomic study of helminth parasites from Oklahoma amphibians [PhD thesis]. Norman (OK): University of Oklahoma. 138 p. Available from: University Microfilms, Ann Arbor, MI.

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

Calhoun DM, Dronen NO. 2012. A reevaluation of specimens of *Mesocoelium monas* (Platyhelminthes: Digenea: Mesocoeliidae) from the Natural History Museum, UK and the United States National Parasite Collection, USA. *Zootaxa* 3589: 1–29.

Dronen NO, Calhoun DM, Simcik SR. 2012. *Mesocoelium* Odhner, 1901 (Digenea: Mesocoeliidae) revisited; A revision of the family and re-evaluation of species composition in the genus. *Zootaxa* 3387:1–96.

Fischthal JH, Kuntz RE. 1965. Digenetic trematodes from amphibians and reptiles from north Borneo (Malaysia). *Proc. Helminthol. Soc. Wash.* 32:124–136.

Fischthal JH, Kuntz RE. 1975. Some trematodes of amphibians and reptiles from Taiwan. *Proc. Helminthol. Soc. Wash.* 42:1–13.

Harwood PD. 1930. A new species of *Oxysomatium* (Nematoda) with some remarks on the genera *Oxysomatium* and *Aplectana*, and observations on the life history. *J. Parasitol.* 17:61–73.

Harwood PD. 1932. The helminths parasitic in the Amphibia and Reptilia of Houston, Texas, and vicinity. *Proc. US Nat. Mus.* 81:1–71.

Jirků M, Bolek MG, Whipps CM, Janovy J Jr, Kent ML, Modrý D. 2006. A new species of *Myxidium* (Myxosporaea: Myxidiidae), from the western chorus frog, *Pseudacris triseriata triseriata*, and Blanchard's cricket frog, *Acris crepitans blanchardi* (Hylidae), from eastern Nebraska: Morphology, phylogeny, and critical comments on amphibian *Myxidium* taxonomy. *J. Parasitol.* 92:611–619.

Kennedy MJ, Killick LM, Beverley-Burton M. 1987. The prevalence of *Paradistomum geckonum*, *Mesocoelium sociale*, and *Postorchigenes ovalis* (Digenea) in lizards

- (Sauria) from Indonesia. *Can. J. Zool.* 65:1292–1294.
- Killick LM, Beverley-Burton M. 1982. Observations on digeneans from lizards (Sauria) in Indonesia (*Paradistomum geckonum*, *Mesocoelium sociale*, and *Postorchigenes ovatus* with a revision of *Paradistomum* Kossack, 1910 (Dicrocoeliidae). *Can. J. Zool.* 65:2093–2106.
- Kuntz RE. 1940. A study of anuran parasites of Comanche County, Oklahoma [MS thesis]. Norman (OK): University of Oklahoma. 93p. Available from: University of Oklahoma Library.
- Kuntz RE. 1941. The metazoan parasites of some Oklahoma Anura. *Proc. Okla. Acad. Sci.* 21:33–34.
- Kuntz RE, Self JT. 1944. An ecological study of the metazoan parasites of the Salientia of Comanche County, Oklahoma. *Proc. Okla. Acad. Sci.* 24:35–38.
- Lemmon EM, Lemmon AR, Collins JT, Cannatella DC. 2008. A new North American chorus frog species (*Pseudacris*: Hylidae: Amphibia) from the south-central United States. *Zootaxa* 1675:1–30.
- McAllister CT, Bursey CR. 2004. Endoparasites of the Sequoyah slimy salamander, *Plethodon sequoyah* (Caudata: Plethodontidae), from McCurtain County, Oklahoma. *Tex. J. Sci.* 56:273–277.
- McAllister CT, Bursey CR, Conn DB. 2005. Endoparasites of Hurter's spadefoot, *Scaphiopus hurterii* and plains spadefoot, *Spea bombifrons* (Anura: Scaphiopodidae), from southern Oklahoma. *Tex. J. Sci.* 43:391–397.
- McAllister CT, Bursey CR, Connior MB, Trauth SE. 2013a. Symbiotic Protozoa and helminth parasites of the Cajun chorus frog, *Pseudacris fouquettei* (Anura: Hylidae), from southern Arkansas and northeastern Texas. *Comp. Parasitol.* 80:96–104.
- McAllister CT, Bursey CR, Connior MB, Trauth SE. 2014. Myxozoan and helminth parasites of the dwarf American toad, *Anaxyrus americanus charlesmithi* (Anura: Bufonidae), from Arkansas and Oklahoma. *Proc. Okla. Acad. Sci.* 94:51–58.
- McAllister CT, Bursey CR, Robison HW, Connior MB. 2013b. Parasites of the Ozark sig-zag salamander, *Plethodon angusticlavius* (Caudata: Plethodontidae), from northern Arkansas. *Comp. Parasitol.* 80:69–79.
- McAllister CT, Bursey CR, Trauth SE. 2008. New host and geographic distribution records for some endoparasites (Myxosporea, Trematoda, Cestoidea, Nematoda) of amphibians and reptiles from Arkansas and Texas, U.S.A. *Comp. Parasitol.* 75:241–254.
- McAllister CT, Trauth SE. 1995. New host records for *Myxidium serotinum* (Protozoa: Myxosporea) from North American amphibians. *J. Parasitol.* 81:485–488.
- McAllister CT, Upton SJ, Conn DB. 1989. A comparative study of endoparasites in three species of sympatric *Bufo* (Anura: Bufonidae), from Texas. *Proc. Helminthol. Soc. Wash.* 56:162–167.
- Norval G, Bursey CR, Goldberg SR, Mao, J-J, Slater J. 2011. Origin of the helminth community of an exotic invasive lizard, the brown anole, *Anolis sagrei* (Squamata: Polychrotidae), in southwestern Taiwan. *Pac. Sci.* 65:383–390.
- Norval G, Goldberg SR, Bursey CR, Mao, J-J, Slater J. 2014. Internal parasites of lizards from Taiwan. *Herpetol. Conserv. Biol.* 9:484–494.
- Sievert G, Sievert L. 2011. A field guide to Oklahoma's amphibians and reptiles. Oklahoma City (OK): Oklahoma Department of Wildlife Conservation. 211 p.
- Tkach VV. 2008. Family Reniferidae Pratt, 1902. In: Gibson DI, Jones A, Bray RA (editors). *Keys to the Trematoda*, Volume 3. Wallingford and London (UK): CABI Publishing and Natural History Museum. p. 411–419.
- Trowbridge AH, Hefley HM. 1934. Preliminary studies of the parasite fauna of Oklahoma anurans. *Proc. Okla. Acad. Sci.* 14:16–19.
- Upton SJ, McAllister CT. 1988. The coccidia (Apicomplexa: Eimeriidae) of Anura, with descriptions of four new species. *Can. J. Zool.* 66: 1822–1830.
- Vhora MS, Bolek MG. 2015. Temporal occurrence and community structure of helminth parasites in southern leopard frogs, *Rana sphenoccephala*, from north central Oklahoma. *Parasitol. Res.* 114:1197–1206.

- Wongsawad C, Sey O, Rojanapaibal A, Chariyahpongpan P, Suwattanacupt S, Marayong T, Wongsawad P, Rojtinnakorn J. 1998. Trematodes from amphibians and reptiles from Thailand. *J. Sci. Soc. Thailand* 24:265–274.
- Zimmerman RM, Brown HP. 1952. Observations on some intestinal Protozoa in Oklahoma lizards, with the description of a new genus, *Biflagella*. *Proc. Okla. Acad. Sci.* 33:103–111.

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# Additional Distribution Records of Scolopendromorph Centipedes in Oklahoma

**David Bass**

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

**Kinsey Tedford**

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

**Courtney Bass Harlin**

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

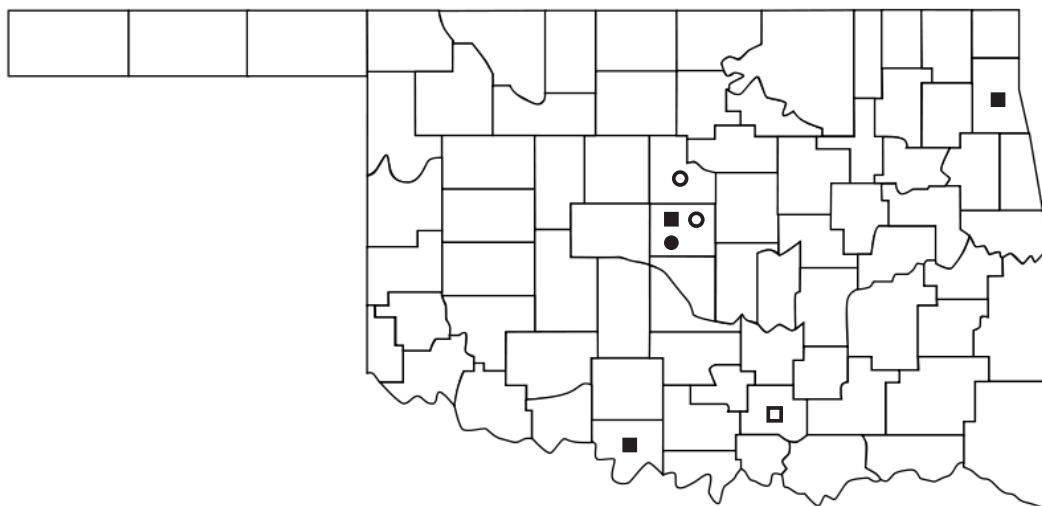
**Rowland M. Shelley**

Florida State Collection of Arthropods, Division of Plant Industry, P. O. Box 147100, Gainesville, FL 32614-7100

Centipede distributions in Oklahoma were largely unknown until the synopsis of North American Scolopendromorpha (Shelley 2002) was published. Subsequent works on this order by McAllister et al. (2003, 2004, 2006, and 2014) have supplemented this knowledge base and provided a more complete documentation of distributions within the state.

Collections in the University of Central

Oklahoma Natural History Museum, the Sam Noble Museum of Natural History, and the Oklahoma State University Entomology Museum were examined, enabling us to report four scolopendromorph species and three families from five Oklahoma counties where centipedes have not been previously recorded (Fig. 1). Additional documentations are expected as research into the Oklahoma centipede diversity and distribution continues.



**Figure 1.** New Oklahoma records of *Hemiscolopendra marginata* (solid dot), *Scolopendra heros* (solid square), *Scolopocryptops rubiginosus* (open dot), and *Theatops posticus* (open square).

## Scolopendridae

*Scolopendra heros* Girard, 1953. The largest-bodied North American centipede, *S. heros*, exhibits a variety of colors and color patterns; in Oklahoma forms, the heads/cephalic plates are generally reddish, along with the 1<sup>st</sup> and 2<sup>nd</sup> tergites (Fig. 2). It is expected statewide in Oklahoma (Shelley 2002, McAllister et al. 2014), as opposed to congeneric species, *S. viridis* Say, 1821 and *S. polymorpha* Wood, 1861, which are unknown from the eastern periphery. Sightings in Oklahoma County (Co.) as far back as seven years ago have been communicated to David Bass, and are documented below:



**Figure 2.** *Scolopendra heros* at Lake Hefner Dam, 24 July 2014.

Oklahoma Co. in general [number of individuals = 4], 29 November 2008, J. Mears (UCONHM 12516), det. DB; Oklahoma City, Lake Hefner Dam, 24 July 2014, CBH (UCONHM 12545), det. DB (Figure 2); Arcadia Lake, Oklahoma Wildlife Department Land, 10 June 2009, W. Lord (UCONHM 12462), det. DB; Oklahoma City, Martin Park Nature Center, 29 May 2015, A. Jones (UCONHM 12546), det. DB.

Jefferson Co., [number of individuals = 4], 5 mi. west of Waurika near State Highway 70, 11 April 2015, E. Van (UCONHM 12536), det. DB.

Delaware Co., [number of individuals = 1], Lat. 36° 32' 56.16" N, Long. 94° Proc. Okla. Acad. Sci. 95: pp 94 - 95 (2015)

58' 3.49" W, 6 September 2015, A. Prince (UCONHM 12775), det. DB.

*Hemiscolopendra marginata* (Say, 1821). Prior records of *H. marginata* have been limited to southeastern Oklahoma (Shelley 2002, McAllister et al. 2014), so its occurrence in Oklahoma Co. is its westernmost documentation. This may reflect an allopatric population in the center of the state, or it may be an artifact of limited collections from those counties lying in between.

Oklahoma Co. in general [number of individuals = 1], 28 April 1965, Dyer (UCONHM 12460), det. RMS.

## Scolopocryptopidae

*Scolopocryptops rubinosus* L. Koch, 1878. This scolopocryptopid occurs throughout the eastern 2/3 of Oklahoma, except for the southeastern corner (Shelley 2002, McAllister et al. 2014), but no records exist for the following two counties:

Logan Co., [number of individuals = 1], 27 September 1987, R. Grantham (UCONHM 12459), det. RMS.

Oklahoma Co., [number of individuals = 1], 28 April 1965, Dyer (UCONHM 12461), det. RMS.

## Plutoniumidae

*Theatops* Newport, 1844, with two species in Oklahoma, is readily distinguished by the enlarged forcipulate caudal in contrast to other Oklahoma scolopendromorphs. *Theatops posticus* (Say, 1821) lacks distomedial preformal spines on the caudal legs, whereas *T. spinicaudus* (Wood, 1862) possess these structures. *T. posticus* has been found previously in southeastern Oklahoma and the following county record may be added:

Johnston Co., Blue River State Recreation Area, [number of individuals = 1], 13 September 1999, DB (UCONHM 12344), det. DB.

## References

McAllister CT, Robison HW, Connior MB.

2014. New geographic distribution records for centipedes (Chilopoda: Scolopendromorpha) from Oklahoma. *Proc. Okla. Acad. Sci.* 94: 44-47.
- McAllister CT, Shelley RM, Cameron ML. 2004. Significant new distribution records for the centipede, *Theatops posticus* (Say) (Chilopoda: Scolopendromorpha: Cryptopidae), from Oklahoma, with four new records from the Ark-La-Tex. *Proc. Okla. Acad. Sci.* 84:73-74.
- McAllister CT, Shelley RM, McAllister JT III. 2003. Geographic distribution records for scolopendromorph centipedes (Arthropoda: Chilopoda) from Arkansas, Oklahoma, and Texas. *J. Ark. Acad. Sci.* 57:111-114.
- McAllister CT, Shelley RM, Robison HW. 2006. Additional distributional records for scolopendromorph centipedes (Chilopoda) from Arkansas, Kansas, Louisiana, New Mexico, Oklahoma, and Texas, with the first report of *Theatops spinicaudus* (Wood) (Cryptopidae) from Texas. *Texas J. Sci.* 58:299-308.
- Shelley RM. 2002. A synopsis of the North American centipedes of the order Scolopendromorpha (Chilopoda). *Virginia Mus. Nat. Hist. Mem. No.* 5:1-108.

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# Macroinvertebrate Community Structure and Physicochemical Conditions of a Southeastern Oklahoma Bog

**Kinsey Tedford**

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

**David Bass**

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

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**Abstract:** An unusual wetland pond, known as a quaking bog, is located on the Oka' Yanahli Preserve in Johnston County, Oklahoma. There is little known about these ecosystems, and none have been described in published studies from Oklahoma. Physicochemical data and aquatic macroinvertebrates were collected in June and December of 2014. With the exception of low dissolved oxygen concentrations, the water quality was capable of supporting a healthy aquatic ecosystem. A total of 10,917 individuals representing 40 taxa were collected during summer and winter. Oligochaetes, bivalves, and chironomids dominated the collections, with 75% of all individuals categorized as detritivores. The highest species richness values and greatest number of individuals occurred in the center of the pond where water levels were the most stable. ©2015 *Oklahoma Academy of Science*

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## Introduction

A quaking bog is a type of wetland pond that possesses a false bottom composed of a thick layer of vegetation floating beneath the water surface. The pond retains water from precipitation, runoff, and groundwater seepage. Aquatic vascular plants will grow toward the surface from the floating vegetation over time as additional water flows into the basin and covers the false bottom. When this kind of bog is walked upon, the bottom “quakes” and occasionally portions of the pond substrate may break loose and drift (Buell and Buell 1941). Bogs with false bottoms are generally found in cool, northern climates due to glacial activity, poor drainage, and eutrophication (Mitsch and Gosselink 2007).

According to Henley and Harrison (2000), several different types of wetlands are recognized in Oklahoma, but none of these have been described as quaking bogs possessing a false bottom. There is little known about these

ecosystems, including the animal communities present or the water quality conditions they possess. The unnamed bog investigated in this project is similar to the nearby larger and younger Boehler Lake studied by Bass and Potts (2001). Passivirta (1988) suggests bogs contain a wide diversity of macroinvertebrates and have much greater productivity than semi-terrestrial sites.

Objectives of this study included determining basic water quality, identifying macroinvertebrates present, estimating population sizes, calculating species diversity, and comparing macroinvertebrate samples from different areas of the bog.

## Study Site

The wetland pond investigated during this study is located in Johnston County of southeastern Oklahoma on the Oka' Yanahli Preserve near the Blue River (4°26'46”N, 96°37'51”W), a property owned and managed

by The Nature Conservancy (Fig. 1). This unnamed bog is small and somewhat circular in shape, measuring approximately 12 meters in diameter. A false-bottom is present a few centimeters below the surface of the water, with approximately three meters of open water existing between the false bottom and the actual pond bottom. Common vegetation in this wetland includes mosses, sedges, rushes, and grasses.

**Methods**

Sampling of this bog occurred in June and December of 2014 to examine conditions occurring during summer and winter, respectfully. Water quality measurements were collected from the center of the pond. Field testing of these samples included measurements of temperature (YSI 550A meter), dissolved oxygen concentration (YSI 550A meter), pH (Hanna pHep meter), and alkalinity (sulfuric acid titration). Additional water samples were transported to the laboratory to determine conductivity levels (Hanna conductivity meter), turbidity (Bausch & Lomb Spectronic 20), and nutrient (ammonia, nitrite, nitrate, and orthophosphate) concentrations (Hach DR 2800 meter). Oxygen saturation (oxygen nomograph) and carbon dioxide levels (Moore’s nomograph) were also determined in the lab.

Macroinvertebrate samples were collected using a petite Ponar bottom grab in a transect

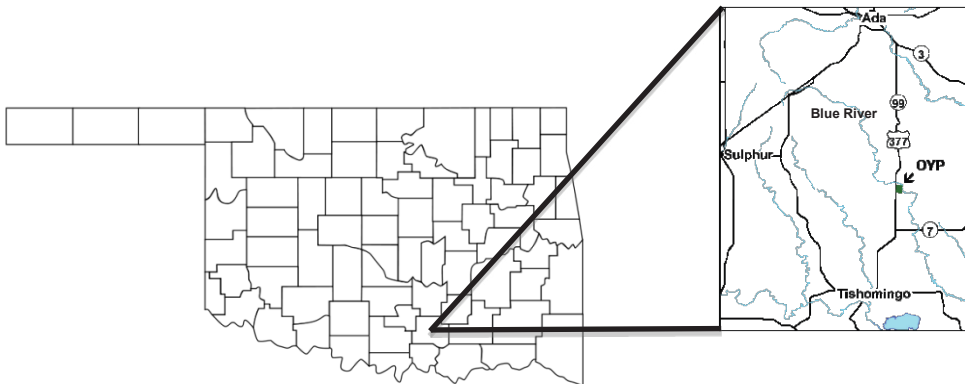
across the bog. Two samples were collected near the edge (outer), two samples were collected half-way to the center (middle), and two samples were collected from the center of the pond. All samples were preserved in the field with a mixture of 10% formalin and rose-bengal dye, and returned to the laboratory where they were sorted using a 250-µm sieve, identified, and enumerated. Identification of macroinvertebrates was determined primarily using keys by Merritt et al. (2008) and Smith (2001). In addition, D-ring net samples were taken at each of those locations to obtain organisms that may have been missed by the petite Ponar grab. However, only petite Ponar samples were used in the statistical analysis. Voucher specimens of macroinvertebrates were deposited into the University of Central Oklahoma Natural History Museum.

Shannon’s index of diversity was used to calculate species diversity and Sorenson’s index of similarity was used to compare macroinvertebrate community similarity.

**Results & Discussion**

Results of the physicochemical analysis are in Table 1. These results generally fall within the normal ranges of aquatic systems in southeastern Oklahoma (Bass and Potts 2001).

The temperature of the bog reflected the



**Figure 1. Map of the field site located in Johnston County, Oklahoma.**

**Table 1. Physicochemical conditions for a quaking bog located in the Oka’ Yanahli Preserve, Johnston County, Oklahoma.**

Parameter	June 2014	December 2014
Water temperature (°C)	23.8	15.6
Dissolved oxygen (mg/L)	2.0	2.2
Oxygen saturation (%)	11	23
pH	7.3	8
Alkalinity (mg/L)	319	320
Carbon dioxide (mg/L)	0	0
Turbidity (JTU)	10	32
Specific conductance (µmohs/cm)	495	545
Ammonia (mg/L)	0.13	0.08
Nitrites (mg/L)	0.26	0.06
Nitrates (mg/L)	0.56	1.71
Orthophosphates (mg/L)	0.01	7.23

seasonal changes of the local area, ranging from 23.8°C in June to 15.6°C in December. Dissolved oxygen concentration only varied by 0.2 mg/L, with June having a lower value of 2.0 mg/L. This is most likely associated with the large amount of decomposing plant debris present during the summer.

Values for pH ranged from 7.3 in June to 8.0 in December. Alkalinity measurements were relatively stable and ranged from 319 mg/L in June to 320 in mg/L in December. These values were expected for Oklahoma and well buffered against the pH changes. There was no free carbon dioxide in the pond, which is supported by the pH and alkalinity readings.

Turbidity measurements varied from 10 JTUs (Jackson Turbidity Units) in June to 32 JTUs in December. These changes may be associated with the amount of seasonal rainfall. Jona Tucker, TNC Oka’ Yanahli Preserve Director (pers. comm), reported that water is usually present in the pond. The drought immediately prior to the June sampling resulted in less than 25 cm of water covering the vegetation, leading to a low turbidity value. Rainfall in an excess of 1.5 inches occurred within a few days prior to the December collection (Oklahoma Climatological Survey, 2015). It

was observed there was a greater amount of water present during this time of the year as compared to the summer. The higher turbidity value in December was due to increased rainfall and runoff. Conductivity readings fell within expected ranges for Oklahoma, 495 µmohs/cm in June, and 545 µmohs/cm in December.

Nutrient values were generally low. Ammonia concentrations ranged from 0.13 mg/L in June to 0.08 in December mg/L. Nitrite values were 0.26 mg/L in June, while only 0.06 mg/L in December. Nitrates ranged from 0.56 mg/L in June to 1.71 mg/L in December. Orthophosphate concentrations were 0.01 mg/L in June, and greatly increased to 7.23 mg/L in December. High orthophosphate levels may have been due to rainfall prior to the sampling period during the winter.

During the course of this study, 10,917 individual macroinvertebrates representing 38 taxa were collected with the petite Ponar grab and identified. Of these, 6,103 (56%) were insects and 2,838 (26%) were oligochaetes. The remaining individuals were turbellarians, nematodes, mollusks, collembolans, hydrachnids, and crustaceans (Table 2). Two additional taxa were collected only in the D-ring net samples.

**Table 2. Macroinvertebrates collected throughout different regions of the bog on the Oka' Yanahli Preserve, Johnston County, Oklahoma.**

Taxa	June 2014			December 2014			Total
	Center	Middle	Outer	Center	Middle	Outer	
<b>Platyhelminthes</b>							
Dugesiiidae*	19	2	1	42	10		74
Unknown Turbellaria	2			9			11
<b>Nematoda</b>							
Unknown Nematoda	46		1	336		4	387
<b>Oligochaeta</b>							
<i>Amphichaeta</i> sp.	5			10		7	22
<i>Dero</i> sp.*	495	6	17	421	12	20	971
<i>Limnodrilus</i> sp.*	1101	5	10	672		2	1790
Unknown Tubificidae	6			46		3	55
<b>Gastropoda</b>							
<i>Physa</i> sp.*	66	1	20	40	6		133
<i>Stagnicola</i> sp.		1	2	1			4
<b>Bivalvia</b>							
<i>Sphaerium</i> sp.*	587	1	48	536	109	40	1321
<b>Crustacea</b>							
Cambaridae				1			1
Cyclopoida				2	9	8	19
<i>Hyaella azteca</i>	3	1	1	1			6
<b>Acarina</b>							
Hydrachnida	2	3	1				6
<b>Collembola</b>							
Isotomidae*		1	3	1	3	6	14
Sminthuridae*							
<b>Odonata</b>							
<i>Amphiagrion</i> sp.				17	1		18
<i>Argia</i> sp.*	54	3	8	54			119
<i>Coenagrion</i> sp.*				25		1	26
<b>Trichoptera</b>							
<i>Helicopsyche</i> sp.				1			1

Continued Taxa	June 2014			December 2014			Total
	Center	Middle	Outer	Center	Middle	Outer	
<b>Coleoptera</b>							
<i>Celina</i> sp.*			1				1
<i>Hydrochus</i> sp.			1				1
<i>Laccobius</i> sp.*	7	1					8
<i>Laccophilus</i> sp.*	17		4	10	2		33
<i>Paracymus</i> sp.*	44	1	2	8			55
<i>Sphaerius</i> sp.*	31		2				33
<i>Tropisternus</i> sp.*	4	2	2	8	3	1	20
<b>Diptera</b>							
<i>Anopheles</i> sp.*							
<i>Chironomus</i> sp.	1	1					2
<i>Chrysops</i> sp.			2	11			13
<i>Corynoneura</i> sp.					2	3	5
<i>Culicoides</i> sp.	2			40		10	52
<i>Dixella</i> sp.	1						1
<i>Larsia</i> sp.*	32	1	2	149	43	72	299
<i>Paratendipes</i> sp.				17		2	19
<i>Polypedilum</i> sp.				3859	46	892	4797
<i>Stratiomys</i> sp.*	53	8		37		3	101
<i>Tanytarsus</i> sp.				468	10	17	495
<i>Tipula</i> sp.				3			3
Unknown Diptera						1	1
Total Individuals	2578	38	128	6825	256	1092	10917
Total Species	22	16	19	29	13	18	38
Species Diversity	1.673	2.459	2.112	1.646	1.776	0.8551	

\* indicates taxon collected in qualitative dip net sample

Dominant taxa (>10%) in the pond included oligochaetes, bivalves, and chironomids. Both seasons had a large amount of oligochaetes, but June had more bivalves, while December contained mostly dipterans. The difference in dominant taxa for each season is likely due to seasonal life cycle changes and water availability.

A non-dominant taxa of particular interest was *Sphaerius* sp. of the family Sphaeriusidae, also referred to as the minute

bog beetles. This insect has been previously collected from Arizona, California, Texas, and Washington, but this is the first report of that family in Oklahoma (Merritt et al. 2008).

Table 2 shows that collectively, Shannon's species diversity value for the June collection (1.757) was slightly higher than that of December (1.618). However, this was a reflection of the lack of evenness in the winter collection. Species richness was actually less in June (27) than in



December (31), but the winter sample had a much greater number of individuals (8,173) than the summer collection (2,744). Diversity values increased from the center toward the middle and outer portions of the bog in the June collection, while the opposite was observed during December. Although the overall diversity values would indicate this aquatic system is stressed, that may not necessarily be the case in this pond.

When comparing the regions of the pond during the two different seasons, it was observed that the center of the pond did not vary in the amount of diversity. In contrast, the middle and outer portions of the bog for the June collection had greater diversity than the December collection. These observations are most likely due to the reduced amount of water concentrating microhabitats in the center of the pond during the summer. In addition, the June collection contained higher species richness in those areas of the pond (Table 2).

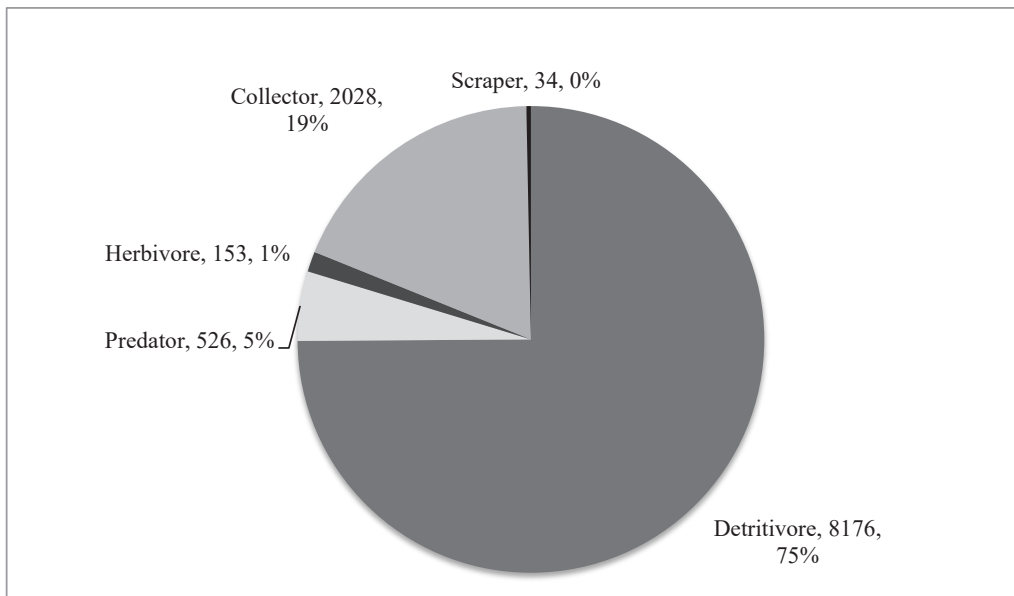
Sorenson’s index of similarity between the two seasons was 0.689, indicating many of the same taxa were present during both seasons (Table 3). This is not unexpected because the pond is a relatively small habitat. It was observed that the presence of water influenced

similarity throughout the different regions of the bog. Collectively, similarity values for the two seasons decreased moving from the center towards the outer edges of the pond. The amount of water present during June fluctuated more in comparison to December. The center of the bog in June was more similar than the middle or outer regions because of the shallow, yet stable amount of water in the center. In contrast, December’s similarity values varied because water had risen immediately prior to that collection due to rainfall, providing more opportunities for organisms to disperse throughout the pond.

When examining trophic structure in the pond, it was observed that 75% of the individuals were detritivores in both collections (Fig. 2). This trophic category included organisms such as oligochaetes, nematodes, and *Polypedilum* sp. Collectors such as the bivalve, *Sphaerium* sp., and many of the dipterans constituted 19% of the individuals. The high numbers in these two groups were attributed to the large amount of detritus present in the pond. Predators, herbivores, and scrapers comprised the remaining 6% of the total individuals collected in both seasons.

**Table 3. Sorenson’s similarity values for macroinvertebrates collected from a quaking bog on the Oka’ Yanahli Preserve, Johnston County, Oklahoma.**

Collection Date	Center	Middle	Outer
June 2014			
Center	—	0.842	0.683
Middle	—	—	0.743
Outer	—	—	—
December 2014			
Center	—	0.572	0.681
Middle	—	—	0.581
Outer	—	—	—
June vs. December 2014			
Center	0.667	—	—
Middle	—	0.552	—
Outer	—	—	0.378



**Figure 2. Macroinvertebrate trophic categories, including number of individuals and percent composition for June and December collections.**

## Summary & Conclusion

Generally high water quality exists within the wetland, except for the low dissolved oxygen concentrations, and these values suggest oxygen is a limiting factor for some aquatic macroinvertebrates. The oligochaetes and chironomids, which dominated most of the collections, are tolerant of reduced oxygen levels. Altogether, a total of 40 taxa and 10,917 individuals of macroinvertebrates were documented from this pond.

Quaking bogs are not only unique ecosystems in Oklahoma, but uncommon in the state. The fragile, unnamed bog on the Oka' Yanahli Preserve showed high water quality and supported a diverse macroinvertebrate community. This research provided a better understanding and additional information regarding freshwater quaking bogs, and this may be used to strengthen measures for their conservation.

## Acknowledgments

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## References

- Bass D, Potts C. 2001. Invertebrate community composition and physicochemical conditions of Boehler Lake, Atoka County, Oklahoma. *Proc Okla Acad Sci* 81:21-29.
- Buell MF, Buell HF. 1941. Surface level fluctuation in Cedar Creek Bog, Minnesota. *Ecology* 22(3):317-321.
- Henley J, Harrison M. 2000. *The Oklahoma Wetlands Reference Guide*. Oklahoma Conservation Commission, Oklahoma City, Oklahoma. 66 p.
- Merritt RW, KW Cummins, MB Berg. 2008. *An Introduction to the Aquatic Insects of North America*. 4<sup>th</sup> ed. Dubuque (IA): Kendall/Hunt Publishing. 1158 p.
- Mitsch W, Gosselink J. 2007. *Wetlands*. 4th ed. Hoboken (NJ): John Wiley & Sons, Inc. 582 p.
- Oklahoma Climatological Survey. 2015. *Climate of Oklahoma* [online]. Available from: <http://climate.ocs.ou.edu>. (Accessed August 12, 2015).

- Paasivirta L, Lahti T, Perätiö T. 1988. Emergence phenology and ecology of aquatic and semi-terrestrial insects on a boreal raised bog in Central Finland. *Holarctic Ecology* 11(2):96-105.
- Smith, DG. 2001. Pennak's Freshwater Invertebrates of the United States. 4<sup>th</sup> ed. New York (NY): John Wiley & Sons, Inc. 638 p.

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# Patterns of Fish Diversity and Community Structure Along the Longitudinal Gradient of the Kiamichi River in Southeastern Oklahoma

**Clayton P. Porter**

Oklahoma Department of Wildlife Conservation, Oklahoma Fishery Research Laboratory, Norman, OK 73072

**Tim M. Patton**

Southeastern Oklahoma State University, Department of Biological Sciences, Durant, OK 74701

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**Abstract:** In riverine systems, patterns of fish community structure and diversity show changes along the longitudinal profile, i.e., from upstream to downstream. Previous research has shown longitudinal patterns may include changes in various measure of species diversity, such as species richness and species abundance, and patterns of species accumulation that often include species addition and/or species zonation. We used seines and electrofishing gear to sample the small-bodied fish community along the longitudinal gradient of the Kiamichi River in southeastern Oklahoma. Our objectives were to look for longitudinal patterns of (1) species diversity and (2) community structure. We sampled at 11 sites during 2012-2013. We standardized sampling based on 30 minutes of electrofishing and 200 meters of seine hauls at each site. We also used experimental gill nets to contribute to the baseline information of large-bodied fishes in the Kiamichi River. A total of 9,620 fish were collected representing 54 species and 15 families. The Kiamichi River showed increasing species diversity along the longitudinal gradient, as well as patterns of species addition. Further, we found four distinct groups of fish along the longitudinal gradient, including ubiquitous species, widespread but scattered species, species restricted to downstream sites, and rare species. ©2015 *Oklahoma Academy of Science*

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## Introduction

One of the key constructs in stream ecology is that of the River Continuum Concept (Vannote et al. 1980), which provided a framework for understanding changes in stream ecology along the longitudinal gradient, that is, how various aspects of stream ecology change from upstream to downstream. While the River Continuum Concept addressed numerous aspects of general stream ecology, including characteristics of organic matter, energy flow, and invertebrate community composition, many subsequent research questions addressed

changes more specific to stream fish community structure along the longitudinal gradient.

Over the past few decades, numerous studies have elucidated how stream fish communities change along the longitudinal gradient, including studies in the United States (e.g., Sheldon 1968; Evans and Noble 1978; Guillory 1982; Rahel and Hubert 1991) and abroad (Ibarra and Stewart 1989; Chadderton and Allibone 2000; Bistoni and Hued 2002). While specific goals and objectives of these studies have varied, most have sought to identify community patterns along the upstream to downstream gradient, and to relate these patterns to environmental

variables. Environmental variables may include abiotic factors such water temperature, depth, substrate, and disturbance, or biotic variables such as predation and competition.

One of the patterns that has emerged from past studies is that species diversity tends to increase along the longitudinal gradient. (Lotrich 1973; Gorman and Karr 1978; Vannotte et al. 1980; Matthews 1985; Rahel and Hubert 1991; Pires et al. 1999). Increases in species diversity are generally attributed to a concomitant increase in habitat diversity along the upstream to downstream gradient, and greater habitat stability in downstream reaches (Gorman and Karr 1978; Schlosser 1987). It is also noteworthy that measuring species diversity may take numerous forms, but most protocols generally rely on measures of the number of species present (species richness), and the relative abundances of individuals within each species (species abundance), or indices that consider richness and abundance simultaneously (Hamilton 2005; McGinley 2014).

In addition to changes in fish species diversity along the longitudinal gradient of streams, another area of research emphasis has been that of describing patterns of species accumulation along that gradient. Rahel and Hubert (1991) note that most studies have attributed longitudinal changes in fish community structure to one of two processes: biotic zonation or continual addition of species in the upstream to downstream direction. The concept of biotic zonation in general suggests that specific groups of organisms are associated with specific areas, or zones, with appreciable replacement of species along some spatial gradient. The concept of zonation has been applied to a variety of taxa including crustaceans on beaches (e.g., Dahl 1953), marine coral (e.g., Alevizon et al. 1985), stream invertebrates (e.g., Statzner and Higler 1986), amphibians and reptiles (e.g., Ravkin et al. 2010), woodland birds (e.g., Colquhoun and Morley 1943), small mammals (e.g., Heaney et al. 1989), and many others. With respect to stream fishes, zonation associated with water temperatures that create distinct temperature-dependent fish zones have been described in

several studies (Rahel and Hubert 1991; Bistoni and Hued 2002; Moyle 2002; Quist et al. 2006; Torgersen et al. 2006; Lasne et al. 2007), but beyond those associated with temperature, causal mechanisms driving zonal associations appear to vary widely among studies. Rahel and Hubert (1991) concisely summarized that, in addition to temperature influences, zonation results from discontinuities in stream geomorphology.

In contrast to biotic zonation, biotic addition implies a continual downstream increase in species richness (Rahel and Hubert 1991), that is, new species are added along the upstream to downstream gradient, with limited species (or community) replacement along spatial gradients. Numerous previous studies have identified patterns of species addition along the longitudinal gradient (e.g., Evans and Noble 1979; Foltz 1982; Petry and Schultz 2006). Interestingly, some researchers report finding indications of both species zonation and species addition within their study sites (Rahel and Hubert 1991; Paller 1994; Williams et al. 1996; Bistoni and Hued 2002). For example Rahel and Hubert (1991) report an upstream cold-water zone and a downstream warm-water zone, with species added within the warm-water zone through the process of species addition.

The Kiamichi River in southeastern Oklahoma is an appropriate study site for investigating patterns of species diversity and community composition along the longitudinal gradient for at least two reasons. First, it is relatively speciose and approximately 100 species of fish have been collected in previous studies (Pigg and Hill 1974; Pyron et al. 1998); however, it should be noted that most previous surveys have included many tributary streams and the main stem of the river does not likely have all of those species. Second, the Kiamichi River has only a single impoundment on the main stem (Hugo Reservoir), and few, if any, water withdrawal structures or major barriers. Additionally, the Kiamichi River is of high conservation interest because it has such a rich diversity of fish (Pigg and Hill 1974; Matthews 1985; Pyron et al. 1998) and freshwater mussels (Vaughan et al. 1996; Gailbraith et al. 2008), and the region of

southeastern Oklahoma that encompasses the Kiamichi River was selected by The Nature Conservancy as a critical area for protecting freshwater diversity (Master et al. 1998).

Our overall goal for this study was to look for patterns of fish diversity and community structure along the longitudinal gradient of the Kiamichi River, thereby leading to a greater understanding of a river system that truly warrants meaningful conservation efforts. The first objective was to describe diversity of the fish community along the longitudinal gradient based on three metrics: species abundance, species richness, and Shannon diversity. The second objective was to see if fish exhibited patterns of biotic zonation or biotic addition along the longitudinal gradient, or other recognizable patterns of community structure.

