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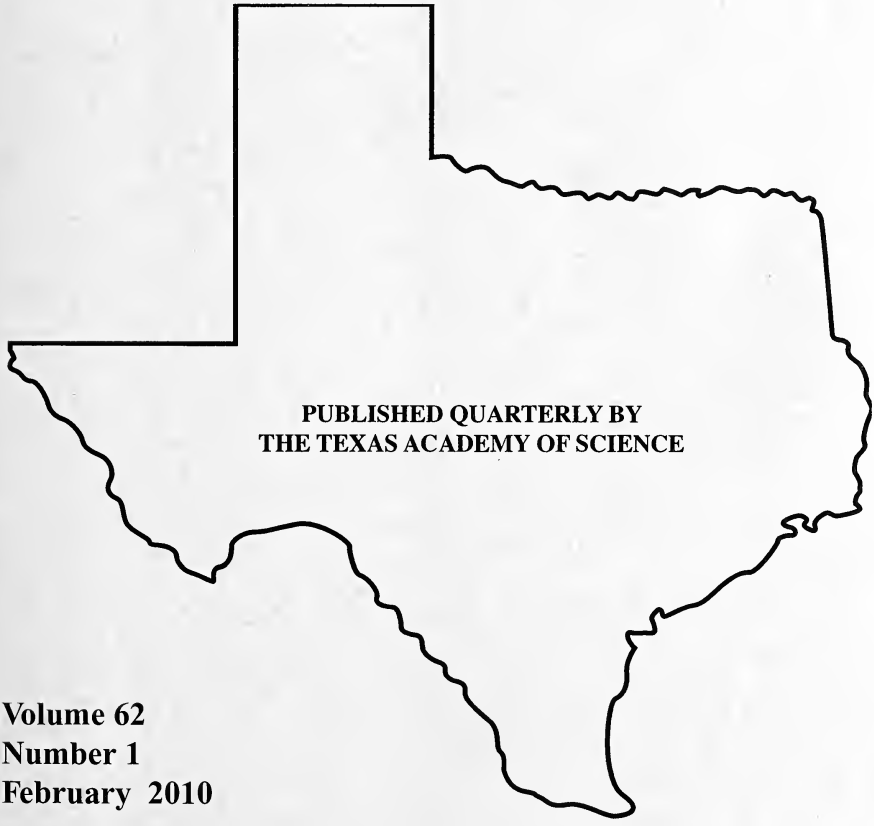

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TEXAS J. OF SCI. 62(1):3-14 FEBRUARY, 2010

DIFFERENTIAL USE OF GRAZED AND UNGRAZED PLOTS BY *DIPODOMYS ELATOR* (MAMMALIA: HETEROMYIDAE) IN NORTH CENTRAL TEXAS

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Abstract.–Two populations of Texas kangaroo rat (*Dipodomys elator*) were compared at a grazed and ungrazed pasture in Wichita County, Texas in order to examine differences in vegetation and Texas kangaroo rat population size. Within vegetation quadrats, species richness, height, percent grass, forbs, woody, and bare ground were examined. Vegetation height and type were examined along transects centered in the quadrat and extending outward in the four cardinal directions. *Dipodomys elator* was found inhabiting areas of short, sparse grasses with little overhead woody cover. Trapping revealed a higher concentration of *D. elator* at the grazed site.

Dipodomys elator is unusual because the habitat in which it is found is not typical among kangaroo rats. *Dipodomys elator* seems to prefer soils with high clay content which support overgrazed or short grasses (Dalquest & Collier 1964; Roberts & Packard 1973; Dalquest & Horner 1984; Schmidly 2004; Goetze et al. 2007) and is rarely recorded in locations with dense vegetation.

The historic range of *D. elator* spanned across the convergence of two physiographic regions, the Rolling Plains to the west, and the West Cross Timbers to the east (Carter et al. 1985; Jones et al. 1988; Martin 2002). Martin (2002) surveyed the entire historic range of *D. elator* and found this species in only five counties in Texas: Archer, Childress, Hardeman, Motley, and Wichita, all within the Rolling Plains region of Texas. The perceived decline in *D. elator* has led to some limited protective status. *D. elator* is listed as a threatened species by the Texas Parks and Wildlife Department (Martin 2002; Schmidly 2004).

Researchers need to quantify habitat critical to *D. elator* survival (Martin & Matocha 1972; Jones et al. 1988). Therefore, vegetation and corresponding population size were compared for two *D. elator* populations found in two habitats whose structure was influenced by grazing, or lack thereof, within Wichita County.

Methods

Study sites.–The grazed site (approximately 15 ha) is located 10 km N of Lake Buffalo Creek Reservoir, Wichita Co., Texas, immediately adjacent to Burnett Ranch Rd. (property entrance Lat: 34.03028709 Long: 98.76656946) (Figure 1). This is a location of previous *D. elator* research involving trapping and burrow surveys, as well as descriptions of the associated habitat (Stangl et al. 1992; Goetze et al. 2007). The grazed site is continuously grazed by cattle at a stocking rate of about 0.83 animals per ha. As a result, vegetation is relatively short and unburned brush piles within this pasture have collapsed over time and accumulated soil, creating mounds which are favored as burrowing sites for *D. elator* (Stangl et al. 1992; Goetze et al. 2007).

The ungrazed site (approximately 16 ha) is located 3.7 km W of the junction of FM 2345 and FM 368, Wichita County, Texas (property entrance Lat: 34.06475002 Long: 98.71983118). This site has not been grazed by livestock since at least 1999 and has not recently been subjected to brush control. As a result, herbaceous vegetation is thick and rank, and residual cover remains from year to year. The dominant woody vegetation is dense, mature mesquite (*Prosopis glandulosa*).