## Methods

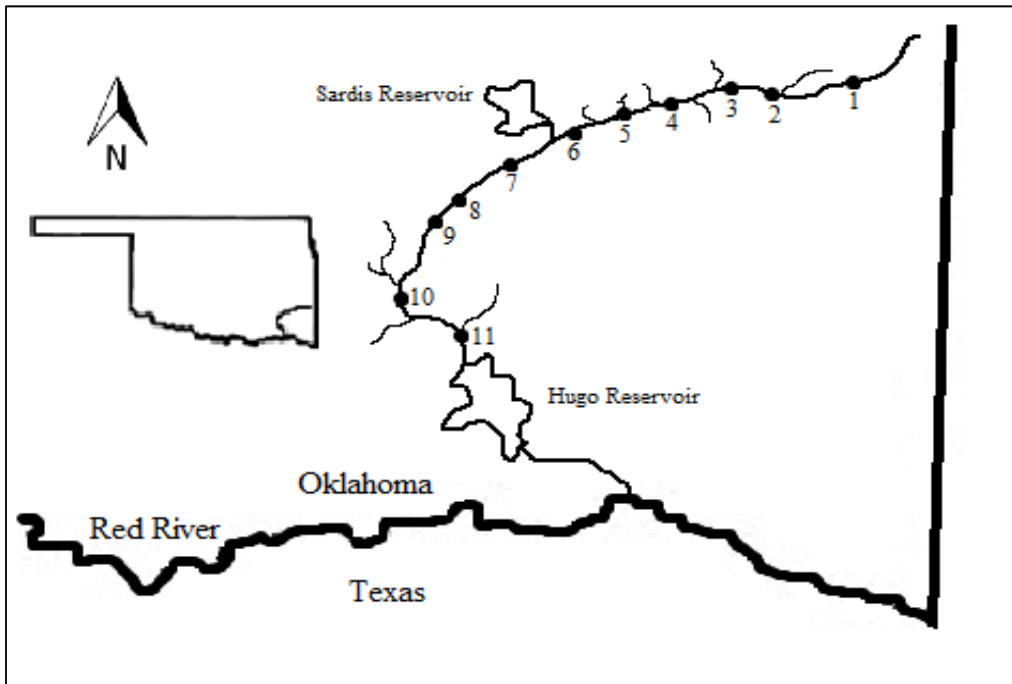
*Study sites-* The Kiamichi River is located in southeastern Oklahoma and flows generally west, then southeasterly, eventually reaching its confluence with the Red River on the Oklahoma-Texas border (Figure 1). The Kiamichi River is 172km long and has an average gradient of 1.46m/Km (Pigg and Hill, 1974). The head waters of the Kiamichi River begins in the Ouachita Mountain range near Big Cedar Oklahoma, with an initial elevation of 367m AMSL and reaching a final elevation of 109m AMSL at its confluence with the Red River. The drainage has been described as crescent shaped (Pigg and Hill 1974), and drains approximately 4739km<sup>2</sup> within seven counties; LeFlore, Latimer, Pittsburg, Atoka, Pushmataha, Choctaw, and McCurtain Counties. The Kiamichi River drainage encompasses a large portion of southeastern Oklahoma, while covering many topographic and ecological features of this area of the state (Figure 1).

There are eight major tributaries to the Kiamichi River, including Jackfork, Buck, Walnut, Buffalo, Cedar, Gates, Anderson, and Pine Creeks. Along with the tributaries, there are two large reservoir impoundments within the watershed (Figure 1). The furthest upstream reservoir is Sardis Lake, which is

an impoundment on Jackfork Creek and was constructed in 1983. Sardis Lake is not on the mainstem of the Kiamichi River but is on a major tributary and contributes considerably to the flow of the Kiamichi River. Further downstream, Hugo Lake was constructed on the mainstem of the Kiamichi River near Hugo Oklahoma in 1974. These reservoirs were constructed primarily for water storage for municipalities throughout southeastern Oklahoma. These tributaries and reservoirs are a vital source of flow regimes for the Kiamichi River and help sustain water levels and base flows. With alteration to natural environments and flow regimes, it follows that the ichthyofauna of the river may be impacted due to these anthropogenic effects. We sampled eleven sites along the longitudinal profile of the Kiamichi River starting at Highway 259 bridge at Big Cedar and extending downstream to Hugo Lake (Figure 1).

*Fish Sampling.* We used three sampling methods for fish collections: seining, electrofishing, and gill netting. Analyses of patterns of fish diversity and community structure were restricted to data collection from seines and electrofishing efforts, therefore, just small-bodied fishes. However, we also wanted to provide some baseline information on the large-bodied fish community of the Kiamichi River, as it has not been previously described in the literature. Therefore, we used experimental gill nets to capture large-bodied fishes.

*Seining-* We sampled eleven sites on the mainstem of the Kiamichi River using 6m x 1.21m seines with a 5mm mesh. We standardized the seining protocol to include twenty seine hauls per sample site, and each seine haul was ten meters in length, for a total of 200 meters/site. A four person crew was used each time that sampling was conducted. Patton et al. (2000) found that that seining four 50 meter seine hauls captured 90% of the species present in prairie streams in Wyoming. However, because substrates in streams of southeastern Oklahoma are likely more complex than those of prairie streams in Wyoming, we also used electrofishing to further sample the small-bodied fish community. Onorato et al.



**Figure 1.** Sample sites along the Kiamichi River in southeastern Oklahoma, October 2012 – January 2013. Numbered sample sites correspond to coordinates in appendix A.

(1998) and Patton et al. (2000) showed that seining was an effective gear, and when paired with electroshocking it can produce more complete samples in terms of species richness and abundance than either gear would have on its own. When arriving at each site, habitat was qualitatively classified for the presence of pools, riffles, runs, backwaters, side channels, or other unique habitat types, and seine hauls were conducted in each habitat type present at each sample location. Kick seining was conducted where riffles were present, wherein the seine was placed at the downstream end of the riffle and a one pass kick method was used to dislodge substrate, thereby washing fish into the seine. Once fish were collected from a site, they were pooled and preserved in a 10% formalin solution, then identified in the laboratory at the Oklahoma Department of Wildlife Conservation (ODWC) at Holdenville Oklahoma. All seine hauls were conducted from October 2012 through January 2013 to avoid high-flow conditions that often occur during the spring.

*Electrofishing-* Electrofishing was conducted using a Halltech HT2000 backpack based electrofishing unit. As with seining, all available habitats were qualitatively classified before electrofishing sampling began. We standardized electrofishing effort by sampling a total of 30 minutes at each location, sampling all available habitats present. While conducting electrofishing, a one pass method was used to collect fish. While many past studies have utilized multiple pass electrofishing methods, an important sampling consideration is the feasibility of time and cost constraints when sampling multiple sites (Meador, et al. 2003). Further, many past studies have shown that a single-pass method is effective, especially if sampling for species richness is a goal. Pusey et al. (1998) found that a single pass method was adequate in collecting a significant proportion of the total species present when using a single pass method vs. multiple pass methods. Meador et al. (2003) showed that with a single pass method, between 80.7-100% of total species present were collected the first pass using backpack

electrofishing units. Bertrand et al. (2006) showed that a single pass electrofishing method was sufficient in sampling streams by collecting all species at 14 of 19 sites on the first pass.

*Gill Netting-* We used 24.4x1.8m experimental gill nets for sampling large-bodied fishes (Hubert 1996). Nets were composed of 8 panels, each 3.1m in length, and ranging in mesh size from 19-63mm. The various mesh panels on each net occurred randomly along the length of the net to allow for more randomization of sampling. We chose these nets because they are used by ODWC as per their standardized sampling protocol for gill netting, and have been shown to be effective for capturing a variety of species and sizes (ODWC 2009). We gill netted at four of the 11 sample sites, plus one site downstream from Hugo Lake, and site selection was based on the presence of large pools to allow for net placement and boat accessibility. At each of the five sample sites, fifteen nets were placed perpendicular to the bank and fished for a 24 hour, equating to 15 net-nights/site and a total of 75 net-nights. All fishes captured were identified to species in the field; live fish were released and dead fish were discarded onto the adjacent stream banks within areas of thick vegetation.

*Data Analysis-* We used linear regression (Zar 1973) to look at patterns of species diversity along the longitudinal gradient. We regressed distance downstream (as the independent variable) against each of three measures of diversity (as the dependent variables): species abundance, species richness, and Shannon diversity (Shannon 1948). All linear regressions were calculated in SAS version 13.2 (SAS 2014). To look for patterns of community composition along the longitudinal gradient, we constructed an arranged data table following Rahel and Hubert (1991). We tried numerous iterations of arrangement to look for patterns, and determined that the most parsimonious interpretation came by sorting data rows by frequency of occurrence, followed by placement of some of the species into like groups.

## Results

*Fish Sampling-* A combined effort of 220 seine hauls were completed, encompassing 2200m of seining at 11 sites (Table 1). Seining resulted in the capture of 3,490 individual fish, representing 30 species (Table 1). Electrofishing for 30 minutes at each of the 11 sites totaled 330 minutes (5.5 hours) of electrofishing effort. Electrofishing resulted in the capture of 5383 individual fish, representing 39 species (Table 1). Combined, seining and electrofishing resulted in the capture of 8,873 individual small-bodied fish, representing 39 species (Table 1). Experimental gill nets were used for a total of 75 net-nights and resulted in the capture of 747 individual large-bodied fish, including 25 species. With all the gears combined we captured a total of 9,620 individual fish representing 54 species.

*Patterns of Diversity-* To assess species diversity along the longitudinal gradient of the Kiamichi River, three components were measured; species abundance, species richness, and Shannon Diversity. With respect to species abundance, we captured an average of 873 individual fish among the 11 sites, with a range of 148 (site 5) to 1,674 (site 8) fish/site (Table 2). Regression analysis indicated that abundance generally increased along the longitudinal gradient ( $R^2 = 0.352$ ) but not significantly ( $P = 0.424$ ; Figure 2a). With respect to species richness, we captured an average of 22.6 species among the 11 sites, with a range of 13 (site 5) to 32 (site 8) species/site (Table 2). Raw values of species richness varied among the sites, but richness was generally lowest at the more upstream sites, highest at sites 7 and 8, and decreased somewhat among the more downstream sites (Table 2). However, regression analyses indicated that species richness increased significantly along the longitudinal gradient ( $R^2 = 0.374$ ,  $p = 0.001$ ; Figure 2b). With respect to Shannon diversity, values did not vary widely along the longitudinal gradient (mean = 2.255, range = 1.82 – 2.58), but regression analysis revealed it was a significant increase  $R^2 = 0.368$ ,  $p = 0.001$ ; Figure 2c).

*Patterns of Community Structure-* We assessed community structure using an arranged data table, which is qualitative approach for



**Table 1. Numbers of fish collected using three sampling gears in the Kiamichi River, southeastern Oklahoma, October 2012 – January 2013.**

Common Name	Scientific Name	Numbered captured by seine	Number captured by electrofishing	Number captured by gill net	Total
Alligator Gar	<i>Atractosteus spatula</i>	0	0	4	4
Bigeye Shiner	<i>Notropis boops</i>	236	165	0	401
Bigmouth Buffalo	<i>Ictiobus cyprinellus</i>	0	0	11	11
Black Bullhead	<i>Ameiurus melas</i>	0	1	0	1
Black Crappie	<i>Pomoxis nigromaculatus</i>	0	0	11	11
Blackside Darter	<i>Percina maculata</i>	3	7	0	10
Blackspotted topminnow	<i>Fundulus olivaceus</i>	89	48	0	137
Blackstripe Topminnow	<i>Fundulus notatus</i>	16	11	0	27
Blue Catfish	<i>Ictalurus furcatus</i>	0	0	119	119
Bluegill	<i>Lepomis macrochirus</i>	110	681	4	795
Bluntnose Darter	<i>Etheostoma chlorosoma</i>	9	8	0	17
Bluntnose Minnow	<i>Pimephales notatus</i>	165	647	0	812
Bowfin	<i>Amia calva</i>	0	0	1	1
Brook Silverside	<i>Labidesthes sicculus</i>	1604	316	0	1920
Channel Catfish	<i>Ictalurus punctatus</i>	0	1	71	72
Channel Darter	<i>Percina copelandi</i>	19	29	0	48
Chestnut Lamprey	<i>Ichthyomyzon castaneus</i>	0	1	0	1
Common Carp	<i>Cyprinus carpio</i>	0	0	8	8
Cypress Darter	<i>Etheostoma proeliare</i>	1	4	0	5
Drum	<i>Aplodinotus grunniens</i>	0	1	9	10
Dusky Darter	<i>Percina sciera</i>	15	21	0	36
Emerald Shiner	<i>Notropis atherinoides</i>	222	94	0	316
Flathead Catfish	<i>Pylodictis olivaris</i>	0	0	13	13
Freckled Madtom	<i>Noturus nocturnus</i>	2	20	0	22
Gizzard Shad	<i>Dorosoma cepedianum</i>	0	3	75	78
Golden Redhorse	<i>Moxostoma erythrurum</i>	0	3	10	13
Green Sunfish	<i>Lepomis cyanellus</i>	25	463	0	488
Hybrid Striped Bass	<i>Morone chrysops x saxatilis</i>	0	0	1	1
Johnny Darter	<i>Etheostoma nigrum</i>	26	60	0	86
Kiamichi Shiner	<i>Notropis ortenburgeri</i>	47	79	0	126
Largemouth Bass	<i>Micropterus salmoides</i>	8	14	1	23
Log Perch	<i>Percina caprodes</i>	7	516	0	523
Longear Sunfish	<i>Lepomis megalotis</i>	32	667	0	699
Longnose Gar	<i>Lepisosteus osseus</i>	0	0	64	64
Mimic Shiner	<i>Notropis volucellus</i>	9	22	0	31
Mosquito Fish	<i>Gambusia affinis</i>	89	177	0	266
Orangebelly Darter	<i>Etheostoma radiosum</i>	135	511	0	646
Orangespotted Sunfish	<i>Lepomis humilis</i>	10	84	0	94

Table 1. Continued

Common Name	Scientific Name	Numbered captured by seine	Number captured by electrofishing	Number captured by gill net	Total
Orangethroat Darter	<i>Etheostoma spectabile</i>	11	32	0	43
Paddlefish	<i>Polyodon spathula</i>	0	0	1	1
Redear Sunfish	<i>Lepomis microlophus</i>	0	20	0	20
Redfin Shiner	<i>Lythrurus umbratilis</i>	110	65	0	175
River Carpsucker	<i>Carpionodes carpio</i>	0	0	14	14
River Redhorse	<i>Moxostoma carinatum</i>	0	1	21	22
Slenderhead Darter	<i>Percina phoxocephala</i>	13	16	0	29
Smallmouth Buffalo	<i>Ictiobus bubalus</i>	0	0	70	70
Spotted Bass	<i>Micropterus punctulatus</i>	4	62	20	86
Spotted Gar	<i>Lepisosteus oculatus</i>	1	2	12	15
Spotted Sucker	<i>Minytrema melanops</i>	0	14	1	15
Steelcolor Shiner	<i>Cyprinella whipplei</i>	390	330	0	720
Stoneroller	<i>Campostoma anomalum</i>	82	180	0	262
Tadpole Madtom	<i>Noturus gyrinus</i>	0	3	0	3
Warmouth Sunfish	<i>Lepomis gulosus</i>	0	4	0	4
White Bass	<i>Morone chrysops</i>	0	0	10	10
White Crappie	<i>Pomoxis annularis</i>	0	0	147	147
Yellow Bass	<i>Morone mississippiensis</i>	0	0	49	49
<b>Total</b>		3490	5383	747	9620

looking for patterns along the longitudinal gradient (Rahel and Hubert 1991). After trying several iterations of arranging the data to look for an interpretable pattern, the most parsimonious interpretation came from sorting the data by frequency of occurrence of species, and then rearranging the tabular position of a few species to fit into appropriate categories. In so doing, and as supported by the regression of species richness (Figure 2b), the Kiamichi River showed a pattern of species addition along the longitudinal gradient (Table 2). Further, by arranging the data as described, we was able to identify several somewhat distinct groups of fishes along the longitudinal gradient, in addition to the broad general pattern of species addition. Specifically, these groups could be described as: (1) a group of 13 species that were ubiquitous, found throughout the longitudinal gradient, and occurred at 90-100% of the sites, (2) a group of nine species that were widespread but scattered along longitudinal gradient, including upstream

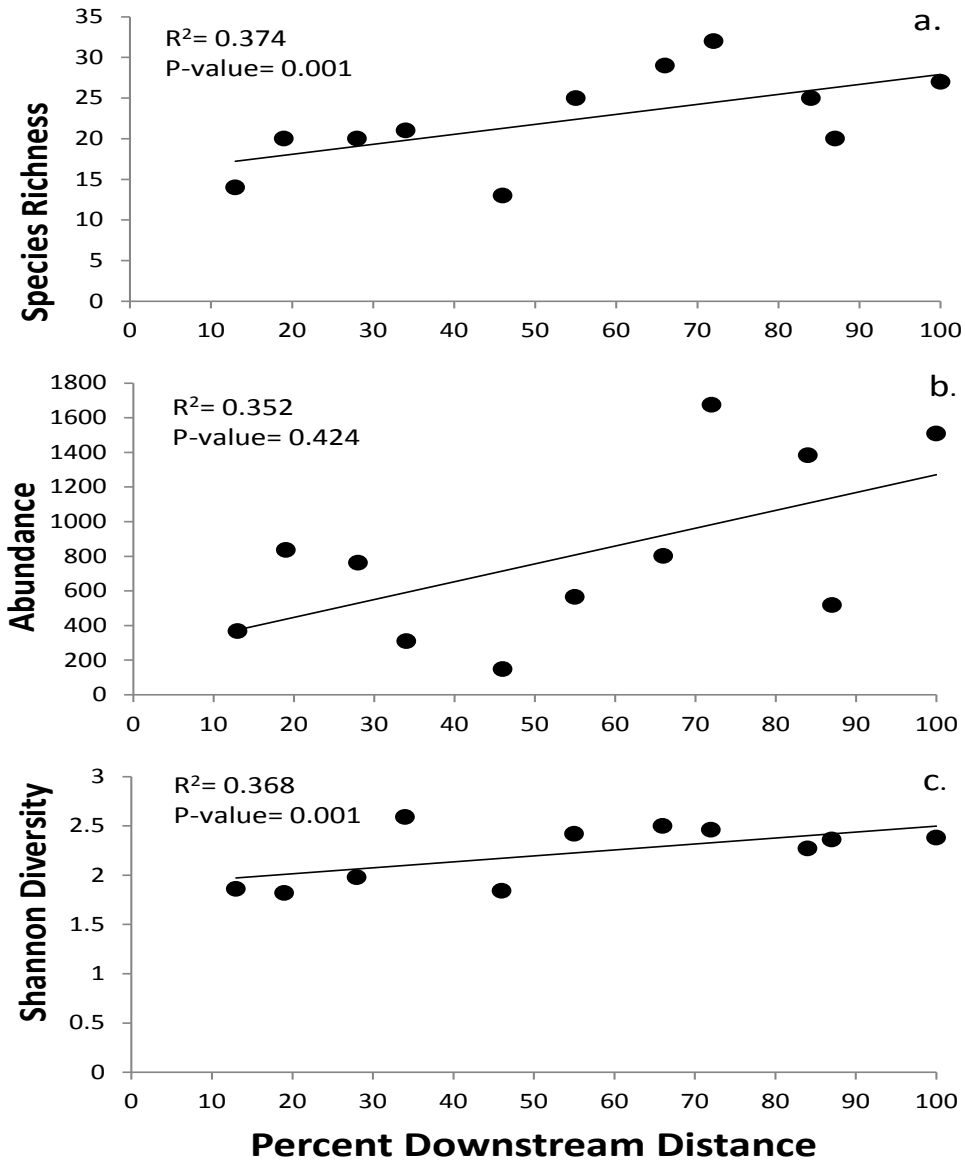
and downstream sites, and occurred at 36-73% of the sites, (3) a group of seven species that showed a pattern of zonation in that they only occurred at downstream sites, and (4) a group of 10 species that were rare, indeed too rare to assign to as particular location along the longitudinal gradient in the catch (Table 2). Species identified as rare were present in only one or two of the 11 sites sampled, and were represented by only 1-20 total individuals ( $\leq 4$  individuals, or 0.05% of the total number of fish captured, were collected among nine out of ten species classified as rare).

## Discussion

*Patterns of Species Richness-* The Kiamichi River showed a statistically significant increase in species diversity along the upstream to downstream gradient, as indicated by species richness and Shannon diversity. These findings are consistent with many previous works (Sheldon 1968; Evans and Noble 1979; Rahel

**Table 2. Numbers of fish captured from 11 sites along the Kiamichi River in Southeastern Oklahoma via seining and electrofishing, October 2012 – January 2013.**

Common Name	Site Number											Total	Frequency
	1	2	3	4	5	6	7	8	9	10	11		
Ubiquitous species, occurred throughout longitudinal gradient													
Brook Silverside	148	391	356	53	4	182	90	141	423	55	81	1924	100
Bluntnose Minnow	9	35	42	28	7	51	80	103	271	62	124	812	100
Bluegill Sunfish	22	137	79	55	2	30	54	152	122	10	128	791	100
Longear Sunfish	36	52	71	23	10	7	131	340	3	7	19	699	100
Green Sunfish	48	77	59	16	14	21	37	55	66	27	68	488	100
Stoneroller	17	12	26	9	21	21	72	53	7	23	1	262	100
Steelcolor Shiner	1	1	11	15		17	102	257	67	77	172	720	91
Orangebelly Darter	2	2	17		7	50	127	303	6	106	26	646	91
Bigeye Shiner	55	34	25	13	6	9		59	76	17	107	401	91
Blackspotted Topminnow	22	45	23	2	3	1	18	1	20		2	137	91
Johnny Darter	2	8	7	5		40	1	7	1	12	3	86	91
Spotted Bass	1	4	11	3	2	1	2	16	11		15	66	91
Mosquitofish		22	9	11	64	42	6	16	46	7	43	266	91
Ubiquitous species, but scattered along the longitudinal gradient													
Orangespotted Sunfish		3		23		9	6	14	26	2	11	94	73
Channel Darter		1		2		1	2	28	1	1	12	48	73
Spotted Sucker		2	1	3		2	1	1	4			14	64
Dusky Darter			6	2		1		19	1	2	5	36	64
Blackstripe Topminnow	2		8	2			2	8			5	27	55
Log Perch		3		1		2	6	20			491	523	55
Orangethroat Darter		4		7			14	6			12	43	45
Largemouth Bass		2	4	14				2			1	23	45
Blackside Darter			3				3	2			2	10	36
Species generally restricted to downstream sites													
Redfin Shiner				22		20	1	24	9	8	91	175	64
Kiamichi Shiner					1	8	12	1	71	19	14	126	64
Slenderhead Darter					7	7	3		2	2	8	29	55
Emerald Shiner						38	12		131	78	57	316	45
Mimic Shiner						2	3	1	16		9	31	45
Freckled Madtom						1	3	15		1	2	22	45
Bluntnose Darter							2	4	8	1	2	17	45
Rare species, occurred in few sites, and very few individuals													
Redear Sunfish								5	15			20	18
Warmouth Sunfish	2							2				4	18
Golden Redhorse								3		1		4	18
Spotted Gar			1						2			3	18
Channel Catfish									1			1	9
Gizzard Shad				3								3	9
Tadpole Madtom									3			3	9
Chestnut Lamprey				1								1	9
Freshwater Drum									1			1	9
Black Bullhead										1		1	9
Total	367	836	762	309	148	565	802	1674	1383	518	1509	8873	
Number of species	14	20	20	21	13	25	29	32	25	20	27	39	



**Figure 2: Regression analysis of species richness (a), abundance (b), and Shannon diversity along the longitudinal gradient of the Kiamichi River, southeastern Oklahoma, October 2012 – January 2013.**

and Hubert 1991; Williams et al. 1996; Bistoni and Hued 2002). And while our results also suggested an increase in overall abundance along the longitudinal gradient ( $R^2=0.352$ ), that increase was not significant ( $P=0.424$ ). It is noteworthy that species richness peaked

before reaching the downstream-most sites, and decreased somewhat among the last few sites. This finding is somewhat inconsistent with other work. However, peak species richness was achieved when several species that were classified as rare (group 4, Table 2) were added

at reaches 7-8; within the last few reaches, these same rare species were not present, thereby contributing to the decrease in species richness among the last few sample sites. It is unlikely that all individuals in a system will be detected during a study (MacKenzie et al. 2005), but inclusion of rare species has been shown to be important for overall assessment of diversity and detection of ecological changes over time (Cao et al. 1998; Cucherousset et al. 2008). Further, in many cases, rare species may provide unique and vulnerable ecological function (Mouillot et al. 2013). While it is beyond the scope of this study to consider each species' ecological function, in this study, consideration of rare species affected the interpretation of total species richness as well as longitudinal patterns of species richness, and may certainly be useful for monitoring change over time.

*Patterns of community structure-* Previous research has shown that, when sampling over some distance with the goal of demonstrating species richness, species accumulation is rapid within the first few sites, and may or may not become asymptotic (Kanno et al. 2009). While the general pattern of community structure indicated species addition along the longitudinal gradient of the Kiamichi River, 36% of all species captured were captured at the first site, 56% of all species had been captured by the time we included the second site, and 67% of all species were captured by the time we included the third site. In that regard, species accumulation was very rapid. Our first sample was ~17km from the uppermost headwaters. Inclusion of samples further upstream may have resulted in the capture of fewer species; however, given the broad range of habitat and stream sizes utilized by the ubiquitous species that we captured at the first few sites, it may require sampling in the smallest headwaters to find areas in which these species do not occur. Further, much of the headwaters of the Kiamichi River is subject to intermittent water and long reaches of desiccation during dry periods (T. Patton, unpublished data).

Though species addition was evident along the longitudinal gradient of the Kiamichi River, four groups of species were detected: (1) a group

of 13 species that were ubiquitous and found throughout the longitudinal gradient, (2) a group of nine species that were widespread but scattered along longitudinal gradient, (3) a group of seven species that showed a weak pattern of zonation in that they only occurred at downstream sites, and (4) a group of 10 species that were rare in our catch. Though it is beyond the scope of this paper to review the specific habitat preferences of each species captures, we addressed the general habitat requirements of species as they relate to the four groups defined above, and referred to Robison and Buchanan (1988) and by Miller and Robison (2004) for general habitat descriptions that may provide insight on species' distributions in the Kiamichi River.

Among the ubiquitous species, a variety of minor habitat preferences are indicated by Robison and Buchanan (1988) and by Miller and Robison (2004); however, these sources suggest that all 10 of the species we classified as ubiquitous are basically habitat generalists, occurring in a variety of habitats, and all 10 are relatively common among streams and rivers in southeastern Oklahoma.

Among the widespread but scattered species, all nine of the species included in this group can be regarded largely as habitat generalists as well (Robison and Buchanan 1988; Miller and Robison 2004), and where specific habitat preference is indicated, those habitats appear to be relatively common in the Kiamichi River. This group also included several species of darters (*Etheostoma* and *Percina*) that are generally common in rivers of southeastern Oklahoma, and that show preference for large substrate size; this may explain why they were captured throughout the longitudinal profile, but not at every location; i.e., they were found where appropriate substrates and other microhabitat features were available. The total number of each species captured within this group also suggests that the widespread but scattered species were not nearly as abundant as the ubiquitous species; reduced overall abundance may also have led to their absence in our catch at some sites.

We captured seven species that were only

present in downstream sites. A review of general stream size preference indicates that six of these seven species are most commonly found in medium to larger streams and rivers, and most of which prefer sluggish pools (Robison and Buchanan 1988; Miller and Robison 2004). This may explain their absence from the upstream sites. Only one species in this group did not fit that general habitat description; freckled madtom (*Noturus nocturnus*) is described as a resident of small-medium sized streams and rivers, where it is usually found over gravel and cobble substrates (Robison and Buchanan 1988; Miller and Robison 2004). Though this species was not abundant in our samples, it was not collected at the more upstream sites, and was found at five out of six of the most downstream sites, suggesting it may be more tolerant of larger rivers than the literature suggests.

We captured 10 species that were classified as rare based on low overall abundance (1 – 20 individuals; usually 1-4 individuals) and their presence at only one or two sites. Among these, four are generally considered large-bodied fishes, which makes them more difficult to capture with sampling gears designed for use while wading; these include golden rehorse (*Moxostoma erythrurum*), spotted gar (*Lepisosteus oculatus*), channel catfish (*Ictalurus punctatus*), and freshwater drum (*Aplodinotus grunniens*). However, we captured each of these species in gill nets at 60-100% of the sites in which we used gill nets, suggesting these species were relatively common, but are more likely to be captured with gill nets than with seining or hand-held electrofishing gear. Gizzard shad (*Dorosoma cepedianum*) were also rare in the seine and electrofishing catch, but common (present in 100%) in our gill net samples. When these five species are excluded due to gear selectivity, only five species were rare in our catch: redear sunfish (*Lepomis microlophus*), warmouth (*Lepomis gulosus*), tadpole madtom (*Noturus gyrinus*), chestnut lamprey (*Ichthyomyzon castaneus*), and black bullhead (*Ameiurus melas*).

*Individual species of interest-* while it was not a specific objective to address questions related to the presence of species of concern or non-

native fishes, it is noteworthy to describe what we found within the constraints of our sampling protocol. We captured two state-sensitive species, Kiamichi shiner (*Notropis ortenburgeri*) and blackside darter (*Percina maculata*), which are classified as Tier I and Tier III, respectively, indicating species of greatest conservation need (CWCS 2005). We captured only 10 blackside darters, and 126 Kiamichi shiners. Among the seine and electrofishing samples we captured no non-native species; however, in the gill net samples we captured two non-native species: bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*). These species were first captured in the Red River Drainage in Oklahoma in 2012 (Patton and Tackett 2012). In this study and in the study by Patton and Tackett (2012), these non-native species were captured downstream from Hugo Reservoir, and were the only two non-native species captured during this study.

In summary, the Kiamichi River showed a pattern of increasing species diversity along the longitudinal gradient, as well as species addition from upstream to downstream. Further, unique fish communities were detected within the overall pattern of species addition. Inclusion of rare species in our analyses facilitated analysis of overall species richness, and affected our interpretation of community patterns.

## Acknowledgements

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## References

- Alevizon WS, Gorham JC, Richardson R, McCarthy SA. 1985. Use of man-made reefs to concentrate Snapper (*Lutjanidae*) and Grunts (*Haemulidae*) in Bahamian waters. Bull. Mar. Sci. 37: 3-10.

- Bistoni MA, Hued AC. 2002. Patterns of Fish Species Richness in Rivers of Central Region of Argentina. *Braz. J. Biol.* 62(4): 753-764.
- Bertrand KN, Gido KB, Guy CS. 2006. An evaluation of single-pass vs. multiple-pass Backpack electrofishing to estimate trends in species abundance and richness in prairie streams. *Trans. Kans. Aca. Sci.* 109(3): 131-138.
- Cao Y, Williams DD, Williams NE. 1998. How important are rare species in aquatic community ecology and bioassessment? *Limn. Ocean.* 43(7):1403-1409.
- Chadderton WL, Allibone RM. 2000. Habitat use and Longitudinal Distributions Patterns of Native Fish from a near Pristine Stewart Island, New Zealand, Stream. *New. Zeal. J. Marine. Fresh. Res.* 34: 487-499.
- Colquhoun MK, Morley A. 1943. Vertical zonation in woodland bird communities. *Anim. Eco.* 12: 75-81.
- Cucherousset J, Santoul F, Figuerola J, Cereghino R. 2008. How do biodiversity patterns of river animals emerge from the distributions of common and rare species? *Biol. Cons.* 141(12):2984-2992.
- CWCS (Comprehensive Wildlife Conservation Strategy). 2005. Oklahoma Department of Wildlife Conservation. <http://www.wildlifedepartment.com/CWCS.htm>. (Accessed May 21, 2015)
- Dahl E. 1953. Some aspects of ecology and zonation of the fauna on sandy beaches. *Oikos* 4(1): 1-27.
- Evans JW, Nobel RL. 1979. The Longitudinal Distribution of Fishes in an East Texas Stream. *Am. Midl. Nat.* 101(2): 333-343.
- Foltz JW. 1982. Fish Species Diversity and Abundance in Relation to Stream Habitat Characteristics. *Proc. Annu. Conf. SEAFWA.* 36:305-311.
- Galbraith HS, Spooner DE, Vaughn CC. 2008. Status of Rare and Endangered Freshwater Mussels in Southeastern Oklahoma. *Southwest. Nat.* 53(1): 45-50.
- Gorman OT, Karr JR. 1978. Habitat structure and stream fish communities. *Eco.* 59:507-515.
- Guillory V. 1982. Longitudinal Gradients of Fishes in Thompson Creek, Louisiana. *Southwest. Nat.* 27(1): 107-115.
- Hamilton AJ. 2005. Species Diversity or Biodiversity? *J. Environ. Man.* 75: 89-92.
- Heaney LR, Heideman PD, Rickart EA, Utzurrum RB, Klompen JH. 1989. Elevational Zonation of mammals in the central Philippines. *J. Trop. Eco.* 5: 259-280.
- Hubert WA. 1996. Passive capture techniques. Pages 157-181 *in* Murphy BR and Willis DW, editors. *Fisheries Techniques*, 2<sup>nd</sup> edition. Am. Fish. Soc. Bethesda, MD.
- Ibarra M, Stewart DJ. 1989. Longitudinal Zonations of Sandy Beach Fishes in the Napo River Basin, Eastern Ecuador. *Copeia* 2: 364-381.
- Kanno Y, Vokoun JC, Dauwalter DC, Hughes RM, Herlihy AT, Maret TR, Patton TM. 2009. Influence of rare species on electrofishing distance when estimating species richness of stream and river reaches. *Trans. Am. Fish. Soc.* 138:1240-1251.
- Lasne E, Bergerot B, Lek S, Laffaille P. 2007. Fish Zonation and Indicator Species for the Evaluation of the Ecological Status of Rivers: Example of the Loire Basin (France) *Riv. Res. Applic.* 23: 1-14.
- Lotrich VA. 1973. Growth, production, and community composition of fishes inhabiting a first, second, and third-order stream of eastern Kentucky. *Ecol. Mon.* 43:377-397.
- MacKenzie DI, Nichols JD., Sutton N, Kawanishi K, Bailey L. 2005. Improving inferences in population studies of rare species that are detected imperfectly. *Ecol.* 86(5):1101-1113.
- Master LL, Flack RS, Stein BA. 1998. Rivers of life: Critical watersheds for protecting freshwater biodiversity. *TNC* 1:20.
- Matthews WJ. 1985. Distributions of Midwestern fishes on multivariate environmental gradients, with emphasis on *Notropis lutrensis*. *Am. Midl. Nat.* 13:225-237.
- McGinley M. 2014. Species Richness. Available from: <http://www.eoearth.org/view/article/156216> (Accessed July 9, 2015).
- Meador MR, McIntyre JP, Pollock KH. 2003. Assessing the efficacy of single-pass backpack electrofishing to characterize fish community structure. *Trans. Am. Fish. Soc.* 132:39-46.

- Miller RJ, Robison HW. 2004. Fishes of Oklahoma. University of Oklahoma Press. Norman, OK.
- Mouillot D, Bellwood DR, Baralato C, Chave J, Galzin R, Harmelin-Vivien M, Kulbicki M, Lavergne S, Lavorel S, Mouquet N, Paine CET, Renaud J, Thuiller W. 2013. Rare species support vulnerable functions in high-diversity ecosystems. *PLoS Biol.* 11(5): e1001569. Doi:10.1371/journal.pbio.1001569 (open access).
- Moyle PB. 2002. Inland fishes of California. University of California Press, Berkeley, CA.
- Oklahoma Department of Wildlife Conservation. 2009. Standard Sampling Protocols for fishes Agency Manual. Oklahoma Department of Wildlife Conservation, Oklahoma City, OK.
- Onorato DP, Angus RA, Marion KR. 1998. Comparison of small-mesh seine and a backpack electroshocker for evaluation of fish populations in a North-Central Alabama stream. *NA. J. Fish. Man.* 18:361-373.
- Paller MH. 1994. Relationships between Fish Assemblage Structure and Stream Order in South Carolina Coastal Plain Stream. *Trans. Am. Fish. Soc.* 123(2): 150-161.
- Patton TM, Hubert WA, Rahel FJ, Gerow KJ. 2000. Efforts needed to estimate species richness in small streams on the Great Plains of Wyoming. *NA. J. Fish. Man.* 20:394-398.
- Patton, T, Tackett, C. 2012. Status of silver carp (*Hypophthalmichthys molitrox*) and bighead carp (*Hypophthalmichthys nobilis*) in southeastern Oklahoma. *Proc. Okla. Acad. Sci.* 92:53-58.
- Petry AC, Schulz UH. 2006. Longitudinal Changes and Indicator Species of the Fish Fauna In the Subtropical Sinos River, Brazil. *J. Fish. Biol.* 69: 272-290.
- Pigg J, Hill JG. 1974. Fishes of the Kiamichi River, Oklahoma. *Proc. Okla. Acad. Sci.* 54:121-130.
- Pires DO, Castro CB, Ratto CC. 1999. Reef coral reproduction in the Abrolhos Reed Complex Brazil. *Mar. Biol.* 135: 463-471.
- Pusey BJ, Kennard MJ, Arthur JM, Arthington AH. 1998. Quantitative Sampling of stream fish assemblages: Single- vs multiple-pass electrofishing. *Aust. J. Eco.* 23:365-374.
- Pyron M, Vaughn CC, Winston MR, Pigg J. 1998. Fish Assemblage Structure from 20 Years Of Collections in the Kiamichi River, Oklahoma. *Southwest. Nat.* 43(3):336-343.
- Quist MC, Bower MR, Hubert WA, Rahel FJ. 2006. Spatial Patterns of Fish Assemblage Structure in a Tributary System of the Upper Colorado River Basin. *J. Fresh. Eco.* 21(4): 673-680.
- Rahel FJ, Hubert WA. 1991. Fish assemblages and habitat gradients in a Rocky Mountain-Great Plains Stream: Biotic zonation and additive patterns of community change. *Trans. Am. Fish. Soc.* 120: 319-332
- Ravkin YS, Bogomolova IN, Chesnokova SV. 2010. Amphibian and reptile biogeographic regions of northern Eurasia mapped separately. *Con. Pro. Eco.* 3(5): 562-571.
- Robison HW, Buchanan TN. 1988. Fishes of Arkansas. University of Arkansas Press, Fayetteville, AR. 526pp.
- SAS Version 13.2. 2012. SAS Institute Inc.
- Schlosser, IJ. 1987. A conceptual framework for fish communities in small warmwater streams. Pages 17-24 in Matthews WJ and Heins DC, editors. *Community and evolutionary ecology of North American stream fishes.* University of Oklahoma Press, Norman, OK
- Shannon CE. 1948. A mathematical theory of communication. *Bell. Tech. J.* 27:379-423.
- Sheldon AL. 1968. Species Diversity and Longitudinal Succession in Stream Fishes. *Ecol.* 49(2): 193-198.
- Statzner B, Higler B. 1986. Questions and Comments on the River Continuum Concept. *Can. J. Fish. Aquat. Sci.* 42: 1038-1044.
- Torgersen CE, Baxter CV, Hiram WL, McIntosh BA. 2006. Landscape Influences on Longitudinal Patterns of River Fishes: Spatially Continuous Analysis of Fish-Habitat Relationships. *Am. Fish. Soc.* 48: 473-492.
- Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. 1980. The River Continuum Concept. *Can. J. Fish. Aquat. Sci.* 37: 130-137.
- Vaughn CC, Mather CM, Pyron M, Mehlhop P, Miller EK. 1996. The Current and Historical Mussel Fauna of the Kiamichi River, Oklahoma. *Southwest. Nat.* 41(3): 325-328.



- Williams LR, Toepfer CS, Martinez AD. 1996. The Relationship between Fish Assemblages And Environmental Gradients in an Oklahoma Prairie Stream. *J. Fresh. Eco.* 11(4): 459-468.
- Zar JH. 1973. *Biostatistical Analysis*, 4<sup>th</sup> ed. Prentice-Hall. Prairie Stream.

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**Appendix A. Site location with UTM Coordinates. The sites are arranged in numerical order from 1-11 stating at the upper reaches of the river moving downstream along the longitudinal gradient.**

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<u>Site Number</u>	<u>Northing</u>	<u>Easting</u>	<u>Description</u>
1	3834061N	15S 348442E	HWY 259 Bridge
2	3836764N	15S 337704E	Muse Bridge
3	3839499N	15S 327293E	Whitesboro Bridge
4	3836970N	15S 312838E	Old Indian HWY
5	3834792N	15S 305506E	Walnut Creek 5
6	3833948N	15S 298335E	1909 RR Site
7	3828321N	15S 285124E	Clayton Site
8	3821093N	15S 269511E	Pine Spur Road
9	3814933N	15S 264796E	Payne Site
10	3812359.35N	15S 263229.18E	Duncan Site
11	3792937.82N	15S 259604.01E	Antlers E-Street

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# Hematozoa (Apicomplexa: Haemogregarinidae, Hepatozoidae) from Two Turtles (Testudines: Chelydridae, Emydidae) and Two Snakes (Ophidia: Colubridae, Viperidae), in Southeastern Oklahoma

Chris T. McAllister

Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

Hematozoans (*Haemogregarina*, *Haemoproteus*, *Hepatozoon* spp.) are intraerythrocytic parasites that infect various vertebrates (Telford 2009). In Oklahoma, hematozoans have been reported from birds (Janovy 1963; Lewis et al. 1975; Bay and Andrews 2009) and mammals (see Allen et al. 2011). Although commonly reported from reptilian hosts in the surrounding states of Arkansas (McAllister and King 1980; Daly et al. 1984; McAllister et al. 1995, 2014), Louisiana (De Giusti and Batten 1951; Herban and Yaeger 1969; Acholonu 1974; Lowichik and Yaeger 1987; Powell and Knesel 1993) and Texas (Hilman and Strandmann 1960; Wang and Hopkins 1965), it is surprising that with the ubiquity of these parasites that no published surveys, to my knowledge, have been conducted on reptiles from Oklahoma. However, one lingering problem has been the taxonomic identity of these hematozoans which requires knowledge of their complete life cycle including developmental stages in vector and definitive host leeches (Siddall and Desser 1991, 2001) and in hematophagous invertebrates (Smith and Desser 1997; Jacobson 2007). Nevertheless, I report new records for some hematozoans from three reptiles of the state, including photomicrographs and select measurements.