Population surveys.–Burrow trapping was conducted by placing three 7.5 by 8.75 by 30 cm Sherman live traps immediately around each active burrow entrance (within 0.10 to 0.50 m of each burrow entrance), with the open end of the trap facing the entrance (Cross & Waser 2000). For the grazed site, trapping was conducted throughout the spring and summer months of 2005 during the following dates (parentheses indicate number of trap nights): 18-24

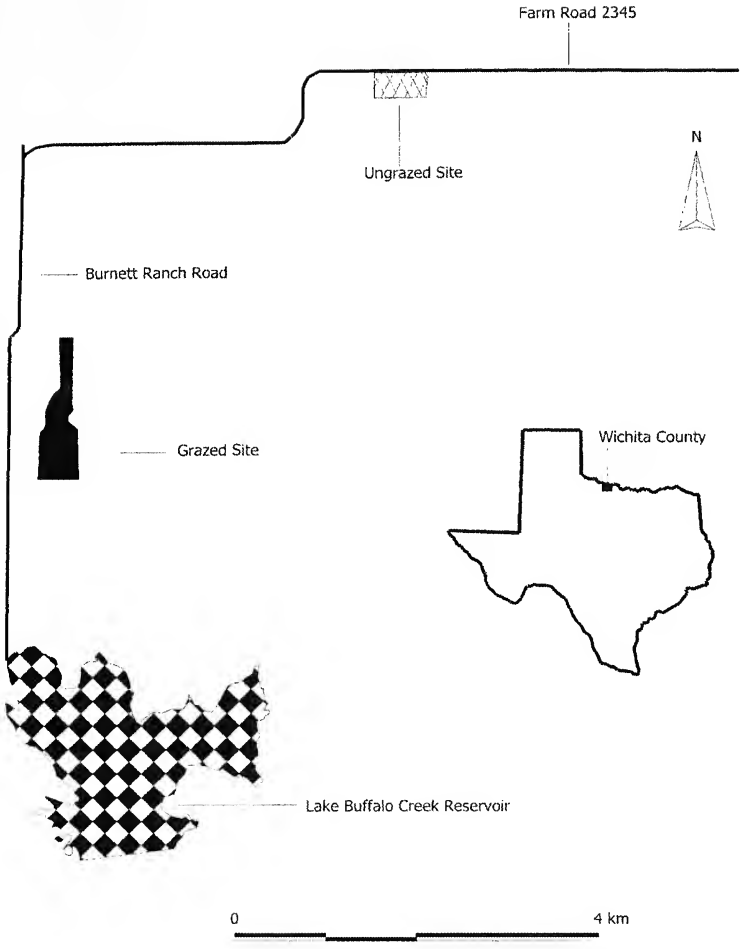

Fig. 1.

May (534), 21-25 June (210), 6-7 July (84), and 19-22 July (168). Trapping was conducted on the ungrazed site throughout the spring and summer months of 2005 during the following dates: 15-18 March (90), 10-12 May (141), 24 May (30), 5-7 June (249), 21-25 June (210), 6-7 July (84), and 19-22 July (168).

Captured *D. elator* were tagged with passive integrated transponder (PIT) tags in order to determine recapture rates. PIT tags

were implanted subcutaneously just posterior to the cranium with a 12ga. syringe fitted with a plunger. In order to minimize handling time and other stresses, anesthesia was not used (Schooley et al. 1993). Syringes were disinfected between implantations with 91% alcohol.

Vegetation.–A survey of vegetation was conducted in May when most species were in bloom. A rectangular quadrat ($1m^2$) was placed directly over each of 10 known *D. elator* burrows at the grazed site. Also, three known *D. elator* burrows and seven additional sites within the ungrazed area were surveyed. Within each quadrat, dominant species were noted, and species richness recorded. Percentage of grass, forb, woody vegetation, bare ground, and average herbaceous vegetation height (obtained by taking four samples 15 cm interior to each corner of the quadrat) were noted. If woody vegetation was present, its height was measured.

To quantify vegetation of the habitat surrounding sampled sites, two 20 m north-south and east-west transects, bisecting each quadrat were evaluated. Vegetation type (grass, forb, bare ground, woody), height, and vertical intercept of woody vegetation (distance from ground to woody species in cm directly over meter-point) was recorded at each meter-point along transects.

Data analysis.–Vegetation data from within quadrats and between quadrats and transects were compared between sites utilizing the Wilcoxon Mann-Whitney test of SAS 8.0 (SAS Institute 1999). Height values were compared with a paired T-test of SAS 8.0 (SAS Institute 1999). Parameters of herbaceous height, and percentages of grasses, forbs, bare ground, woody, and species richness were all evaluated for statistically significant ($P<0.05$) differences.

Vegetation data recorded along transects was compared between sites, percentage vegetation type, height, transect direction (North,

East, South or West), and transect meter-point distance (1-10 m), using the generalized linear model procedure (Proc GLM) of SAS 8.0 (SAS Institute 1999). Parameters of herbaceous height and percentages of grasses, forbs, bare ground, woody and other were all evaluated for statistically significant ($P<0.05$) differences across direction, distance, and site.

RESULTS

Burrow trapping.–There was a total of 1968 trap nights (trap night = 1 trap/night) for the two sites combined. At the grazed site, 996 trap nights over 18 trapping periods resulted in a total of 67 captures, 45 (67%) of which were *D. elator*. Eighteen different individuals accounted for the 45 *D. elator* captures, caught at 22 different burrows. There were 22 non-target captures (species other than *D. elator*) at the grazed site representing four species of rodents. Of the non-target species, *Chaetodipus hispidus* was the most common (10 captures), followed by *Spermophilus tridecemlineatus* (six captures), *Neotoma micropus* (four captures), and *Peromyscus leucopus* (two captures).

At the ungrazed site, 972 trap nights resulted in a total of 220 captures, of which eight were *D. elator* (Table 1). Two different individuals accounted for the eight *D. elator* captures caught at three different burrows. There were 212 non-target captures at the ungrazed site with the most common species being *Sigmodon hispidus* (119 captures) followed by *Peromyscus maniculatus* (36 captures), *P. leucopus* (28 captures), *N. micropus* (16 captures), and *C. hispidus* (13 captures).

Vegetation.–Little barley (*Hordeum pusillum*) was the dominant grass species recorded at all ten quadrats within the grazed site. Virginia pepperweed (*Lepidium virginicum*) was the dominant forb the majority of the time (six quadrats). Other dominant forbs within quadrats at the grazed site were western ragweed (*Ambrosia psilostachya*), common broomweed (*Gutierrezia dracunculoides*), and hog potato (*Hoffmannseggia glauca*). One mesquite (*Prosopis*

Table 1. Average vegetative height, mean richness and mean percent cover of quadrats compared between sites. Statistics reported for percentages and richness were evaluated by a Wilcoxon Mann-Whitney test (*SD*). Statistics reported for height data were compared by a paired T-test (*SD*).

Parameter	Grazed Site	Ungrazed Site	*P*
Mean % Bare Ground	49.9 (± 24.0)	18.2 (± 25.5)	0.027*
Mean % Forbs	17.0 (± 12.9)	13.8 (± 17.7)	0.168
Mean % Grasses	25.1 (± 18.9)	61.5 (± 31.1)	0.010*
Mean % Woody	6.0 (± 15.8)	4.5 (± 12.6)	0.479
Mean Richness	5.8 (± 2.2)	6.5 (± 2.8)	0.230
Avg. Herbaceous Height (cm)	7.1 (± 6.8)	27.7 (± 11.0)	<0.001*
Avg. Woody Height (cm)	15.9(± 38.8)	20.1 (± 44.4)	0.842

* Indicates statistical significance at ($P<0.05$).

glandulosa) and one lotebush (*Ziziphus obtusifolia*) occurred in two of the quadrats at the grazed site.

Within the ungrazed site, Japanese brome (*Bromus japonicus*) was the dominant grass species in nine out of ten quadrats. Little barley was the dominant grass in one quadrat. Western ragweed was the dominant forb in most quadrats (eight quadrats). Other dominant forbs were common broomweed and nightshade (*Solanum* spp.). Mesquite was the only woody species occurring in two quadrats.

Significant differences were observed in the quadrats sampled at each site (Table 1). Percentage bare ground was significantly higher within quadrats at the grazed site (mean 50%) than at the ungrazed site (mean 18%). The mean percentage of grass at the ungrazed site was significantly different than the grazed site (62% as compared to 25%). Herbaceous height within quadrats was significantly different between sites and was, on average, three times higher within the ungrazed site than quadrats within the

grazed site (average herbaceous height 27.7 cm and 7.1 cm respectively). Percentage coverage of forbs and woody vegetation and of woody vegetation height did not differ significantly between sites. Species richness was not significantly different between sites.

Within the grazed site there was significantly more bare ground within quadrats (49.9%) than along transects (22.3%) (Table 2). Also, there was significantly less grass within quadrats (25.1%) than along transects (54.3%). At the grazed site, there were no significant differences between average herbaceous and woody height, percent forbs, and woody vegetation within quadrats and along transects. Within the ungrazed site, there were no significant differences between vegetation sampled within quadrats and along transects (Table 2).

Discussion

Species composition of the rodent populations at the two sites is different. The grazed site is predominately inhabited by *D. elator*, whereas the ungrazed site is predominately inhabited by *Sigmodon hispidus* and *Peromyscus* spp. The high population of *S. hispidus* (119 captures) and extremely low occurrence of *D. elator* (2) at the ungrazed site concurs with Packard & Roberts (1973) observation that the Texas kangaroo rat and hispid cotton rat rarely co-occur. Other notable species compositional differences between sites were the absence of *P. maniculatus* and the presence of *S. tridecemlineatus* at the grazed site. These species reflect the habitat differences between the sites. *Peromyscus maniculatus* prefers areas where vegetation offers concealment, such as tall forbs and grasses (Schmidly 2004). *Spermophilus tridecemlineatus* is an inhabitant of short-grass prairies (Schmidly 2004) and prefers heavily grazed pastures (Jones 1964; Streubel & Fitzgerald 1978). Therefore, it is reasonable to conclude that the grazed site is more indicative of suitable *D. elator* habitat.

At the grazed site, *D. elator* utilized existing mounds (old brush piles, fence rows, and abandoned farm equipment that has

Table 2. Mean percentages bare ground, forbs, grasses, and woody vegetation as well as average herbaceous and woody vegetation height compared between quadrats and transects. Statistics reported for percentages were evaluated by a Wilcoxon Mann-Whitney test (*SD*). Statistics reported for height data were compared by a paired T-test (*SD*).

	Grazed Site			Ungrazed Site		
Parameter	Quadrats	Transects	*P*	Quadrats	Transects	*P*
Mean % Bare Ground	49.9 (± 24.0)	22.3 (± 8.7)	0.010*	18.2 (± 25.5)	17.5 (± 20.4)	0.197
Mean % Forbs	17.0 (± 12.9)	22.9 (± 9.8)	0.177	13.8 (± 17.7)	13.0 (± 5.9	0.119
Mean % Grasses	25.1 (± 18.9)	54.3 (± 9.5)	0.002*	61.5 (± 31.1)	67.9 (± 22.2)	0.500
Mean % Woody	6.0 (± 15.8)	0.5 (± 1.6)	0.256	4.5 (± 12.6)	0.7 (± 1.2	0.442
Avg. Herbaceous Height (cm)	7.1 (± 6.7)	7.2 (± 3.7)	0.934	27.7 (± 11.0)	27.6 (± 11.5)	0.957
Avg. Woody Height (cm)	15.9 (± 38.8)	10.2 (± 32.3)	0.700	20.1 (± 44.4)	32.5 (± 58.2)	0.112

* Indicates statistical significant diffenence ($P<0.05$)

accumulated soil) for burrow construction, but it was observed that *D. elator* will readily excavate burrows on natural, slightly raised areas. Thirty-three percent of the burrows at the grazed site were associated with these types of areas. Contrary to Dalquest & Collier (1964), who invariably associated *D. elator* burrows with mesquite at their study site, only 3.0% of burrows at the grazed site were associated with mesquite. When burrows associated with lotebush (*Zizyphus obtusifolia*) are added, the percentage of burrows associated with woody species rises to 7.5%. Goetze et al. (2007) found burrows associated with mesquite 6.0% of the time. When burrows associated with lotebush were added, the percentage of burrows associated with woody species rose to 15.0% (Goetze et al. 2007). Other researchers have noted an association of *D. elator* with mesquite (Dalquest & Collier 1964; Chapman 1972; Roberts

& Packard 1973; Carter et al. 1985; Schmidly 2004). However, this investigation concurs with Stangl et al. (1992) and Goetze et al. (2007) in suggesting that woody vegetation is not essential for *D. elator* burrows. Burrow site selection by *D. elator* seems to be based primarily on a disturbance regime and the presence of bare ground (mean 49.9% at the grazed site), in agreement with Stangl et al. (1992), Martin (2002) and Goetze et al. (2007). Within the grazed site, 50% of burrows were associated with disturbance (fence rows, brush piles, and/or human structures). Goetze et al. (2007) reported 56.0% of burrows associated with disturbance.