Between June 2013 and October 2015, the following 12 reptiles were collected from McCurtain County and, as part of a survey of their helminth parasites, were at the same time also examined for hematozoans: two each of

common snapping turtle (*Chelydra serpentina*), Mississippi mud turtle (*Kinosternon subrubrum hippocrepsis*), common musk turtle (*Sternotherus odoratus*), and eastern cooter (*Pseudemys concinna concinna*), and one each of southern copperhead (*Agkistrodon contortrix contortrix*), western cottonmouth (*Agkistrodon piscivorus leucostoma*), timber rattlesnake (*Crotalus horridus*), and western rat snake (*Pantherophis obsoletus obsoletus*). Reptiles were overdosed with an intraperitoneal injection of sodium pentobarbital (Nembutal®). The plastron was removed from turtles with a bone saw and a midventral incision was made on snakes to expose the viscera. Blood was obtained from their exposed heart by obtaining a sample using ammonium heparinized (75 mm long) capillary tubes and thin films were air-dried, fixed for 1 min in absolute methanol, stained for 20–30 min with Wright-Giemsa stain, and rinsed in phosphate buffer (pH = 7.0). Slides were scanned at 100× or 400× and when infected cells were found, photographs were taken and length measurements were made on intraerythrocytic parasites (20/form) using a calibrated ocular micrometer under a 1,000× oil immersion lens and are reported in micrometers as means ±1SD followed by the ranges. Host vouchers are deposited in the Arkansas State University Museum of Zoology (ASUMZ) Herpetological Collection, State University, Arkansas. Voucher slides of hematozoans are deposited in the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska.

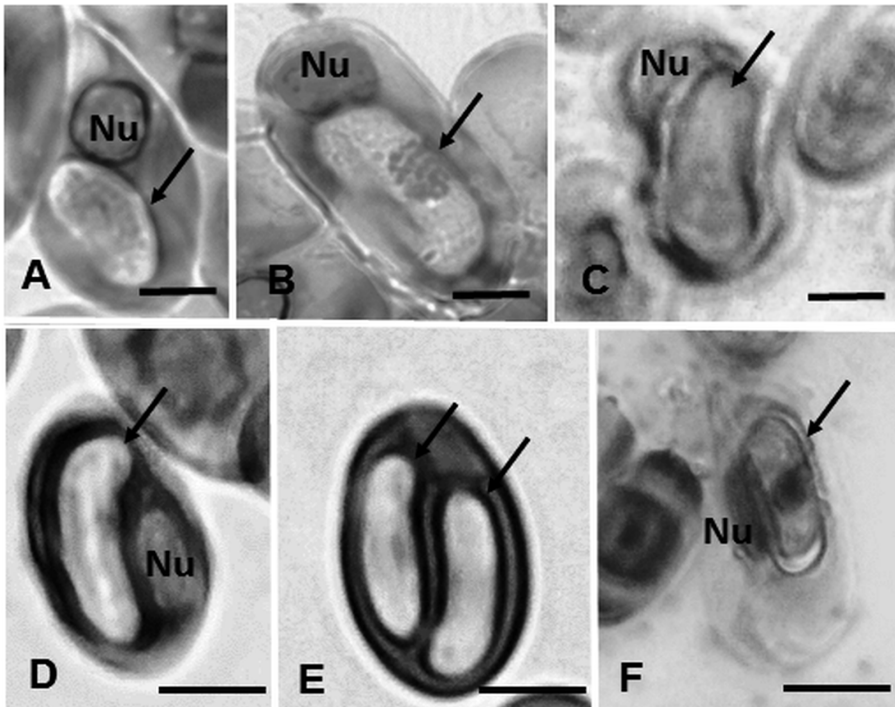
Four (33%) of the 12 individual reptiles, including *C. s. serpentina*, *P. c. concinna*, *C. horridus* and *P. obsoletus* were found to harbor intraerythrocytic hematozoans. The following were negative: *K. s. hippocrepis*, *S. odoratus*, *A. c. contortrix* and *A. p. leucostoma*. Data is presented below in an annotated format.

**Apicomplexa: Adeleorina:  
Haemogregarinidae**

*Haemogregarina* sp. Danilewsky, 1885 – About 5% of the red blood cells (rbc's) of a *C. serpentina* (adult male, 350 mm carapace length [CL], collected in April 2015 from the vicinity of Holly Creek, 33.96814°N, 94.804255°W) contained an intraerythrocytic hematozoan thought to belong to the genus *Haemogregarina* (HWML 101841). Three morphological types (Figs. 1A–B) were observed and measurements are given for small, medium and large types (Table 1). Ovoidal to bean-shaped gamonts were most often observed (Fig. 1A). These were very similar to morphological types described, but

not figured, from Arkansas red-eared sliders (*Trachemys scripta elegans*) by McAllister and King (1980). McAllister et al. (1995, Fig. 3) also reported large immature gamonts from an alligator snapping turtle (*Macrochelys temminckii*) from Arkansas similar of those from *C. serpentina*. The common snapping turtle has been commonly studied and previously reported as a host of various hematozoans from Illinois, Iowa, Kentucky, Louisiana, Massachusetts, Ohio, Tennessee, Texas, and Ontario, Canada (Hahn 1909; Roudabush and Coatney 1937; Edney 1949; Wang and Hopkins 1965; Marquardt 1966; Herban and Yeager 1969; Desser 1973; Acholonu 1974; Paterson and Desser 1976; Strohle and Christensen 1984; Siddall and Desser 1991, 1992; Brown et al. 1994).

Less than 1% of the rbc's of an adult male (270 mm CL) *P. c. concinna* collected in September 2015 from 8.0 km N of Broken Bow off US 259 (34.091873°N, 94.739463°W) harbored a hematozoan (HWML 101842) also



**Figure 1.** Photomicrographs of hematozoans (arrows) from four Oklahoma reptiles. A–B. Gamonts from *Chelydra serpentina*. C. Gamont from *Pseudemys concinna concinna*. D–E. Single (D) and pair (E) of macrogamonts from *Pantherophis obsoletus obsoletus*. F. Gamont from *Crotalus horridus*. Abbreviation: Nu (nucleus of host rbc). Scale bars = 10  $\mu$ m.

**Table 1.** Length measurements of hematozoans found in *Chelydra serpentina* and *Pantherophis obsoletus obsoletus* from Oklahoma.

Host/morphological type	Mean length ( $\mu\text{m}$ )	Size range ( $\mu\text{m}$ )
<b><i>C. serpentina</i></b>		
Small	12.2 $\pm$ 1.2	10–15
Medium	20.0 $\pm$ 1.2	16–21
Long	31.6 $\pm$ 2.2	28–37
<b><i>P. o. obsoletus</i></b>		
Long slender	18.2 $\pm$ 1.4	17–20

thought to belong to *Haemogregarina* (Fig. 1C). River cooters have previously been reported to be infected with hematozoans from Louisiana (Herban and Yeager 1969; Acholonu 1974), Illinois (Marquardt 1966), Tennessee (Edney 1949) and Texas (Hopkins 1965). Haemogregarines are most commonly reported from aquatic turtles with leeches serving as the only known invertebrate hosts and vectors (Telford 2009). We report these hematozoans from two turtles in Oklahoma for the first time.

### Hepatozoidae

*Hepatozoon* sp. Miller, 1908 – About 10% of the rbc's of a *P. o. obsoletus* (adult male, 793 mm snout-vent length [SVL], collected in October 2013 from Beavers Bend State Park, 34.126576°N, 94.674858°W) contained an intraerythrocytic hematozoan (HWML 101843) thought to belong to the genus *Hepatozoon* (Figs. 1D-E). Measurements of a single form of the parasite is provided in Table 1. Hematozoans were reported previously from various subspecies of *P. obsoletus* from Arkansas, Florida, Illinois and Louisiana (Marquardt 1966; Daly et al. 1984; Lowichik and Yeager 1987; Telford et al. 2001, 2004, 2005) and from captive rat snakes from zoos in Illinois and Ohio (Hull and Camin 1960).

In addition, a single *C. horridus* (adult male, 1,030 mm SVL, collected on 3 June 2013 from the Eastern Oklahoma State College Campus,

Idabel, 33.920662°N, 94.777173°W) was found to be infected (~ 1% of rbc's) with another hematozoan (HWML 101844), also thought to belong to the genus *Hepatozoon* (Fig. 1F). Hematozoans were reported previously from 4% of the erythrocytes of *C. horridus* from the Catskill Mountains (specific locality unknown) (Fantham and Porter 1954) and *Hepatozoon horridus* and *H. sauritus* was documented in one of eight (13%) timber rattlesnake from Florida (Telford et al. 2008). Gamonts of *H. horridus* and *H. sauritus* were reported by these authors to measure 15.7  $\times$  5.1 (13–17  $\times$  4–6) and 16.6  $\times$  4.1 (15–19  $\times$  3.5–6)  $\mu\text{m}$  respectively, and, although there were not enough infected erythrocytes in the present sample for comparative measurements, length of two gamonts were well within their ranges.

Smith (1996) considered all hemogregarines of snakes to be members of the genus *Hepatozoon* even in the absence of life-cycle data to the contrary. However, with little evidence that snakes are infected by species of *Haemogregarina*, Telford et al. (2001) described, with some reservation, *Haemogregarina floridana* from Florida green water snake (*Nerodia floridana*), Florida water snake (*Nerodia fasciata pictiventris*), and North Florida swamp snake (*Liodytes pygaea*) from Florida and South Carolina. I therefore document the two hematozoans from Oklahoma

snakes herein to represent *Hepatozoon* spp.

Turtles and snakes are hosts of numerous described and potentially undescribed hematozoans (Ernst and Ernst 1979; Telford 2009). Since Oklahoma supports 18 species and subspecies of turtles and 46 species and subspecies of snakes (Sievert and Sievert 2011), additional surveys on larger samples of turtles and snakes from the state need to be done as several species should be examined for hematozoans. Moreover, the inclusion of molecular characterization (DNA sequences) would be particularly helpful to identify some hematozoans (see Allen et al. 2011; Cook et al. 2014; Maia et al. 2014) which have limited morphological traits. As such, new host and distributional records could be found, including the possibility of discovering new species.

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### References

- Acholonu AD. 1974. *Haemogregarina pseudemydis* n. sp. (Apicomplexa: Haemogregarinidae) and *Pirhemocytion chelonarum* n. sp. in turtles from Louisiana. *J. Protozool.* 21:659–664.
- Allen KE, Yabsley MJ, Johnson EM, Reichard MV, Panciera RJ, Ewing SA, Little SE. 2011. Novel *Hepatozoon* in vertebrates from the southern United States. *J. Parasitol.* 97:648–653.
- Bay MD, Andrews KD. 2009. Prevalence of hematozoan parasites (Apicomplexa) in some common passerine birds (Passeriformes) from east-central Oklahoma. *Tex. J. Sci.* 61:311–318.
- Brown GP, Brooks RJ, Siddall ME, Desser SS. 1994. Parasites and reproductive output in the snapping turtle, *Chelydra serpentina*. *Copeia* 1994:228–231.
- Cook CA, Lawton SP, Davies AJ, Smith NJ. 2014. Reassignment of the land tortoise haemogregarine *Haemogregarina fitzsimonsi* Dias 1953 (Adeleorina: Haemogregarinidae) to the genus *Hepatozoon* Miller 1908 (Adeleorina: Hepatozoidae) based on parasite morphology, life cycle and phylogenetic analysis of 18S rDNA sequence fragments. *Parasitol.* 13:1–10.
- Daly JJ, McDaniel RC, Townsend JW, Calhoun CH Jr. 1984. Alterations in the plasma membranes of *Hepatozoon*-infected snake erythrocytes as evidenced by differential staining. *J. Parasitol.* 70:151–153.
- De Giusti DL, Batten PJ Jr. 1951. Notes on *Haemoproteus metchnikovi* in turtles from Wisconsin, Michigan and Louisiana. *J. Parasitol.* 37(Suppl.):12.
- Desser SS. 1973. A description of intraerythrocytic schizonts and gametocytes of a haemogregarine of the snapping turtle *Chelydra serpentina*. *Can. J. Zool.* 52:431–433.
- Edney JM. 1949. *Haemogregarina stepanowi* Danilewsky (1885) in middle Tennessee turtles. *J. Tenn. Acad. Sci.* 24:220–223.
- Ernst CH, Ernst EM. 1979. Synopsis of protozoans parasitic in native turtles of the United States. *Bull. Maryland Herpetol. Soc.* 15:1–15.
- Hahn CW. 1909. The stages of *Haemogregarina stepanovi* Danilewsky found in the blood of turtles, with special reference to changes in the nucleus. *Arch. Protistenkd.* 17:307–376.
- Herban NL, Yeager RG. 1969. Blood parasites of certain Louisiana reptiles and amphibians. *Amer. Midl. Nat.* 82:600–601.
- Hilman JL, Strandtmann RW. 1960. The incidence of *Hepatozoon serpentium* in some Texas snakes. *Southwest. Nat.* 5:226–228.
- Hull RW, Camin JH. 1960. Haemogregarines in snakes: The incidence and identity of the erythrocytic stages. *J. Parasitol.* 46:515–523.
- Jacobson ER. 2007. Parasites and parasitic diseases of reptiles. In: Jacobson ER (editor): *Infectious diseases and pathology of reptiles: Color atlas and text*. Boca Raton (FL): CRC Press, p. 566–571.
- Janovy J Jr. 1963. A preliminary survey of blood parasites of Oklahoma birds. *Proc. Okla. Acad. Sci.* 43: 59–61.

- Lewis JC, Carpenter JW, Morrison JA. 1975. Hemoprotozoa in mourning doves and other small birds of western Oklahoma. *J. Wildl. Dis.* 11:537–539.
- Lowichik A, Yeager RG. 1987. Ecological aspects of snake hemogregarine infections from two habitats in southeastern Louisiana. *J. Parasitol.* 73:1109–1115.
- Maia JP, Crottini A, Harris DJ. 2014. Microscopic and molecular characterization of *Hepatozoon domerguei* (Apicomplexa) and *Foleyella furcata* (Nematoda) in wild endemic reptiles from Madagascar. *Parasite* 21:47–58.
- Marquardt WC. 1966. Haemogregarines and *Haemoproteus* in some reptiles in southern Illinois. *J. Parasitol.* 52:823–824.
- McAllister CT, Bursley CR, Robison, HW, Connor MB, Barger MA. 2014. *Haemogregarina* sp. (Apicomplexa: Haemogregarinidae), *Telorchis attenuata* (Digenea: Telorchhiidae) and *Neoechinorhynchus emydis* (Acanthocephala: Neoechinorhynchidae) from map turtles (*Graptemys* spp.), in northcentral Arkansas. *J. Ark. Acad. Sci.* 68:154–157.
- McAllister CT, King AW. 1980. Hemogregarines in the red-eared slider, *Chrysemys scripta elegans* (Wied) from Arkansas. *Proc. Ark. Acad. Sci.* 34:124.
- McAllister CT, Upton SJ, Trauth SE. 1995. Hemogregarines (Apicomplexa) and *Falcaustra chelydrae* (Nematoda) in an alligator snapping turtle, *Macroclemys temminckii* (Reptilia: Testudines), from Arkansas. *J. Helminthol. Soc. Wash.* 62:74–77.
- Paterson WB, Desser SS. 1976. Observations of *Haemogregarina balli* sp. n. from the common snapping turtle, *Chelydra serpentina*. *J. Protozool.* 23:294–301.
- Powell SC, Knesel JA. 1993. Preliminary analysis of peripheral blood components of *Macroclemys temminckii*. *Herpetol. Rev.* 24:81–82.
- Roudabush RL, Coatney GR. 1937. On some blood Protozoa of reptiles and amphibians. *Trans. Amer. Microsc. Soc.* 56:291–297.
- Sievert G, Sievert L. 2011. A field guide to Oklahoma's amphibians and reptiles. Oklahoma City (OK): Oklahoma Department of Wildlife Conservation. 211 p.
- Siddall ME, Desser SS. 1991. Merogonic development of *Haemogregarina balli* (Apicomplexa: Adeleina: Haemogregarinidae) in the leech *Placobdella ornata* (Glossiphoniidae), its transmission to a chelonian intermediate host and phylogenetic implications. *J. Parasitol.* 77:426–436.
- Siddall ME, Desser SS. 2001. Transmission of *Haemogregarina balli* from painted turtles to snapping turtles through the leech *Placobdella ornata*. *J. Parasitol.* 87:1217–1218.
- Siddall ME, Desser SS. 2002. Prevalence and intensity of *Haemogregarina balli* (Apicomplexa: Adeleina: Haemogregarinidae) in three turtle species from Ontario, with observations on intraerythrocytic development. *Can. J. Zool.* 70:123–128.
- Smith TG. 1996. The genus *Hepatozoon* (Apicomplexa: Adeleina). *J. Parasitol.* 82:565–585.
- Smith TG, Desser SS. 1997. Phylogenetic analysis of the genus *Hepatozoon* Miller, 1908 (Apicomplexa: Adeleorina). *Syst. Parasitol.* 36:213–221.
- Strohlein DA, Christensen BM. 1984. *Haemogregarina* sp. (Apicomplexa: Sporozoa) in aquatic turtles from Murphy's Pond, Kentucky. *Trans. Amer. Microsc. Soc.* 103:98–101.
- Telford SR Jr. 2009. Hemoparasites of the Reptilia: Color atlas and text. Boca Raton (FL): CRC Press, 376 p.
- Telford SR Jr, Butler JF, Moler PE. 2005. Two additional *Hepatozoon* species (Apicomplexa: Hepatozoidae) from the southern black racer, *Coluber constrictor priapus* (Serpentes: Colubridae), in northern Florida. *J. Parasitol.* 91:139–143.
- Telford SR Jr, Ernst JA, Clark AM, Butler JF. 2004. *Hepatozoon sauritus*: A polytopic hemogregarine in three genera and four species of snakes in northern Florida, with specific identity verified from genome analysis. *J. Parasitol.* 90:352–358.
- Telford SR Jr, Moler PE, Butler JF. 2008. *Hepatozoon* species of the timber rattlesnake in northern Florida: Description of a new species, evidence of salivary gland oocysts,

- and a natural cross-familial transmission of an *Hepatozoon* species. J. Parasitol. 94:520–523.
- Telford SR Jr, Wozniak EJ, Butler JF. 2001. Haemogregarine specificity in two communities of Florida snakes, with descriptions of six new species of *Hepatozoon* (Apicomplexa: Hepatozoidae) and a possible species of *Haemogregarina* (Apicomplexa: Haemogregarinidae). J. Parasitol. 87:890–905.
- Wang CC, Hopkins SH. 1965. *Haemogregarina* and *Haemoproteus* (Protozoa, Sporozoa) in blood of Texas freshwater turtles. J. Parasitol. 51:781–787.

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# Helminth Parasites (Trematoda, Cestoda, Nematoda, Acanthocephala) of Herpetofauna from Southeastern Oklahoma: New Host and Geographic Records

**Chris T. McAllister**

Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

**Charles R. Bursey**

Department of Biology, Pennsylvania State University-Shenango, Sharon, PA 16146

**Matthew B. Connior**

Life Sciences, Northwest Arkansas Community College, Bentonville, AR 72712

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**Abstract:** Between May 2013 and September 2015, two amphibian and eight reptilian species/subspecies were collected from Atoka ( $n = 1$ ) and McCurtain ( $n = 31$ ) counties, Oklahoma, and examined for helminth parasites. Twelve helminths, including a monogenean, six digeneans, a cestode, three nematodes and two acanthocephalans was found to be infecting these hosts. We document nine new host and three new distributional records for these helminths. Although we provide new records, additional surveys are needed for some of the 257 species of amphibians and reptiles of the state, particularly those in the western and panhandle regions who remain to be examined for helminths. ©2015 Oklahoma Academy of Science

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## Introduction

In the last two decades, several papers from our laboratories have appeared in the literature that has helped increase our knowledge of the helminth parasites of Oklahoma's diverse herpetofauna (McAllister and Bursey 2004, 2007, 2012; McAllister et al. 1995, 2002, 2005, 2010, 2011, 2013, 2014a, b, c; Bonett et al. 2011). However, there still remains a lack of information on helminths of some of the 257 species of amphibians and reptiles of the state (Sievert and Sievert 2011). Here, we attempt to augment that void with several new host and distributional records for select herpetofauna from southeastern Oklahoma.

## Methods

Between May 2013 and September 2015, 11 Sequoyah slimy salamander (*Plethodon sequoyah*), nine Blanchard's cricket frog (*Acris blanchardii*), two eastern cooter (*Pseudemys concinna concinna*), two common snapping turtle (*Chelydra serpentina*), two Mississippi mud turtle (*Kinosternon subrubrum hippocrepis*), two western cottonmouth (*Agkistrodon piscivorus leucostoma*), one southern black racer (*Coluber constrictor priapus*), one diamondback watersnake (*Nerodia rhombifer*), one Midland brown snake (*Storeria dekayi wrightorum*), and one western ribbon snake (*Thamnophis proximus proximus*),

were collected by hand or tong from Atoka ( $n = 1$ , *S. d. wrightorum* only) and McCurtain counties. Specimens were placed in collection bags, taken to the laboratory for necropsy within 24 hr and killed by prolonged immersion with a concentrated chloretone® (chlorobutanol) solution (amphibians) or injection of sodium pentobarbital (reptiles). For turtles, a bone saw was used to remove the plastron and expose the viscera which was removed and placed in a Petri dish. For intravascular and ocular trematodes in turtles we followed methods of Snyder and Clopton (2005). The gastrointestinal tract was split lengthwise and examined as well as other organs, including the lungs, liver, and gonads. For other herptiles, a mid-ventral incision was made to expose the viscera and the entire gastrointestinal tract and other organs were examined for helminths. Trematodes and cestodes were fixed in hot tap water without coverslip pressure, stained with acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate (trematodes only) and mounted in Canada balsam. Nematodes were fixed in hot tap water and studied as temporary mounts on a microscopic slide in a drop of glycerol. Acanthocephalan cystacanths were placed in 70% ethanol and studied as temporary mounts in glycerol. Helminth voucher specimens were deposited in the Harold W. Manter Laboratory of Parasitology (MWML), Lincoln, Nebraska. If HWML numbers are not listed for a particular parasite, specimens are being retained for future morphological and molecular studies. Host voucher specimens were deposited in the Arkansas State University Museum of Zoology (ASUMZ), Herpetological Collection, State University, Arkansas.

## Results and Discussion

Of the 33 individual amphibians and reptiles examined, ten (30%) harbored at least one helminth. One turtle (*C. serpentina*) from McCurtain County was multiply infected with four helminths (a monogenean, two digeneans, and a nematode), another turtle (*P. c. concinna*) from McCurtain County harbored two helminths (a nematode and an acanthocephalan) and a single snake (*S. d. wrightorum*) from

Atoka County had two helminths (one digenean and one nematode). The helminths found in these Oklahoma herpetofauna are presented below in annotated format.

### Monogenea: Polystomatidae

#### *Neopolystoma* sp. (Fig. 1A)

Two monogeneans, *Neopolystoma* sp. were found in the conjunctival sacs of an adult (310 mm carapace length [CL]) male *C. serpentina* collected on 15 April 2015 near Holly Creek off Tebo Jones Road, McCurtain County (33.968086°N, 94.816722°W). *Neopolystoma* spp. is found in the cloaca, urinary and accessory bladders, oral, pharyngeal and nasal cavities, as well as in the conjunctival cavities of freshwater chelonians (Pichelin 1995). There is a high degree of site-specificity for *Neopolystoma* spp. (Du Preez and Lim 2000). In the United States, Mexico, and Central America, eight *Neopolystoma* are known, including *Neopolystoma domitalae* (Caballero, 1938) Price, 1939, *Neopolystoma elizabethae* Platt, 2000, *Neopolystoma fentoni* Platt, 2000, *Neopolystoma grossi* Du Preez and Morrison, 2015, *Neopolystoma moleri* Du Preez and Morrison, 2015, *Neopolystoma orbiculare* (Stunkard, 1916) Price, 1939, *Neopolystoma rugosa* (MacCallum, 1918), *Neopolystoma terrapenis* (Harwood, 1932) and an undescribed *Neopolystoma* sp. Héritier, Badets, Du Preez, Aisien, Lixian, Combes and Verneau (Thatcher 1964; Platt 2000a, b; Du Preez and Morrison 2015; Héritier et al. 2015). Of these, only *N. elizabethae* from the western painted turtle, *Chrysemys picta belli* from Indiana, Michigan, and Wisconsin, *N. fentoni* from white-lipped mud turtle, *Kinosternon leucostomum* and painted wood turtle, *Rhinoclemmys pulcherrima* from Costa Rica, *N. moleri* from Florida softshell turtle, *Apalone ferox*, *N. grossi* from Florida cooter, *Pseudemys concinna floridana* from Florida and an unknown species of *Neopolystoma* sp. (C. s.) from *C. serpentina* from Nebraska (O. Verneau, pers. comm.) are known from the conjunctival sac of the eye (Platt 2000a, b; Du Preez and Morrison 2012; Héritier et al. 2015). However, we document a new host as well as the first time a *Neopolystoma* sp. from the eye of a turtle has been reported from Oklahoma.

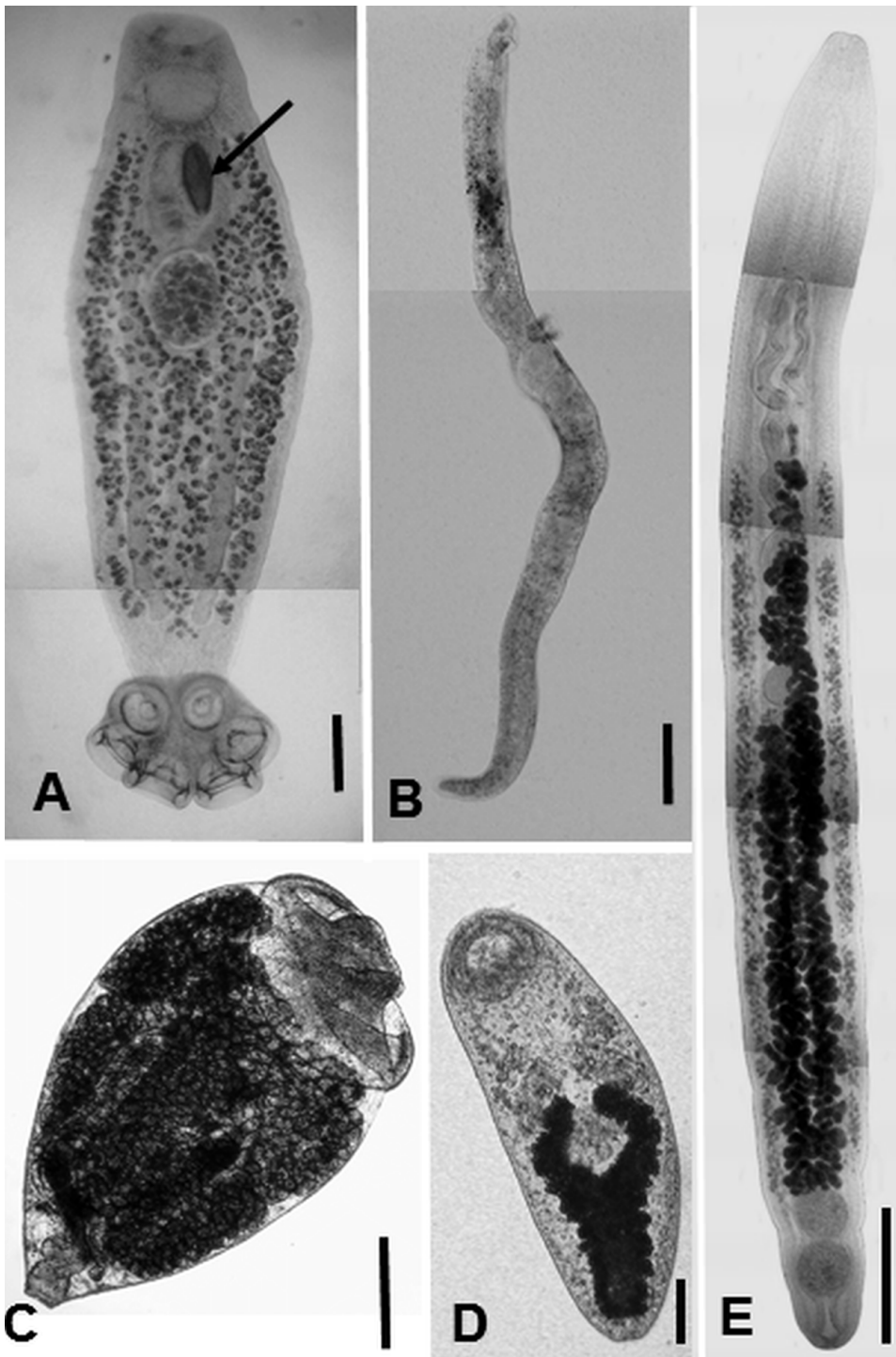


Figure 1. Select helminths of Oklahoma herpetofauna. A. *Neopolystoma* sp. from conjunctival sac of *Chelydra serpentina*. Arrow = egg. Scale bar = 300  $\mu$ m. B. Immature *Hapalorhynchus* from blood vascular system of *C. serpentina*. Scale bar = 100  $\mu$ m. C. *Megalodiscus* sp. from rectum of *Acris blanchardii*. Scale bar = 400  $\mu$ m. D. Metacercariae of *Dasymetra* sp. from esophagus of *Thamnophis proximus proximus*. Note Y-shaped cecum. Scale bar = 50  $\mu$ m. E. *Telorchis corti* from intestinal tract of *Kinosternon subrubrum hippocrepis*. Scale bar = 1 mm.

Our specimens ( $n = 2$ ) of *Neopolystoma* sp. can be morphologically distinguished from *N. moleri* in having a smaller body length (2,968–3,043  $\mu\text{m}$  as opposed to 3,249–7,944  $\mu\text{m}$ ) and possession of 7–8 as opposed to 12–13 genital spines; from *N. grossi* in having intestinal diverticula, a smaller body length (2,968–3,043  $\mu\text{m}$  compared to 3,298–4,873  $\mu\text{m}$ ), a wider pharynx (280–283 vs. 209–246  $\mu\text{m}$ ), a wider genital bulb (67–69  $\mu\text{m}$  as opposed to 58–60  $\mu\text{m}$ ), longer and wider testis (length 378–413  $\mu\text{m}$  compared to 186–293  $\mu\text{m}$ , width 287–367  $\mu\text{m}$  vs. 149–245  $\mu\text{m}$ ), and an ovary that is only 100–108  $\mu\text{m}$  wide compared to 118–135  $\mu\text{m}$  wide; from *N. elizabethae* in having a single egg (see Fig. 1A) that is 280–303  $\mu\text{m}$  in length vs. three eggs that are 322–367  $\mu\text{m}$  long and more narrow haptorial suckers 227–235  $\mu\text{m}$  wide vs. 344–408  $\mu\text{m}$  wide; and from *N. fentoni* in possessing shorter genital spines (9 compared to 11), in having a body length that is 2,968–3,043  $\mu\text{m}$  as opposed to 1,500–2,450  $\mu\text{m}$ , and in having the vitellarium and ceca not extending posteriorly to the haptor. Given these morphological differences with congeners also parasitizing the conjunctival sac of turtles, we suspect that our species is new and are currently in the process of collecting additional specimens for a description.

**Trematoda: Digenea: Brachycoeliidae**  
*Brachycoelium* cf. *salamandrae* (Frölich, 1789) Dujardin, 1845

One of 11 (%) *P. sequoyah* (adult male, 52 mm snout-vent length [SVL]) collected on 15 December 2013 from Beavers Bend State Park, McCurtain County (34.124837°N, 94.670665°W) was found to harbor three *Brachycoelium* cf. *salamandrae* in its small intestine. McAllister and Bursey (2012) previously reported this digenean from central newt, *Notophthalmus viridescens louisianensis* from McCurtain County. In addition, McAllister and Bursey (2004) did not find this trematode in a previous survey of helminths of this endemic salamander.

A single *S. d. wrightorum* (adult female, 140 mm SVL) collected on 8 March 2013 from McGee Creek State Park, Atoka County (34.327533°N, 95.914099°W) was infected

in the small intestine with a single *B. cf. salamandrae* (HWML 91938). Harwood (1932) previously reported *B. salamandrae* from Texas brown snake, *S. d. texana* from Houston, Texas. Interestingly, very few snakes have been reported as hosts of this trematode (Bursey et al. 2012).

McAllister et al. (2014b) recently noted they had serious doubts about Old World and New World *B. salamandrae* being conspecific (see summary by Bursey et al. 2012), and suggested caution with their former conclusions (McAllister et al. 2013) until a molecular approach was completed (V.V. Tkach, *pers. comm.*). Regardless of what species is eventually verified, we document two new host records for the genus *Brachycoelium*.

**Telorchidae**

*Telorchis corti* Stunkard, 1915 (Fig. 1E)

A single *K. s. hippocrepis* (adult male, 83 mm CL) collected on 20 June 2015 from the Little River off US 259, McCurtain County (33.942941°N, 94.759119°W) was infected with four *Telorchis corti* (HWML 101025) in the small intestine. This is a common digenean in various turtles and localities including those in Alabama, Arkansas, California, Florida, Idaho, Illinois, Iowa, Louisiana, Nebraska, North Carolina, Texas, Virginia, and Washington, D.C., and British Columbia, Canada (Ernst and Ernst 1977; Macdonald and Brooks 1989). It has also been reported in Oklahoma from *C. serpentina* (Williams 1953), red-eared slider, *Trachemys scripta elegans* (Everhart 1958) and an unknown host from Lake Texoma, Marshall County (Macdonald and Brooks 1989). In addition, *T. corti* was previously reported in an unpublished thesis by McKnight (1958) from *K. s. hippocrepis* from Lake Texoma; however, we document the first published record in this turtle.

**Schistosomatoidea: Spirorchidae**

*Spirorchis haematobius* (Stunkard 1922) Price, 1934

Two (one adult, one immature) blood flukes belonging to *Spirorchis* (HWML 101024) were found in the same *C. serpentina* harboring the *Neopolystoma* sp. Numerous *Spirorchis* spp. have been reported from various turtles (Ernst

and Ernst 1977), including two species from *C. serpentina*: *S. haematobius* (Stunkard 1922) Price, 1934 from Indiana, Iowa, Louisiana, Mississippi, Nebraska, New Jersey, New York, North Carolina, Ohio, Oklahoma, and Tennessee (Stunkard 1923; Byrd 1939; Rausch 1947; Williams 1953; Ulmer 1959; Brooks 1979; Brooks and Mayes 1975) and *S. magnitestis* Byrd, 1939 from Illinois and Tennessee (Byrd 1939; Martin 1973). In Oklahoma, *S. artericola* (Ward, 1921) Stunkard, 1923, *S. elegans* Stunkard, 1923 and *S. scripta* Stunkard, 1923 has been reported from *T. s. elegans* (Harwood 1931; McKnight 1958; Everhart 1975), and *S. innominatus* Ward, 1921 has been documented from *P. concinna* (Harwood 1931). Platt's (1993) key to North American species of *Spirorchis* identifies our specimens as *S. haematobius* given their possession of 10 testes, a testicular field beginning at the midbody, and their large size. This is only the second time in over 75 yr that this blood fluke has been reported from the common snapping turtle from Oklahoma.

#### **Haplorhynchus sp. (Fig. 1B)**

Ten immature *Haplorhynchus* sp. (HWML 101023) was found in the blood vascular system of the same *C. serpentina* above. These blood digeneans are commonly found in turtles (Ernst and Ernst 1977), including at least four species in *C. serpentina*: *H. brooksi* Platt, 1988 and *H. foliorchis* Brooks and Mayes, 1975 from Nebraska (Brooks and Mayes 1975; Platt 1988), *H. gracilis* Stunkard, 1922 from Indiana (Stunkard 1922; Platt 1988) and Wisconsin (Guilford 1959), and *H. stunkardi* Byrd, 1939 from Nebraska (Brooks and Mayes 1976). To our knowledge, there are no previous reports of *Haplorhynchus* spp. from Oklahoma so we document a new distribution record for this blood fluke in the state. Unfortunately, the immature nature of our specimens prevents any specific identification.

#### **Ochetosomatidae**

##### ***Dasymetra* sp. (Fig. 1C)**

Over 100 immature flukes (metacercaria) thought to represent *Dasymetra* sp. was found in the esophagus of an adult (270 mm SVL) *T. p. proximus* collected on 7 September 2013 from Lukfata, McCurtain County (34.006658°N,

94.764592°W). The only previously reported trematode from this host is *Paralechiorchis megacystis* (Ochetosomatidae) from Kansas (Stewart 1960). *Dasymetra longicirrus* (Odlaug, 1938) Denton, 1938, originally described from banded water snake (*Nerodia sipedon*) from Louisiana, has been reported previously from the related eastern garter snake, *Thamnophis sirtalis* (Ernst and Ernst 2006). In addition, McAllister and Bursey (2012) reported *D. conferta* Nicoll, 1991 from *N. rhombifer* from McCurtain County. We document a new host record for *Dasymetra* sp.

#### **Echinostomatiformes: Diplodiscidae** ***Megalodiscus* sp. (Fig. 1D)**

One of nine (11%) *A. blanchardi* (male, 25 mm SVL) collected on 13 October 2013 from Hochatown, McCurtain County (34.171077°N, 94.75184°W) harbored a single *Megalodiscus* sp. in its rectum. *Megalodiscus temperatus* (Stafford, 1905) Harwood, 1932 is a common trematode of North American anurans (see Bolek and Janovy 2008). In Oklahoma, *M. temperatus* has been previously reported from southern leopard frog, *Lithobates sphenoccephalus utricularius* (Trowbridge and Hefley 1934; Kuntz and Self 1944; Vhora and Bolek 2015). We were not, however, able to identify this helminth to species until a molecular analysis can be completed. However, this is the first time *Megalodiscus* sp. has been reported in *A. blanchardi*.

#### **Cestoda: Eucestoda: Bothriocephalidea: Proteocephalidae**

##### ***Ophiotaenia marenzelleri* (Barrois, 1898) Railliet, 1899**

A single *O. marenzelleri* (HWML 101840) was removed from the small intestine of a juvenile (335 mm SVL) *A. p. leucostoma* collected on 8 October 2014 from Lukfata, McCurtain County (34.01445°N, 94.774386°W). This tapeworm has been reported previously from *A. piscivorus* from Alabama (Detterline et al. 1984), Louisiana (Brooks 1978; Fontenot and Font 1996), North Carolina (Collins 1969), and Texas (Harwood 1933) and an unknown locality in the Catskill Mountains (Fantham and Porter 1954). This is the first time *O. marenzelleri* has been reported from Oklahoma.

**Nematoda: Spirurida:****Gnathostomatidae*****Spiroxys contorta* (Rudolphi, 1819) Hedrick, 1935**

Nematodes matching the description of *S. contorta* (HWML 91949) were found in the stomach of an adult male (745 mm SVL) *N. rhombifer* collected on 6 August 2014 from Yashau Creek, McCurtain County (33.987161°N, 94.743637°W). This nematode has been reported previously in Oklahoma from eastern river cooter, *Pseudemys concinna concinna* (Harwood 1931) and *T. s. elegans* (Everhart 1958) from Oklahoma. This is the first time this nematode has been reported from *N. rhombifer*. However, it is possible the snake obtained the infection from eating prey (fishes, amphibians, turtles) normally associated with the *S. contorta* life cycle (Hedrick 1935).

**Camallanidae*****Serpinema trispinosus* (Leidy, 1852) Yeh, 1960**

Two female *S. trispinosus* (HWML 91939) were found in the small intestine of *P. c. concinna* collected on 10 September 2015 from off US 259 N of Broken Bow (34.094728°N, 94.739301°W). In addition, 43 *S. trispinosus* were taken from the small intestine of the same *C. serpentina* noted herein multiply infected with *Neopolystoma* and blood flukes. To our knowledge, this nematode has not been previously reported in the literature from Oklahoma. It was, however, noted in the unpublished thesis of McKnight (1959) where he reported the synonym *Camallanus trispinosus* from four species of turtles. In addition, *S.* (= *Camallanus*) *microcephalus* (Dujardin, 1845) Yeh, 1960 has been reported from the state and elsewhere in turtles (Harwood 1931; Williams 1953; Everhart 1958); however, this parasite is an Asian and European species of turtles (Baker 1987). Various Nearctic turtles have been reported as hosts of *S. trispinosus*, including *C. serpentina* and *P. concinna* (Baker 1987) and commonly (98%) in *T. s. elegans* from neighboring Arkansas (Rosen and Marquardt 1978)

**Ascaridida: Cosmocercidae*****Cosmocercoides variabilis* (Harwood, 1930)****Travassos, 1931**

A single *S. d. wrightorum*, the same host of *B. cf. salamandrae* above, was infected with an immature female *C. variabilis* (HWML 91950). Harwood (1930, 1932) previously reported *C. variabilis* from *S. d. texana* from Houston, Texas, and Rau and Gordon (1980) reported this nematode from 8% northern brown snakes, *S. d. dekayi* from Montreal, Quebec, Canada. There are several other snakes reported as hosts and the parasite has a vast range in the Neotropical and Nearctic regions (see Bursey et al. 2012). In Oklahoma, *C. variabilis* has been reported from *P. sequoyah* (McAllister and Bursey 2004), American bullfrog, *Lithobates catesbeianus* (Trowbridge and Hefley 1934), Hurter's spadefoot, *Scaphiopus hurterii* (McAllister et al. 2005) and dwarf American toad, *Anaxyrus americanus charlesmithi* (McAllister et al. 2014a). We document a new host record for this nematode.

**Centrorhynchidae gen. sp. Van Cleave, 1916****Undetermined cystacanth**

Two acanthocephalan cystacanths (HWML 91951) were found in the coelomic cavity of an adult male (810 mm SVL) *C. constrictor priapus* collected on 28 May 2013 from the Eastern Oklahoma State College Campus-Idabel, McCurtain County (33.920565°N, 94.77717°W). The only acanthocephalans (Oligacanthorhynchidae) reported previously from the species were from a northern black racer (*C. constrictor constrictor*) and include *Macracanthorhynchus ingens* (Linstow, 1879) Meyer, 1932 from Louisiana and Pennsylvania (Elkins and Nickol 1983; Bolette 1998) and a *Macracanthorhynchus* sp. from New York (see Ernst and Ernst 2006). Juvenile stages of centrorhynchid acanthocephalans have been found in several amphibians and reptiles (see summary in Bursey et al. 2012) and these are considered paratenic hosts. We document the first report of a centrorhynchid cystacanth from this host and the initial record, to our knowledge, for any snake from the Nearctic zoogeographical region (Bursey et al. 2012).

**Neoechinorhynchidae**

*Neoechinorhynchus* sp.

A single adult male *P. c. concinna* (270 mm CL) collected on 10 September 2015 from off US 259 N of Broken Bow (34.094728°N, 94.739301°W) harbored 12 acanthocephalans (HWML 91960) in its small intestine belonging to the genus *Neoechinorhynchus*. However, because no fully developed eggs were found, identification to species is problematic. Based on the posterior end of worms, specific identity is thought to be *N. emyditoides* Fisher, 1960 or *N. pseudemydis* Cable and Hopp, 1954 (M. Barger, pers. comm.). Both of these *Neoechinorhynchus* have been previously reported from the state, *N. emyditoides* from McIntosh County and Lake Talawanda, Pittsburg County, and *N. pseudemydis* from an unspecified locality in Oklahoma (Barger 2004). However, either species represents a new host record for *P. concinna* (Barger 2004).

In summary, we have documented additional new host and distributional records for some amphibians and reptiles of the state. Future work should target those species for which we know very little about, many of which occur in the western portion and Panhandle of Oklahoma where the ecoregions (Central Great Plains and High Plains) differ considerably from those in the current study of McCurtain County.

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**References**

- Baker MR. 1987. Synopsis of the Nematoda parasitic in amphibians and reptiles. Mem. Univ. Newfoundl. Occ. Pap. Biol. 11:1–325.
- Barger MA. 2004. The *Neoechinorhynchus* of turtles: Specimen base, distribution, and host use. Comp. Parasitol. 71:118–129.
- Bolek MG, Janovy J Jr. 2008. Alternative life cycle strategies of *Megalodiscus temperatus* in tadpoles and metamorphosed tadpoles. Parasite 15:396–401.
- Bolette DP. 1998. Natural occurrence of *Macracanthorhynchus ingens* (Acanthocephala: Oligacanthorhynchidae) in a northern black racer *Coluber constrictor constrictor* (Serpentes: Colubridae). Tex. J. Sci. 50:85–89.
- Bonett RM, Steffen MA, Trujano-Alvarez AL, Martin SD, Bursley CR, McAllister CT. 2011. Distribution, abundance, and genetic diversity of *Clinostomum* spp. metacercariae (Trematoda: Digenea) in a modified Ozark stream system. J. Parasitol. 97:177–184.
- Brooks DR. 1978. Systematic status of proteocephalid cestodes from reptiles and amphibians in North America with descriptions of three new species. Proc. Helminthol. Soc. Wash. 45:1–28.
- Brooks DR. 1979. New records for amphibian and reptile trematodes. Proc. Helminthol. Soc. Wash. 46:286–289.
- Brooks DR, Mayes MA. 1975. Platyhelminths of Nebraska turtles with descriptions of two new species of spirorchiids (Trematoda: Spirorchiidae). J. Parasitol. 61:403–406.
- Brooks DR, Mayes MA. 1976. *Telorchis gutturossi* sp. n. (Trematoda: Telorchidae) from *Graptomys pseudogeographica* Gray in Nebraska, with reports of additional species of trematodes from Nebraska turtles. J. Parasitol. 62:901–905.
- Bursley CR, Goldberg SR, Telford SR Jr, Vitt LJ. 2012. Metazoan endoparasites of 13 species of Central American anoles (Sauria: Polychrotidae: *Anolis*) with a review of the helminth communities of Caribbean, Mexican, North American, and South American anoles. Comp. Parasitol. 79:75–132.
- Byrd EE. 1939. Studies on the blood flukes of

- the family Spirorchidae. Part II. Revision of the family and description of new species. *J. Tenn. Acad. Sci.* 14:116–161.
- Collins RF. 1969. The helminths of *Natrix* spp. and *Agkistrodon piscivorus piscivorus* (Reptilia: Ophidia) in eastern North Carolina. *J. Elisha Mitchell Sci. Soc.* 85:141–144.
- Detterline JL, Jacob JS, Wilhelm WE. 1984. A comparison of helminth endoparasites in the cottonmouth (*Agkistrodon piscivorus*) and three species of water snakes (*Nerodia*). *Trans. Amer. Micros. Soc.* 103:137–143.
- Du Preez LH, Lim LHS. 2000. *Neopolystoma liewi* sp. n. (Monogenea: Polystomatidae) from the eye of the Malayan box turtle (*Cuora amboinensis*). *Fol. Parasitol.* 47:11–16.
- Du Preez LH, Morrison L. 2012. Two new polystomes (Monogenea: Polystomatidae) from the eyes of North American freshwater turtles. *Zootaxa* 3392:47–59.
- Elkins CA, Nickol BB. 1983. The epizootiology of *Macranthorhynchus ingens*. *J. Parasitol.* 69:951–956.
- Ernst CH, Ernst EM. 1977. Synopsis of helminths endoparasitic in native turtles of the United States. *Bull. Maryland Herp. Soc.* 13:1–75.
- Ernst CH, Ernst EM. 2006. Synopsis of helminths endoparasitic in snakes of the United States and Canada. *Soc. Stud. Amph. Rept. Circ.* 34:1–86.
- Everhart BA. 1958. Notes on the helminths of *Pseudemys scripta elegans* (Wied, 1838) in areas of Texas and Oklahoma. *Proc. Okla. Acad. Sci.* 38:38–43.
- Fantham HB, Porter A. 1954. The endoparasites of some North American snakes and their effects on the Ophidia. *Proc. Zool. Soc. London* 123:867–898.
- Fontenot LW, Font WF. 1996. Helminth parasites of four species of aquatic snakes from two habitats in southeastern Louisiana. *J. Helminthol. Soc. Wash.* 63:66–75.
- Guilford HG. 1959. Some helminth parasites found in turtles from northeastern Wisconsin. *Trans. Wisconsin Acad. Sci. Arts Lett.* 48:121–124.
- Harwood PD. 1930. A new species of *Oxysomatium* (Nematoda) with some remarks on the genera *Oxysomatium* and *Aplectana*, and observations on the life history. *J. Parasitol.* 17:61–73.
- Harwood PD. 1931. Some parasites of Oklahoma turtles. *J. Parasitol.* 18:98–101.
- Harwood PD. 1932. The helminths parasitic in the Amphibia and Reptilia of Houston, Texas, and vicinity. *Proc. US Nat. Mus.* 81:1–71.
- Harwood PD. 1933. The helminths parasitic in the water moccasin, with a discussion of the characters of Proteocephalidae. *Parasitol.* 25:130–142.
- Hedrick LR. 1935. Taxonomy of the nematode genus *Spiroxys* (family Spiruridae). *J. Parasitol.* 21:397–409.
- Héritier L, Badets M, Du Preez LH, Aisien MSO, Lixian F, Combes C, Verneau O. 2015. Evolutionary processes involved in the diversification of chelonian and mammal polystomatid parasites (Platyhelminthes, Monogenea, Polystomatidae) revealed by palaeoecology of their hosts. *Mol. Phyl. Evol.* 92:1–10.
- Kuntz RE, Self JT. 1944. An ecological study of the metazoan parasites of the Salientia of Comanche County, Oklahoma. *Proc. Okla. Acad. Sci.* 24:35–38.
- Macdonald CA, Brooks DR. 1989. Revision and phylogenetic analysis of the North American species of *Telorchis* Luehe, 1899 (Cercomeria: Trematoda: Digenea: Telorchidae). *Can. J. Zool.* 67:2301–2320.
- Martin DR. 1973. Distribution of helminth parasites in turtles native to southern Illinois. *Trans. Illinois Acad. Sci.* 65:61–67.
- McAllister CT, Bursey CR. 2004. Endoparasites of the Sequoyah slimy salamander, *Plethodon sequoyah* (Caudata: Plethodontidae), from McCurtain County, Oklahoma. *Tex. J. Sci.* 56:273–277.
- McAllister CT, Bursey CR. 2007. First report of the nematode *Physaloptera squamatae* (Spirurida: Physalopteridae) in Oklahoma with a summary of hosts. *Proc. Okla. Acad. Sci.* 87:65–67.
- McAllister CT, Bursey CR. 2012. New host and distributional records for helminth parasites (Trematoda, Cestoidea, Nematoda) of herpetofauna from southeastern Oklahoma. *Proc. Okla. Acad. Sci.* 92:29–35.
- McAllister CT, Bursey CR, Conn DB. 2005.



- Endoparasites of Hurter's spadefoot, *Scaphiopus hurterii* and plains spadefoot, *Spea bombifrons* (Anura: Scaphiopodidae), from southern Oklahoma. *Tex. J. Sci.* 43:391–397.
- McAllister CT, Bursey CR, Connior MB, Trauth SE. 2014a. Myxozoan and helminth parasites of the dwarf American toad, *Anaxyrus americanus charlesmithi* (Anura: Bufonidae), from Arkansas and Oklahoma. *Proc. Okla. Acad. Sci.* 94:51–58.
- McAllister CT, Bursey CR, Connior MB, Durden LA, Robison HW. 2014b. Helminth and arthropod parasites of the ground skink, *Scincella lateralis* (Sauria: Scincidae), from Arkansas and Oklahoma. *Comp. Parasitol.* 81:210–219.
- McAllister CT, Bursey CR, Steffen MA. 2010. Nematode parasites of the many-ribbed salamander, *Eurycea multiplicata* (Caudata: Plethodontidae), from Arkansas and Oklahoma. *Proc. Okla. Acad. Sci.* 90:69–73.
- McAllister CT, Bursey CR, Steffen MA, Martin SE, Trujano-Alvarez AL, Bonett RM. 2011. *Sphyranura euryceae* (Monogenoidea: Polystomatoinea: Sphyranuridae) from the grotto salamander, *Eurycea spelaea* and Oklahoma salamander, *Eurycea tynerensis* (Caudata: Plethodontidae), in northeastern Oklahoma, U.S.A. *Comparative Parasitology* 78:188–192.
- McAllister CT, Bursey CR, Upton SJ, Trauth SE, Conn DB. 1995. Parasites of *Desmognathus brimleyorum* (Caudata: Plethodontidae) from the Ouachita Mountains of Arkansas and Oklahoma. *J. Helminthol. Soc. Wash.* 62:150–156.
- McAllister CT, Connior MB, Bursey CR, Robison HW. 2014c. A comparative study of helminth parasites of the many-ribbed salamander, *Eurycea multiplicata* and Oklahoma salamander, *Eurycea tynerensis* (Caudata: Plethodontidae), from Arkansas and Oklahoma. *J. Ark. Acad. Sci.* 68:87–96.
- McAllister CT, Bursey CR, Robison HW, Connior MB. 2013. Endoparasites of the spotted dusky salamander, *Desmognathus conanti* (Caudata: Plethodontidae), from southern Arkansas, U.S.A. *Comp. Parasitol.* 80:60–68.
- McAllister CT, Upton SJ, Trauth SE. 2002. Parasites of four species of endemic *Plethodon* from Arkansas and Oklahoma. *J. Ark. Acad. Sci.* 56:239–242.
- McKnight TJ. 1959. A taxonomic study of the helminth parasites of the turtles of Lake Texoma [PhD thesis]. Norman (OK): University of Oklahoma. 55 p. Available from: University Microfilms, Ann Arbor, MI: 59–2966.
- Pichelin S. 1995. The taxonomy and biology of the Polystomatidae (Monogenea) in Australian freshwater turtles (Chelidae, Pleurodira). *J. Nat. Hist.* 29: 1345–1381.
- Platt TR. 1988. *Hapalorhynchus brooksi* sp. n. (Trematoda: Spirorchidae) from the snapping turtle (*Chelydra serpentina*), with notes on *H. gracilis* and *H. stunkardi*. *Proc. Helminthol. Soc. Wash.* 55:317–323.
- Platt TR. 1993. Taxonomic revision of *Spirorchis* MacCallum, 1919 (Digenea: Spirorchidae). *J. Parasitol.* 79:337–346.
- Platt TR. 2000a. *Neopolystoma fentoni* n. sp. (Monogenea: Polystomatidae) a parasite of the conjunctival sac of freshwater turtles in Costa Rica. *Mem. Inst. Oswaldo Cruz* 96:833–837.
- Platt TR. 2000b. Helminth parasites of the western painted turtle, *Chrysemys picta belli* (Gray), including *Neopolystoma elizabethae* n. sp. (Monogenea: Polystomatidae), a parasite of the conjunctival sac. *J. Parasitol.* 86:815–818.
- Rau ME, Gordon DM. 1980. Host specificity among the helminth parasites of four species of snakes. *Can. J. Zool.* 58:929–930.
- Rausch R. 1947. Observations on some helminths parasitic in Ohio turtles. *Amer. Midl. Nat.* 38:434–442.
- Rosen R, Marquardt WC. 1978. Helminth parasites of the red-eared turtle (*Pseudemys scripta elegans*) in central Arkansas. *J. Parasitol.* 64:1148–1149.
- Sievert G, Sievert L. 2011. A field guide to Oklahoma's amphibians and reptiles. Oklahoma City (OK): Oklahoma Department of Wildlife Conservation. 211 p.
- Snyder SD, Clopton RE. 2005. Collection and preservation of spirorchid and polystomatid parasites from turtles. *Comp. Parasitol.* 72:102–107.

- Stewart PL. 1960. Lung-flukes of snakes, genera *Thamnophis* and *Coluber*, in Kansas. Univ. Kansas Sci. Bull. 41:877–890.
- Stunkard HW. 1922. Two new genera of North American blood flukes. Amer. Mus. Novit. 39:1–8.
- Stunkard HW. 1923. Studies on North American blood flukes. Bull. Amer. Mus. Nat. Hist. 48:165–221.
- Thatcher VE. 1964. Estudios sobre los trématodos de reptiles de Tabasco, Mexico: Lista de huéspedes y sus parasitos. An. Esc. Nac. Cienc. Biol. Mexico 13:91–96.
- Trowbridge AH, Hefley HM. 1934. Preliminary studies of the parasite fauna of Oklahoma anurans. Proc. Okla. Acad. Sci. 14:16–19.
- Ulmer MJ. 1959. Studies on *Spirorchis haematobium* (Stunkard, 1922) Price, 1934 (Trematoda: Spirorchidae) in the definitive host. Trans. Amer. Micros. Soc. 78:81–89.
- Vhora MS, Bolek MG. 2015. Temporal occurrence and community structure of helminth parasites in southern leopard frogs, *Rana sphenocephala*, from north central Oklahoma. Parasitol. Res. 114:1–11.
- Williams RW. 1953. Helminths of the snapping turtle, *Chelydra serpentina*, from Oklahoma, including the first report and description of the male of *Capillaria serpentina* Harwood, 1932. Trans. Amer. Micros. Soc. 72:175–178.

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# Observational Changes to the Natural Flow Regime in Lee Creek in Relation to Altered Precipitation Patterns and its Implication for Fishes

**Michael R. Gatlin**

Oklahoma Cooperative Fish and Wildlife Research Unit, Department of Natural Resource Ecology and Management, Oklahoma State University Stillwater, OK 74078

**James M. Long**

U.S. Geological Survey, Oklahoma Cooperative Fish and Wildlife Research Unit, Department of Natural Resource Ecology and Management, Oklahoma State University, Stillwater, OK 74078

**Donald J. Turton**

Department of Natural Resource Ecology and Management, Oklahoma State University, Stillwater, OK 74078

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**Abstract:** The natural flow regime is important for structuring streams and their resident ichthyofauna and alterations to this regime can have cascading consequences. We sought to determine if changes in hydrology could be attributed to changes in precipitation in a minimally altered watershed (Lee Creek). The stream flow regime was analyzed using Indicators of Hydrologic Alteration (IHA) software, and data from a nearby climate station were used to summarize concurrent precipitation patterns. We discovered that Lee Creek hydrology had become flashier (i.e., increased frequency of extreme events of shorter duration) since 1992 coincident with changes in precipitation patterns. Specifically, our results show fewer but more intense rain events within the Lee Creek watershed. Our research provides evidence that climate-induced changes to the natural flow regime are currently underway and additional research on its effects on the fish community is warranted.

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## Introduction

A variety of perturbations can alter stream hydrology but precipitation is the ultimate factor and human-mediated climate change is predicted to affect precipitation patterns (Solomon et al. 2007). Significant deviations in the timing and magnitude of precipitation events could affect stream hydrology (Poff et al. 1997, Groisman et al. 2001). The effects of climate change are expected to vary across regions (Whitfield 2010), although some general trends are expected (Solomon et al. 2007), such as increased

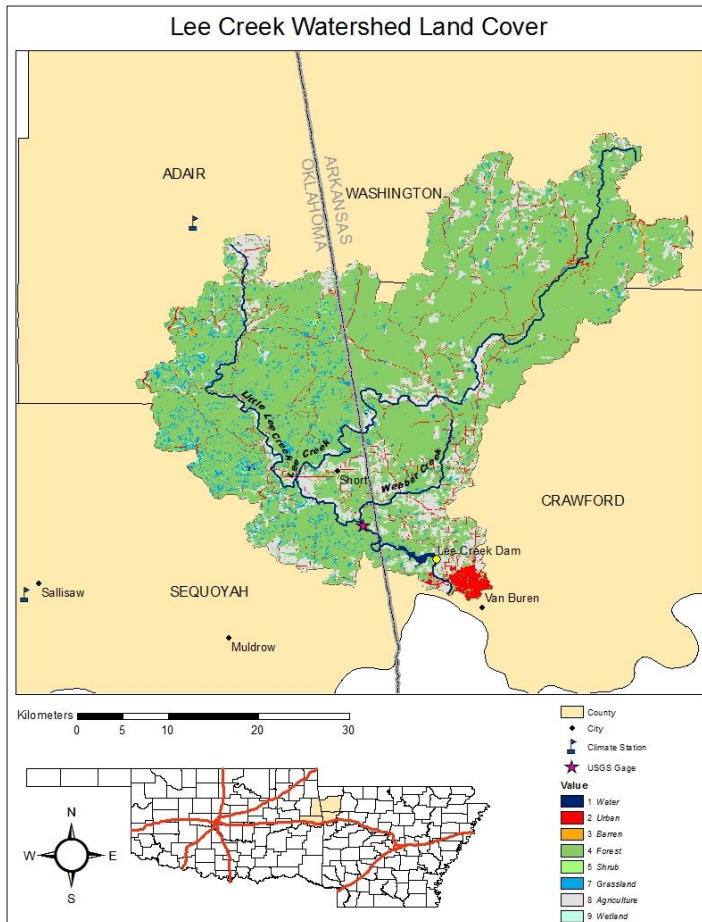
intensity of rain storms coupled with decreased frequency of rain events (Karl and Knight 1998, Easterling et al. 2000, Singh et al. 2013).

Modifications to precipitation patterns that ultimately affect streamflow will likely impact fishes and other aquatic organisms. For instance, a shift in flood timing could affect fish reproduction by inadvertently triggering spawning cues or causing larval mortality (Bunn and Arthington 2002, Lytle and Poff 2004). Changes in the natural flow regime are of particular concern for fishes of conservation need, especially those that occur on the edge of

their natural range and are highly adapted to flow.

With the wide-ranging availability of precipitation data, it is possible to examine this force on streams. Lee Creek and its watershed in Oklahoma and Arkansas is a system where these criteria are mostly met and have implications for future decision makers to consider. Lee Creek is a 5th order stream and one of six scenic rivers in Oklahoma with head-waters in the Boston Mountains of northwestern Arkansas, which flows through Oklahoma and confluences with Arkansas River near Van Buren, AR (Figure 1). The watershed of Lee Creek is mostly forested (76.8%) with minimal change or disturbance (Gatlin 2013), making this an ideal

setting to isolate the role of precipitation on hydrology. However, in 1992, the Lee Creek Dam and Reservoir was constructed lower in the watershed. Because it was constructed near the mouth of the creek, we limited our hydrological analyses to above the reservoir. The construction of Lee Creek Dam was thought to threaten the persistence of many fish species of “greatest conservation need” such as the wedgespot shiner *Notropis greenei*; Ozark minnow *Notropis nobilis*; sunburst darter *Etheostoma mihileze*; blackside darter *Percina maculate* and longnose darter *Percina nasuta* (ODWC 2005). Longnose darter in particular was of interest (FERC 1987) because Lee Creek was the last remaining river in Oklahoma that contained this species since it



**Figure 1. Map depicting the location, extent, and land cover types of the Lee Creek watershed in Oklahoma and Arkansas. Locations of climate stations and USGS stream flow gage station are provided.**

became extirpated from the Poteau River after that system was dammed (Wagner 1985). As a result, the longnose darter is considered by the state of Oklahoma as “endangered”; the only fish species to be classified as such. As a result, understanding the role that climate change may have on the hydrology of Lee Creek would be beneficial to these future decisions. Thus, we sought to determine if changes in hydrology above the dam before and after 1992 would mirror any concomitant patterns in precipitation.

## Methods

### Hydrological analysis

To determine alterations in streamflow, we obtained daily streamflow data from the USGS gaging station on Lee Creek near Short, OK above Lee Creek Dam (gage# 07249985; Figure 1), split the data into pre- (1970-1991) and post-impoundment (1992-2013) periods, and used Indicators of Hydrologic Alteration (IHA) software to determine differences (Richter et al. 1996, Mathews and Richter 2007). The IHA analysis calculates 32 ecologically significant parameters for each period, based on the water-year (Oct. 1-Sept. 31), and was used to determine how flow duration, magnitude, frequency, or timing might have changed (Richter et al. 1996). Because the data were not normally distributed, we reported percentiles and medians (TNC 2009). High- and low-flow conditions were defined as median flow  $\pm$  25%. Streamflow that exceeded the two year return interval was considered a small flood; flow that exceeded the ten year return interval was a large flood. Extreme low-flows were defined as any flow that was in the lower 10% of the daily flows for the period. Range of variability analysis (RVA) bounds, which determine “natural” flow conditions based on the pre-impoundment period, were set to  $\pm$  17% of the median value for the period. We used significant count values ( $< 0.05$ ) to determine statistical significance of IHA parameters (TNC 2009).

### Precipitation Analysis

To determine changes in precipitation patterns, we obtained precipitation data for the

period 1970-2010, which coincided with the hydrology analysis, from the nearest station that had a long-term record (Sallisaw, OK, Oklahoma Climatological Survey). Only those years with complete records were used. We defined a precipitation event as any day with precipitation  $\geq 0.025$  mm and used linear regression to determine the relationship between the number of precipitation events per year and the mean event magnitude. Differences in mean annual precipitation, mean number of precipitation events, and mean precipitation event magnitude were determined with a t-test (when data were normally distributed) or Mann-Whitney U test (when data were not normally distributed) pre- and post-impact (1992) to match the IHA analysis. All statistical analyses were performed using SigmaPlot v12.5 software and considered significant at  $P \leq 0.05$ .

## Results

### Hydrological analysis

Lee Creek’s hydrology changed since 1992, mainly with increased frequency of high flows that were more variable in nature (Table 1). For example, during 1970-1991, the median frequency of high flows (median flow + 25%) was eight times per year, with 25th and 75th percentiles ranging from 7 to 11 (Figure 2). During the subsequent period (1992-2013), the median frequency of high flows increased significantly (significance count  $< 0.05$ ) to 10.5 times per year, with an increased range of percentiles of these flows occurring (25th = 6.75 and 75th = 12.25). Other items that were found to occur significantly greater than chance (significance counts  $< 0.05$ ) during the post-impact period compared to the pre-impact period included high pulse counts, high flow fall rate coefficient of dispersion (CD), and large flood rise rate CD; all of which support the findings of increased frequencies of high flows that were more variable. Related, 1-day minimum flows became more variable (CD from 3.286 to 15.9600), indicating further that the flow regime in Lee Creek had become more dynamic after 1992.

**Table 1. Indicators of Hydrologic Alteration (IHA) scorecard generated from stream flow data collected at USGS gage #07249985 on Lee Creek near Short, OK investigating differences in hydrology before and after Lee Creek Dam was constructed in 1992. Significance counts can be interpreted similarly to a p-value where < 0.05 indicates significant deviation between pre- and post-impoundment values (bold).**

IHA Group	Medians		Coefficient of Dispersion (CD)		Deviation Factor		Significance Count	
	Streamflow (m <sup>3</sup> )		Pre-impact	Post-impact	Medians	CD	Medians	CD
	Pre-impact	Post-impact						
<b>Group 1: Monthly</b>								
October	0.623	1.203	7.281	1.903	0.932	0.739	0.153	0.177
November	3.589	4.212	6.085	2.827	0.174	0.535	0.771	0.426
December	6.853	8.226	2.538	1.760	0.200	0.307	0.749	0.572
January	5.536	6.499	2.224	2.774	0.174	0.247	0.732	0.582
February	11.780	13.830	0.885	0.822	0.174	0.071	0.483	0.894
March	24.820	12.980	0.789	1.429	0.477	0.811	0.333	0.120
April	17.290	16.120	0.998	1.161	0.068	0.163	0.891	0.654
May	11.670	9.826	0.751	1.666	0.158	1.218	0.305	0.070
June	3.617	3.157	1.568	2.692	0.127	0.717	0.762	0.315
July	0.411	1.076	2.229	2.943	1.621	0.320	0.079	0.535
August	0.197	0.231	3.442	3.690	0.173	0.072	0.897	0.895
September	0.166	0.248	2.917	7.550	0.489	1.588	0.295	0.230
<b>Group 2: Magnitude and duration of annual extremes</b>								
1-day minimum	0.015	0.007	<b>3.286</b>	<b>15.900</b>	0.524	<b>3.839</b>	0.560	<b>0.010</b>
3-day minimum	0.016	0.010	3.210	12.750	0.396	2.970	0.840	0.062
7-day minimum	0.017	0.015	3.313	8.838	0.108	1.667	0.948	0.106
30-day minimum	0.039	0.078	2.954	2.394	0.995	0.190	0.164	0.724
90-day minimum	0.595	1.090	1.754	2.247	0.831	0.281	0.099	0.493
1-day maximum	468.60	392.20	0.814	1.064	0.163	0.307	0.548	0.415
3-day maximum	288.70	234.60	0.689	0.774	0.188	0.123	0.483	0.760
7-day maximum	155.40	140.70	0.674	0.685	0.095	0.017	0.836	0.963
30-day maximum	63.680	68.60	0.520	0.687	0.077	0.322	0.608	0.299
90-day maximum	38.940	43.580	0.417	0.484	0.119	0.160	0.284	0.674
Base flow index	0.001	0.001	5.775	8.217	0.542	0.423	0.580	0.557

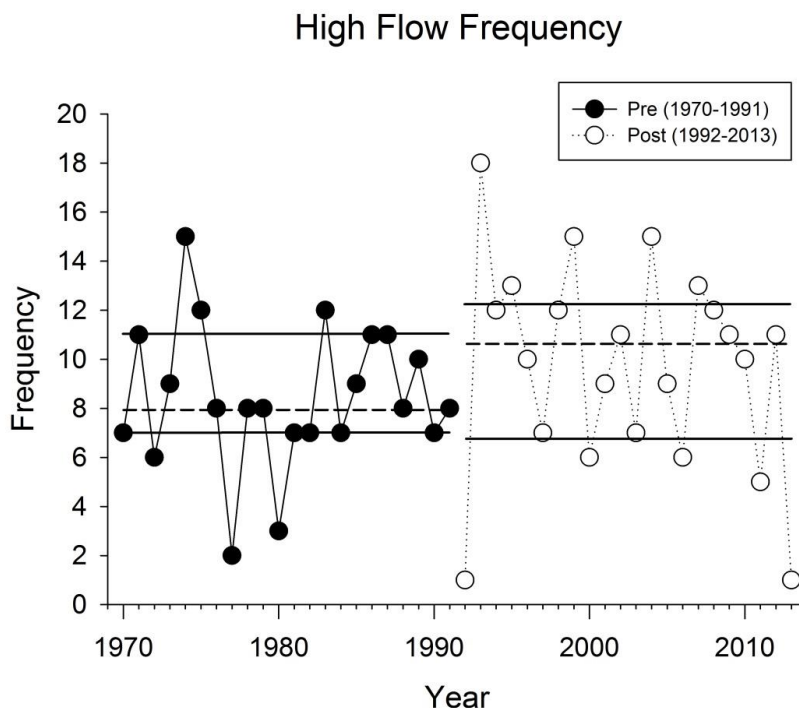
Table 1. Continued.

IHA Group	Medians		Coefficient of Dispersion (CD)		Deviation Factor		Significance Count	
	Streamflow (m <sup>3</sup> )		Pre-impact	Post-impact	Medians	CD	Medians	CD
	Pre-impact	Post-impact						
<b>Group 3: Timing of annual extremes</b>								
Julian date of minimum	255	249.5	0.131	0.148	0.030	0.136	0.733	0.654
Julian date of maximum	123	114	0.389	0.253	0.049	0.351	0.718	0.572
<b>Group 4: Frequency and duration of high and low pulses</b>								
Low pulse count	3	3	0.667	0.417	0.00	0.375	0.288	0.404
Low pulse duration	18.00	22.00	1.569	2.165	0.222	0.379	0.628	0.431
High pulse count	<b>8.00</b>	<b>11.00</b>	0.625	0.546	<b>0.375</b>	0.127	<b>0.019</b>	0.515
High pulse duration	6.50	5.00	0.404	0.650	0.231	0.610	0.065	0.115
Low Pulse Threshold	0.570							
High Pulse Threshold	16.310							
<b>Group 5: Rate and frequency of change in conditions</b>								
Rise rate	1.104	0.998	1.470	2.205	0.096	0.500	0.831	0.289
Fall rate	-0.467	-0.531	-1.106	-1.260	0.136	0.139	0.754	0.726
Number of reversals	73.50	75.50	0.201	0.192	0.027	0.043	0.571	0.889
<b>Environmental Flow Component (EFC) Results</b>								
<b>EFC Low flows</b>								
October	0.793	1.161	3.625	1.820	0.464	0.498	0.323	0.249
November	2.294	3.469	3.568	2.140	0.512	0.400	0.390	0.403
December	6.173	6.683	1.095	1.230	0.083	0.123	0.803	0.717
January	4.863	5.097	1.596	1.397	0.048	0.125	0.768	0.642
February	8.814	10.260	0.560	0.563	0.165	0.004	0.135	0.992
March	10.730	9.741	0.546	0.688	0.092	0.259	0.712	0.586
April	10.730	8.169	0.582	0.964	0.239	0.656	0.542	0.098
May	7.030	6.180	0.713	1.079	0.121	0.515	0.550	0.127
June	2.945	2.534	1.238	1.781	0.139	0.438	0.719	0.180
July	0.411	1.034	2.134	2.451	1.517	0.149	0.076	0.776
August	0.368	0.312	1.907	2.607	0.154	0.367	0.867	0.403
September	0.249	0.312	3.341	8.491	0.250	1.541	0.818	0.116

Table 1. Continued.

IHA Group	Medians		Coefficient of Dispersion (CD)		Deviation Factor		Significance Count	
	Streamflow (m <sup>3</sup> )		Pre-impact	Post-impact	Medians	CD	Medians	CD
	Pre-impact	Post-impact						
<b>EFC Parameters</b>								
Extreme low peak	0.040	0.030	0.748	1.202	0.240	0.607	0.294	0.216
Extreme low duration	11.750	9.750	1.457	1.256	0.170	0.138	0.735	0.775
Extreme low timing	245.50	247.80	0.145	0.082	0.012	0.435	0.853	0.289
Extreme low freq.	1.500	2.00	1.333	1.500	0.333	0.125	0.465	0.953
High flow peak	71.450	55.220	0.585	0.708	0.227	0.211	0.077	0.548
High flow duration	5.750	5.00	0.457	0.650	0.130	0.424	0.340	0.264
High flow timing	65.750	64.50	0.210	0.258	0.007	0.226	0.997	0.312
High flow frequency	<b>8.00</b>	<b>10.50</b>	0.500	0.524	<b>0.313</b>	0.048	<b>0.044</b>	0.853
High flow rise rate	25.690	24.960	0.581	0.866	0.028	0.490	0.696	0.333
High flow fall rate	-10.690	-10.00	<b>-0.386</b>	<b>-0.744</b>	0.064	<b>0.928</b>	0.225	<b>0.033</b>
Small flood peak	546.50	626.50	0.487	0.228	0.146	0.531	0.426	0.203
Small flood duration	22.00	13.50	1.295	1.917	0.386	0.480	0.657	0.332
Small flood timing	301.00	122.00	0.234	0.398	0.978	0.702	0.092	0.145
Small flood rise rate	105.20	307.20	3.848	1.033	1.919	0.732	0.207	0.402
Small flood fall rate	-46.390	-55.660	-0.778	-1.000	0.200	0.285	0.494	0.582
Large flood peak	1007.00	1079.00	0.250	0.173	0.072	0.311	0.515	0.500
Large flood duration	24.00	27.00	0.0	0.630	0.125		0.554	
Large flood timing	326.50	115.50	0.014	0.148	0.847	9.850	0.152	0.057
Large flood rise rate	201.00	109.90	<b>0.250</b>	<b>1.533</b>	0.453	<b>5.137</b>	0.460	<b>0.024</b>
Large flood fall rate	-49.570	-66.630	-0.254	-0.568	0.344	1.234	0.239	0.067





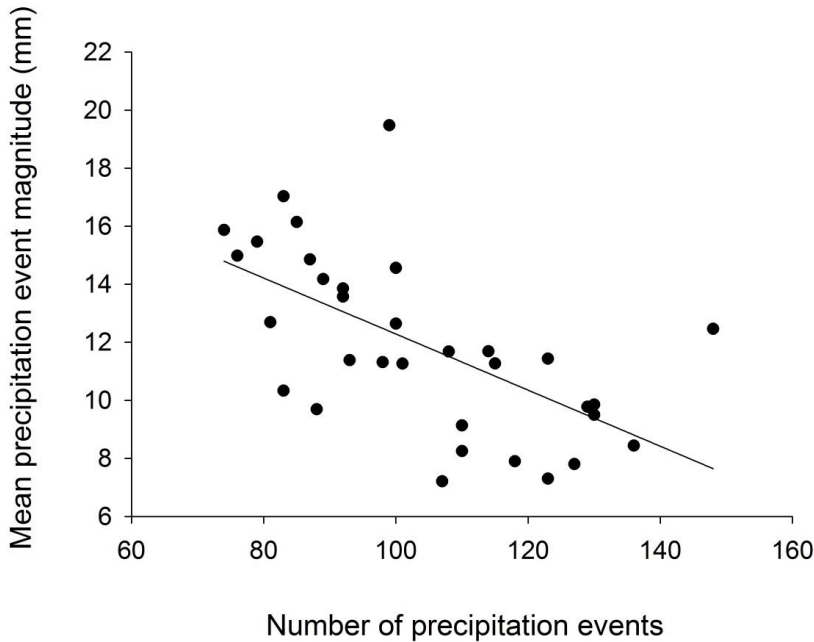
**Figure 2. High flow frequency of Lee Creek, Oklahoma pre-impact (1970-1991) and post-impact (1992-2013). The dashed line is the median for each period and the solid lines are the 25th and 75th percentiles.**

### Precipitation Analysis

Full precipitation records existed from 1970 to 1990 for the pre-impact period and from 1996 to 2010 for the post-impact period. Precipitation varied according to the number of events and their magnitude. The mean magnitude of precipitation events was significantly related to the number of those events, declining at a rate of 0.1 mm per event ( $r^2 = 0.37$ ;  $P < 0.01$ ; Figure 3). The mean annual precipitation did not change significantly between pre- and post-impact periods (Mann-Whitney  $U = 121.0$ ;  $P = 0.63$ ; Figure 4), with both periods receiving approximately 1200 mm of precipitation annually. However, the mean annual number of precipitation events significantly decreased from a mean of 114 pre-impact to 92 post-impact ( $t = 3.96$ ;  $df = 31$ ;  $P < 0.01$ ) whereas the mean precipitation event magnitude increased from a mean of 10.8 mm/event pre-impact to 13.3 mm/event post-impact (Mann-Whitney  $U = 60.0$ ;  $P < 0.01$ ).

### Discussion

Since 1992, high-flow events in Lee Creek above the impoundment have changed, resulting in a flashier system (Baker et al. 2004). The altered streamflow appears to be driven by long-term changes in precipitation (i.e., increased rain-event magnitude coupled with decreased rain-event frequency). While we did not examine the role of land cover changes on hydrology, previous analyses showed the watershed to be mostly forested (76.8%) with minimal change (less than 2 percentage points in any category from 1992 to 2006; Gatlin 2013) and would likely not have affected hydrology. These findings are consistent with predictions of climate change (Easterling et al. 2000, Meehl et al. 2000, Dore 2005, Solomon et al. 2007, Cheng et al. 2012), but because we only considered 40 years of record (20 years pre- and post-impoundment), these changes may not reflect climate-change *per se*. Precipitation



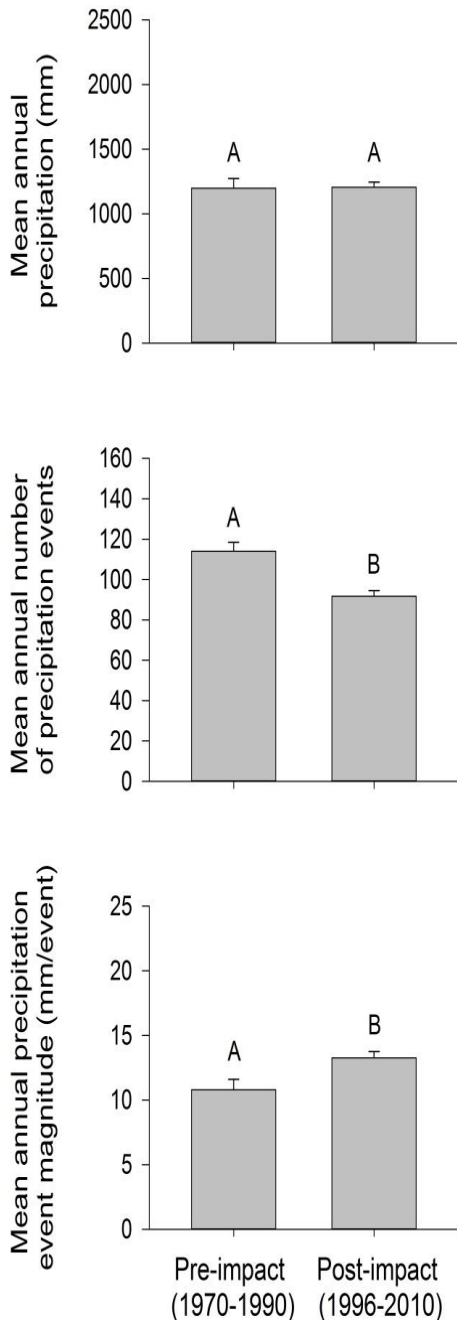
**Figure 3. Relationship between number of precipitation events per year and mean annual event magnitude from 1970 to 2010 at Sallisaw, OK.**

has fluctuated in this part of Oklahoma since records began in 1895 (OCS 2013) and whether the changes we observed are indicative of long-term climate changes are unknown. Moreover, we were only able to gather useful precipitation data from one point-location due to a lack of other long-term stations. As a result, we were unable to directly relate precipitation in the entire watershed to the hydrology, which would be more useful. Estimates of precipitation from RADAR coupled with GIS would improve this ability, but these data have only been available since the early 2000s (Zhang et al. 2011). Precipitation patterns are ultimately responsible for flow variability in streams (Hynes 1975, Changnon and Kunkel 1995, Poff et al. 1997) and stream flashiness has been shown to increase in concert with rain-event magnitude (Groisman et al. 2001, Kokkonen et al. 2004). If the pattern of decreased precipitation event frequency coupled with increased event magnitude patterns continues in the Lee Creek watershed, the resultant increased stream flashiness will likely have repercussions for the resident biota.