Overall, *D. elator* habitat is dominated by short vegetation (between 3.5 cm and 10.9 cm in height at the grazed site) with very little overhead woody cover (grazed site < 1.0% coverage). The microhabitat immediately around burrows at the grazed site contained significantly more bare ground and less grass than transects sampled further from the burrows (Table 2). The overall habitat values from this investigation compare favorably with percent grass (avg. 75.5%), forbs (avg. 15.8%), and bare ground (avg. 18.9%) data collected in Hardeman County, Texas for *D. elator* habitat (Martin 2002). There is general agreement that *D. elator* requires a sparse, short-grassland habitat (Dalquest & Collier 1964; Roberts & Packard 1973; Carter et al. 1985; Stangl et al. 1992; Martin 2002; Goetze et al. 2007).

Much land is under cultivation within the range of *D. elator*. Routine tillage and the resulting agronomic monocultures render such areas uninhabitable to this species (Stangl et al. 1992). Many areas that are not in crop production have been developed for gas and oil exploration. Associated disturbances, such as road construction and discarded equipment that accumulates soil, are thought to be beneficial for kangaroo rats (Roberts & Packard 1973; Stangl & Schafer 1990; Stangl et al. 1992; Martin 2002; Goetze et al. 2007). The use of fire to control woody species is uncommon and mechanical means are often too costly. These circumstances allow areas to develop dense stands of mesquite and the herbaceous

understory to become dense, as noted at the ungrazed site in this study. Based upon rodent species composition at the two sites, lack of grazing and these other extrinsic factors may have favored dense mid-grass to woodland vegetational communities that include *S. hispidus* and *P. maniculatus* as noted by Schmidly (2004). In 1985, *D. elator* was recorded from two separate locations in Hardeman County, Texas. When these sites were visited again in 1990, the vegetation had become denser and *D. elator* had been extirpated from both locations (Stangl & Schafer 1990).

It is speculated that historically the short, sparse, grassland habitat that *D. elator* requires was maintained by buffalo (*Bos bison*), and/or prairie dogs (*Cynomys ludovicianus*) and naturally-occurring wildfires (Stangl et al. 1992). Currently, neither of these species or fires have a major impact on the environment. To develop and maintain favorable habitat for *D. elator*, systems of moderate to intense grazing pressure need to be implemented that mimic grazing and disturbance by bison and prairie dogs (Stangl et al. 1992; Nelson et al. 2009).

In addition to these management practices, continued research of *D. elator* is needed to assure the future stability of the species. Specific habitat requirements need to be defined from additional localities within the geographical range of *D. elator*. Genetic variation and minimum sustainable population size will need to be assessed.

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Sincere thanks are owed to Ernest, Oscar and Edith Goetze for access to the study sites located in Wichita County, Texas. We also thank Mike Miller of the Texas Parks and Wildlife Department and Roger Wittie in the College of Agriculture and Human Sciences at Tarleton State University who provided field equipment and technical advice. We thank Robert E. Martin and an anonymous reviewer for their thoughtful comments that improved this manuscript.

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TEXAS J. OF SCI. 62(1):15-24 FEBRUARY, 2010

DISTRIBUTION OF THE PALLID SHINER, *HYBOPSIS AMNIS* (CYPRINIFORMES: CYPRINIDAE), IN ARKANSAS

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Abstract.—The pallid shiner (*Hybopsis amnis*) is a fish rarely collected in Arkansas. It is distributed throughout the Coastal Plain with disjunct populations in the extreme northeastern part of the state. Prior to the present study, *H. amnis* had not been collected from the former lowland range in Arkansas. This study updates the geographic distribution of the pallid shiner in Arkansas, from collections taken between 1988 and 2008, and provide nine new county records and 46 new localities. Although populations of *H. amnis* are quite localized, they appear to be stable in Arkansas, particularly within the southern tier of counties in the Little Red, Ouachita, Red, and Saline rivers. However, disjunct populations of *H. amnis* in the upper St. Francis River of extreme northeastern Arkansas may no longer exist and require further study.

The pallid shiner, *Hybopsis amnis* (Hubbs & Greene) is a slender, slightly compressed minnow of moderate size with a subterminal and horizontal mouth. The species ranges in the Mississippi River basin from Wisconsin and Minnesota south to Louisiana, extending up the Cumberland River to south-central Kentucky and in the Arkansas and Red River drainages to eastern Oklahoma, and in Gulf Slope drainages from the Amite River in Louisiana to the Guadalupe River of Texas (Clemmer 1980; Page & Burr 1991; Ross 2001). This shiner prefers medium to large-sized sand-silty streams and rivers but also is common in some reservoirs and oxbow lakes (Hubbs 1951; Robison & Buchanan 1988; Etnier & Starnes 1993; Miller & Robison 2004). It appears to be intolerant of excessive siltation and turbidity and avoids strong currents (Pflieger 1997).

In Arkansas, *H. amnis* is distributed sporadically throughout the Coastal Plain of the state, from the Black, lower White, St. Francis,

lower Arkansas, Poteau, Little, Red, Saline, and Ouachita rivers (Robison & Buchanan 1988; Buchanan et al. 2003). The Nature Conservancy (NatureServe 2008) reported the species to be absent in recent collections from its former lowland range in Arkansas.

There are only eight pre-1960 records of *H. amnis* in Arkansas (Robison & Buchanan 1988): two from the extreme western (Poteau River), three from the eastcentral (lower White River), and three from the extreme northeastern (Spring and St. Francis rivers and Middle Slough) parts of the state. Robison & Buchanan (1988) added 17 more sites and Buchanan et al. (2003) reported the first occurrence of *H. amnis* from the Arkansas segment of the Red River. Two *H. amnis* were collected by the Lower Ouachita River Work Group (LORWG) in 1991 at Reach 3 (between Arkadelphia and Camden) on the lower Ouachita River in Dallas County, and a single *H. amnis* was taken by the LORWG in 1992 at Reach 8 below Felsenthal Lock and Dam of the lower Ouachita River, just north of the Louisiana border in Union County (Wise et al. 1993). In addition, Buchanan (2005) reported a remarkable collection of 3,697 specimens of *H. amnis* from rotenone sampling in eight reservoirs of the state, including DeGray, Dierks, Felsenthal, Georgia-Pacific, Gillham, Hinkle, Millwood, and Pool 7 of the Arkansas River. However, number per reservoir, county of occurrence, and collection date were not reported. More recently, McAllister et al. (2009) reported the first vouchered specimen of *H. amnis* from the Strawberry River.