Most studies that investigated the effects of

flooding on aquatic ecology describe negative effects on spawning behavior, success, and overall recruitment (see Poff and Zimmerman 2010) because larval fishes experience high mortality and displacement during flood events (Harvey 1987, Filipek et al. 1991, Jellyman and McIntosh 2010). Species that require specific substrate types for ovipositing, or require nest building to complete spawning, may experience reproductive failure during flooding because of reconfiguration of substrate (Jager et al. 1997, Carline and McCullough 2003). The redistribution of bed-load materials during flooding can destroy fish eggs deposited in or on the substrate (Swanston 1991). For instance, rock bass (*Ambloplites rupestris*) in streams had to repeatedly rebuild nests that were destroyed during spring flooding events (Noltie and Keenleyside 1986), decreasing nest success. A change in high flows would be of particular concern for fish species that reproduce in spring when these flows are more common.

Several fish species in Lee Creek spawn during the spring, including many that are of greatest conservation need in Oklahoma (i.e.,



**Figure 4.** Measures of precipitation (mean  $\pm$  1 SE) pre- and post-impact at Sallisaw, OK. Different letters indicate significant difference between periods for each measure.

Ozark minnow *Notropis nubilus*, longnose darter *Percina nasuta*, and sunburst darter *Etheostoma mihileze*; Miller and Robison 2004) but data are lacking on how flooding will affect reproduction or recruitment for these species. Though it is unclear how climate-mediated changes in hydrology may affect population dynamics of Lee Creek's fishes, it may be substantial for imperiled species. For example, Lee Creek is the last remaining stream system within Oklahoma to support the longnose darter (Gatlin and Long 2011), which has very low fecundity (i.e., females produce  $< 4$  eggs per day) and spawns intermittently (Anderson et al. 1998). Information on the natural breeding behavior for longnose darter is lacking (Anderson et al. 1998), but similar species (*Percina spp*) depend on gravel and cobble substrate for spawning. For example, shield darter (*P. pellata*), dusky darter (*P. sciera*), and leopard darter (*P. pantherina*) require small gravel and cobble for burying eggs, which can be disturbed during flooding (New 1966, James and Maughan 1989, James et al. 1991, Labay et al. 2004). The increased flashiness of annual flood events could affect longnose darter from Oklahoma by limiting their spawning success due to flooding related nest failure, although additional research on factors affecting longnose darter persistence would be needed to establish the level of this risk.

Research investigating the effects of climate change on fishes and fisheries has primarily focused on temperature (Tonn, 1990, Pörtner and Peck 2010), particularly for several salmonid species (Jonsson and Jonsson 2010, Wenger et al. 2011, Isaak et al. 2012); however, a paucity of information exists regarding warm-water fishes. It is imperative to consider how changing precipitation patterns as a predicted consequence of climate change will alter stream hydrology because it may determine a species' ability to persist (Poff et al. 1997). Our research provides evidence that climate-induced changes to the natural flow regime are currently underway and may negatively affect the fish community in an eastern Oklahoma scenic river, but more work is needed to reliably predict these effects across multiple systems.

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## References

- Anderson KA, Rosenblum PM, Whiteside BG, Standage RW, Brandt TM. 1998. Controlled spawning of longnose darters. *Prog Fish-Cult* 60:137-145.
- Baker DB, Richards RP, Loftus TT, Kramer JW. 2004. A new flashiness index: characteristics and applications to Midwestern rivers and streams. *J Am Water Resour As* 40:503-522.
- Bunn SE, Arthington AH. 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environ Manage* 30:492-507.
- Carline RF, McCullough BJ. 2003. Effects of floods on brook trout populations in the Monongahela National Forest, West Virginia. *T Am Fish Soc* 132:1014-1020.
- Changnon SA, Kunkel KE. 1995. Climate-related fluctuations in Midwestern floods during 1921-1985. *J Water Res Pl-ASCE* 121:326-334.
- Cheng CS, Auld H, Li Q, G Li. 2012. Possible impacts of climate change on extreme weather events at local scale in south-central Canada. *Climatic Change* 112:963-979.
- Dore MHI. 2005. Climate change and changes in global precipitation patterns: what do we know? *Environmental International* 31:1167-1181.
- Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO. 2000. Climate extremes: observations, modeling, and impacts. *Science* 289:2068-2074.
- Federal Energy Regulatory Committee (FERC). 1987. Lee Creek Project, FERC No. 5251, Final Environmental Impact Statement, FERC/FEIS-0043. Washington, D.C. 521 p.
- Federal Register. 2010. Notices. *Federal Register* 75:65613-65614.
- Filipek SP, Armstrong MA, Claybrook LG. 1991. Effects of a hundred-year flood on the smallmouth bass population of the upper Caddo River, Arkansas. In: Jackson DC, editor. *First International Smallmouth Bass Symposium*. Bethesda (MD): American Fisheries Society. p. 84-89.
- Gatlin MR. 2013. The effects of hydrologic alteration on stream fish community structure in Lee Creek, Oklahoma [MSc thesis]. Stillwater (OK): Oklahoma State University. 96 p.
- Gatlin MR, Long JM. 2011. Persistence of the longnose darter (*P. nasuta*) in Lee Creek, Oklahoma. *Proc Okla Acad Sci* 91:11-14.
- Groisman PY, Knight RW, Karl TR. 2001. Heavy precipitation and high streamflow in the contiguous United States: trends in the twentieth century. *B Am Meteorol Soc* 82:219-246.
- Harvey BC. 1987. Susceptibility of young-of-year fishes to downstream displacement by flooding. *T Am Fish Soc* 116:851-855.
- Hynes HBN. 1975. A stream and its valley. *Verhandlungen, Internationale Vereinigung für Theoretische und Angewandte Limnologie* 19:1-15.
- Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, editors.. 2007. *Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge: Cambridge University Press. 996 p.
- Isaak DJ, Wollrab S, Horan D, Chandler G. 2012. Climate change effects on stream and river temperatures across the northwest U.S. from 1980-2009 and implications for salmonid fishes. *Climatic Change* 113:499-524.
- Jager HI, Cardwell HE, Sale MJ, Bevelhimer MS, Coutant CC, Van Winkle W. 1997.

- Modelling the linkages between flow management and salmon recruitment in rivers. *Ecol Model* 103:171-191.
- James PW, Maughan OE. 1989. Spawning behavior of the threatened leopard darter, *Percina pantherina*. *Southwest Nat* 34:298-301.
- James PW, Maughan OE, Zale AV. 1991. Life history of the leopard darter *Percina pantherina* in the Glover River, Oklahoma. *Am Midl Nat* 125:173-179.
- Jellyman PG, McIntosh AR. 2010. Recruitment variation in a stream galaxiid fish: multiple influences on fry dynamics in a heterogeneous environment. *Freshwater Biol* 55:1930-1944.
- Jonsson B, Jonsson N. 2010. A review of the likely effects of climate change on anadromous Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*, with particular reference to water temperature and flow. *J Fish Biol* 75:2381-2447.
- Karl TR, Knight RW. 1998. Secular trends of precipitation amount, frequency, and intensity in the USA. *B Am Meteorol Soc* 79:231-241.
- Kokkonen T, Koivusalo H, Karvonen T, Croke B, Jakeman A. 2004. Exploring streamflow response to effective rainfall across event magnitude scale. *Hydrol Process* 18:1467-1486.
- Labay AA, Standage RW, Brandt TM. 2004. Selected methods for dusky darter captive propagation. *N Am J Aquacult* 66:146-152.
- Lytle DA, Poff NL. 2004. Adaptation to natural flow regimes. *Trends Ecol Evol* 19:94-100.
- Mathews R, Richter BD. 2007. Application of the Indicators of Hydrologic Alteration software in environmental flow setting. *J Am Water Resour As* 43:1400-1413.
- Meehl GA, Zwiers F, Evans J, Knutson T, Mearns L, Whetton P. 2000. Trends in extreme weather and climate events: issues related to modeling extremes in projections of future climate change. *B Am Meteorol Soc* 81:427-436.
- Miller RJ, Robison HW. 2004. Fishes of Oklahoma. Norman (OK): University of Oklahoma Press. 450 p.
- New JG. 1966. Reproductive behavior of the shield darter, *Percina peltata peltata*, in New York. *Copeia* 1966:20-28.
- Noltie DB, Keenleyside MHA. 1986. Breeding ecology, nest characteristics, and nest-site selection of stream- and lake-dwelling rock bass, *Ambloplites rupestris* (Rafinesque). *Can J Zoolog* 65:379-390.
- Oklahoma Climatological Survey (OCS). 2013. Precipitation history – annual, east central district [online]. Available from [http://climate.ok.gov/index.php/climate/climate\\_trends/precipitation\\_history\\_annual\\_statewide/CD06/prcp/Annual](http://climate.ok.gov/index.php/climate/climate_trends/precipitation_history_annual_statewide/CD06/prcp/Annual). (Accessed July 12, 2013).
- Oklahoma Department of Wildlife Conservation (ODWC). 2005. Oklahoma comprehensive wildlife conservation strategy: planning for the future for Oklahoma's wildlife Final Report T-2-P-1, Oklahoma City, Oklahoma. 435 p.
- Poff NL, Allen JD, Bain MB, Karr JR, Prestegard KL, Richter BD, Sparks RE, Stromberg JC. 1997. The natural flow regime. *BioScience* 47:769-784.
- Poff NL, Zimmerman JKH. 2010. Ecological responses to altered flow regimes: a literature review to inform science and management of environmental flows. *Freshwater Biol* 55:194-205.
- Pörtner HO, Peck MA. 2010. Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. *J Fish Biol* 77:1745-1779.
- Richter BD, Baumgartner JV, Powell J, Braun DP. 1996. A method for assessing hydrologic alteration within ecosystems. *Conserv Biol* 10:1163-1174.
- Singh D, Tsiang M, Rajaratnam B, Diffenbaugh NS. 2013. Precipitation extremes over the continental United States in a transient, high-resolution, ensemble climate model experiment. *J Geophys Res-Atmos* 118:7063-7086.
- Swanston DN. 1991. Natural processes. In: Meehan WR, editor. Influences of forest and rangeland management on salmonid fishes and their habitat. Bethesda (MD): American Fisheries Society, Special Publication 19. p. 139-179.
- The Nature Conservancy (TNC). 2009. Indicators of hydrologic alteration version 7.1 user's manual. 76 p.
- Tonn WM. 1990. Climate change and fish

- communities: a conceptual framework. *T Am Fish Soc* 119:337-352.
- Wagner BA, Echelle AA, Maughan OE. 1985. Status and distribution of the longnose darter, *Percina nasuta*, and the Neosho madtom, *Noturus placidus*, in Oklahoma. *Proc Okla Acad Sci* 65:59-60.
- Wenger SJ, Isaak DJ, Luce CH, Neville HM, Fausch KD, Dunham JB, Dauwalter DC, Young MY, Elsner ME, Rieman BE, Hamlet AF, Williams JE. 2011. Flow regime, temperature, and biotic interactions drive differential declines of trout species under climate change. *P Natl Acad Sci USA* 108:14175-14180.
- Whitfield PH. 2010. Floods in future climates: a review. *J Flood Risk Manage* 5:336-365.
- Zhang J, Howard K, Langston C, Vasiloff S, Kaney B, Arthur A, Van Cooten S, Kelleher K, Kitzmiller D, Ding F, Deo D-J, Wells E, Dempsey C. 2011. National mosaic and multi-sensor QPE (NMQ) system: Description, results, and future plans. *B Am Meteorol Soc* 92:1321-1338.

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# The Effect of Oxygen Supply on Nitrite Reduction by Tallgrass Prairie Soil Bacteria

**Samer M. AbuBakr**

Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, 73019

**Kathleen E. Duncan**

Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, 73019

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**Abstract:** Nitrite reduction, catalyzed by nitrite reductase, is a key step in the denitrification pathway because it catalyzes the reduction of soluble nitrite ( $\text{NO}_2^-$ ) into nitric oxide gas ( $\text{NO}$ ). The production of nitric oxide gas therefore decreases the amount of nitrogen in soils. Other studies have investigated the effect of oxygen on denitrification in a few specific microorganisms (e.g. *Aquaspirillum magnetotacticum*, *Thiosphaera pantotropha*, and *Paracoccus denitrificans*). In this study, we tested the effect of oxygen on nitrite reduction in seven strains representing 5 different genera obtained from Tallgrass Prairie soil. The strains were chosen based on positive detection of at least one functional gene in the denitrification pathway along with positive results for nitrate ( $\text{NO}_3^-$ ) and/or nitrite reduction after growth in nitrate broth in a microtiter plate assay. Under these conditions which did not totally exclude oxygen, three strains were able to reduce nitrite while four strains did not reduce nitrite. All seven strains were retested for the production/consumption of nitrite under strictly anaerobic conditions using nitrate as the electron acceptor. In addition, the strains were screened for genes encoding the copper nitrite reductase (*nirK*) and the cytochrome *cd<sub>1</sub>*-nitrite reductase (*nirS*) and other functional markers of denitrification pathway, namely nitric oxide reductase gene (*norB*) and nitrous oxide ( $\text{N}_2\text{O}$ ) reductase gene (*nosZ*) by PCR amplification using specific primers. Our results show that under strict anaerobic conditions, two additional strains demonstrated nitrite reduction. Although none of the molecular markers showed perfect correlation with the ability to reduce nitrite, *nirS/nirK*, commonly used to screen environmental samples for denitrifying bacteria, was detected in only two of the five strains shown in this study to reduce nitrite. More nitrite reducing strains were correctly identified when both the *cnorB* and *nosZ* primer sets were used. ©2015 Oklahoma Academy of Science

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## Introduction

Nitrite reductase is a significant enzyme in the denitrification pathway as it converts nitrite, a toxic intermediate, to nitric oxide, the first gaseous product of denitrification. Primers designed to detect *nirS* nitrite reductase gene (Braker et al. 1998) and *nirK* nitrite reductase gene (Qiu et al. 2004) have often been used

to detect the presence of denitrifying (DN) bacteria. Molecular detection of denitrification genes has become the norm (Oakley et al., 2007; Dandie et al., 2008) while studies evaluating the phylogenetic range of DN species detected by molecular means have lagged. Even more critically, there are few recent studies of the environmental conditions under which denitrification enzymes are active, and those focus on a relatively narrow

group of microorganisms (Saleh-Lakha et al., 2009a; Saleh-Lakha et al., 2009b). This study contributes to a better understanding of the effect of environmental conditions on the activity of denitrification enzymes by determining the effect of oxygen on the ability of a group of strains to reduce nitrite, and also evaluates how well molecular detection of various denitrification genes serves to identify denitrifying bacteria.

To express the trait of denitrification, microorganisms must have the genetic capacity (e.g. denitrification genes) that are transcribed and translated into the denitrification enzymes, and those enzymes must be active under the given environmental conditions. Denitrification is known to be an environmentally-regulated process with respect to oxygen supply, the presence and nature of an N oxide, and possibly additional external factors such as metal ions (Zumft, 1997). Various studies have investigated the effect of oxygen on denitrification in specific microorganisms. It was thought that synthesis of denitrifying enzymes only occurs when nitrate is present and oxygen is absent and that oxygen is a repressor of enzyme synthesis in many denitrifiers (Payne, 1973). However, it was shown that little or no repression of denitrifying enzymes was caused by oxygen in nitrifying activated sludge systems (Simpkin and Boyle, 1988). Moreover, several studies showed the production of nitrogen-containing gases under aerobic conditions. For example, *Aquaspirillum magnetotacticum*, a bacterium that has an obligate requirement for oxygen, was shown to reduce nitrate to nitrous oxide and dinitrogen in the presence of low levels (0.2-1.0%) of oxygen (Bazylinski and Balkemore, 1983). Also, the presence of nitrate reductase and the production of nitrogen-containing gases from nitrate by *Thiosphaera pantotropha* were demonstrated at dissolved O<sub>2</sub> concentrations of up to 90% air saturation (Robertson and Kuenen 1983; 1984a). In addition, the consumption of NO<sub>3</sub><sup>-</sup> and production of nitrogen-containing gases by the facultative anaerobes *Pseudomonas aeruginosa* and *Paracoccus denitrificans* in the presence of relatively high concentrations of dissolved O<sub>2</sub> (150 μM or 195 μM, respectively) provides strong additional proof that denitrification

may not strictly require anaerobic conditions. Indeed, with anaerobically grown *P. aeruginosa* washed cell suspension, not only were N<sub>2</sub> and N<sub>2</sub>O produced after the addition of O<sub>2</sub>, but also their production increased (Davies et al., 1989). Furthermore, several nitrifying strains were found to denitrify via nitrite in the presence of oxygen which indicates that they have an oxygen-tolerant denitrification system (Matsuzaka et al., 2003). However, the effect of oxygen on denitrification has not been systematically examined simultaneously on a wide variety of strains.

Many of the strains in this study were obtained from tallgrass prairie soils contaminated by spills of crude oil or oil field brine (Sublette et al., 2005; Sublette et al., 2007). In fact, oxygen levels decrease as oil contaminants are degraded (Duncan et al., 1999; Ehrenreich et al., 2000). This decrease in oxygen levels may give an advantage to facultative anaerobes such as DN bacteria and strict anaerobes over strict aerobes. If then the rate of nitrate reduction/denitrification increases, nitrogen will be lost from soil. One objective of this study was to compare the ability of DN bacteria to reduce nitrite in a microtiter plate system containing nitrate broth (e.g. microtiter plate assay) versus inoculation and growth under strictly anaerobic conditions. Since oxygen is known to regulate denitrification (Zumft, 1997), and since oxygen levels decrease when oil contaminants are degraded, we hypothesized that the low oxygen levels also increase the loss of nitrogenous compounds due to the creation of conditions more favorable to denitrification.

Seven strains were chosen for investigation of the effect of oxygen levels on nitrite reduction. These seven strains reduced nitrate and/or nitrite using the microtiter plate assay. Also, they all were shown by PCR amplification with primers specific for functional genes in the denitrification pathway and sequencing of the PCR product to possess one or more functional genes in the denitrification pathway. These seven strains represented 5 different genera, namely: *Pseudomonas* strains I-1 and I-65, *Ensifer* strain I-4, *Achromobacter* strains I-5 and I-49, *Aeromonas* strain I-6, and *Enterobacter* strain I-25.



## Sites Description

The sampling sites used in this work are located in the Tallgrass Prairie Preserve (TPP) in Osage County, Oklahoma. Nitrate reducing and DN bacteria were isolated from a total of 2 contaminated sites and 2 uncontaminated sites. The contaminated sites were contaminated with crude oil or with a mixture of oil/brine (e.g. salt water) due to accidental spills as described previously (Sublette et al., 2005; Sublette et al., 2007; Duncan et al., 1998; Duncan et al., 1999).

## Methods

### Sampling soils for the seven strains

The seven strains were obtained from tallgrass prairie soils. Strains I-1, I-4, I-5, and I-6 were isolated from G7 (brine contaminated) in July 21, 2005. Strain I-25 was sampled from LF (oil contaminated) in August 2, 2005. Strain I-49 was sampled from G7P (prairie, not contaminated) in July 21, 2005. And finally, strain I-65 was isolated from LFP (prairie, not contaminated) in August 2, 2005. In brief, soils were homogenized, serially diluted and added to microtiter plates containing nitrate broth (Difco), and after two weeks of incubation at room temperature (23-25°C), all wells were examined for evidence of growth (turbidity) and tested for nitrate reduction and nitrite reduction using Griess reagents (Smibert and Krieg, 1994). Based on positive scoring for growth and nitrate or nitrite reduction in the microtiter plates, nutrient agar plates (Nutrient Agar, Difco, Detroit, MI) were inoculated with 25 µL from microtiter plate wells, chosen randomly from 10<sup>-1</sup> to 10<sup>-4</sup> dilutions that scored positive for growth and nitrate reduction or nitrite reduction. The nutrient agar plates were incubated at 30°C. A colony chosen from a plate was streaked at least three times in succession from a single colony to a fresh plate in order to obtain pure cultures. All seven strains were retested using the microtiter plate assay to confirm the ability to reduce nitrate or nitrite once purified. In addition, the following strains of known phenotype were included as controls: *P. aeruginosa* ATCC 27853 (Denitrifier), *P. putida* pG7 (not a nitrate reducer), *S. maltophilia*

ATCC 13637 (Nitrate reducer), and uninoculated wells containing sterile nitrate broth.

### DNA extraction from isolates

A single colony was used to inoculate 1-3 mL nutrient broth, and grown overnight at 30°C. The culture was pelleted by centrifugation, then DNA was extracted using a commercial DNA extraction kit, FastDNA®SPIN Kit (QBIogene, Solon, Ohio, USA).

### Molecular detection of 16S rRNA gene sequences and denitrification pathway functional genes

Universal bacterial primers 27F/1492R (Wilson et al., 1990) for 16S rRNA gene were used to amplify ~1400 bp. 16S rRNA gene was amplified from the seven strains. In addition, the seven strains were screened for the following functional markers: *nirS*, *nirK*, *cnorB*, *qnorB*, and *nosZ* using the primers listed in Table 1. To amplify nitrite reductase genes, primer pairs *nirS* 1F/*nirS* 6R (Braker et al., 1998) and *nirK* F/*nirK* R (Qiu et al., 2004) were used to amplify fragments of *nirS* and *nirK*, respectively. Fragments from *cnorB* gene were amplified using primer pairs *cnorB* 2F/*cnorB* 6R, the *qnorB* gene using primer pairs *qnorB* 2F/*qnorB* 7R (Braker and Tiedje, 2003), and the *nosZ* gene using primer pairs *nosZ*-F-1181/ *nosZ*-R-1880 (Rich et al., 2003). Primers used for detecting denitrification functional genes are listed in Table 1. PCR reaction mixtures consisted of 0.5 µL (10 mM stock) deoxynucleotide triphosphate mixture of all four nucleotides (dNTP), 1 µL (50 mM stock) MgCl<sub>2</sub>, 2.5 µL (5.0 M stock) Betaine monohydrate (Henke et al., 1997) (Sigma-Aldrich Corporation, St. Louis, MO, USA), 5.0 ng/ µL- 50 pg/ µL DNA, 0.125 µL of Invitrogen Taq Polymerase (0.625 U). In addition, 5 pM/µL of *nirS* and *nirK* primers; 50 pM/µL of *cnorB* and *qnorB*, or 0.2 µM of *nosZ* primer were used in a buffer containing 50 mM Tris Cl, 50 mM KCL, and 0.01% Triton-X100 for a final volume of 25 µL. The master mix of the PCR reaction, e.g. all the above ingredients minus DNA, but with an additional 1 µL of PCR water was used as a negative control in all PCR reactions. All thermal cycling conditions included initial denaturation at 94°C for four minutes (exception: 5 minutes

**Table 1. Summary of primers used to detect denitrification functional genes.**

Primer <sup>a</sup>	Primer sequence (5'-3')	Organism <sup>b</sup>	Nucleotide	Size of the	Annealing	Reference
		(Accession #)	position <sup>c</sup>	PCR fragment (bp)	temperature (°C)	
nirS 1F	CCTAYTGGCCGCCRCART	<i>Pseudomonas stutzeri</i> ZoBell (X56813)	763-780	890	Touchdown	1
nirS 6R	CGTTGAACTTRCCGGT		1638-1653		56-51°C	
nirK F	TCATGGTCCTGCCGCGYGACGG	<i>Alcaligenes faecalis</i> (D13155)	1319-1337	329	Touchdown	2
nirK R	GAACCTGCCGGTNGCCAGAC		1668-1648		63-53°C	
cnorB 2F	GACAAGNNNACTGGTGGT	<i>Pseudomonas denitrificans</i> Pd1222 (U28078)	553-571	389	Touchdown	3
cnorB 6R	GAANCCCCANACNCCNGC		942-925		57-52.5°C	
qnorB 2F	GGNCAYCARGGNTAYGA	<i>Ralstonia eutropha</i> H16 (AF002661)	1204-1220	637	Touchdown	3
qnorB 7R	GGNGRITDATCADGAANCC		1841-1822		57-52.5°C	
nosZ-F-1181	CGTGTTCTTCGACAGYCAG	<i>Pseudomonas stutzeri</i> (M22628)	1463-1482	680	56°C	4
nosZ-R-1880	ATGTGCAKIGCRTGGCAGAA		2162-2143			

<sup>a</sup>Forward and reverse primers are indicated by F and R as the last letter, respectively.

<sup>b</sup>Strains used for PCR positive control as indicated in the references.

<sup>c</sup>Positions in the nitrate reducing/denitrifying functional genes of the corresponding positive control microorganisms.

1. Braker et al. (1998).
2. Qiu et al. (2004).
3. Braker and Tiedje (2003).
4. Rich et al. (2003).

denaturation for *cnorB* and *qnorB* genes) and a final extension at 72°C for 10 minutes. Annealing temperatures for all reactions are listed in Table 1. Thermocyclers used were PE9600 thermocycler (Perkin Elmer Cetus, Gene Amp model PCR System 9600) Perkin Elmer, Foster City, CA, USA and RoboCycler Gradient 40 Thermal Cycler (Stratagene, Inc., La Jolla, CA, USA). Three strains were obtained from ATCC for use as positive controls for PCR detection of denitrification functional genes. *P. aeruginosa* ATCC 27853 as the positive control for *nirS* and *cnorB*, *Alcaligenes faecalis* ATCC 8750 as the positive control for *nirK* and *qnorB*, and *P. stutzeri* ATCC 17588 as the positive control for *nosZ*. The PCR products were visualized using agarose gel electrophoresis technique.

### DNA sequencing and analysis

PCR products were prepared for sequencing using ExoSAP-IT (Bell, 2008). Sequence chromatograms were analyzed using Sequencher (Windows version 4.2; Gene Codes

Corp., Ann Arbor, MI). The sequences of the 16S rRNA gene and denitrifying functional genes were compared with the Genbank nt/nr database using BLASTN searches (Basic Local Alignment Search Tool) (Altschul et al., 1990). The taxonomic assignment of 16S rRNA gene sequence was determined by the Naïve Bayesian rRNA Classifier of the Ribosomal Database Project II (RDP) (Wang et al., 2007).

### Nitrate reduction/denitrification under strictly anaerobic conditions

The ability of seven strains to reduce nitrate/nitrite under strict anaerobic conditions was tested. All seven strains reduced nitrate/nitrite using the microtiter plate assay and contained sequences with high similarity to one or more of the denitrifying functional genes. The chosen strains were: *Pseudomonas* strains I-1 and I-65; *Ensifer* strain I-4; *Achromobacter* strain I-5; *Aeromonas* strain I-6; *Enterobacter* strain I-25; and *Achromobacter* strain I-49; along with the following control strains: *S. maltophilia* ATCC

13637 (nitrate reducer), *P. aeruginosa* ATCC 27853 (denitrifier), *P. putida* F1 ATCC 17485 (neither nitrate reducer nor denitrifier). The strains and the controls were streaked on Nutrient agar plates overnight followed by transferring a loopful into 15 mL nutrient broth under aerobic conditions. After incubating overnight at 30°C with shaking, 15 mL was transferred to sterile anaerobic 125-mL serum bottles followed by changing the gas phase using N<sub>2</sub>/CO<sub>2</sub> gas. Each bottle was used as an inoculum (1.5 mL/bottle) to inoculate 5 serum bottles containing 50 mL minimal medium prepared anaerobically. This medium contains 1g NaCl, 0.5g NH<sub>4</sub>Cl, 0.3g KH<sub>2</sub>PO<sub>4</sub>, 0.4g MgCl<sub>2</sub>·6H<sub>2</sub>O, 0.15g CaCl<sub>2</sub>·2H<sub>2</sub>O, 1g of 10 mM KNO<sub>3</sub>, 5.0g yeast extract, and 10 mL RST metals (Tanner, 1997) per liter. After adjusting the pH to 7.2, the medium was boiled and gassed with N<sub>2</sub>/CO<sub>2</sub> in an ice bath followed by the addition of 3g/L NaHCO<sub>3</sub>. Finally, aliquots of 15 mL/serum bottle were prepared and autoclaved for sterilization.

Two out of the 5 bottles were autoclaved and used as sterile controls in the experiment. Bottles were incubated at room temperature (23-25°C). Samples (0.2 mL) were taken and frozen at time zero and subsequent time points for two weeks. Optical density (O.D.) readings at 600 nm were recorded to monitor the growth of those strains. Nitrate and nitrite concentrations were measured by ion chromatography (model DX500 fitted with an AS-4A column; Dionex Corp., Sunnyvale, CA). Subsequently, second and third transfers of 1.5 mL to fresh bottles containing 50 mL minimal medium prepared anaerobically were performed to obtain well-established anaerobic cultures. O.D readings and sampling for Ion chromatography were performed for all transfers. Results are reported for the third transfer only in order to ensure adaption to anaerobic conditions had occurred.

## Results

### Summary of 16S rRNA phylogeny

Based on the 16S rRNA sequence analyses, the seven strains included four  $\gamma$ -proteobacteria belonging to the genera

*Pseudomonas*, *Aeromonas*, or *Enterobacter*, one  $\alpha$ -proteobacterium (*Ensifer*), and two  $\beta$ -proteobacteria (*Achromobacter*) at the 99% threshold of similarity (RDP Classifier, Wang et al., 2007) (Table 2).

### Molecular detection of denitrifying functional genes

*nirS/nirK* genes were detected in three strains (Table 2). The PCR primers successfully amplified 890-bp *nirS* fragments from *Pseudomonas* strain I-1. Sequences were 96% similar to *nirS* from *Pseudomonas migulae* (DQ518189). Also, 514-bp fragments from *nirK* were successfully amplified from *Enterobacter* strain I-3 and *Achromobacter* strain I-49. The sequences obtained matched 77% to *nirK* from *Rhizobium* sp. R-24654 (AM230814) and 90% to *nirK* from *Alcaligenes* sp. STC1 (AB046603), respectively.

A PCR product with highest similarity to the *cnorB* gene sequence was obtained from the *Pseudomonas* strain I-1 and was 75% similar to *cnorB* from *P. stutzeri* (AY957389). In addition, the *cnorB* sequence from *Pseudomonas* strain I-65 was 86% similar to *cnorB* from *P. fluorescens* (AF197467). *Ensifer* strain I-4 *cnorB* sequence showed 93% similarity to *cnorB* from *Achromobacter cycloclastes* (AJ298324).

Primers specific for nitrous oxide reductase (Rich et al., 2003) were successful in amplifying a *nosZ* fragment from *Pseudomonas* strain I-1. The sequence was 95% similar to *nosZ* from *Pseudomonas* sp. PD 22 (DQ377794). *Pseudomonas* strain I-65 *nosZ* sequence was 94% similar to *nosZ* from *P. fluorescens* (AF197468). In addition, *Aeromonas* strain I-6 *nosZ* sequence was 84% similar to *nosZ* from *Pseudomonas stutzeri* A1501 (CP000304). Finally, the *nosZ* PCR product from *Achromobacter* strain I-5 was 93% similar to *nosZ* sequence from *Achromobacter* sp. PD 25 (DQ377797) while *Achromobacter* strain I-49 *nosZ* sequence was 91% similar to *nosZ* sequence from *A. xylosoxidans* (AY072227). In summary, *nirS/nirK* was amplified from three strains, *cnorB* from three strains, and *nosZ* from five strains.

### Nitrate reduction/denitrification physiology

**Table 2: Nitrite reduction and detected denitrification functional genes.**

Group #	Strain #	Genus [% similarity]* (Class)	Nitrite reduced <sup>1</sup>	Nitrite reduced <sup>2</sup>	<i>nirS/nirK</i>	<i>cnorB</i>	<i>nosZ</i>
1	1	<i>Pseudomonas</i> [100%] ( $\gamma$ )	Yes	Yes	<i>nirS</i>	Yes	Yes
	65	<i>Pseudomonas</i> [100%] ( $\gamma$ )	Yes	Yes	No	Yes	Yes
	4	<i>Ensifer</i> [99%] ( $\alpha$ )	Yes	Yes	No	Yes	No
2	6	<i>Aeromonas</i> [100%] ( $\gamma$ )	No	No	No	No	Yes
	25	<i>Enterobacter</i> [99%] ( $\gamma$ )	No	No	<i>nirK</i>	No	No
3	5	<i>Achromobacter</i> [99%] ( $\beta$ )	No	Yes	No	No	Yes
	49	<i>Achromobacter</i> [100%] ( $\beta$ )	No	Yes	<i>nirK</i>	No	Yes

\*Threshold level of similarity as determined by The Ribosomal Database Project (RDS) Classifier program.

NR: nitrate reduction.

DN: nitrite reduction.

<sup>1</sup>not strictly anaerobic, microtiter plate assay.

<sup>2</sup>strictly anaerobic.

$\gamma$  =  $\gamma$ -proteobacteria.

$\alpha$  =  $\alpha$ -proteobacteria.

$\beta$  =  $\beta$ -proteobacteria.

*nirS*: cytochrome cd1-nitrite reductase.

*nirK*: copper nitrite reductase.

*cnorB*: nitric oxide reductase gene.

*nosZ*: nitrous oxide reductase gene.

### A. Microtiter plate assay: initially aerobic

In the microtiter plate assay, bacteria were inoculated into nitrate broth and incubated for two weeks. Cells are exposed to the ambient level of oxygen in the microtiter plate wells at the beginning of the experiment and the microtiter plate was presumed to become anaerobic as the microorganism grew and consumed oxygen. Although oxygen level was not monitored, the control microorganisms gave the expected results regarding nitrate/nitrite reduction which suggests that the concentration of oxygen decreased over time. Under these

conditions, *Aeromonas* strain I-6, *Enterobacter* strain I-25, and *Achromobacter* strains I-5 and I-49 reduced nitrate to nitrite. However, *Pseudomonas* strains I-1 and I-65; and *Ensifer* strain I-4 were able to reduce nitrate and nitrite under the same conditions, suggesting the ability to perform nitrite reduction (Table 2).

### B. Strictly anaerobic conditions

The seven strains and the control strains *S. maltophilia* ATCC 13637 (nitrate reducer), *P. aeruginosa* ATCC 27853 (denitrifier), and *P. putida* F1 ATCC 17485 (neither nitrate reducer nor denitrifier) were tested for the ability to

reduce nitrate/nitrite under strict anaerobic conditions. Based on the results of nitrate/nitrite reduction under both conditions (e.g. microtiter plate assay and strictly anaerobic conditions), the seven strains were clustered into three groups.

### Group 1

Three strains (e.g. strains I-1, I-65, and I-4) behaved similarly to denitrifying bacterium *P. aeruginosa* ATCC 27853. When inoculated with *P. aeruginosa* ATCC 27853, all nitrate was depleted after one day of incubation and there was no significant nitrite accumulation at any time during 14 days of incubation (data not shown). *Pseudomonas* strain I-1 (*nirS*<sup>+</sup>, *norB*<sup>+</sup>, and *nosZ*<sup>+</sup>) was able to reduce nitrite when grown in nitrate broth in the microtiter plate assay. It also showed its ability to reduce nitrite when grown under strictly anaerobic conditions. Nitrate was completely depleted after three days and there was no significant accumulation of nitrite when *Pseudomonas* strain I-1 was grown under nitrate reducing conditions. The largest detectable amount of nitrite was around 0.25 mM after one day of incubation (Figure 1.a). *Pseudomonas* strain I-65 (*cnorB*<sup>+</sup>, *nosZ*<sup>+</sup>) and *Ensifer* strain I-4 (*cnorB*<sup>+</sup>) were able to reduce nitrate and nitrite under nitrate reducing conditions. The same results were obtained from microtiter plate assay. *Pseudomonas* strain I-65 was a very fast nitrite reducer, similar to the denitrifier control *P. aeruginosa* ATCC 27853. After one day of incubation, all nitrate was depleted and there was no significant nitrite accumulation (Figure 1.b). On the other hand, *Ensifer* strain I-4, there was no significant loss of nitrate or accumulation of nitrite after seven days of incubation. However, after 14 days of incubation, all nitrate was depleted and there was no significant accumulation of nitrite (Figure. 1.c).

### Group 2

Two strains (e.g. strains I-6 and I-25) showed a loss of nitrate and an accumulation of nitrite. In *Aeromonas* strain I-6 (*nosZ*<sup>+</sup>), after 14 days of incubation, nitrate was depleted to around 3.5 mM and nitrite was accumulated to the same value (e.g. 3.5 mM). These two values remained almost the same even after 53 days of incubation (Figure 1.d). This strain also reduced nitrate to

nitrite in the microtiter plate assay. *Enterobacter* strain I-25 (*nirK*<sup>+</sup>) was able to reduce nitrate to nitrite under strictly anaerobic conditions (Figure 1.e). After three days, all nitrate was depleted and nitrite was accumulated to around 7.0 mM and remained around this value after 14 days (*Enterobacter* strain I-25, Figure 1.e). In comparison, the nitrate reducer control strain (*S. maltophilia* ATCC 13637), depleted all nitrate and accumulated nitrite to around 8.3 mM in four days (data not shown). *Enterobacter* strain I-25 also reduced nitrate to nitrite when the microtiter plate assay was used (Table 2).

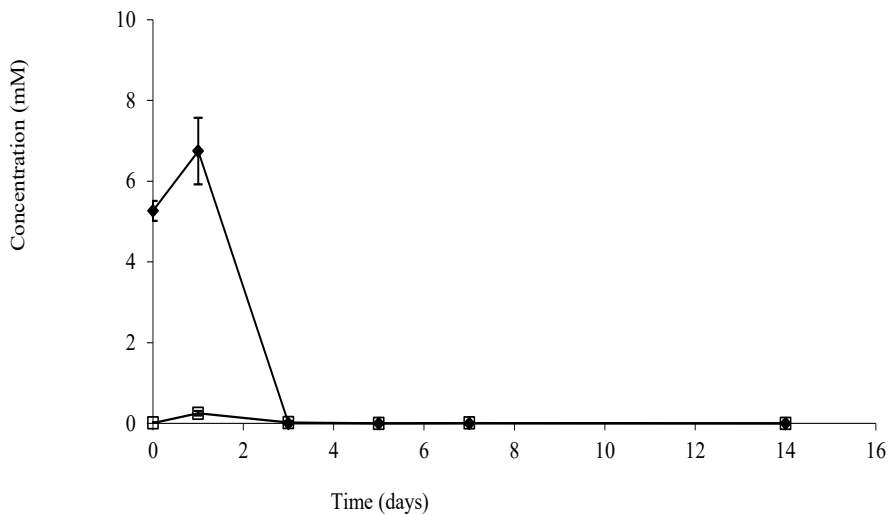
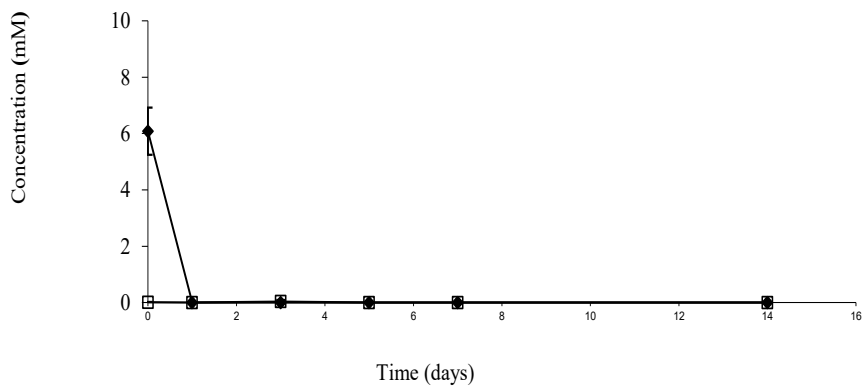
### Group 3

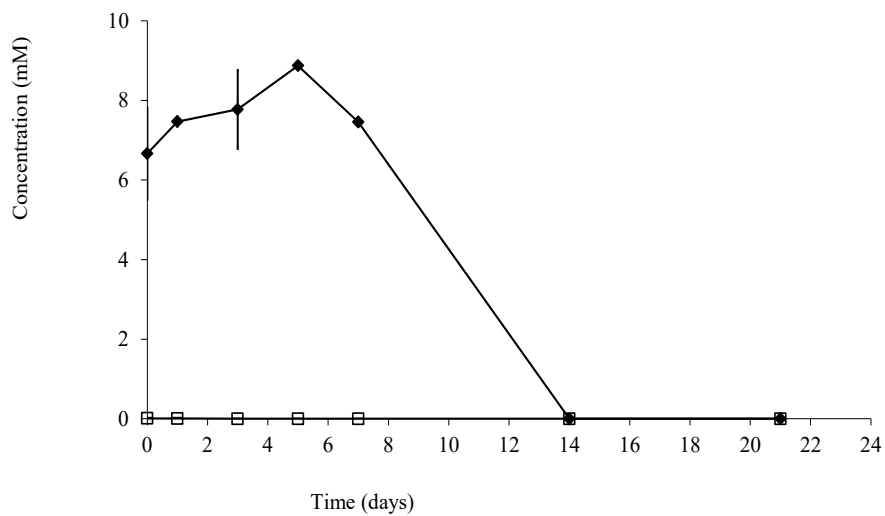
Finally, based on the microtiter plate assay, *Achromobacter* strains I-5 and I-49 had the ability to reduce nitrate to nitrite, but did not reduce nitrite. However, under strictly anaerobic conditions they were able to reduce both nitrate and nitrite. In *Achromobacter* strain I-5 (*nosZ*<sup>+</sup>), after one day of incubation, nitrate was depleted to around 1.4 mM and nitrite accumulated to around 4.8 mM. After three days of incubation, almost all nitrate and nitrite were depleted (Figure 1.f). Also, in *Achromobacter* strain I-49 (*nirK*<sup>+</sup>, *nosZ*<sup>+</sup>), after one day of incubation, nitrate was depleted to around 2.9 mM and nitrite was accumulated to around 5.0 mM. After three days of incubation, almost all nitrate was depleted and nitrite was depleted to around 2.8 mM. Nitrate and nitrite were completely depleted after five days of incubation (Figure 1.g). The negative control *P. putida* pG7 did not show any significant depletion of nitrate or accumulation of nitrite even after 14 days of incubation (data not shown).