To update the status of this uncommonly collected shiner, this study sampled streams throughout Arkansas and mapped museum collection data along with providing detailed collection data not reported previously by Buchanan (2005).

Materials and Methods

Between June 1998 and June 2008 pallid shiners were collected with standard nylon seines (1.8 by 0.5 m and 2.7 by 0.5 m of 3.2 mm mesh) or via two-day rotenone sampling. In addition, mini-

Fyke nets were set on 2 May 2006, and trawls were done on 9 February and 12 June 2008 in the lower Ouachita River. Fishes were preserved in 10% formalin and later transferred to 45% isopropanol. Specimens were field identified, verified in the laboratory, and vouchers deposited in the collections at Southern Arkansas University, Magnolia, Arkansas (SAU), the University of Louisiana-Monroe Museum of Natural History, Monroe, Louisiana (NLU), and the University of Arkansas at Fort Smith, Fort Smith, Arkansas (UA-FS). Detailed data provided on the 46 new sites are as follows: (county, specific locality [township, section, and range or latitude and longitude when available], date, museum accession number (if known), and number of specimens in parentheses).

Material examined.–The following is a list of collection localities of *H. amnis* in Arkansas. County names followed by an asterisk (*) represent new county records.

ARKANSAS COUNTY (n = 32): Cook's Lake. 17 August 1998. UA-FS (29); White River at RM 12 of Gunbarrel Reach. 19 October 1998. (3).

ASHLEY COUNTY (n = 314): Lake Georgia Pacific. 14-15 July 1997. UA-FS (266); Bayou Bartholomew at St. Hwy. 160, W of Portland. 9 October 2003. (48).

BRADLEY COUNTY (n = 76): Saline River at St. Hwy 15 Access, 8.0 km NE of Warren. 4 & 10 April 1992. (1, 5); Saline River, 11.3 km S of Johnsville on County Road (CR). 56. 16 September 1995, 19 October 1995, 16 March 1996 & 19 October 1996 (31, 14, 8, 15); Ouachita River at Moro Bay State Park (Sec. 28, T16S, R12W). 10 June 2005. SAU (1); Ouachita River, downstream backwater from Pine Prairie (33.25746°N, 92.23820°W). 12 June 2008. (1).

CHICOT COUNTY* (n = 1): Mississippi River at NM 512 along dike. 30 July 2003. UA-FS (1).

CLARK COUNTY* (n = 3,040): Caddo River at U.S. 67 bridge at Caddo Valley (Sec. 31, T6S, R19W). 22 June 1993. SAU (2); Lake DeGray near Arlie Moore Recreation Area. 13-14 July 1998. UA-FS (3038).

DALLAS COUNTY* (n = 5): 1.6 km upstream and downstream from Dallas County access area on Ouachita River. 17 July 1991. NLU 20420 (5).

DESHA COUNTY* (n = 102): White River, 1.6 km W of Whiskey Lake at Gunbarrel Reach. 18 August 1998. (44); Arkansas River, Cowpen Bendway, RM 14.2. 18 August 1998 & 19-20 October 1998. (55, 3).

DREW COUNTY* (n = 9): Saline River at Ozment Bluff Recreation Area, 19.3 km SW Monticello (Sec. 14, T14S, R9W). 13 July 1998. UA-FS (6); St. Hwy. 138 N of Selma at Able's Creek. 7 September 2000. NLU 40301 (3).

GARLAND COUNTY (n = 1): 2.1 km N of Crystal Springs at Crystal Springs Creek (Sec. 27, T2S, R22W). 30 September 1989. NLU 63635 (1).

GRANT COUNTY* (n = 8): Lost Creek, 7.2 km W of Cross Roads on St. Hwy. 35. 5 March 1992. (8).

HEMPSTEAD COUNTY (n = 23): Little River backwater, 1.6 km below G&FC Allen Ferry Access (Sec. 13, T13S, R27W). 26 July 1995. UA-FS (3); Millwood Lake along riprap dike near Okay Landing. 27 July 1998. UA-FS (18); Red River, 16.1 km upstream from Garland City Access. 19 July 2000. UA-FS (2)

HOT SPRING COUNTY* (n = 4): Lake DeGray at Point Cedar. 7-8 July 1998. UA-FS (4).

HOWARD COUNTY (n = 11): Millwood Lake near mouth of Saline River. 29 July 1998. UA-FS (6); Saline River at St. Hwy. 24 (Sec. 23, T9S, R29W). 24 October 1990. SAU (1); Cossatot River at St. Hwy. 24 (Sec. 23, T9S, R29W). 18 June 2000. SAU (2); Plum Creek, 0.8 km above Lake Millwood near OK Access. 14 July 2004. UA-FS (2).

JEFFERSON COUNTY (n = 1): 3.2 km S of Ladd off St. Hwy. 425 at Bayou Bartholomew. 2 September 1999. NLU 40286 (1).

LITTLE RIVER COUNTY (n = 72): Little River, 1.6 km N of Billingley's Corner (Sec. 4, T10S, R32W). 16 September 1997. SAU (3); Millwood Lake, Little River arm near Yarborough Landing. 27 July 1998. UA-FS (63); Millwood Lake near Jack's Isle. 28 July 1998. UA-FS (1); Millwood Lake near Horseshoe Lake area at mile marker 15. 28 July 1998. UA-FS (1); Millwood Lake, Little River arm at US 71 Access. 8 July 2003. UA-FS (4).

MILLER COUNTY* (n = 1): Red River, 8.0 km upstream from Garland City Access. 19 July 2000. UA-FS (1).

MONROE COUNTY (n = 10): Roc Roe Bayou at U.S. 79 bridge, 4.0 km SW Clarendon. 4 June 1988 & 10 November 1989. UA-FS (6, 3); White River, Horseshoe Lake side. 25 June 1996. (1).

OUACHITA COUNTY (n = 2): Ouachita River at jct. of Little Missouri River at Tate's Bluff (Sec. 1, T11S, R18W). 9 October 1989. SAU (2).

PERRY COUNTY (n = 125): Pool 7 of Arkansas River near mouth of Fourche La Fave River. 23-24 August 2000, 21-22 August 2002 & 26-27 August 2004. UA-FS (33, 37, 55).

PRAIRIE COUNTY (n = 27): White River, 1.6 km N De Valls Bluff at St. Hwy. 70 bridge. 20 August 1998. (21); White River at Brunt Bayou Bend. 20 August 1998. (6).

POLK COUNTY (n = 1): Gillham Lake. 6 August 1998. UA-FS (1).

SCOTT COUNTY (n = 22): Lake Hinkle. 13-14 July 1999 & 11 July 2000. UA-FS (15, 7).

SEVIER COUNTY (n = 124): CR 3, N of West Otis at Rolling Fork River (Sec. 20, T9S, R23W). 17 August 1995. SAU (1); Rolling Fork River, 1.6 km NW of De Queen (Sec. 14, T8S, R32W). 7 September 1995. SAU (1); Lake Millwood near mouth of Cossatot River at US 71. 29 July 1998. UA-FS (50); Dierk's Lake. 20 July 1998. UA-FS (68); Rolling Fork River at gravel road, 8.0 km SW of De Queen (Sec. 20, T9S, R32W). 21 October 2003. SAU (2); Cossatot River, 3.2 km above Lake Millwood near OK Access. 13 July 2004. UA-FS (2).

UNION COUNTY (n = 304): Ouachita River at Felsenthal (Sec. 15, T19S, R10W). 9 November 1989. SAU (2); Shallow Lake at Felsenthal National Wildlife Refuge. 18 September 1997, 18-19 August 1998 & 17-18 August 1999. UA-FS (1, 37, 100); Ouachita River at Beryl Anthony Lower Ouachita River Wildlife Management Area (33.00863°N, 92.06939°W). 2 May 2006. (6); Ouachita River, above Moro Bay (33.30187°N, 92.35697°W). 9 February 2008. (158).

WOODRUFF COUNTY* (n = 21): near Cotton Plant at Cache River. 2 July 1997. 22132 (1); Cache River at Gregory DEQ 210. 29 October 2003. (20).

Results and Discussion

This study documents the collection of 4,336 *H. amnis* from 25 of 75 counties (33%) of Arkansas, including Arkansas, Ashley, Bradley, Chicot, Clark, Dallas, Desha, Drew, Garland, Grant,

Hempstead, Hot Spring, Howard, Jefferson, Little River, Miller, Monroe, Ouachita, Perry, Prairie, Polk, Scott, Sevier, Union, and Woodruff (Fig. 1). Of those, 3,697 (85%) were reported previously by Buchanan (2005) but without specific collection data; 639 specimens are reported herein for the first time. Not counting the 3,038 specimens collected by Buchanan (2005) at Lake DeGray in Clark County from two-day rotenone sampling, 158 *H. amnis* (48-57 mm SL) were taken by trawl at a depth of 2.4 m on 9 February 2008 from the Ouachita River (above Moro Bay) in Union County. The next largest collection was 55 pallid shiners taken by seine on 18 August 1998 from the Arkansas River in Desha County.

Except for the St. Francis River, pallid shiners were collected from all major rivers within the Gulf Coastal Plain (GCP) and Mississippi Alluvial Plain (MAP) physiographic provinces of Arkansas, including the Arkansas, Ouachita, lower Mississippi, Red, Saline, and White rivers and their tributaries. The recent collection of a single *H. amnis* from the Strawberry River in Lawrence County (McAllister et al. 2009) suggests the pre-1960 historic site in the Current River system might yield additional specimens with intensive collection efforts. However, collecting during May and June 2006 in the St. Francis River, above and below Lake City did not yield *H. amnis* (B. Layher, pers. comm.) nor were any pallid shiners collected during this study from the same; therefore, the pre-1960 disjunct populations in the upper St. Francis River reported by Robison & Buchanan (1988) in extreme northeastern Arkansas may no longer exist. Numerous collections by Etnier & Starnes (1993) from the Mississippi River main channel of Tennessee have yielded only two specimens. Indeed, Pflieger (1997) reported a drastic reduction in the historic range of *H. amnis* in Missouri and suggested the species may have been extirpated. The species may be on the decline in the Midwest as well (Warren & Burr 1988). Furthermore, Ross (2001) reported populations in the northern part of the range also have greatly declined or have been extirpated, apparently due to heavy siltation and pollutants.

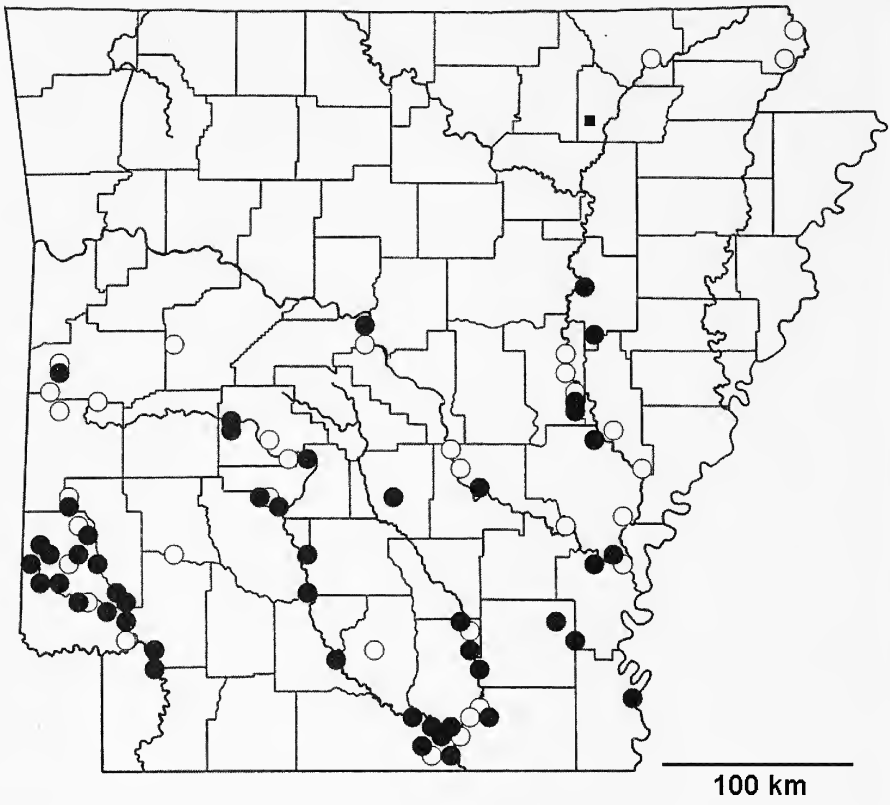

Figure 1. Records of the pallid shiner (*H. amnis*) in Arkansas. Open circles (○) are historic records referenced herein; closed circles (●) are current records; closed square (■) is from McAllister et al. (2009).