## Discussion

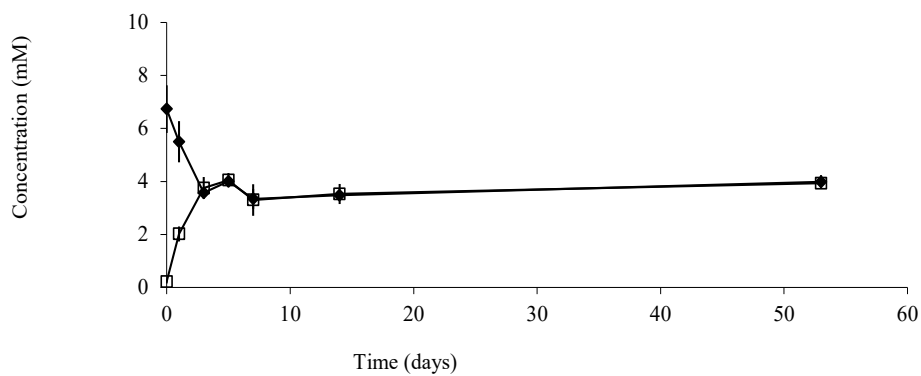
### The effect of oxygen on nitrite reduction

This study compared the effect of oxygen on the production/loss of nitrite in seven strains from five different genera of bacteria. We found that nitrate/nitrite reduction in five strains was relatively insensitive to oxygen. However, nitrite reduction in two strains of *Achromobacter* only occurred under strictly anaerobic conditions. We conclude that oxygen levels are

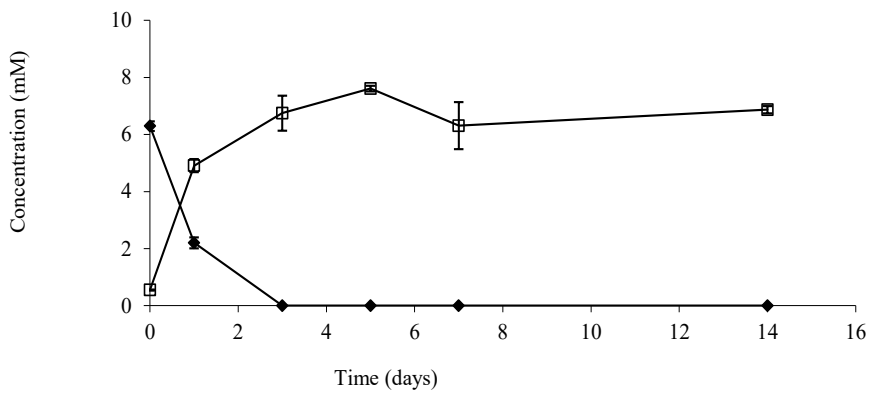
1.a. *Pseudomonas* strain I-1.1.b. *Pseudomonas* strain I-65.



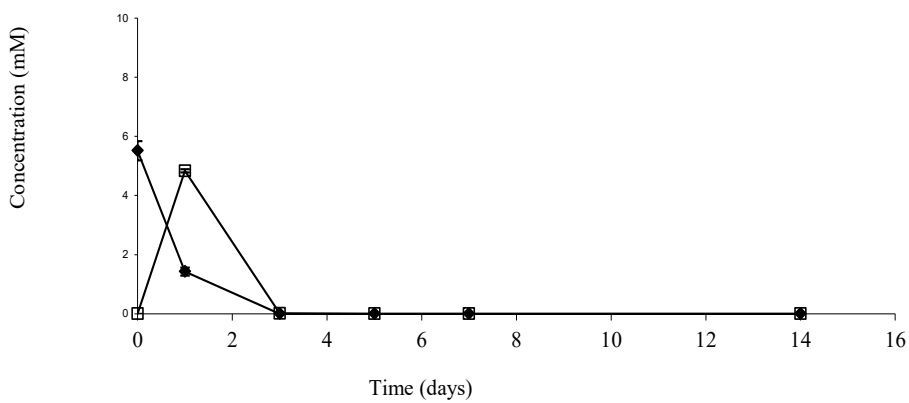
1.c. *Ensifer* strain I-4.



1.d. *Aeromonas* strain I-6.

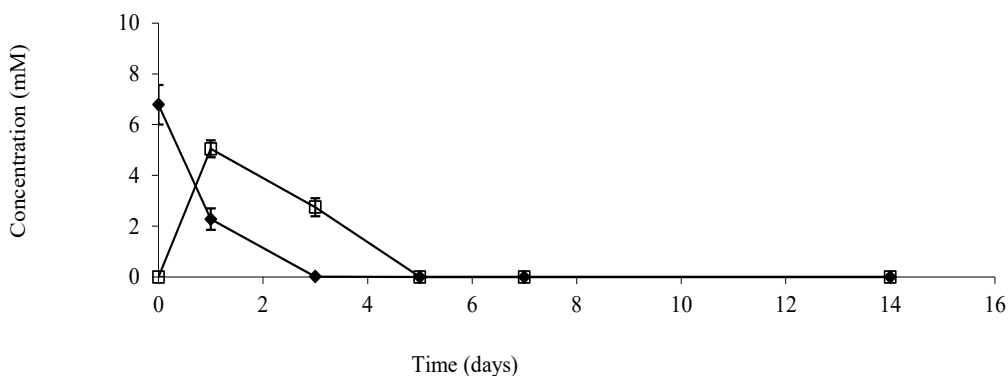


1.e. *Enterobacter* strain I-25.



1.f. *Achromobacter* strain I-5.





1. g. *Achromobacter* strain I-49.

**Figure 1. Nitrate or nitrate/nitrite reduction under strictly anaerobic conditions for seven strains. Time in days is plotted on the X axis and nitrate (diamonds)/nitrite (hollow squares) concentration in micromolar is plotted on the Y axis. Each data point represent the average of 3 data points. The bars represent one standard deviation. 1.a. *Pseudomonas* strain I-1; 1.b. *Pseudomonas* strain I-65; 1.c. *Ensifer* strain I-4; 1.d. *Aeromonas* strain I-6; 1.e. *Enterobacter* strain I-25; 1.f. *Achromobacter* strain I-5; 1.g. *Achromobacter* strain I-49.**

important in the expression of nitrite reduction genes in some strains or genera of bacteria commonly found in Tallgrass Prairie soil.

Based on the effect of oxygen on nitrite reduction, we can categorize the seven strains into three groups. Group 1 includes *Pseudomonas* strains I-1 and I-65, and *Ensifer* strain I-4. This group showed no effect of the two test regimens on nitrite reduction, nitrite reduction appeared to be relatively oxygen-insensitive. Group 2 includes *Aeromonas* strain I-6 and *Enterobacter* strain I-25. This group, which reduced nitrate but did not reduce nitrite under either oxygen regimen, showed that there was no effect of strict oxygen exclusion on nitrite reduction. Finally, Group 3 includes *Achromobacter* strains I-5 and I-49. Nitrite reduction in this group appears to be oxygen sensitive because nitrite was consumed only under strictly anaerobic conditions.

### Group 1: Nitrite reduction under both microtiter plate assay and strict anaerobic conditions

Strain I-1 (99% similar to 16S rRNA sequence from *Pseudomonas* sp. MT14, accession number

DQ647192) showed its ability to reduce nitrate and nitrite when microtiter plate assay was used and it reduced nitrite under strictly anaerobic conditions which may indicate that the nitrite reductase of some *Pseudomonas* strains is not sensitive to oxygen. In support, it was shown that *Pseudomonas* sp. strain K50 produced N<sub>2</sub> even under oxic conditions indicating that it is an O<sub>2</sub> resistant, aerobic denitrifier (Takaya et al., 2003). Also, oxygen was shown to have no effect on nitrite production/loss in *Pseudomonas* strain I-65 (99% similar to the 16S rRNA sequence of *Pseudomonas* sp. strain MTQ15, accession number HQ143608) that reduced nitrate/nitrite when microtiter plate assay was used and under strictly anaerobic conditions. However, not all *Pseudomonas* strains are capable of denitrification or even nitrate reduction. For example, *Pseudomonas* sp. S-JS-8 (FJ529034) does not reduce nitrate (Huang et al., 2010) while *Pseudomonas* sp. ONBA-17 (DQ079062) reduces nitrate to nitrite under aerobic conditions (Fang-Bo et al., 2006).

*Ensifer* strain I- 4 (100% similar to the 16S rRNA sequence of *Ensifer* sp. TA12-B, accession

number HM219616) reduced nitrate/nitrite when microtiter plate assay was used and under strictly anaerobic conditions which indicates some degree of oxygen tolerance on nitrite reduction in this strain. In fact, *Ensifer adhaerens* strains 2FB8 and 4FB6 *were shown* to utilize different halobenzoates under aerobic and denitrifying conditions, but the metabolic pathways were not investigated (Song et al., 2000).

### **Group 2: No nitrite reduction under either oxygen level**

Strain I-6 (99% similar to the 16S rRNA sequence of *Aeromonas punctata* strain JA11, accession number GU205200) and strain I-25 (99% similar to the 16S rRNA sequence of *Enterobacter cloacae* strain P04, HM854373) were able to reduce nitrate when the microtiter plate was assayed but nitrite was not consumed. The same results were obtained for these two strains under strictly anaerobic conditions. However, strain I-6 (*nosZ*<sup>+</sup>) and strain I-25 (*nirK*<sup>+</sup>) each possesses a sequence highly similar to one denitrification gene.

### **Group 3: Nitrite reduction only under strict anaerobic conditions**

Oxygen was shown to play a role in the production/loss of nitrite in *Achromobacter* strains I-5 and I-49. Strain I-5 (99% similar to the 16S rRNA sequence of *Achromobacter xylosoxidans*, accession number AF531768) possesses *nosZ* and strain I-49 (99% similar to the 16S rRNA sequence of *Achromobacter* sp. MMD19, accession number JN546225) possesses *nirK* and *nosZ*. In fact, *A. xylosoxidans* strains are known to vary in their ability to reduce nitrate. Tatum divided *A. xylosoxidans* based on its ability to reduce nitrate into two groups: one group reduces nitrate to nitrite while the other reduces nitrate to nitrogen gas (Tatum et al., 1974). Also, Shigeta found the same results where in different strains of *A. xylosoxidans* varied in their ability to reduce nitrate. Some *A. xylosoxidans* strains reduced nitrate to nitrogen gas, whereas other strains reduced nitrate to nitrite only (Shigeta et al., 1983). On the other hand, *Achromobacter insolitus* strain LMG 6003 (NR\_025685) reduces nitrate but does not reduce nitrite and does not denitrify

(Coenye et al., 2003). Our results showed that both *Achromobacter* strain I-5 and strain I-49 have the ability to reduce nitrate when using the microtiter plate assay. However, under strictly anaerobic conditions, *Achromobacter* strain I-5 and strain I-49 were able to reduce both nitrate and nitrite. These results are consistent with our hypothesis that in certain groups, the production/loss of nitrite is regulated by oxygen supply which may indicate that certain denitrifying genes are expressed only under strictly anaerobic conditions. This regulation may be important in the transition from aerobic to anaerobic conditions, such as produced in the bioremediation of contaminated soils. In fact, a consortium that contained *A. xylosoxidans* from weathered, oil-contaminated soil was shown to degrade hydrocarbons in oxygen-limited environments (Medina-Moreno et al., 2005).

Stres et al. (2008) showed that some environmental factors (e.g. absence of plant cover, changes in water content and temperature) played a minor role in shaping bacterial and denitrifiers community structures but significantly altered their activity. *Achromobacter* strain I-5 was isolated from the brine contaminated G7 site which was periodically watered to enhance remediation. *Achromobacter* strain I-49 was obtained from the uncontaminated G7P site which depended on natural rainfall. Apparently, *Achromobacter* strains could be isolated from both the relatively more aerobic uncontaminated prairie as well as from intermittently anaerobic soil, but may be able to reduce nitrite only when the soil is flooded producing oxygen deficient soil conditions.

### **The power of molecular detection in identifying denitrifying bacteria**

We detected nitrite reductase genes (e.g. *nirS*<sup>+</sup> or *nirK*<sup>+</sup>) in only two out of the five strains that showed nitrite reduction under strictly anaerobic conditions. However, when we used *cnorB* and *nosZ* primers to detect these two functional genes, the additional three nitrite reducing strains (*nirS*<sup>+</sup> and *nirK*<sup>+</sup>) were identified as denitrifying bacteria by at least one of these two denitrifying genes (e.g. *cnorB*<sup>+</sup> and/or *nosZ*<sup>+</sup>). These results indicate that the more denitrifying genes we

target in the denitrification pathway using the PCR-approach, the higher chance in identifying denitrifying bacteria since nitrite reductase genes may not be sufficient by themselves to identify denitrifying bacteria. In fact, another study (Hallin and Lindgren, 1999) showed that two sets of PCR primers were used to amplify *nirS* and *nirK* from nitrite reducing bacteria where in some cases only one of these two sets of primers was able to amplify the expected fragment of the corresponding gene. However, in some other cases, neither of these two primer sets was able to detect the nitrite reductase genes.

## References

- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J Mol Biol.* 215:403–410.
- Bazylnski, D. A., and R. P. Blakemore. 1983. Denitrification and Assimilatory nitrate reduction in *Aquaspirillum magnetotacticum*. *Appl Environ Microbiol.* 46:1118-1124.
- Bell, J. 2008. A simple way to treat PCR products prior to sequencing using ExoSAP-IT. *BioTechniques.* 44:834.
- Braker, G., A. Fesefeldt, and K. P. Witzel. 1998. Development of PCR primer systems for amplification of nitrite reductase genes (*nirK* and *nirS*) to detect denitrifying bacteria in environmental samples. *Appl Environ Microbiol.* 64:3769-3775.
- Coenye, T., M. Vancanneyt, E. Falsen, J. Swings, and P. Vandamme. 2003. *Achromobacter insolitus* sp. nov. and *Achromobacter spanius* sp. nov., from human clinical samples. *Int J Syst Evol Microbiol.* 53:1819-1824.
- Dandie, C. E., D. L. Burton, B. J. Zebarth, S. L. Henderson, J. T. Trevors, and C. Goyer. 2008. Changes in bacterial denitrifier community abundance over time in an agricultural field and their relationship with denitrification activity. *Appl Environ Microbiol.* 74:5997-6005.
- Davies, K. J., D. Lloyd, and L. Boddy. 1989. The effect of oxygen on denitrification in *Paracoccus denitrificans* and *Pseudomonas aeruginosa*. *J Gen Microbiol.* 135:2445-2451.
- Duncan, K., E. Jennings, S. Hettenbach, W. Potter, K. Sublette, G. Subramaniam, and R. Narasimhan. 1998. Nitrogen cycling and nitric oxide emissions in oil-impacted prairie soils. *Bioremediation Journal.* 1:195-208.
- Duncan, K. E., R. Kolhatkar, G. Subramaniam, R. Narasimhan, E. Jennings, S. Hettenbach, A. Brown, C. McComas, W. Potter, and K. Sublette. 1999. Microbial dynamics in oil-impacted prairie soil. *Appl Biochem Biotechnol.* 77-79:421-434.
- Ehrenreich, P., A. Behrends, J. Harder, and F. Widdel. 2000. Anaerobic oxidation of alkanes by newly isolated denitrifying bacteria. *Arch Microbiol* 173:58–64
- Fang-Bo, Y., S. Biao, and L. Shun-Peng. 2006. Isolation and characterization of *Pseudomonas* sp. strain ONBA-17 degrading o-nitrobenzaldehyde. *Curr Microbiol.* 53:457-461.
- Hallin, S., and P. E. Lindgren. 1999. PCR detection of genes encoding nitrite reductases in denitrifying bacteria. *Appl Environ Microbiol.* 65:1652-1657.
- Huang, X. F., W. Guan, J. Liu, L. J. Lu, J. C. Xu, and Q. Zhou. 2010. Characterization and phylogenetic analysis of biodeulsifier-producing bacteria. *Bioresour Technol.* 101:317-323.
- Matsuzaka, E., N. Nomura, T. Nakajima-Kambe, N. Okada, and T. Nakahara. 2003. A simple screening procedure for heterotrophic nitrifying bacteria with oxygen-tolerant denitrification activity. *J Biosci Bioeng.* 95:409-411.
- Medina-Moreno, S. A., S. Huerta-Ochoa, and M. Gutiérrez-Rojas. 2005. Hydrocarbon biodegradation in oxygen-limited sequential batch reactors by consortium from weathered, oil-contaminated soil. *Can J Microbiol.* 51:231-239.
- Oakley, B. B., C. A. Francis, K. J. Roberts, C. A. Fuchsman, S. Srinivasan, and J. T. Staley. 2007. Analysis of nitrite reductase (*nirK* and *nirS*) genes and cultivation reveal depauperate community of denitrifying bacteria in the Black Sea suboxic zone. *Environ Microbiol.* 9:118-130.
- Payne, W. J. 1973. Reduction of nitrogenous oxides by microorganisms. *Bacteriol Rev.* 37:409-452.
- Qiu, X. Y., R. A. Hurt, L. Y. Wu, C. H. Chen,

- J. M. Tiedje, and J. Z. Zhou. Detection and quantification of copper-denitrifying bacteria by quantitative competitive PCR. 2004. *J Microbiol Methods*. 59:199-210.
- Rich, J. J., R. S. Heichen, P. J. Bottomley, K. Cromack Jr, and D. D. Myrold. 2003. Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. *Appl Environ Microbiol*. 69:5974-5982.
- Robertson, L. A., and J. G. Kuenen. 1983. *Thiosphaera pantotropha* gen. nov. sp. nov., a facultatively anaerobic, facultatively autotrophic sulphur bacterium. *J Gen Microbiol*. 129:2847-2855.
- Robertson, L. A., and J. G. Kuenen. 1984a. Aerobic denitrification—a controversy revived. *Arch Microbiol*. 139: 351-354.
- Saleh-Lakha, S., K. E. Shannon, S. L. Henderson, C. Goyer, J. T. Trevors, B. J. Zebarth, and D. L. Burton. 2009. Effect of pH and temperature on denitrification gene expression and activity in *Pseudomonas mandelii*. *Appl Environ Microbiol*. 75:3903-3911.
- Saleh-Lakha, S., K. E. Shannon, S. L. Henderson, B. J. Zebarth, D. L. Burton, C. Goyer, and J. T. Trevors. 2009. Effect of nitrate and acetylene on *nirS*, *cnorB*, and *nosZ* expression and denitrification activity in *Pseudomonas mandelii*. *Appl Environ Microbiol*. 75:5082-5087.
- Shigeta, S., S. Hyodo, E. Chonan, and E. Yabuuchi. 1983. Serological classification of *Achromobacter xylosoxidans*. *J Clin Microbiol*. 17:181-186.
- Simpkin, T. J., and W. C. Boyle. 1988. The lack of repression by oxygen of the denitrifying enzymes in activated sludge. *Water research*. 22:201-206.
- Smibert, R. M., and N. R. Krieg. 1994. Phenotypic characterization. In: Gerhardt, P., R. G. E. Murray, W. A. Wood, and N. R. Krieg, Editors, *Methods for General and Molecular Bacteriology*, American Society for Microbiology, p. 649.
- Song, B., N. J. Palleroni, and M. M. Häggblom. 2000. Isolation and characterization of diverse halobenzoate-degrading denitrifying bacteria from soils and sediments. *Appl Environ Microbiol*. 66:3446-3453.
- Stres, B., T. Danevcic, L. Pal, M. M. Fuka, L. Resman, S. Leskovec, J. Hacin, D. Stopar, I. Mahne, and I. Mandic-Mulec. 2008. Influence of temperature and soil water content on bacterial, archaeal and denitrifying microbial communities in drained fen grassland soil microcosms. *FEMS Microbiol Ecol*. 66:110-122.
- Sublette, K., E. Jennings, C. Mehta, K. Duncan, J. Brokaw, T. Todd, and G. Thoma. 2007. Monitoring soil ecosystem recovery following bioremediation of a terrestrial crude oil spill with and without a fertilizer amendment. *Soil & Sediment Contamination*. 16:181-208.
- Sublette, K. L., A. Moralwar, L. P. Ford, K. Duncan, G. Thoma, and J. Brokaw. 2005. Remediation of a spill of crude oil and brine without gypsum. *Environmental Geosciences*. 12: 115-125.
- Takaya, N., M. A. Catalan-Sakairi, Y. Sakaguchi, I. Kato, Z. Zhou, and H. Shoun. 2003. Aerobic denitrifying bacteria that produce low levels of nitrous oxide. *Appl Environ Microbiol*. 69:3152-3157.
- Tanner, R. 1997. Cultivation of bacteria and fungi. In *Manual of Environmental Microbiology*, pp. 52-60. Edited by C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stetzenbach & M. V. Walter. Washington, DC: American Society for Microbiology.
- Tatum, H. W., W. H. Ewing, and R. E. Weaver. 1974. Miscellaneous gram-negative bacteria, p. 270-294. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
- Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol*. 73:5261-5267.
- Wilson, K. H., R. B. Blitchington, and R. C. Greene. 1990. Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. *J Clin Microbiol*. 28:1942-1946.
- Zumft, W. G. 1997. Cell biology and molecular basis of denitrification. *Microbiol Mol Biol Rev*: MMBR. 61:533-616.

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# Test of Polyaromatic Hydrocarbon Degradation by Nitrate-reducing Microorganisms Isolated from Tallgrass Prairie Soils

**Samer M. AbuBakr**

Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, 73019

**Irene A. Davidova**

Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK 73019

**Kathleen E. Duncan**

Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK 73019

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**Abstract:** Soils are frequently contaminated with hydrocarbons such as polyaromatic hydrocarbons (PAH). This contamination inhibits the growth of some microorganisms in the contaminated soils. However, the contamination may select for microorganisms capable of hydrocarbon degradation under aerobic or anaerobic conditions. Forty five strains of bacteria isolated from tallgrass prairie soil samples were screened for the ability to grow with naphthalene as sole carbon source under aerobic conditions by culturing on minimal medium containing naphthalene as the sole carbon source. Our results showed that none of the 45 strains were able to grow on naphthalene under these conditions. Three out of the 45 strains, e.g. one strain each classified as *Ensifer*, *Stenotrophomonas*, or *Serratia*, were tested for the ability to degrade naphthalene under nitrate reducing conditions. All three strains were facultative anaerobes and showed the physiology of nitrate or nitrate/nitrite reduction when grown under nitrate-reducing conditions in medium containing yeast extract. Two strains (e.g. *Stenotrophomonas* and *Serratia*) were tested for the ability to grow on naphthalene, gasoline, or benzene under nitrate-reducing conditions. The *Serratia* grew poorly on the hydrocarbons, but *Stenotrophomonas* reached its highest O.D. values on naphthalene. However, upon re-testing a well-washed cell suspension of the *Stenotrophomonas*, no loss of naphthalene was found when grown under nitrate-reducing conditions. Although it might be expected that crude-oil contamination would select for PAH degraders, we did not find the nitrate reducing/nitrite reducing isolates to be capable of naphthalene-degradation under aerobic or nitrate-reducing conditions. ©2015 Oklahoma Academy of Science

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## Introduction

Petroleum contamination results regularly from leaks and accidental spills during production, transportation, and storage of petroleum and petroleum products. Since accumulation of pollutants in animals and in plant tissue may cause death or mutations,

soil contaminated with hydrocarbons causes extensive damage of local systems (Alvarez and Vogel, 1991). Polyaromatic hydrocarbons (PAHs) have been found to show toxic and hazardous properties (Mihelcic and Luthy, 1988). The Environmental Protection Agency has listed 16 PAH compounds, including naphthalene, as priority pollutants to be monitored in industrial effluents (Mihelcic and Luthy, 1988).

Naphthalene and its methylated derivatives are among the most toxic compounds found in the water-soluble fraction of petroleum (Heitkamp et al., 1987). In fact, several physicochemical and biological factors affect the degradation of petroleum hydrocarbons in soil such as: 1- the number and types of microbial species present, 2- the nature, amount, and bioavailability of contaminants, 3- the existing environmental conditions for microbial degradation (e.g., oxygen, temperature, pH, and nutrient levels), 4- soil characteristics (e.g., organic matter and soil aggregates) (MacNaughton et al., 1999; Röling et al., 2002; and Smith et al., 2008). Even worse, some high molecular weight PAHs, such as fluoranthene and benzo[*a*]pyrene (BaP), may not be degraded at all (Atlas and Bragg, 2009).

PAHs can be biodegraded in the presence of oxygen (Ni Chadhain et al., 2006), or under anaerobic condition such as nitrate reducing (e.g. NR) (Al-Bashir et al., 1990), sulfate-reducing (Coates et al., 1997; Davidova et al. 2007), or methanogenic (Godsy et al., 1992) conditions. Microbial degradation of naphthalene has been investigated and it is well documented in the literature (Al-Bashir et al., 1990; Ensley et al., 1982; Mihelcic and Luthy, 1988; Rockne et al., 2000). However, most of the studies were performed under aerobic conditions. In the classical pathway of naphthalene degradation under aerobic conditions, naphthalene dioxygenase (NDO), the multicomponent enzyme system, initiates the metabolism of low-molecular-weight PAHs (Ensley et al., 1982). The classical pathway of naphthalene degradation is via dihydroxylation and cleavage of the first ring and removal of the resulting aliphatic side chain to produce salicylate (2-hydroxybenzoate). Salicylate is then converted by the action of salicylate 1-hydroxylase to catechol (1,2-dihydroxybenzene). Finally, catechol undergoes extradiol cleavage via the same route used for a wide range of other aromatic compounds such as toluene, phenol, and xylenes (Zhou et al., 2002).

Some initial chemical transformations of naphthalene might take place even if strictly aerobic naphthalene degraders are killed

by the initial spill of crude oil. Enzymatic activity has been shown to persist in soil for several weeks (Renella et al., 2007; Stursova and Sinsabaugh, 2008), therefore lysed cells could possibly be a source of NDO or similar oxygenase enzymes under natural conditions. Purified NDO was shown to transform the model substrates naphthalene and indole in the presence of humic acids when incubated for 2 weeks at room temperature which might indicate that NDO maintains some enzymatic activity over days (AbuBakr et al., 2008).

Although the emphasis has been on naphthalene degradation under aerobic conditions, there are some studies about the microbial degradation of naphthalene under anaerobic conditions. Mihelcic and Luthy showed the first evidence of microbial degradation of unsubstituted PAH compounds under denitrifying conditions (Mihelcic and Luthy, 1988). Indeed, denitrifying conditions could play an important role in the decomposition of PAHs in nature, and naphthalene biodegradation rates obtained under denitrifying conditions have been shown to be in the same range as those obtained under aerobic conditions (Al-Bashir et al., 1990). Rockne et al showed that naphthalene was mineralized to carbon dioxide under nitrate reducing (NR) conditions in pure cultures, and that the ability to degrade naphthalene under NR conditions was found in a wide diversity of  $\gamma$ -Proteobacteria including *Pseudomonas* and *Vibrio* species (Rockne et al., 2000).

Previous studies showed that NR and denitrifying (DN) bacteria are abundant in the Tallgrass Prairie Preserve (TPP), which has experienced hydrocarbon contamination from surface spills of crude oil and/or salt water (brine) (Sublette et al, 2007b). Remediation treatments commonly included the application of nitrate-containing fertilizer. Higher levels of culturable aerobic naphthalene degrading bacteria were found in oil contaminated sites the first year of treatment with nitrogen compared to the uncontaminated prairie. However, the naphthalene-degrading bacteria were not identified (Duncan et al., 1999). Since NR bacteria, DN bacteria, and hydrocarbons are

abundant in the TPP, we hypothesized that many of the NR/DN bacteria from TPP have the ability to degrade hydrocarbons under NR/DN conditions. However, as facultative anaerobes, nitrate reducers could degrade PAH under aerobic or NR conditions, if the strains possessed the necessary degradation genes. Therefore, this study also tested the ability of 45 strains to degrade naphthalene under aerobic conditions. The 45 strains (Table 1) included the strains that were confirmed to possess the nitrate reducing (NR) or nitrite reducing (DN) phenotype and were chosen to be representative of the 16S rRNA phylogenetic diversity plus all the remaining *Stenotrophomonas* and *Pseudomonas* strains from the total set of 75 strains. The 75 strains were isolated from the same sites described in the Materials and Methods and by the same isolation procedure as a part of a wider effort to characterize species composition of culturable nitrate reducing/denitrifying bacteria isolated from contaminated and uncontaminated tallgrass prairie soil. Strains of *Stenotrophomonas* and *Pseudomonas* were chosen because they have been shown in previous studies to be hydrocarbon degraders (Gibson et al., 1974; Jung and Kafkewitz, 1996; Jeong et al., 2006; Lee et al., 2002; Shim and Yang, 1999).

## Methods

### Sites Description

The sampling sites used in this work are located in the Tallgrass Prairie Preserve (TPP) in Osage County, Oklahoma. Briefly, strains were obtained from 5 contaminated (e.g. G5, G7, LF, J6F, and J6NF) and 4 uncontaminated (e.g. G5P, G7P, LFP, and J6P) sites. More than 90% of the crude oil in TPP was aliphatic compounds, and less than 10% were aromatic compounds such as polyaromatic hydrocarbons (PAH) (e.g. naphthalene) (Sublette et al., 2007a).

### Sampling soils, isolating bacteria and testing their ability to reduce nitrate/nitrite

Soils were sampled from all 9 sites and serially diluted and scored for growth and nitrate/nitrite reduction. Briefly, soils were homogenized, serially diluted and added to microtiter plates

containing nitrate broth (Difco), after 2 weeks of incubation at room temperature (23-25°C), all wells were examined for evidence of growth (turbidity) and tested for nitrate reduction and nitrite reduction using Griess reagents (Smibert and Krieg, 1994). Based on positive scoring for growth and nitrate or nitrite reduction in the microtiter plates, nutrient agar plates (Nutrient Agar, Difco, Detroit, MI) were inoculated with 25 µL from microtiter plate wells, chosen randomly from 10<sup>-1</sup> to 10<sup>-4</sup> dilutions that scored positive for growth and nitrate reduction or nitrite reduction. The nutrient agar plates were incubated at 30°C. Pure strains were obtained from the microtiter plate wells containing nitrate broth followed by at least three rounds of streaking for single colonies on nutrient agar plates. The purified strains were tested on their ability to reduce nitrate or nitrite after incubation into nitrate broth in microtiter plates and the presence of nitrate/nitrite assayed using Griess reagents (Smibert and Krieg, 1994). In addition, the following strains of known phenotype were included as controls: *P. aeruginosa* ATCC 27853 (Denitrifier), *P. putida* pG7 (not a nitrate reducer), *S. maltophilia* ATCC 13637 (Nitrate reducer), and uninoculated wells containing sterile nitrate broth.

API 20 NE strips (bioMérieux, France) were used to perform different biochemical tests on a subset of three strains, including nitrate/nitrite reduction and glucose utilization.

### DNA extraction from strains

Genomic DNA was isolated from cells using a bead beating method. Briefly, each of the 45 strains was streaked on nutrient agar plate incubated one to three days at 30°C until good colony growth was observed, then a single colony from each plate was used to inoculate 5 mL nutrient broth and incubated at 30°C until turbid growth was seen. A sufficient volume of culture (1 to 2 ml) was centrifuged at 6000xg for 10 minutes to provide a pellet size of approximately 10<sup>9</sup> bacteria. Pellets were resuspended in sterile isotonic saline (0.85%) to give a maximum suspension volume of 200 µL. A commercially available DNA extraction kit, FastDNA®SPIN Kit (QBIogene, Solon, OH,

Table 1. Characteristics of the 45 strains used in this study.

Strain #*	Isolation site characteristics	RDP Classifier**	NR/DN
I-1	Brine	<i>Pseudomonas</i> [100%] ( $\gamma$ )	DN
I-2	Brine	<i>Pseudomonas</i> [100%]( $\gamma$ )	DN
I-3	Brine	<i>Pseudomonas</i> [100%] ( $\gamma$ )	NR
I-4	Brine	<i>Ensifer</i> [99%] ( $\alpha$ )	DN
I-5	Brine	<i>Achromobacter</i> [99%] ( $\beta$ )	NR
I-6	Brine	<i>Aeromonas</i> [100%] ( $\gamma$ )	NR
I-10	Brine	<i>Phenylobacterium</i> [100%] ( $\alpha$ )	NR
I-11	Brine	<i>Stenotrophomonas</i> [100%] ( $\gamma$ )	NR
I-12	Brine	<i>Pseudomonas</i> [95%] ( $\gamma$ )	NR
I-13	Brine	<i>Stenotrophomonas</i> [100%] ( $\gamma$ )	NR
I-18	Brine	<i>Stenotrophomonas</i> [100%] ( $\gamma$ )	NR
I-21	Oil	<i>Stenotrophomonas</i> [100%] ( $\gamma$ )	NR
I-23	Oil	<i>Stenotrophomonas</i> [100%] ( $\gamma$ )	NR
I-24	Oil	<i>Serratia</i> [100%] ( $\gamma$ )	NR
I-25	Oil	<i>Enterobacter</i> [99%] ( $\gamma$ )	NR
I-26	Oil	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	None
I-27	Oil	<i>Stenotrophomonas</i> [100%] ( $\gamma$ )	None
I-28	Oil	<i>Stenotrophomonas</i> [100%] ( $\gamma$ )	None
I-30	Oil	<i>Pseudomonas</i> [100%] ( $\gamma$ )	NR
I-34	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	NR
I-35	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	NR
I-36	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	NR
I-37	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	NR
I-38	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	NR
I-39	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	NR
I-40	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	NR
I-41	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	NR
I-42	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	NR
I-49	Prairie	<i>Achromobacter</i> [100%] ( $\beta$ )	NR
I-50	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	None



Strain #*	Isolation site characteristics	RDP Classifier**	NR/DN
I-51	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	None
I-52	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	None
I-53	Prairie	<i>Brevibacillus</i> [100%] (Bacilli)	NR
I-57	Prairie	<i>Stenotrophomonas</i> [100%] ( $\gamma$ )	DN
I-61	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	NR
I-62	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	None
I-63	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	None
I-64	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	None
I-65	Prairie	<i>Pseudomonas</i> [100%]( $\gamma$ )	DN
I-67	Prairie	<i>Pseudomonas</i> [100%]( $\gamma$ )	NR
I-68	Prairie	<i>Pseudomonas</i> [100%]( $\gamma$ )	NR
I-70	Prairie	<i>Acinetobacter</i> [100%] ( $\gamma$ )	NR
I-71	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	None
I-73	Prairie	<i>Pseudomonas</i> [100%]( $\gamma$ )	NR
I-74	Prairie	<i>Stenotrophomonas</i> [100%]( $\gamma$ )	NR

Strains were tested for nitrate/nitrite loss using the microtiter plate assay.

NR: Nitrate Reduction using microtiter plate assay.

DN: Nitrite reduction/denitrification using microtiter plate assay.

None: No reduction of nitrate or nitrite using microtiter plate assay.

\*Strain #: Represents the designated numbers from the original set of the 75 strains.

\*\*RDP Classifier: Ribosomal Database Project Classifier.

**Table 2. The distribution of 45 nitrate reducing bacteria. (NR), nitrite reducing bacteria (DN), and the strains that do not reduce nitrate/nitrite (None) along with the distribution of *Pseudomonas* and *Stenotrophomonas* among the three different soil sites.**

Site	# NR	# DN	#None	# <i>Pseudomonas</i>	# <i>Stenotrophomonas</i>
Brine	8	3	0	4	3
Oil	5	0	3	1	5
Uncontaminated	17	2	7	4	19

USA), was used to extract DNA from the cell suspensions by following the manufacturer's directions. The protocol combined mechanical (e.g., bead-beating) and chemical means to lyse the cells. Extracted DNA was stored at  $-20^{\circ}\text{C}$ . Controls for contamination included processing one reagent control to which sterile water rather than cells was added.

### **Molecular detection of 16S rRNA gene**

Universal bacterial primers for 16S rRNA were used to amplify an approximately 1400 bp fragment. Extracted DNA was used as the template in a polymerase chain reaction (PCR) using primer pair 27F/1492R (Wilson et al., 1990). The PCR products were visualized using agarose gel electrophoresis technique.

### **DNA sequencing and analysis**

PCR products were prepared for sequencing using ExoSAP-IT (USBiochemicals) to eliminate unincorporated primers and dNTPs so that they cannot interfere with downstream sequencing reactions (Bell, 2008). Sequencing was performed by the Oklahoma Medical Research Foundation (OMRF) DNA Sequencing facility by using an ABI 3730 capillary sequencer with primer pair 27F/1492R. Sequence chromatograms were analyzed to produce a consensus sequence, and compared to the GenBank nt/nr database BLASTN searches (Basic Local Alignment Search Tool) (Altschul et al., 1990).

### **Testing the ability to degrade naphthalene under aerobic conditions**

To test the ability of the 45 strains to degrade naphthalene under aerobic conditions, mineral salt medium plates without any carbon source were prepared (Youssef et al., 2004). Each of the 45 strains was streaked on a mineral salt medium plate and naphthalene crystals were added to the plate lids. The plates were incubated at room temperature for 2 weeks in a closed plastic container. *Pseudomonas putida* pG7, which contains the NAH7 naphthalene-degradation plasmid (Dunn and Gunsalus, 1973), was used as a positive control. Each of the 45 strains was also streaked on mineral salt medium plus glucose (0.2%) to test the ability of

each of the 45 strains to grow on the mineral salt medium with a commonly used carbon source.

### **Molecular detection of aerobic naphthalene degradation functional genes**

Three strains were further examined, namely: strain I-4 (*Ensifer*), strain I-21 (*Stenotrophomonas*), and strain I-24 (*Serratia*) (Table 1). For the chosen three strains, PCR was used to amplify a portion of the *nahAc* gene which encodes the large subunit of the iron-sulfur protein component of naphthalene dioxygenase, the initial enzyme in the aerobic naphthalene catabolic pathway (Simon et al., 1993). The primer pair used was nahAcl(5'-G1TTGCAGCTATCACGGCTGGGGCTTCGGC-3') corresponding to nucleotides 794 to 823 of the *nahAC* sequence, and nahAc3(5'-TTTCGACAATGGCGTAGGTCCAGACCTCGGT-3') corresponding to nucleotides 1495 to 1466 (Kurkel et al., 1988). The amplicon size with the primer pair nahAcl/nahAc3 is 701-bp (Herrick et al., 1993). DNA extracted from *Pseudomonas putida* G7 containing the NAH7 plasmid was used as a positive control.

### **Nitrate depletion/nitrite accumulation stoichiometry of cell suspensions**

Strains *Ensifer* I-4, *Stenotrophomonas* I-21, and *Serratia* I-24 which had shown their ability to reduce nitrate/nitrite after growth in nitrate broth in a microtiter plate were chosen to test their ability to reduce nitrate/nitrite under NR conditions. Nitrate broth medium (5.0 g  $\text{NaNO}_3/\text{L}$  plus 8.0g Nutrient broth/L) was prepared under strictly anaerobic conditions in which it was boiled and gassed with  $\text{N}_2/\text{CO}_2$  in an ice bath. Each of the three chosen strains was cultivated under NR (e.g. strictly anaerobic) conditions. The cultures were routinely grown in 125-mL serum bottles containing 50 mL of Nitrate broth medium. Two bottles of a well established culture (three transfers) from each strain were used to inoculate, anaerobically, two 1-L bottles each containing 500 mL nitrate broth. After overnight incubation at room temperature, the cells were anaerobically collected in the late exponential phase by centrifugation ( $10,000 \times g$ , 15 min,  $6^{\circ}\text{C}$ ) in sealed bottles and washed once

in anoxic 25 mM morpholinepropanesulfonic acid (MOPS) buffer (pH 7.2). The washed cells were resuspended in 20 mL of the same buffer and divided into two aliquots. Each aliquot was centrifuged again and suspended in MOPS buffer (pH 7.2) and normalized to similar O.D.<sub>600</sub> (~0.36). Glucose (20 mM) was provided as a substrate and the reactions were initiated with nitrate (5mM). Samples taken at various time points were filter-sterilized immediately and nitrate/nitrite concentrations were monitored by Ion Chromatography (model DX500 fitted with an AS-4A column; Dionex Corp., Sunnyvale, CA) as described in Caldwell et al. (1998).

### **Growth on hydrocarbons under NR conditions**

Growth (e.g. increase in optical density "O.D." at 600 nm) on hydrocarbons under NR conditions was tested using strains that showed the ability to reduce nitrate to nitrite under NR conditions. The tested strains were grown in 125-mL serum bottles containing 50 mL of the mineral medium (Widdel and Bak, 1992). One liter of this medium contains 1g NaCl, 0.5g NH<sub>4</sub>Cl, 0.3g KH<sub>2</sub>PO<sub>4</sub>, 0.4g MgCl<sub>2</sub>·6H<sub>2</sub>O, 0.15g CaCl<sub>2</sub>·2H<sub>2</sub>O, 1g of 10 mM KNO<sub>3</sub>, 5.0g yeast extract, and 10 mL RST metals (Tanner, 1997). Well-established cultures were used as inocula. The following substrates were tested: Gasoline (2 µl/50 mL medium), Naphthalene (10 mg/mL in 2,2,4,4,6,8,8-heptamethyl-nonane (HMN)), Benzene (0.225 mM), and Glucose (20 mM). Glucose was shown to be a good substrate for all three strains based on the API 20 NE strips (bioMérieux) results. Unamended substrate (no glucose or hydrocarbons) was used as a control and autoclaved cultures were used as negative controls. Cell densities, which were used to indicate growth and to infer substrate utilization, were determined by O.D. readings at 600 nm. Samples were taken at various time points and filter-sterilized immediately to be used in the following steps.

### **Nitrate depletion/nitrite accumulation when grown on a hydrocarbon substrate**

Nitrate/nitrite concentrations in the cultures of the strains that showed good growth on a hydrocarbon were monitored by Ion

Chromatography (model DX500 fitted with an AS-4A column; Dionex Corp., Sunnyvale, CA) as described (Caldwell et al., 1998).

### **Monitoring naphthalene depletion using Gas Chromatography**

Naphthalene depletion was monitored under nitrate-reducing conditions in the strain that showed nitrate depletion/nitrite accumulation when naphthalene was the carbon source. Mineral medium was prepared under NR conditions. Five milliliters of cell suspension were used to inoculate 40 mL mineral medium plus 3 mL overlay of naphthalene (5 mg/mL) dissolved in HMN. Five milliliters autoclaved inoculum, as a sterile control, was used to inoculate 40 mL minimal medium plus 3 mL overlay of naphthalene (5 mg/mL) in HMN. For unamended controls, 5 mL inoculum was used to inoculate 45 mL minimal medium. Blanks containing 45 mL minimal medium were overlaid with 3 mL naphthalene (5 mg/mL) in HMN. All samples were prepared in triplicates. The concentration of naphthalene in the incubations was measured by periodic sampling of the HMN overlay and injection of a small portion (1 µL) directly into an Agilent Technologies 5973 Network Gas Chromatograph (GC) equipped with a HP5-MS column (30 m x 0.25 mm, 0.25µm film) and a mass selective (MS) detector. The oven temperature was held at 100°C for 2 min, then increased at 5°C per min to 160°C and then raised at a rate 20°C per minute to 220°C. The injector temperature was 250°C. Helium was used as a carrier gas at a flow rate of 1.2 mL/min.

A parallel experiment was conducted in which the wash medium was made from the mineral medium without nitrate and without yeast extract to avoid any traces of yeast extract in the cell suspension. Briefly, after 5 transfers, 18 bottles (50 mL of anaerobic culture/bottle) were pooled under anaerobic conditions in 2 centrifuge bottles. The bottles were centrifuged for 20 min at 6000 xg at 6°C, subsequently centrifuged in smaller bottles for 10 min at 10000 xg at 6°C. The collected cells were washed twice with a mineral medium minus yeast extract and minus nitrate. Cell pellets were resuspended in 60 mL of wash medium. The following mixtures

were prepared: mixtures 1 and 2 (replicates) contained 10 mL of the cell suspension plus 3 mL of naphthalene (0.5 mg/ mL in HMN) and 0.5 mL of KNO<sub>3</sub> (20.2 mg/ mL), mixture 3 contained 10 mL of the cell suspension plus 3 mL of naphthalene (0.5 mg/ mL in HMN) (e.g. “No nitrate”), mixture 4 contained 10 mL of the cell suspension plus 0.5 mL of KNO<sub>3</sub> (20.2 mg/ mL), (e.g. “No naphthalene”), mixture 5 contained 10 mL of sterile (e.g. autoclaved) cell suspension plus 3 mL of naphthalene (0.5 mg/ mL) and 0.5 mL of KNO<sub>3</sub> (20.2 mg/ mL), mixture 6 contained 10 mL of the cell suspension plus 1 mL of glucose (20 mM) and 0.5 mL of KNO<sub>3</sub> (20.2 mg/ mL), mixtures 7 and 8 (replicates) contained 10 mL of wash medium plus 3 mL of naphthalene (0.5 mg/ mL) and 0.5 mL of KNO<sub>3</sub> (20.2 mg/ mL). Samples were taken for both Ion Chromatography (nitrate depletion and nitrite accumulation) and Gas Chromatography (naphthalene concentration) at 0, 5, and 10 days. A naphthalene standard curve was made using naphthalene concentrations at 0.5, 1.0, 3.0, and 5.0 mg/mL in HMN.

## Results

### Characteristics of the 45 strains

Out of a total of 75 strains obtained, 45 strains were chosen that included a subgroup representing the range of taxonomic diversity plus all the remaining *Stenotrophomonas* and *Pseudomonas* strains based on their 16S rRNA gene sequence as determined by the Ribosomal Database Project (RDP) Classifier (Wang et al., 2007; Cole et al, 2009). Most of the 45 strains were members of the genus *Stenotrophomonas* (e.g. 61%). The genus *Pseudomonas* represented 20% of the 45 strains. Proportionately more strains from these genera were chosen because previous studies have shown *Stenotrophomonas* and *Pseudomonas* to be hydrocarbon degraders (Gibson et al., 1974; Jung and Kafkewitz, 1996; Jeong et al., 2006; Lee et al., 2002; Shim and Yang, 1999). Other genera represented lower percentages (Table 1).

The majority (67%) of the 45 strains were nitrate reducers, according to colorimetric

detection of nitrate/nitrite by Griess reagents after inoculation into nitrate broth and growth in microtiter plates (Smibert and Krieg, 1994). On the other hand, 22% did not show any nitrate/nitrite reduction while 11% reduced both nitrate and nitrite (Table 2).

### Testing the ability to degrade naphthalene under aerobic conditions

None of the 45 strains grew on mineral salt medium plus naphthalene under aerobic conditions, although the positive control (e.g. *P. putida* G7) grew well. However, all 45 strains grew on mineral salt medium plus glucose. These results indicate that the 45 strains did not utilize naphthalene under these aerobic conditions.

### Molecular detection of aerobic naphthalene degradation functional genes

Three strains (*Ensifer* I-4, *Stenotrophomonas* I-21, and *Serratia* I-24) were tested using specific PCR primers for the presence of one functional gene of aerobic naphthalene degradation. Our PCR results showed that we did not detect the naphthalene dioxygenase gene in any of the three strains when we used nahAcl/nahAc3 primer pair. However, we obtained amplicons from all three strains. The amplicons obtained from *Ensifer* strain I-4 and *Serratia* strain I-24 were larger in size than the expected nahAc size (701 bp) but after sequencing, *Ensifer* strain I-4 and *Serratia* strain I-24 sequences were not significantly similar to nahAc of *P. putida* G7 or to any sequence in the GenBank database. The amplicon from *Serratia* strain I-21 was similar in size to the nahAc. But, after sequencing, the top two matches (93%) were similar to succinate dehydrogenase, a flavoprotein subunit in *Burkholderia* sp. JV3 (CP002986), and to a dehydrogenase, a flavoprotein subunit in *S. maltophilia* R5S1-3 (CP001111).

### Nitrate depletion/nitrite accumulation under NR conditions

Since none of the 45 strains utilized naphthalene under aerobic conditions, a subset of three strains, namely *Ensifer* strain I-4, its 16S rRNA gene sequence was 100% similar to that of *Ensifer* sp. TA12-B (HM219616),

*Stenotrophomonas* strain I-21, its 16S rRNA gene sequence was 100% similar to that of *Stenotrophomonas maltophilia* strain YNA104-1 (JN867123), and *Serratia* strain I-24, its 16S rRNA gene sequence was 99% similar to that of *Serratia marcescens* strain NBRC 3736 (AB680122) was chosen to test their ability to degrade different hydrocarbons (HC) including naphthalene under NR conditions. Before testing their ability to degrade hydrocarbons, we tested the ability of the three strains to reduce nitrate/nitrite under NR conditions with yeast extract as their carbon source. Ion chromatography was used to study nitrate reduction under NR conditions. After providing cell suspension of *Serratia* I-24 with 5 mM nitrate, nitrate was depleted down to 2.17 mM and nitrite was accumulated up to 2.17 mM after 120 minutes of incubation at room temperature (23-25°C) under NR conditions (Figure 1.a). After further incubation for 24 hours nitrate was completely depleted (e.g. 0 mM) and nitrite was accumulated up to 1.3 mM (data not shown). However, in *Ensifer* strain I-4, the 5mM nitrate was depleted completely after 36 hours but nitrite did not accumulate to any significant level. The highest level nitrite reached was 0.15 mM after 36 hours then reduced to 0 mM on hour 39 (Figure 1.b). Finally, in *Stenotrophomonas* strain I-21, the 5mM nitrate was almost depleted completely (e.g. 0.01 mM) after 7 hours and the nitrite accumulated up to 3.8 mM after 9 hours. Subsequently, nitrite started to decrease slowly until it reached 3 mM after 22 hours (Figure 1.c).

Based on the depletion of nitrate and accumulation of nitrite, *Serratia* strain I-24 and *Stenotrophomonas* strain I-21 were confirmed as nitrate reducers. However, *Ensifer* strain I-4 did not show any significant accumulation of nitrite during sampling points although all nitrate was depleted after 36 hours suggesting *Ensifer* reduced nitrite and therefore might be a denitrifier.

### **Growth on hydrocarbons under NR conditions**

The two strains that showed nitrate reduction under NR conditions (e.g. *Stenotrophomonas* strain I-21 and *Serratia* strain I-24) were tested for growth in the presence of different hydrocarbons,

namely gasoline, benzene, or naphthalene, under NR conditions. *Serratia* strain I-24 showed little growth on any of the hydrocarbons used, though it grew well on glucose. On glucose, the O.D. reached its highest value (0.611) after 2 days (Figure 2.a). Naphthalene was the best substrate among the hydrocarbons with an O.D. of 0.26 after 20 days (Figure 2.a). In contrast, *Stenotrophomonas* strain I-21 grew best on naphthalene reaching even higher densities than those on glucose. Its O.D. on naphthalene reached 0.202 after 41 days, while the highest O.D. on glucose was 0.069 after 13 days of incubation (Figure 2.b). Since this study focused on hydrocarbon degradation, and since *Serratia* strain I-24 did not show good growth on any of the hydrocarbon used as substrates, *Serratia* strain I-24 was eliminated from further tests.

### **Nitrate depletion/nitrite accumulation in *Stenotrophomonas* strain I-21 when grown on naphthalene**

Glucose, naphthalene, and unamended medium (e. g. no added carbon source) were used in monitoring nitrate depletion/nitrite accumulation in *Stenotrophomonas* strain I-21. Although well-established cultures (5 transfers) were used in this study, there was still nitrate reduction/nitrite accumulation in the unamended medium samples (Figure 3) which indicates an unknown carbon source was utilized at the expense of nitrate. In fact, extracellular materials were visible inside the culture bottles which could serve as an alternative carbon source. We hypothesized that *Stenotrophomonas* strain I-21 degraded naphthalene under NR conditions. To test this hypothesis, naphthalene depletion in *Stenotrophomonas* strain I-21 was monitored using Gas Chromatography to follow the loss of naphthalene at the expense of nitrate. An alternative hypothesis was that extracellular materials or media components might serve as substrates under NR conditions. To test this hypothesis, we monitored nitrate loss and nitrite accumulation in unamended substrate samples using cells washed in medium without nitrate or yeast extract.

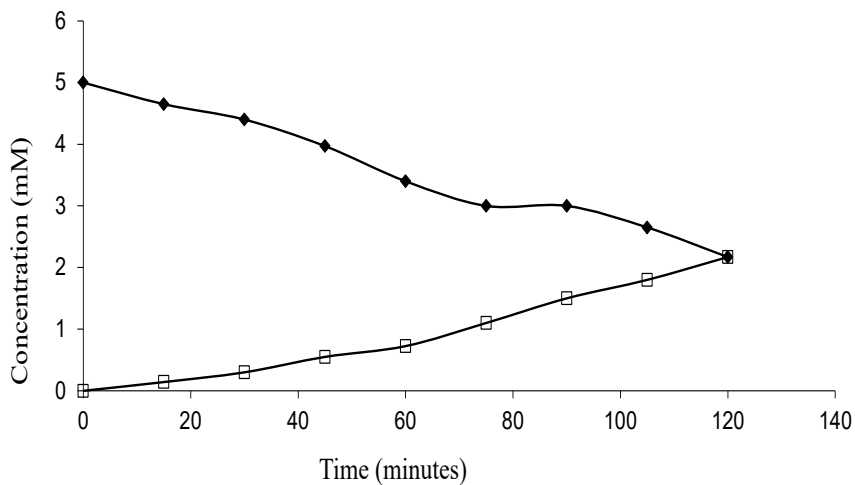


Figure 1.a. Nitrate/Nitrite Stoichiometry of *Serratia* strain I-24.

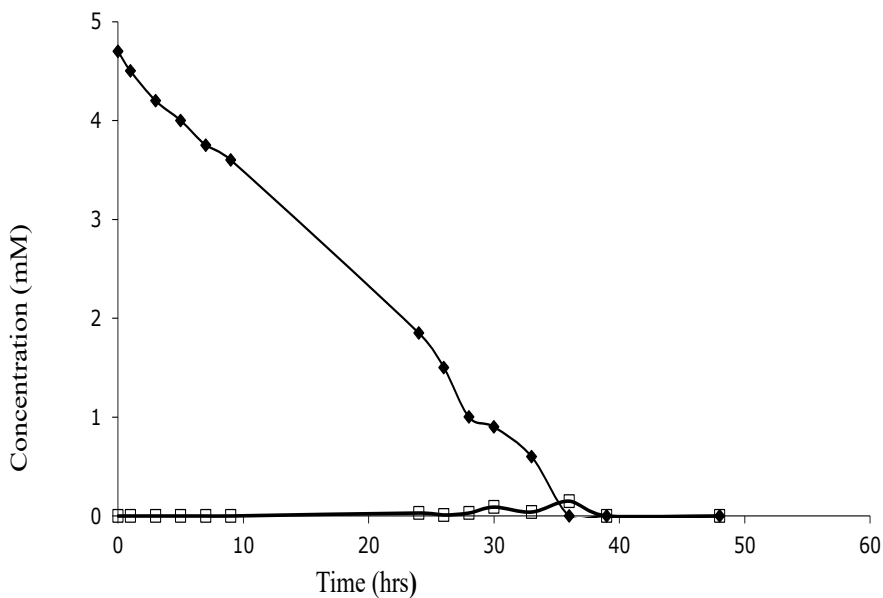


Figure 1.b. Nitrate/Nitrite Stoichiometry of *Ensifer* strain I-4.

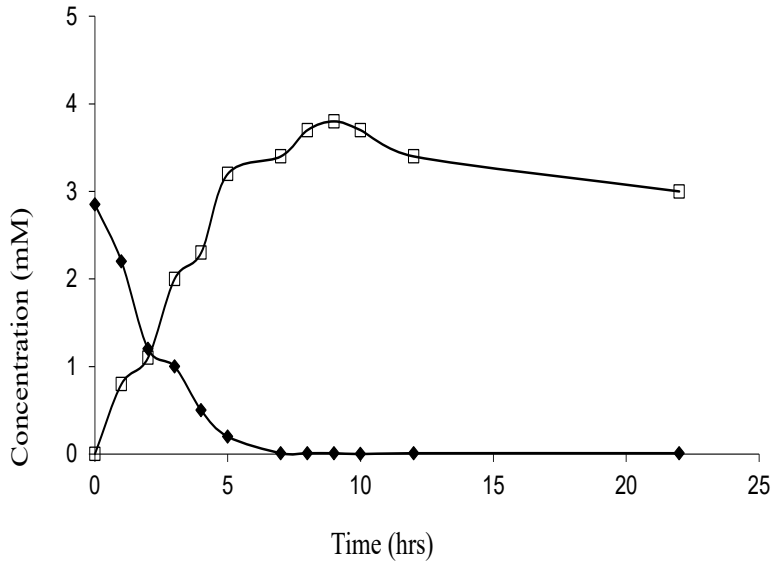


Figure 1.c. Nitrate/Nitrite Stoichiometry of *Stenotrophomonas* strain I-21.

**Figure 1. Nitrate depletion and nitrite accumulation.** A cell suspension was obtained after an overnight incubation of a well-established culture (three transfers) for three strains grown under strictly anaerobic conditions. Glucose (20 mM) was provided as a substrate and the reactions were initiated with 5 mM nitrate. Two replicates for each strain were pooled together to obtain one cell suspension for each strain. Time in minutes is plotted on the X axis and the nitrate (diamonds)/nitrite (hollow squares) concentration in micromolars is plotted on the Y axis. a. *Serratia* strain I-24. b. *Ensifer* strain I-4. c. *Stenotrophomonas* strain I-21.

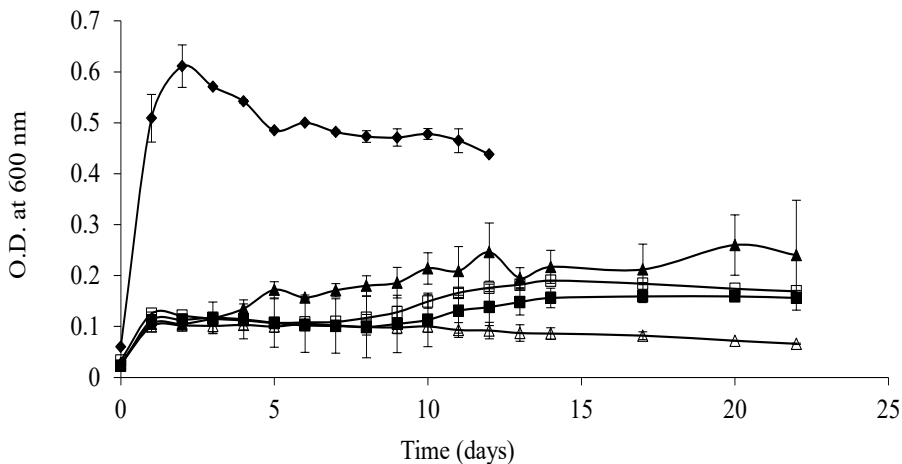


Figure 2.a. Growth of *Serratia* strain I-24 on various substrates under NR conditions.

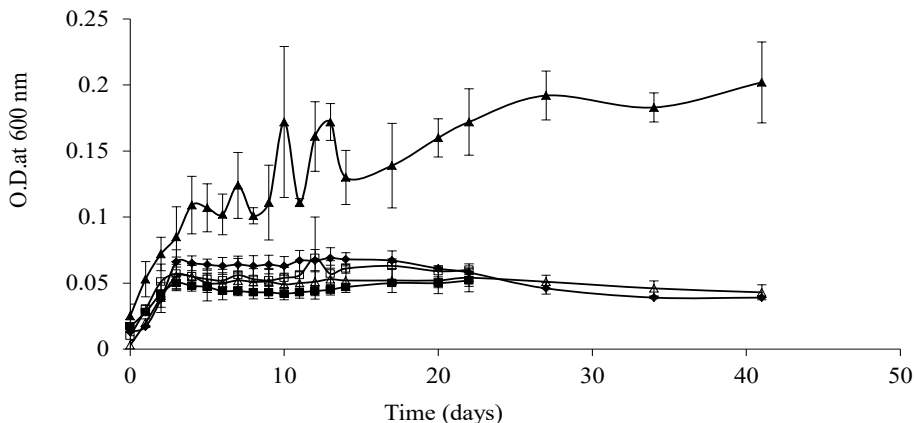


Figure 2.b. Growth of *Stenotrophomonas* strain I-21 on various substrates under NR conditions.

Figure 2. Growth curve on different substrates: Glucose (20 mM) (diamonds, average of 2 samples), naphthalene (10 mg/mL in 2,2,4,4,6,8,8-heptamethyl-nonane (HMN)) (filled triangles, average of 3), benzene (0.225 mM) (hollow squares, average of 2), gasoline (2 µl/50 mL medium) (filled squares, average of 3), and unamended (hollow triangles, average of 3). Time in days is plotted on the X axis and the O.D. at 600 nm is plotted on the Y axis. a. *Serratia* strain I-24. b. *Stenotrophomonas* strain I-21.

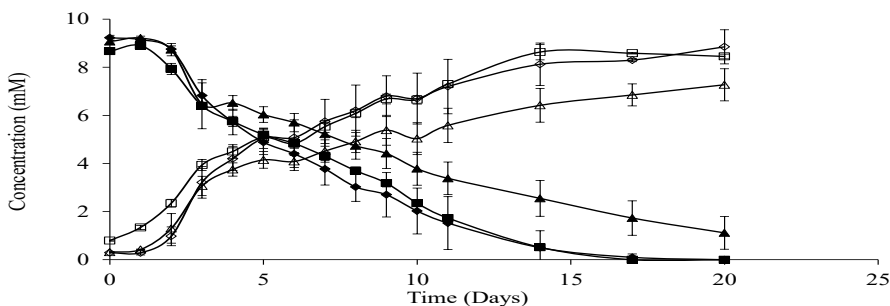


Figure 3. Nitrate depletion/nitrite accumulation by *Stenotrophomonas* strain I-21 on various substrates.

Figure 3. Nitrate depletion/nitrite accumulation by *Stenotrophomonas* strain I-21 on various substrates: Glucose (20 mM) (average of 2 samples). Nitrate as filled diamonds and nitrite as hollow diamonds, naphthalene (10 mg/mL in 2,2,4,4,6,8,8-heptamethyl-nonane (HMN)) (average of 3). Nitrate as filled squares and nitrite as hollow squares, unamended (average of 3). Nitrate as filled triangles and nitrite as hollow triangles. Time in days is plotted on the X axis and the nitrate/nitrite concentrations (µM) is plotted on the Y axis.



**Table 3. Stoichiometry of naphthalene loss coupled to nitrate reduction by *Stenotrophomonas* strain I-21.**

Naphthalene mineralization:  $C_{10}H_8 + 24NO_3^- \rightarrow 10CO_2 + 24 NO_2 + 4H_2O$

Nitrate loss (mM)	6.2 ± 0.05
Total naphthalene (mM)	1.5 ± 0.05
Naphthalene consumed (mM)	0.15
Theoretical consumed naphthalene (mM)	0.25
% of expected	60%

After four weeks of incubation; initial naphthalene level was 5 mg/mL.

$NO_3^-$  consumed from the unamended was 5 mM ± 0.2.

### Monitoring naphthalene depletion in *Stenotrophomonas* strain I-21 using Gas Chromatography

Naphthalene loss at the expense of nitrate in *Stenotrophomonas* strain I-21 was monitored using Gas Chromatography. The actual naphthalene consumed was estimated to be 0.15 mM (Table 3). However, the theoretical naphthalene consumed based on the naphthalene mineralization equation ( $C_{10}H_8 + 24NO_3^- \rightarrow 10CO_2 + 24 NO_2 + 4H_2O$ ) (Rockne et al., 2000) should have been 0.25 mM, based on the loss of nitrate. So, the actual naphthalene lost is 60% of the theoretical. This means that there was more nitrate reduced than naphthalene depleted. The excess of the reduced nitrate might drive the degradation of media components or the extracellular products that were produced by *Stenotrophomonas* strain I-21. This hypothesis was supported by the fact that 5 mM nitrate was consumed even in the unamended substrate culture (Table 3).

To avoid having traces of media components such as yeast extract even after 5 transfers, the same experiment above was repeated with the following changes. The wash medium was made this time from the mineral medium without nitrate and without yeast extract. Almost 50% of nitrate was reduced in the first five days in samples 1 and 2, containing cells, naphthalene, and nitrate (Table 4). However, the GC results showed that no loss of naphthalene during the course of the experiment. So, it appears that there was some other electron donor, perhaps extracellular

materials and/or medium components drove the reduction of nitrate to nitrite. Samples 7 and 8, which did not contain viable cells, showed a similar pattern, suggesting abiotic loss of nitrate. The existence of an electron donor other than naphthalene was confirmed because nitrate was lost from sample 4 (with nitrate without naphthalene) for the first five days. Between days 5 and 10 there was no more loss of nitrate, nitrate remained almost the same from day 5 to day 10. This means that the electron donor was not sufficient to support nitrate reduction for more than 5 days. Sample 6 that contained glucose continued nitrate depletion until nitrate was completely utilized which means that microorganisms consumed their substrates at the expense of nitrate. We are unable to account for some of the initial differences in nitrate level and the loss shown on days 5 and 10 in control samples 7 and 8 (e.g. without cells).

## Discussion

### PAH degradation under aerobic conditions

None of the 45 strains tested were able to grow in mineral medium containing naphthalene as the sole carbon source under aerobic conditions. Thirty six of the strains were *Stenotrophomonas* or *Pseudomonas*, both genera are known to contain many strains with the ability to degrade different hydrocarbons (Gibson et al., 1974; Jung and Kafkewitz, 1996; Jeong et al., 2006; Lee et al., 2002; Shim and Yang, 1999). For example, complete mineralization of aromatic compounds

under aerobic conditions was performed by *Stenotrophomonas* HPC383 (Verma et al., 2011). Different microorganisms may use different pathways for hydrocarbon degradation (Fuchs et al., 2011). PAH degradation under aerobic conditions by gram-positive bacteria has not been studied as extensively as that in gram-negative bacteria under the same conditions. Recent studies documented genetic and biochemical analysis of PAH aerobic degradation by *Rhodococcus*, *Mycobacterium*, *Terrabacter*, and *Nocardioideis* (Boyd et al., 1997; Brezna et al., 2003; Khan et al., 2001; and , Nojiri et al., 2001). Although one of our 75 strains is *Rhodococcus*, we did not test its ability to utilize naphthalene under aerobic or NR conditions.

### Detecting naphthalene aerobic degradation genes

The *nahAc* gene coding for a subunit of naphthalene dioxygenase was not detected in any of the three tested strains using the primer pair nahAcl/nahAc3. Although it may be that the primers did not amplify the *nahAc* gene in our strains if they had a variant *nahAc* sequence, it is more likely that these genes were absent, given the lack of growth on naphthalene vapors under aerobic conditions. Polyaromatic hydrocarbon degradation pathways are well studied in *Pseudomonas* (Bosch et al., 1999; Simon et al., 1993). The best studied PAH dioxygenase is naphthalene dioxygenase from *Pseudomonas putida* NCIB 9816-4 (Kauppi et al., 1998; Lee et al., 1997; Parales et al., 2000), encoded by the NAH plasmid pDTG1 (Dennis et al., 2004). The *nah* genes have been found in a broad variety of bacteria such as *Pseudomonas*, *Burkholderia*, and *Ralstonia* and have been found in strains originating from many different geographic locations (Ahn et al., 1999; Ferrero et al., 2002; Lloyd-Jones et al., 1999, Tuomi et al., 2004; Widada et al., 2002).

Indeed, as more PAH-degrading bacteria have been isolated and characterized, it has become obvious that *Pseudomonas* and the *nah*-like genes represent only a fraction of the PAH degradation picture. However, surveys of PAH degradation potential frequently rely on *nah*-based primers or probes to assess biodegradation

potential in the environment (Ahn et al., 1999; Stapleton et al., 1998; Wilson et al., 1999).

The *phn* genes, possessing activity similar to that of the *nah* genes, are more distantly related PAH degradation genes and have also been studied. *Burkholderia* sp. strain RP007 that contains the *phn* genes was isolated from a PAH-contaminated site based on its ability to degrade phenanthrene (Laurie and Lloyd-Jones., 1999). It was shown that *Pseudomonas*-type *nah* genes are not always dominant in the environment and that the *phn*-type genes can have a greater ecological significance than the *nah*-like genotype (Laurie and Lloyd-Jones, 2000). Although we did not test the presence of *phn* genes in the three strains, again the lack of growth on naphthalene under aerobic conditions is a more comprehensive test than detection using specific primers.

### Degradation of polyaromatic hydrocarbons under nitrate-reducing conditions

We acknowledge that our results do not represent an extensive study of the anaerobic degradation of polyaromatic hydrocarbons (see Fuchs et al., 2011 for a review). Nevertheless, none of the tested strains (e.g. *Stenotrophomonas* strain I-21 and *Serratia* strain I-24) degraded the tested hydrocarbons under NR conditions. However, nitrate depletion and nitrite accumulation were shown in *Stenotrophomonas* strain I-21 and *Serratia* strain I-24 under NR conditions. Strains of *Stenotrophomonas maltophilia* (Kaparullina et al., 2009) and *Serratia marcescens* (Borsodi et al., 2010) were previously shown to have the ability to reduce nitrate. In addition, the ability of *Ensifer* strain I-4 to reduce nitrite suggests its ability to denitrify. In fact, it was shown that two *Ensifer adhaerens* strains were able to utilize different substrates such as benzoate, 3-hydroxybenzoate, and 2-fluorobenzoate under aerobic and DN conditions (Song et al., 2000). The ability of *Ensifer* strain I-4 to degrade hydrocarbons under NR conditions was not studied.

To the best of our knowledge, strains of *S. marcescens* have never been shown to degrade hydrocarbons which is consistent with our

**Table 4. Naphthalene and nitrate levels over time in cultures of *Stenotrophomonas* strain I-21 grown without yeast extract.**

Day	Day 0		Day 5		Day10	
	Naph (mg/mL)	Nitrate (mM)	Naph (mg/mL)	Nitrate (mM)	Naph (mg/mL)	Nitrate (mM)
1 <sup>a</sup>	0.62	18.6	0.6	8.7	0.64	7.7
2 <sup>a</sup>	0.58	19	0.61	8.8	0.59	7.7
3 <sup>b</sup>	0.66	0	0.57	0	0.56	0
4 <sup>c</sup>	ND <sup>g</sup>	14.6	ND	9	ND	7.8
5 <sup>d</sup>	0.65	11.3	0.47	11.2	0.6	10.5
6 <sup>e</sup>	ND	2.9	ND	3.4	ND	0.49
7 <sup>f</sup>	0.58	22.3	0.4	9.8	0.59	9
8 <sup>f</sup>	0.63	19.3	0.37	9.9	0.61	9.1

<sup>a</sup> Cell suspension + naphthalene + KNO<sub>3</sub>.

<sup>b</sup> No nitrate: cell suspension + naphthalene.

<sup>c</sup> No naphthalene: cell suspension + KNO<sub>3</sub>.

<sup>d</sup> Sterile cell suspension + naphthalene + KNO<sub>3</sub>.

<sup>e</sup> Cell suspension + glucose + KNO<sub>3</sub>.

<sup>f</sup> Wash medium + naphthalene + KNO<sub>3</sub>.

<sup>g</sup> ND: Not determined because sample did not contain naphthalene.

findings. However, the ability of *S. marcescens* to degrade different contaminants such as the organophosphate insecticide, diazinon (O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate) was shown before (Abo-Amer, 2011). On the other hand, *S. maltophilia* strains have been isolated from oil-polluted soils (Duarte et al., 2001) which may indicate the ability to degrade hydrocarbons. However, our final Gas Chromatography results showed that our *Stenotrophomonas* strain I-21 was not able to mineralize naphthalene under NR conditions and that the reduction of nitrate in our previous experiments might have been driven by the degradation of extracellular materials observed in the culture bottles. Therefore, one must be cautious before stating a microorganism's ability to mineralize hydrocarbons based on its isolation from hydrocarbon-contaminated sites. Also, our GC results (Table 4) showed that measuring naphthalene loss is a necessary complement to measuring nitrate loss since our results showed around 50% loss of nitrate in the control samples 7 and 8 (e.g. no cell suspension), while documenting

no loss of naphthalene occurred (Table 4).

### Loss of plasmid-borne naphthalene degradation genes

It is possible that at least some of the strains were capable of naphthalene degradation but lost the ability during the process of isolation. Pathways for aerobic degradation are often borne on conjugative plasmids (Basta et al., 2004). In a previous study, a plasmid-borne naphthalene degradation phenotype was transferred from *P. putida* CSV86 to *S. maltophilia* CSV89 by conjugation. However, the transferred property was lost completely when transconjugants were grown on glucose or on a medium that contained tryptone, yeast extract, and NaCl. This loss indicates that the degradation property was not stable and it was lost when more favorable substrate was available (Basu and Phale, 2008). In other cases, a transferred plasmid (e.g. NAH7) was shown to be rapidly selected to become more stable (Duncan et al., 1995). We did not make any attempt to screen for plasmids in our strains, or test for growth on naphthalene immediately upon isolation of the strains.

## Response of soil microbial communities to contamination by crude oil

The structure of microbial communities in soil changes due to changes in environmental conditions such as oil contamination that can cause drastic effects on microbial diversity in soil. The supposed shifts in community structure caused by selective pressure (e.g. oil) are important for the catabolism of soil pollutants (Powell et al., 2006). In fact, the degradation of PAH from geographically diverse soils suggests that this degradation is associated with distinct genera, notably *Sphingomonas* (formerly *Pseudomonas*) and *Burkholderia*, independent of geographic location (Mueller et al., 1997). A different study showed a shift in microbial community structure from alpha- and betaproteobacteria to gammaproteobacteria when a microbial community was exposed to a mixture of aromatic hydrocarbons (Stoffels et al., 1998). Other factors that alter the catabolism of soil pollutants and possibly select for NR/DN bacteria are the application of fertilizer containing nitrogenous compounds. In fact, fertilization increased hydrocarbon degradation in both anaerobic and aerobic soils, especially anaerobic soils. This increase of hydrocarbon degradation in the anaerobic soils corresponded with a shift in the denitrifier community composition and an increased abundance of denitrifiers and benzoyl-CoA reductase (Powell et al., 2006). However, in our study we found no evidence that PAH degradation was performed by culturable NR/DN bacteria from the Tallgrass Prairie sites.

## References

- Abo-Amer, A. 2011. Biodegradation of diazinon by *Serratia marcescens* DI101 and its use in bioremediation of contaminated environment. *J Microbiol Biotechnol.* 21:71-80.
- AbuBakr, S., S. L. Macmil, M. A. Nanny, and K. E. Duncan. 2008. Enzymatic transformation of humic substances by NDO. *Soil Biol Biochem.* 40: 2055-2062.
- Ahn, Y., J. Sanseverino, and G. S. Sayler. 1999. Analyses of polycyclic aromatic hydrocarbon-degrading bacteria isolated from contaminated soils. *Biodegradation.* 10:149-157.
- Al-Bashir, B., T. Cseh, R. Leduc, and R. Samson. 1990. Effect of soil/contaminant interactions on the biodegradation of naphthalene in flooded soils under denitrifying conditions. *Appl Microbiol Biotech.* 34:414-419.
- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J Mol Biol.* 215:403-410
- Alvarez, P. J. J., and T. M. Vogel. 1991. Substrate interactions of benzene, toluene, and paraxylene during microbial degradation by pure cultures and mixed culture aquifer slurries. *Appl Environ Microbiol.* 57:2981-2985.
- Atlas, R., and J. Bragg. 2009. Bioremediation of marine oil spills: when and when not—the Exxon Valdez experience. *Microb Biotechnol.* 2:213-221.
- Basta, T., A. Keck, J. Klein, and A. Stolz. 2004. Detection and characterization of conjugative degradative plasmids in xenobiotic-degrading *Sphingomonas* strains. *J Bacteriol.* 186:3862-3872.
- Basu, A., and P. S. Phale. 2008. Conjugative transfer of preferential utilization of aromatic compounds from *Pseudomonas putida* CSV86. *Biodegradation.* 19:83-92.
- Bell, J. 2008. A simple way to treat PCR products prior to sequencing using ExoSAP-IT. *BioTechniques.* 44:834.
- Borsodi, A. K., R. I. Kiss, G. Cech, B. Vajna, E. M. Tóth, and K. Márialigeti. 2010. Diversity and activity of cultivable aerobic planktonic bacteria of a saline Lake located in Sovata, Romania. *Folia Microbiol (Praha).* 55:461-466.
- Bosch, R., E. García-Valdés, and E. R. Moore. 1999. Genetic characterization and evolutionary implications of a chromosomally encoded naphthalene-degradation upper pathway from *Pseudomonas stutzeri* AN10. *Gene.* 236:149-157.
- Boyd, C., M. J. Larkin, K. A. Reid, N. D. Sharma, and K. Wilson. 1997. Metabolism of naphthalene, 1-naphthol, indene, and indole by *Rhodococcus* sp. strain NCIMB 12038. *Appl Environ Microbiol.* 63:151-155.
- Brezna, B., A. A. Khan, and C. E. Cerniglia. 2003. Molecular characterization of dioxygenases from polycyclic aromatic hydrocarbon-degrading *Mycobacterium* spp. *FEMS Microbiol Lett.* 223:177-183.

- Caldwell, M. E., R. M. Garrett, R. C. Prince, and J. M. Suffita. 1998. Anaerobic biodegradation of long-chain *n*-alkanes under sulfate-reducing conditions. *Environ Sci Technol.* 32: 2191-2195.
- Coates, J. D., J. Woodward, J. Allen, P. Philp, D. R. Lovley. 1997. Anaerobic degradation of polycyclic aromatic hydrocarbons and alkanes in petroleum-contaminated marine harbor sediments. *Appl Environ Microbiol.* 63:3589-3593.
- Cole, J. R., Q. Wang, E. Cardenas, J. Fish, B. Chai, R. J. Farris, A. S. Kulam-Syed-Mohideen, D. M. McGarrell, T. Marsh, G. M. Garrity, J. M. Tiedje. 2009. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucl Acids Res.* 37(Database issue):D141-D145.
- Davidova, I. A., L. M. Gieg, K. E. Duncan, and J. M. Suffita. 2007. Anaerobic phenanthrene mineralization by a carboxylating sulfate-reducing bacterial enrichment. *ISME J.* 1:436-42.
- Dennis, J. J., and G. J. Zylstra. 2004. Complete sequence and genetic organization of pDTG1, the 83 kilobase naphthalene degradation plasmid from *Pseudomonas putida* strain NCIB 9816-4. *J Mol Biol.* 341:753-768.
- Duarte, G. F., A. S. Rosado, L. Seldin, W. de Araujo, and J. D. van Elsas. 2001. Analysis of bacterial community structure in sulfurous-oil-containing soils and detection of species carrying dibenzothiophene desulfurization (*dsz*) genes. *Appl Environ Microbiol.* 67:1052-1062.
- Duncan, K. E., N. Ferguson, and C. A. Istock. 1995. Fitnesses of a conjugative plasmid and its host bacteria in soil microcosms. *Molecular Biology and Evolution* 12:1012-1021.
- Duncan, K. E., R. Kolhatkar, G. Subramaniam, R. Narasimhan, E. Jennings, S. Hettenbach, A. Brown, C. McComas, W. Potter, and K. Sublette. 1999. Microbial dynamics in oil-impacted prairie soil. *Appl Biochem Biotechnol.* 77-79:421-434.
- Dunn, N. W., and I. C. Gunsalus. 1973. Transmissible plasmid coding early enzymes of naphthalene oxidation in *Pseudomonas putida*. *J Bacteriol.* 114:974-979.
- Ensley, B. D., D. T. Gibson, and A. L. Laborde. 1982. Oxidation of naphthalene by a multicomponent enzyme system from *Pseudomonas* sp. strain NCIB 9816. *J Bacteriol.* 149:948-954.
- Ferrero, M., E. Llobet-Brossa, J. Lalucat, E. García-Valdés, R. Rosselló-Mora, and R. Bosch. 2002. Coexistence of two distinct copies of naphthalene degradation genes in *Pseudomonas* strains isolated from the western Mediterranean region. *Appl Environ Microbiol.* 68:957-962.
- Gibson, D. T., V. Mahadevan, and J. F. Davey. 1974. Bacterial metabolism of *para*- and *meta*-xylene: oxidation of the aromatic ring. *J Bacteriol.* 119:930-936.
- Godsy, E. M., D. F. Goerlitz, and D. Grbic-Galic. 1992. Methanogenic biodegradation of creosote contaminants in natural and simulated groundwater ecosystems. *GroundWater* 30:232-242.
- Heitkamp, M. A., J. P. Freeman, and C. E. Cerniglia. 1987. Naphthalene biodegradation in environmental microcosms: estimates of degradation rates and characterization of metabolites. *Appl Environ Microbiol.* 53:129-136.
- Herrick, J. B., E. L. Madsen, C. A. Batt, and W. C. Ghiorse. 1993. Polymerase chain reaction amplification of naphthalene-catabolic and 16S rRNA gene sequences from indigenous sediment bacteria. *Appl Environ Microbiol.* 59:687-694.
- Jeong, E., M. Hirai, and M. Shoda. 2006. Removal of p-xylene with *Pseudomonas* sp. NBM21 in biofilter. *J Biosci Bioeng.* 102:281-287.
- Jung, J. and D. Kafkewitz. 1996. Toluene and xylene degradation by a denitrifying strain of *Xanthomonas maltophilia* with limited or no oxygen. *Chemosphere.* 32:1843-1850.
- Kaparullina, E., N. Doronina, T. Chistyakova, and Y. Trotsenko. 2009. *Stenotrophomonas chelatiphaga* sp. nov., a new aerobic EDTA-degrading bacterium. *Syst Appl Microbiol.* 32:157-162.
- Kauppi, B., K. Lee, E. Carredano, R. E. Parales, D. T. Gibson, H. Eklund, and S. Ramaswamy. 1998. Structure of an aromatic-ring-hydroxylating dioxygenase-naphthalene 1,2-dioxygenase. *Struct Fold Des.* 6:571-586.