Compared with the records in Robison and Buchanan (1988), this study adds nine new county records of *H. amnis*, including the southeasternmost and southwesternmost range extensions in the state for Chicot and Miller counties, respectively. Further, numerous other collections are noted of the species from new localities in counties with historic records. Most specimens originated from habitats within the GCP and MAP, although several *H. amnis* were taken well above the interface of the GCP and the Ouachita Mountains uplift in Garland and Scott counties. Because present data indicate that *H. amnis* is more widespread and common

as previously known, it is believed that its populations in Arkansas are stable but still quite localized. In addition to conventional seining and rotenone sampling, it is suggested that alterative techniques for collecting *H. amnis* be used, including usage of boat-mounted electrofishing devices, mini-Fyke and hoop nets, and trawling. A combination of these collecting efforts may reveal whether the current Arkansas distribution of *H. amnis* is definitive or an artifact of inadequate surveying.

ACKNOWLEDGMENTS

Much appreciation is extended to Dr. N. H. Douglas, University of Louisiana at Monroe (NLU), B. Layher (Pine Bluff, AR), and B. K. Wagner (Arkansas Game & Fish Commission) for use of collection records of *H. amnis* in their care. Also, thanks to previous SAU Vertebrate Natural History and Field Biology classes, and former SAU students K. Ball, C. Brummett, N. Covington, L. Crump, and J. Rader for assistance in collecting. We further acknowledge the Arkansas Game & Fish Commission for scientific collecting permits issued to TMB and HWR, and Dr. W. C. Starnes (North Carolina State Museum of Natural Sciences, Raleigh) for providing helpful comments on the ms.

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A COMPARISON OF LITTER PRODUCTION IN YOUNG AND OLD BALDCYPRESS (*TAXODIUM DISTICHUM* [L.]) STANDS AT CADDO LAKE, TEXAS

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Abstract.–Aboveground primary productivity for cypress forests was assessed from measurements of litter production in two age groups and in two hydrological regimes (standing water and free-flowing). Caddo Lake, located in northeast Texas on the Texas-Louisiana border, offered a unique study site since it is dominated by extensive stands composed entirely of *Taxodium distichum* (L.) Rich. (baldcypress) in different age groups. Young stands (approximately 100 years old) are found along the shoreline and on shallow flooded islands. Old stands (~150 to 300 years old) are found in deeper water where they were continuously flooded. Litter production over three years from October 1998 to September 2001 was measured. Litter consisting of leaves, twigs, bark, reproductive parts, and *Tillandsia usneoides* (L.) L. (Spanish moss) was collected monthly using 0.5 m^2 floating traps. Tree diameters were measured within 200 m^2 circular plots in each stand. The young stands supported densities greater than 2,000 stems/ha and a mean stand basal area of 72.3 m^2/ha, whereas old stands supported lower densities of about 500 stems/ha but with a similar mean stand basal area of 73.3 m^2/ha. There was a significant difference between old and young stands for overall yearly litter production, averaging about 670 g/m^2/yr in the young stands and 460 g/m^2/yr in the old stands. Leaves and twigs were significantly greater in the young stands, while reproductive parts were higher in old stands. Litter collections between years or hydrological regimes were not significantly different.

Litter production is useful in ecological assessments of ecosystem function and health for determining the aboveground primary productivity. Primary productivity may be influenced by factors such as hydrological regime and nutrient content, where productivity was compared for bottomland forests in stagnant floodwaters or long-term inundated areas and forests in free-flowing or seasonally-flooded areas. Productivity, litter production, and nutrient levels were associated as functions of differences in flooding regime (Conner et al. 1981; Gomez & Day 1982). Diverse

Florida cypress systems subjected to various water flows and nutrients were examined in Brown (1981) and compared with other studies; nutrient-enriched cypress domes in still water had higher productivity than flowing water stands. Specific stem densities and basal areas are not necessarily associated with certain hydrological regimes. Brown et al. (1979) showed high stem densities and basal areas associated with inundated areas. Gomez & Day (1982) reported the converse where litter production in frequently-flooded stands at Dismal Swamp, Virginia, exceeded other periodically flooded or stagnant water ecosystems, such as the flowing water (periodically flooded) ecosystems of Lac des Allemands Swamp in Louisiana (Conner & Day 1976; Conner et al. 1981), very-slowly flowing cypress ecosystems at Okefenokee Swamp, Georgia (Schlesinger 1978) or in Illinois (Mitsch et al. 1977), and seasonally-flooded alluvial swamps in North Carolina (Brinson et al. 1980). Mitsch et al. (1991) likewise ascertained a definite pattern of progressively higher to lower litter production along a light-to-heavy flooded gradient in Kentucky. Litter production was also greater in a regularly drained crayfish pond and natural swamp area than in an impounded area at Barataria Basin, Louisiana (Conner & Day 1992). Brown & Peterson (1983), however, reported no significant differences in litter production for mixed-species bottomland forests in Illinois for flowing water and in stagnant floodwaters.

Past studies focused on hydrological regimes and they were conducted in mixed-species forests of undetermined age or of predominantly old or young growth stands only. Conner & Day (1976) suggested that maturity of trees may be a factor influencing overall biomass productivity or litter production. They studied two highly-diverse ecosystems of second-growth young stands of less than 30 years for the bottomland hardwoods (23 woody species) and between 50 and 95 years old for cypress swamps (nine woody species). The standing biomass of cypress swamps was twice that of younger bottomland hardwoods. Although average litter production was not significantly different between these two

ecosystems, Conner & Day (1976) observed that the sparse, larger trees of the cypress swamp yielded more litter than dense younger bottomland hardwoods collectively.