- Khan A. A., R. F. Wang, W. W. Cao, D. R. Doerge, D. Wennerstrom, and C. E. Cerniglia. 2001. Molecular cloning, nucleotide sequence, and expression of genes encoding a polycyclic aromatic ring dioxygenase from *Mycobacterium* sp. strain PYR-1. *Appl Environ Microbiol.* 67:3577-3585.
- Kurkela, S., H. Lehvälaiho, E. T. Palva, and T. H. Teeri. 1988. Cloning, nucleotide sequence and characterization of genes encoding naphthalene dioxygenase of *Pseudomonas putida* strain NCIB9816. *Gene.* 73:355-362.
- Laurie, A. D., and G. Lloyd-Jones. 1999. The *phn* genes of *Burkholderia* sp. strain RP007 constitute a divergent gene cluster for polycyclic aromatic hydrocarbon catabolism. *J Bacteriol.* 181:531-540.
- Laurie, A. D., and G. Lloyd-Jones. 2000. Quantification of *phnAc* and *nahAc* in contaminated New Zealand soils by competitive PCR. *Appl Environ Microbiol.* 66:1814-1817.
- Lee, E. Y., Y. S. Jun, K. S. Cho, and H. W. Ryu. 2002. Degradation characteristics of toluene, benzene, ethylbenzene, and xylene by *Stenotrophomonas maltophilia* T3-c. *J Air Waste Manag Assoc.* 52:400-406.
- Lee, K., B. Kauppi, R. E. Parales, D. T. Gibson, and S. Ramaswamy. 1997. Purification and crystallization of the oxygenase component of naphthalene dioxygenase in native and selenomethionine-derivatized forms. *Biochem Biophys Res Commun.* 241:553-557.
- Lloyd-Jones, G., A. D. Laurie, D. W. F. Hunter, and R. Fraser. 1999. Analysis of catabolic genes for naphthalene and phenanthrene degradation in contaminated New Zealand soils. *FEMS Microbiol Ecol.* 29:69-79.
- MacNaughton, S. J., J. R. Stephen, A. D. Venosa, G. A. Davis, Y. J. Chang, and D. C. White. 1999. Microbial population changes during bioremediation of an experimental oil spill. *Appl Environ Microbiol.* 65: 3566-3574.
- Mihelcic, J. R., and R. G. Luthy. 1988. Degradation of polycyclic aromatic hydrocarbon compounds under various redox conditions in soil-water systems. *Appl Environ Microbiol.* 54:1182-1187.
- Mihelcic, J. R., and R. G. Luthy. 1988. Microbial degradation of acenaphthene and naphthalene under denitrification conditions in soil-water systems. *Appl Environ Microbiol.* 54:1188-1198.
- Mueller, J. G., R. Devereux, D. L. Santavy, S. E. Lantz, S. G. Willis, and P. H. Pritchard. 1997. Phylogenetic and physiological comparisons of PAH-degrading bacteria from geographically diverse soils. *Antonie Van Leeuwenhoek.* 71:329-343.
- Ni Chadhain, S. M. N., R. S. Norman, K. V. Pesce, J. J. Kukor, and G. J. Zylstra. 2006. Microbial dioxygenase gene population shifts during polycyclic aromatic hydrocarbon biodegradation. *Appl Environ Microbiol.* 72:4078-4087.
- Nojiri, H., H. Habe, and T. Omori. 2001. Bacterial degradation of aromatic compounds via angular dioxygenation. *J Gen Appl Microbiol.* 47:279-305.
- Parales, R. E., K. Lee, S. M. Resnick, H. Jiang, D. J. Lessner, and D. T. Gibson. 2000. Substrate specificity of naphthalene dioxygenase: effect of specific amino acids at the active site of the enzyme. *J Bacteriol.* 182:1641-1649.
- Powell, S. M., S. H. Ferguson, I. Snape, and S. D. Siciliano. 2006. Fertilization stimulates anaerobic fuel degradation of Antarctic soils by denitrifying microorganisms. *Environ Sci Technol.* 40:2011-2017.
- Renella, G., U. Szukics, and L. Landi. 2007. Quantitative assessment of hydrolase production and persistence in soil. *Biology and Fertility of Soils* 44:321-329.
- Rockne, K. J., J. C. Chee-Sanford, R. A. Sanford, B. P. Hedlund, J. T. Staley, and S. E. Strand. 2000. Anaerobic naphthalene degradation by microbial pure cultures under nitrate-reducing conditions. *Appl Environ Microbiol.* 66:1595-1601.
- Röling, W. F., M. G. Milner, M. Jones, K. Lee, F. Daniel, R. J. P. Swannell, and I. M. Head. 2002. Robust hydrocarbon degradation and dynamics of bacterial communities during nutrient-enhanced oil spill bioremediation. *Appl Environ Microbiol.* 68: 5537-5548.

- Shim, H., and S. T. Yang. 1999. Biodegradation of benzene, toluene, ethylbenzene, and o-xylene by a coculture of *Pseudomonas putida* and *Pseudomonas fluorescens* immobilized in a fibrous-bed bioreactor. *J Biotechnol.* 67:99-112.
- Simon, M. J., T. D. Osslund, R. Saunders, B. D. Ensley, S. Suggs, A. Harcourt, W. Suen, D. Cruden, D. T. Gibson, and G. Zylstra. 1993. Nucleotide sequences encoding the genes for naphthalene dioxygenase in *Pseudomonas putida* strain G7 and *Pseudomonas putida* strain NCIB. *Gene.* 127:31-37.
- Smibert, R. M., and N. R. Krieg. 1994. Phenotypic characterization. In: Gerhardt, P., R. G. E. Murray, W. A. Wood, and N. R. Krieg, Editors, *Methods for General and Molecular Bacteriology*, American Society for Microbiology, p. 649.
- Smith, J. M., S. J. Green, C. A. Kelly, L. Prufert-Bebout, and B. M. Bebout. 2008. Shifts in methanogen community structure and function associated with long-term manipulation of sulfate and salinity in a hypersaline microbial mat. *Environ Microbiol.* 10: 386-394.
- Song, B., N. J. Palleroni, and M. M. Häggblom. 2000. Isolation and characterization of diverse halobenzoate-degrading denitrifying bacteria from soils and sediments. *Appl Environ Microbiol.* 66: 3446-3453.
- Stapleton, R. D., and G. S. Sayler. 1998. Assessment of the Microbiological Potential for the Natural Attenuation of Petroleum Hydrocarbons in a Shallow Aquifer System. *Microb Ecol.* 36:349-361.
- Stoffels, M., R. Amann, W. Ludwig, D. Hekmat, and K. H. Schleifer. 1998. Bacterial community dynamics during start-up of a trickle-bed bioreactor degrading aromatic compounds. *Appl Environ Microbiol.* 64:930-939.
- Stursova, M., and R. L. Sinsabaugh. 2008. Stabilization of oxidative enzymes in desert soil may limit organic matter accumulation. *Soil Biol Biochem.* 40: 550-553.
- Sublette, K., E. Jennings, C. Mehta, K. Duncan, J. Brokaw, T. Todd, and G. Thoma. 2007. Monitoring soil ecosystem recovery following bioremediation of a terrestrial crude oil spill with and without a fertilizer amendment. *Soil & Sediment Contamination.* 16:181-208.
- Sublette, K. L., J. B. Tapp, J. B. Fisher, E., Jennings, K. Duncan, G. Thoma, J. Brokaw, and T. Todd. 2007. Lessons Learned in Remediation and Restoration in the Oklahoma Prairie: A Review. *Applied Geochemistry.* 22:2225-2239.
- Tuomi, P. M., J. M. Salminen, and K. S. Jørgensen. 2004. The abundance of *nahAc* genes correlates with the 14C-naphthalene mineralization potential in petroleum hydrocarbon-contaminated oxic soil layers. *FEMS Microbiol Ecol.* 51:99-107.
- Verma, V., S. C. Raju, A. Kapley, V. C. Kalia, G. S. Kanade, H. F. Dagainawala, and H. J. Purohit. 2011. Degradative potential of *Stenotrophomonas* strain HPC383 having genes homologous to *dmp* operon. *Bioresour Technol.* 102:3227-3233.
- Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive bayes classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol.* 73:5261-5267.
- Widada, J., H. Nojiri, K. Kasuga, T. Yoshida, H. Habe, and T. Omori. 2002. Molecular detection and diversity of polycyclic aromatic hydrocarbon-degrading bacteria isolated from geographically diverse sites. *Appl Microbiol Biotechnol.* 58:202-209.
- Widdel, F., and F. Bak. 1992. Gram-negative mesophilic sulfate-reducing bacteria, p. 3352-3378. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), *The prokaryotes*, 2nd ed. Springer-Verlag, New York, N.Y.
- Wilson, M. S., C. Bakermans, and E. L. Madsen. 1999. In situ, real-time catabolic gene expression: extraction and characterization of naphthalene dioxygenase mRNA transcripts from groundwater. *Appl Environ Microbiol.* 65:80-87.
- Wilson, K. H., R. B. Blitchington, and R. C. Greene. 1990. Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. *J Clin Microbiol.* 28:1942-1946.
- Youssef, N., K. E. Duncan, D. Nagle, K. N. Savage, R. Knapp, and M. J. McInerney. 2004. Comparison of methods to detect biosurfactant production by diverse microorganisms. *J Microbiol. Methods* 56:339-347

Zhou, N. Y., J. Al-Dulayymi, M. S. Baird, and P. A. Williams. 2002. Salicylate 5-hydroxylase from *Ralstonia* sp. strain U2: a monooxygenase with close relationships to and shared electron transport proteins with naphthalene dioxygenase. *J Bacteriol.* 184:1547-1555.

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# Longitudinal Development of Attention to Hemispheric Lateralization in Emotion Perception

**Robert D. Mather**

Department of Psychology, University of Central Oklahoma, Edmond, OK 73034

**Cidnee Ray**

Department of Psychology, University of Central Oklahoma, Edmond, OK 73034

**Katherine Jones**

Department of Psychology, University of Central Oklahoma, Edmond, OK 73034

**Erin McReynolds**

Department of Psychology, University of Central Oklahoma, Edmond, OK 73034

## Introduction

The human visual system plays an important role in the dynamics of social interaction. The direction of eye gaze and amount of pupil dilation can indicate attention and provide insight into the cognitive processes that may affect the initial elements of a decision. Farroni, Massaccesi, Pividori, and Johnson (2004) tracked the eye movements of infants and found that newborns can attend to the visual systems of target faces, which indicates that humans join this social interaction system immediately. Dodd, Van der Stigchel, and Hollingworth (2009) tracked the eye movements of adults and found the complexities of the visual system extend to visual search strategies (inhibition of return and facilitation of return) where people return faster or slower to a previously viewed part of a scene depending on context, which demonstrates that visual search is not random. This is important in being able to prioritize elements of a scene to examine, particularly during a limited viewing.

Emotional expressions serve to communicate our emotional state to others (Wilson, 2006), deceive others regarding our emotional expressions (Feldman, 2009), and give us information through which to interpret our own emotions (Levenson, Ekman, & Friesen, 1990).

There are six basic emotions and expressions (anger, fear, disgust, sadness, happiness, and surprise) as well as others that occur cross-culturally (Ekman, 1992), and, to make things more complicated, we must deal with affect blends (Szameitat et al., 2009) and ambivalent emotions (Larsen, McGraw, & Cacioppo, 2001; Larsen, To, & Fireman, 2007). It is important to a perceiver to accurately perceive the emotions of others (though it may be important to the other person to be deceptive with their emotional expression), as this can communicate intentions and give the perceiver an opportunity to predict the behavior of the expresser.

Over the last two decades, there has been an increase in interest in eye movement research, but very few of these studies have focused on typically-developing infants and toddlers. The majority of infant and toddler studies have been with inanimate objects that are unfamiliar to the participants. Studies that have included familiar stimuli lack the benefit of using eye tracking technology.

The aim of this study is to investigate the development of human emotion perception. The rationale is that emotion perception is dynamic, rather than stable, and changes across time as children get more experience with a variety of subtle adult expressions. We hypothesize that

children focus on very specific cues that reveal information about the emotional state of the target.

The current longitudinal case study provides new information of a child's interest in familiar scenes and objects. The researchers presented the participant with still images of scenes and objects from the participant's home. While viewing these images, the participant's eye movements were recorded by an eye tracking device. The participant was presented with these images in a video at the age of 15 months, and with still-frame images at the ages of 27 months, 33 months, 38 months, and 47 months.

## Methods

### Participant

After gaining parental consent, one male participant was assessed at the ages of 15 months, 27 months, 33 months, 38 months, and 47 months.

### Procedure

An Applied Sciences Laboratory Series 5000 eye tracker captured the participant's eye movements, including eye fixation coordinates and gaze trajectories. The parents of the participant recorded approximately 11 minutes of digital video footage at the infant's home. The video footage included objects and scenes frequently encountered by the participant. The original video was made into slides containing images of scenes and objects that the participant encountered frequently, omitting images that were encountered less frequently. The calibration procedure consisted of Sesame Street characters appearing on the screen one at a time on each of the nine calibration points. The character appeared on each point long enough for calibration, and then disappeared so there was only one point of focus on the screen at a time.

The parent held him while the participant watched approximately 11 minutes of video footage (15 months) and 8 minutes of image slides (27, 33, 38, and 47 months) consisting of scenes and objects from the participant's home. Each slide appeared on the screen for approximately

5 s. Each slide was followed by a buffer screen that readjusted the participant's pupil diameter and prepared him to view the next slide. The slides were watched in one session at each age. After the participant viewed all of the slides, the parents and the researchers discussed each slide.

## Results and Discussion

### General Gaze Patterns

At 15 months, the participant's gaze fixations were focused on facial features of the objects in the video. At 27 months, the participant's gaze patterns fixated less on facial expressions, but included fixations on the body parts of the images. At 33 months, the participant called the objects out by name with no instruction from his parent or the researcher. The participant reached out for certain objects as they appeared on the screen as if they were in the room. The participant showed ownership of objects that he frequently encountered by referring to them as "mine." In certain images, the participant recognized emotional expressions. At 38 months, the participant's eye movements were more methodical and there were fewer fixations. The participant was able to correctly identify objects without fixating on the image, as he looked at the objects peripherally. At 47 months, data collection problems and equipment malfunction occurred during the session, making it difficult to evaluate cognitive development based on eye movement patterns. The participant identified objects in the images upon prompting by the parent. Unlike previous sessions, the participant did not discuss the objects.

### Emotion Gaze Patterns

Most interesting was the participant's development of perception of emotion at 33 months. When viewing pictures of his parents' faces smiling and frowning, there were substantial shifts in the gaze patterns across time (Table 1).

The participant focused his gaze patterns on two key regions for emotional expression: the zygomaticus major muscle and the corrugator supercilli muscle on the left side of each target's

**Table 1. Fixations on familiar faces at 33 months.**

	<u>Right Side of Target Face</u>	<u>Left Side of Target Face</u>
Mom (smiling)	3	272
Dad (smiling)	3	174
Mom (frowning)	4	273
Dad (frowning)	6	250
Self (crying)	7	203

face. The zygomaticus major muscle is engaged in a Duchenne's smile, while the corrugator supercilli is engaged in frowning. Both are commonly measured in facial electromyography as measures of attitude (cf., Mather & Romo, 2007; Tassinari & Cacioppo, 2000). This is a very effective area to attend to in perceiving emotions in others. Research has established hemispheric lateralization in humans (Corballis, 2009) and chimpanzees (Hopkins, Russell, & Cantalupo, 2007), and that the left side of the human face is more expressive than the right side due to being connected to the more expressive right hemisphere (Cacioppo & Petty, 1981; Sackeim & Gur, 1978).

## Conclusions

The main findings of this study are the evidence at age 33 months of perception of cues consistent with the attention to hemispheric lateralization of emotion in a target. The location of these cues may be the most effective place to attend to decode emotional expressions in adults (or at minimum the parents who were used in the photos), and their frequency of use may be a result of experience. The appearance of this pattern at 33 months indicates that cognitive processes developed during that time period.

The major limitation of this study was the fact that it was a case study design and thus

lacks external validity. However, this design allowed for repeated assessment of personalized stimuli in a controlled laboratory setting. Future research should test the development of gaze patterns across early development as well as differences in response to different types of emotion. This design should be used in a variety of samples, including young children with varying symptoms of autism spectrum disorder, in which social perception deficits occur.

Emotion perception is important for social interaction. The current findings contribute to the growing body of developmental eye tracking research with young children that builds our knowledge of development of human social perception.

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## References

- Cacioppo JT, Petty RE. 1981. Lateral asymmetry in the expression of cognition and emotion. *Journal of Experimental Psychology: Human Perception and Performance* 7:333-341.

- Corballis MC. 2009. The evolution and genetics of cerebral asymmetry. *Philosophical Transactions of the Royal Society B* 364:867-879.
- Dodd, MD, Van der Stigchel, S, Hollingworth, A. 2009. Novelty is not always the best policy: Inhibition of return and facilitation of return as a function of visual task. *Psychological Science* 20:333-339.
- Ekman, P. 1992. An argument for basic emotions. *Cognition and Emotion*, 6:169-200.
- Farroni, T, Massaccesi, S, Pividori, D, Johnson, MH. 2004. Gaze following in newborns. *Infancy* 5:39-60.
- Feldman, R. 2009. *The liar in your life: The way to truthful relationships*. New York: Twelve. 292 p.
- Hopkins WD, Russell JL, Cantalupo C. 2007. Neuroanatomical correlates of handedness for tool use in chimpanzees (*Pan Troglodytes*): Implications for theories on the evolution of language. *Psychological Science* 18:971-977.
- Larsen, JT, McGraw, AP, Cacioppo, JT. 2001. Can people feel happy and sad at the same time? *Journal of Personality and Social Psychology* 81:684-696.
- Larsen, JT, To, YM, & Fireman, G. 2007. Children's understanding and experience of mixed emotions. *Psychological Science* 18:186-191.
- Levenson, RW, Ekman, P, Friesen, WV. 1990. Voluntary facial action generates emotion-specific autonomic nervous system activity. *Psychophysiology* 27:363-384.
- Mather RD, Romo A. 2007. *Automaticity and cognitive control in social behavior*. Southlake (TX): Fountainhead.
- Sackiem HA, Gur RC. 1978. Lateral asymmetry in intensity of emotional expression. *Neuropsychologia* 16:473-481.
- Szameitat, DP, Alter, K, Szameitat, AJ, Darwin, CJ, Wildgruber, D, Dietrich, S., Sterr, A. 2009. Differentiation of emotions in laughter at the behavioral level. *Emotion* 9:397-405.
- Tassinary LG, Cacioppo JT. 2000. The skeletomotor system: Surface electromyography. In: Cacioppo JT, Tassinary LG, Bernston GG, editors. *Handbook of psychophysiology* (2<sup>nd</sup> ed.). New York (NY): Cambridge University Press. p. 163-199.
- Wilson, EO editor. 2006. *From so simple a beginning: The four great books of Charles Darwin*. New York: Norton. 1706 p.

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Oklahoma City University – Oklahoma City**

**‘IN-SILICO’ CHEMOTAXONOMY; A NEW TOOL FOR MICROBIAL SYSTEMATICS?**

**Nisha B. Patel, Krithivasan Sankaranarayanan, Cecil M. Lewis Jr., & Paul A. Lawson,**  
University of Oklahoma, Norman, OK,

Characterizations of novel microorganisms require a polyphasic approach that include phylogenetic, biochemical and chemotaxonomic investigations. The analysis of various cellular components such as polar lipid, fatty acid, and peptidoglycan types may be used in order to better differentiate between nearest neighbors. These chemotaxonomic traits help augment the data obtained from physiological tests to achieve a complete profile in microbial taxonomy. However, the costly and time consuming nature of these tests coupled with the lack of curated databases to compare the data accurately makes reproducibility difficult. Furthermore, few laboratories now have the skills to perform extensive chemotaxonomic studies which are required by many peer-reviewed journals and the description of novel taxa. A newly described bacterium from our laboratory recovered from the human gut, *Ezakiella peruensis* will serve as a model for a proposed study for “in-silico” chemotaxonomy. Polar lipids, respiratory quinones and peptidoglycan will be analyzed via traditional methods and the data will be compared to its genome. We hypothesize that embracing the information contained in the genome and identifying genes responsible for the metabolic pathways that lead to the production of important taxonomic and diagnostic cell-wall and plasma-membrane components, can be a powerful tool in identification and classification systems. This “in-silico” chemotaxonomy will compliment existing laboratory protocols using chemical and physiological tests to aid in the identification of microorganisms.

**EFFECT OF EXTRINSIC REWARDS AND SOCIAL COMPARISON ON INHIBITORY CONTROL IN ADULTS WITH ADHD**

**William Scott Sims & Tephillah Jeyaraj-Powell,** University of Central Oklahoma, Edmond, OK

Multiple studies show that motivation can improve inhibitory control in children with ADHD, but there is little evidence demonstrating similar effects in adult populations. We attempted to understand the effects of tangible rewards (like money) and upward social comparisons on behavior inhibition in adults with ADHD. False information regarding the performance of other participants was used to elicit an upwards social comparison. In this study, 46 participants were prescreened for ADHD symptoms, randomly assigned to one of three monetary reward conditions (none, low, and high), and assessed for correct inhibition using a Stop-Signal Task (SST). In a second session, they performed the SST after being informed of what other participants had scored (i.e. false feedback). Participants showed strongest inhibitory control in the low-reward/feedback condition, and weakest inhibitory control in the no-reward/feedback condition. Overall, participants exhibited better inhibitory control when monetary rewards were higher. Unexpectedly, false feedback did not appear to improve inhibitory control, with the exception of the low-reward/feedback condition. There was some evidence of ceiling effects in the data, so further investigation is needed to examine the relationship between rewards, social comparison, and inhibitory control.

## EFFECTS OF $\beta$ -FNA ON TAK-1 PHOSPHORYLATION IN NORMAL HUMAN ASTROCYTES

**Eric Bates, Kelly McCracken, D.J. Buck, & R.L. Davis,** Oklahoma State University Center for Health Sciences, Tulsa, OK

**Introduction:** Neuroinflammation is a component of many neurological disorders including CNS infections, neurodegenerative diseases, depression and other mental health disorders.

$\beta$ -funaltrexamine ( $\beta$ -FNA) shows promise as an effective means to combat neuroinflammation. More specifically,  $\beta$ -FNA inhibits interleukin-1 $\beta$  (IL-1 $\beta$ )-induced chemokine expression in normal human astrocytes (NHA).  $\beta$ -FNA is best characterized as a *mu*-opioid receptor (MOR) antagonist but, the anti-inflammatory actions identified thus far are MOR-independent. Our objective was to better understand the mechanism by which  $\beta$ -FNA inhibits inflammatory signaling by assessing its effects on the activation (phosphorylation) of transforming growth factor beta-activated kinase 1 (TAK-1).

**Methods:** NHA were maintained in cell culture with media replenished every 48-72 h until 80% confluence was reached. Cells were exposed to IL-1 $\beta$  (3 ng/ml),  $\beta$ -FNA (10  $\mu$ M), IL-1 $\beta$  +  $\beta$ -FNA or untreated for 30-120 minutes. Western Blot analysis was used to measure phosphorylated TAK-1 (p-TAK-1), TAK-1 and  $\beta$ -tubulin expression in whole cell lysates.

**Results:** A trend toward increased p-TAK-1 in IL-1 $\beta$  treated cells was observed, but did not reach the level of statistical significance. Similarly,  $\beta$ -FNA tended to decrease IL-1 $\beta$ -induced TAK-1 phosphorylation, but again not to the level of significance.

**Conclusion:**  $\beta$ -FNA may decrease TAK-1 activation by interrupting phosphorylation mechanisms, but further experiments are required, likely with modified experimental conditions (e.g., increased IL-1 $\beta$  concentration and earlier time points).

**Funding:** This project was supported in part by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447 and OCAST HR14-007 (RLD).

## PRODUCING RECOMBINANT PROTEINS OF EMCV FOR ASSAYS

**Javad, Arkam, Reddig, B.J. & Blewett, Earl L.,** Oklahoma State University – Center for Health Sciences, Tulsa, OK

The purpose of this research project is to produce a safe, inexpensive recombinant protein assay to detect antibodies against encephalomyocarditis virus (EMCV). EMCV is a cardio virus present in rodents and known to spread to larger animals proving lethal for hippos and elephants. By procuring effective recombinant proteins we will eliminate the need for the current infected cell protein assay allowing for a more effective, inexpensive, and safer alternative to detect the presence of EMCV. The first phase of the project involved the PCR amplification of EMCV primers MV012/MV013 and MV014/MV015. Thereon, using the cloning and verification of PCR Product into pTOPO as an intermediary phase, the objective was to insert the DNA into pBAD/His A plasmid vector for protein expression. The penultimate phase involved expression of pBAD/His A proteins using an arabinose promoter. Lastly, the final phase of the project involved running an ELISA of the yielded recombinant proteins to determine their effectiveness as an assay. Overall we were successfully able to yield PCR product, clone DNA into pTOPO for replicating and verifying the DNA sequence, and clone the product into pBAD/His A vector as our goal remains to optimize protein expression and purification methods and repeat our ELISAs.

## SECURITY METRICS AND DATA LOSS

**Patrick Harrington**, Northeastern State University, Tahlequah, OK

We address the issue of security metrics as a technique for improving overall security and explore some of the problems with its use. Solutions are explored in the context of a small-scale approach for computer security evaluation, versus the contemporary application to modeling and improving complex systems.

## CULTIVATION STUDIES ON THE GASTROINTESTINAL TRACT FROM AN INDIGENOUS PERUVIAN COMMUNITY YIELDS SEVERAL NOVEL BACTERIAL TAXA \*

**N. B. Patel, C. M. Lewis Jr., & P. A. Lawson**, University of Oklahoma, Norman, OK

**R. Y. Tito, A. J. Obregón-Tito, L. Troncoso-Corzo, E. Guija-Poma**, Universidad Científica del Sur, Lima, Peru

**O. Trujillo-Villaroel & L. Marin-Reyes**, Instituto Nacional de Salud, Lima, Peru

While the literature contains many examples of studies focused on the human gut microbiome of individuals from western populations, indigenous populations with a “non-western” diet and lifestyles are underrepresented. In order to truly determine if there is a core human microbiome, individuals with a variety of diets and geographic regions also need to be included in these investigations. The primary purpose of this study is to test the hypothesis that traditional communities from remote regions harbor novel microorganisms influenced by diet, health, and environmental conditions. We used rRNA-based road maps generated in our laboratories to target previously uncultivated bacterial groups to investigate their phylogenetic, physiological, biochemical, and chemotaxonomic properties. Freshly voided fecal samples were collected from members of the Afro-Peruvian community of Cruz Verde in Tambo de Mora, region Ica, in Peru. Multiple enrichments using an array of substrates were constructed and inoculated with 1 ml of fecal slurry. All isolates recovered from the enrichments were maintained on blood agar plates and were screened using 16s gene sequence analysis. A number of isolates yielded relatively low sequence similarity values to those in DNA databases; phylogenetic tree topologies demonstrated that a number of isolates belonged to a group of organisms known as the anaerobic Gram-positive cocci. The nearest relatives included members of the genera *Peptoniphilus*, *Finegoldia*, *Gallicola* and *Parvimonas*. To date, our studies have identified two novel genera and a new species belonging to the genus *Peptoniphilus* recovered from a single individual. Our investigations demonstrate that remote indigenous communities harbor novel microbial taxa and further studies employing culture-based approaches of human gut microbiomes of diverse communities are encouraged to augment the insights provided by molecular investigations. Cultivation and characterization of novel organisms from these unique communities will help to gain a deeper understanding of ecological and functional diversity of the gastrointestinal tract of indigenous communities.

\*Presented at 2014 OAS Technical Meeting

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**OKLAHOMA ACADEMY OF SCIENCE**  
**STATEMENT OF REVENUES COLLECTED AND EXPENSES PAID**  
**FOR THE YEAR ENDED DECEMBER 31, 2014**

**REVENUES COLLECTED**

Contributions		
Donations		<u>\$ 100.00</u>
Membership Dues and assessments		
Dues		<u>5,497.86</u>
Investment Income		
Interest Earned	91.14	<u>91.14</u>
Other Income		
<i>POAS</i> Income	2,979.85	
<i>Woody Plants</i>	120.00	<u>3,099.85</u>
Meetings		
Registration- Spring Meeting	1,314.00	
Registration - Fall Meeting	10,810.00	
Registration - Technical Meeting	6,042.00	<u>18,066.00</u>
Total revenue collected		<u>\$26,854.85</u>

**EXPENSES PAID**

Grants and Similar		
Awards		
Stipends and Other Compensation		
Stipends	6,141.24	
Social Security	824.60	
Medicare	192.84	<u>\$7,158.68</u>
Professional Fees		
Audit	200.00	
Tax Preparation	945.00	<u>1,145.00</u>

**STATEMENT OF REVENUES COLLECTED AND EXPENSES PAID  
FOR THE YEAR ENDED DECEMBER 31, 2014 (Continued)**

Other Expenses		
Spring Meeting	1,995.00	
Fall Meeting	8,174.50	
Technical Meeting	1,708.97	<u>11,878.47</u>
AAAS	99.00	
NAAS	259.00	
POAS	2,623.53	
Others	824.25	<u>3,796.78</u>
Total Expenses		<u>\$23,978.93</u>
<b>Revenues Collected over Expenses Paid</b>		<b><u>\$2,875.92</u></b>

**OKLAHOMA ACADEMY OF SCIENCE**  
**STATEMENT OF ASSETS, LIABILITIES AND FUND BALANCE**  
**ARISING FROM CASH TRANSACTIONS**  
**DECEMBER 31, 2014**

**ASSETS**

Cash:

Checking account	\$24,726.35	
Savings account	1,187.26	
Savings account	3,274.09	
		\$29,187.70

Investments:

Certificate of Deposit	60,000.00	\$60,000.00
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Total Assets \$89,187.70

**LIABILITIES AND FUND BALANCE**

Liabilities \$ 0.00

Fund balance:

Beginning operation fund balance	\$86,311.78
Excess revenues collected over expenses	<u>2,875.92</u>
	\$89,187.70
	<u>\$89,187.70</u>

## INDEPENDENT AUDITORS' REPORT

August 30, 2015

Executive Committee  
The Oklahoma Academy of Science

I have audited the accompanying statements of assets, liabilities and fund balance arising from cash transactions of the Oklahoma Academy of Science as of December 31, 2014, and the related statements of revenue collected and expenses paid for the year then ended. These financial statements are the responsibility of the Company's management. My responsibility is to express an opinion on these financial statements based on the audit.

I have conducted an audit in accordance with generally accepted auditing standards. An audit to obtain reasonable assurance about whether the financial statements are free of material misstatement and examining, on a test basis evidence supporting the amounts and disclosures in the financial statements. These financial statements were prepared on the basis of cash receipts and disbursements and this report prepared only for the internal use of the Executive Committee of the Oklahoma Academy of Science.

I find the financial statements referred to above present fairly, in all material respects, the assets, liabilities and fund balance arising from cash transactions of The Oklahoma Academy of Science as of December 31, 2014 and its revenue collected and expenses paid during the year then ended.

E. Pace, Retired

Assistant County Auditor

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All manuscripts must be refereed critically. The *POAS* Editors have an obligation to the membership of the Academy and to the scientific community to insure, as far as possible, that the *Proceedings* is scientifically accurate. Expert refereeing is a tested, effective method by which the scientific community maintains a standard of excellence. In addition, expert refereeing frequently helps the author(s) to present the results in a clear, concise form that exceeds minimal standards.

The corresponding author is notified of the receipt of a manuscript, and the Editor sends the manuscript to at least two reviewers, anonymous to the author(s). After the initial review, the Editor either accepts the manuscript for publication, returns it to the author for clarification or revision, sends it to another referee for further review, or declines the manuscript.

A declined manuscript will have had at least two reviews, usually more. The Editors examine such manuscripts very carefully and take full responsibility. There are several grounds for declining a manuscript: the substance of the paper may not fall within the scope of the *Proceedings*; the work may not meet the standards that the *Proceedings* strives to maintain; the work may not be complete; the experimental evidence may not support the conclusion(s) that the author(s) would like to draw; the experimental approach may be equivocal; faulty design or technique may vitiate the results; or the manuscript may not make a sufficient contribution to the overall understanding of the system being studied, even though the quality of the experimental work is not in question.

A combination of these reasons is also possible grounds for declining to publish the MS. In most cases, the Editors rely on the judgment of the reviewers.

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We thank the reviewers who contribute so much to the quality of these *Proceedings*. They must remain anonymous to assure their freedom in making recommendations. The responsibilities or obligations of these reviewers are

- Because science depends on peer-reviewed publications, every scientist has an obligation to do a fair share of reviewing.
- A reviewer who has a conflict of interest or a schedule that will not allow rapid completion of the review will quickly return the manuscript; otherwise, the review will be completed and returned promptly.
- A reviewer shall respect the intellectual independence of the author(s). The review shall be objective, based on scientific merit alone, without regard to race, religion, nationality, sex, seniority, or institutional affiliation of the author(s). However, the reviewer may take into account the relationship of a manuscript under consideration to others previously or concurrently offered by the same author(s).
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- Reviewers should explain and support their judgments and statements, so both the Editor and the author(s) may understand the basis of their comments.

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**poas@okstate.edu**

Prospective authors should note carefully the policy statement “Policies of the *Proceedings*” on page ii.

The Editors review the MS and carefully select other reviewers as described in “Editorial Policies and Practices” (see p. 194); all referee and editorial opinions are anonymous. Send a resubmitted and/or revised manuscript and a point-by-point response to the reviewers’/Editor’s comments.

All authors should approve all revisions (the corresponding author is responsible for insuring that all authors agree to the changes). A revised paper will retain its original date of receipt only if the revision is received by the Editor within two months after the date of the letter to the author(s).

### B. Types of Manuscripts.

A manuscript may be a paper (report), review, note (communication), a technical comment, or a letter to the editor.

*Paper* (a report; traditional research paper). A Paper may be of any length that is required to describe and to explain adequately the

experimental observations.

*Review.* The Editor will usually solicit review articles, but will consider unsolicited ones. The prospective writer(s) of reviews should consult the Editor; in general, the Editor needs a synopsis of the area proposed for review and an outline of the paper before deciding. Reviews are typically peer-reviewed.

*Note* (Communication). The objective of a *Note* is to provide an effective form for communicating new results and ideas and/or describing small but complete pieces of research. Thus, a *Note* is either a preliminary report or a complete account of a small investigation. *Notes* must not exceed four printed pages including text, figures, tables, and references. One journal page of standard text contains about 600 words; hence, there is space for presentation of considerable experimental detail. *Notes* are peer-reviewed.

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The same guidelines that apply to manuscripts and notes submitted for peer-review, also apply to abstracts submitted for print. Just as manuscripts and notes are subject to thorough testing, so are comments written in abstracts (supported by data). The Proceedings understands that all disciplines are in a search for a deeper understanding of the world some of which are through creative expression and personal interpretation. Science is a system by which one discovers and records physical phenomena, dealing with hypotheses that are testable. The domain of “science” while working within nature is restricted to the observable world. There are many valid and important questions to be answered but lie outside the realm of science.

### C. Manuscript Organization.

#### 1. General organization.

For papers (reports), the subsections should typically include the following: Abstract, Introduction, Experimental Procedures (or Methods), Results, Discussion, Acknowledgments (if any), and References. In the case of notes or short papers, you may combine some headings, for example, “Results and Discussion”:

- I. The title should be short, clear, and informative; it should not exceed 150 characters and spaces (three lines in the journal), and include the name of the organism, compound, process, system, enzyme, etc., that is the major object of the study.
- II. Provide a running title of fewer than 60 characters and spaces.
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- IV. Every manuscript (including Notes) must begin with a brief Abstract (up to 200 words) that presents clearly the plan, procedure, and significant results of the investigation. The Abstract should be understandable alone and should provide a comprehensive overview of the entire research effort.
- V. The Introduction should state the purpose of the investigation and the relationship with other work in the same field. It should not be an extensive review of literature, but provide appropriate literature to demonstrate the context of the research.
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- VII. You may present the Results in tables or figures or both, but note that it is sometimes simpler and clearer to state the observations and the appropriate experimental values directly in the text. Present a given set of results *in only one form*: in a table, or figure, or the text.
- VIII. The Discussion section should interpret the Results and how these observations fit with the results of others. Sometimes the combination of Results and Discussion can give a clearer, more compact presentation.
- IX. Acknowledgments of financial support and other aid are to be included.
- X. References are discussed below.
  1. References



figure legends include the surname of the author or authors of the cited document and the year of publication. The references are listed alphabetically by authors' surnames in the reference list found at the end of the text of the article. Below are given several examples of correct formats for citing journal articles, books, theses and web resources. For Additional information regarding the name- year system, consult the CBE Manual [Scientific *Style and Format: The CBE Manual for Authors, Editors, and Publishers*, 6th edition]. Abbreviate journal names according to the *International List of Periodical Title Word Abbreviations*.

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### Examples of References in CBE Style and Format

#### *Journal Articles*

Miller LF, Chance CJ. 1954. Fishing in the tail waters of TVS dams. *Prog Fish-Cult* 16:3-9.

Ortenburger AI, Hubbs CL. 1927. A report on the fishes of Oklahoma, with descriptions of new genera and species. *Proc Okla Acad Sci* 6:123-141.

#### **Books**

##### *Book with Authors:*

Miller RJ, Robison HW. 1980. The fishes of Oklahoma. Stillwater (OK): Oklahoma State University Press. 246 p.

##### *Book with Editors:*

Gilman AG, Rall TW, Nies AS, Taylor P, editors. 1990. The pharmacological basis of therapeutics. 8th ed. New York: Pergamon. 1811 p.

##### *Book with Organization as Author:*

International Union of Pure and Applied Chemistry, Physical Chemistry Division. 1993. Quantities, units, and symbols in physical chemistry. 3rd. Oxford (UK): Blackwell Science. 166 p.

##### *Chapter in Book with Editors:*

Hamilton K, Combs DL, Randolph JC. 1985. Sportfishing changes related to hydro- power generation and non-generation in the tailwater of Keystone Reservoir, Oklahoma. In: Olsen FW, White RG, Hamre RH, editors. Proceedings of the symposium on small hydropower and fisheries. Bethesda (MD): American Fisheries Society. p 145-152.

*Theses:* Knapp MM. 1985. Effects of exploitation on crappie in a new reservoir [MSc thesis]. Stillwater (OK): Oklahoma State University. 84 p. Available from: OSU Library.

*Internet:* Oklahoma Climatological Survey. 2003. Climate of Oklahoma [online]. Available from: <http://climate.ocs.ou.edu>. (Accessed August 15, 2005).

#### **D. Review Process.**

The Editors review the MS and carefully select reviewers for all submitted manuscripts. All referee and editorial opinions are anonymous. A decision to accept, revise, or reject the manuscript is made by the editor after careful consideration of reviewers' comments and recommendations. If a "revise" decision is reached, the authors will be allowed to resubmit a revised version of the manuscript within a given time window. The authors are considered to address all reviewers' comments and concerns, or provide compelling reasons to explain why they chose not to do so. A point-by-point rebuttal letter is required with each revised manuscript, which clearly indicates the nature and locations of corrections within the revised manuscript. All authors should approve all revisions, with the corresponding author being responsible for insuring that all authors agree to the changes.

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The OAS will publish accepted MSs with the implicit understanding that the author(s) will pay a charge per published page. Page charges are billed at the cost per page for the given issue: current rates of \$90 per page for nonmembers of the Academy and \$35 for members. All authors are expected to honor these page charges. Billing for page charges and receipt of payment are handled by the

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