In addition, the mixed forest species stands in these studies can influence overall stand litter production. Stands with multiple species were noted in Conner & Day (1976), but litter was not differentiated by species in their study. Litter was sorted by species groups in Brown (1981) and Deghi (1977). Gomez & Day (1982) offer a comprehensive separation of litter production by four to six dominant species in four forest types at the Great Dismal Swamp, Virginia, that included a cypress swamp. Since dissimilar species will exhibit different litter production phenologies (Burns & Honkala 1990a; 1990b), the monthly or yearly patterns of overall stand litter production can be subsequently affected. Thus, a selection of forest stands with fewer species may help control for confounding litter production effects of diverse species.

A monotypic tree species ecosystem would aid research to examine the influence of multiple species on primary litter production. Furthermore, ecosystems with discrete age stands would allow maturity of stands to be also studied as a factor in litter production. Caddo Lake is a naturally-occurring permanent lake of 10,200 ha in Texas (Fig. 1), and is a locale uniquely dominated by baldcypress (*Taxodium distichum*) with different age stands. There are distinct water depth areas present in the lake that include deep water (>1.5 m) without baldcypress, deep water (1−1.5 m) with older baldcypress stands, and shallow water (<0.5 m) with baldcypress stands. Most of Caddo Lake was formed by the "Great Raft", a 266-km log jam on the Red River and Atchafalaya River. The Great Raft accreted around 1100 AD and was cleared by Henry Shreve from 1832 to 1839 (Triska 2008). Presently, the water level at Caddo Lake is controlled by a weir (66.3 m MSL), constructed in 1971 on the Louisiana side of the lake. Water levels are also affected by releases from Lake O' the Pines reservoir, located about 26 km upstream on Big Cypress Bayou.

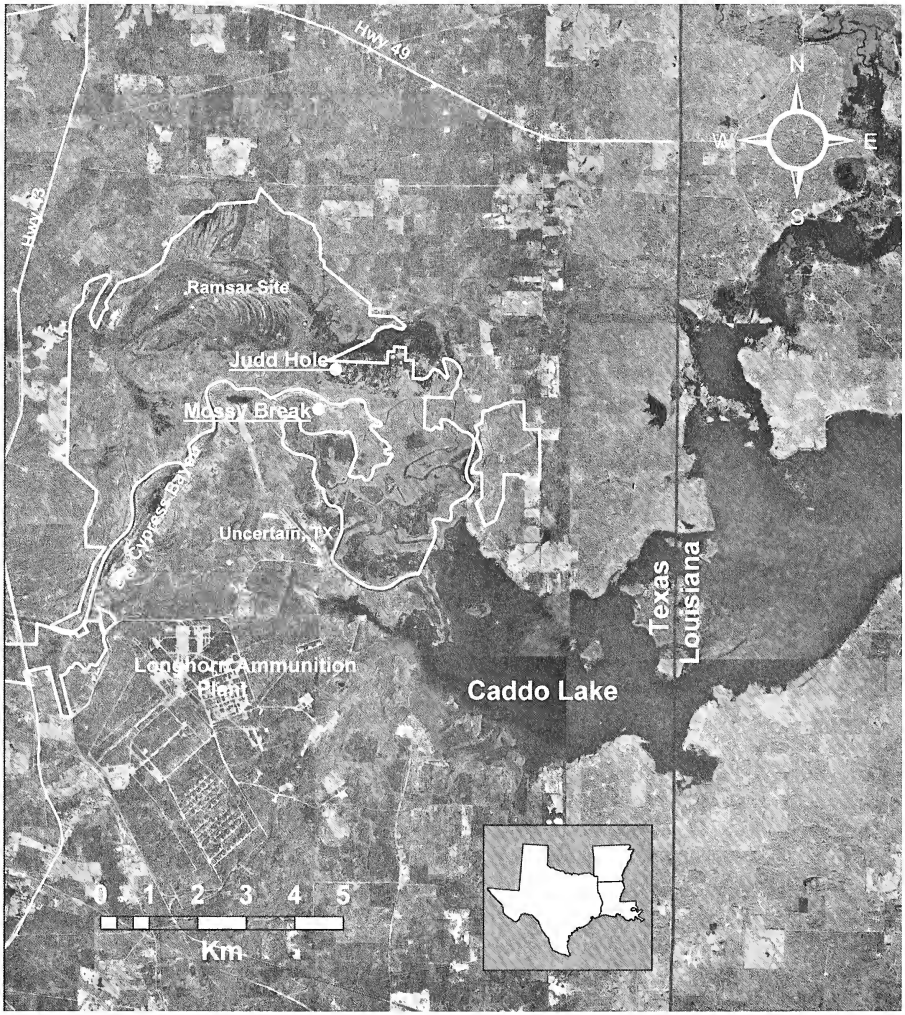

Figure 1. Map of Caddo Lake, Texas. The Ramsar Wetland Site boundary is delineated by the white line. Inset map shows approximate location of study area. Mossy Break and Judd Hole are indicated with white dots. The Texas side of Caddo Lake contains most of the baldcypress stands while the Louisiana side is deeper with more open water.

Preservation of this natural lake was undertaken by The Nature Conservancy in 1992, when it purchased 3,200 ha of Caddo Lake and then donated the land to the Texas Parks and Wildlife

Department (Texas Parks and Wildlife Department 2008). In 1993, this area was declared a Wetland of International Importance under the Ramsar Convention (Texas Parks and Wildlife Department 2008). Although the lake is dominated by baldcypress only, the area surrounding Caddo Lake supports about 120 woody and over 300 herbaceous plant species, as well as 450 species of animals (fish, amphibians, reptiles, birds, and mammals), of which 44 are threatened or endangered (Sierra Club 2000's).

Although baldcypress can cohabitate with species such as *Nyssa biflora* Walter (swamp tupelo) or *Nyssa aquatica* L. (water tupelo) (Brandt & Ewel 1989), Caddo Lake contains exclusive baldcypress stands of relatively younger (about 100 years) and older trees (150 to 300 years in age) (Keeland et al. 1997). Caddo Lake also has two main hydrological regimes, standing water and free-flowing. This study examined differences in forest characteristics and litter production between the young and old stands in two water flow regimes for a monotypic species (baldcypress) at Caddo Lake over multiple yearly time periods. Three specific objectives were tested: (1) to determine whether litter production varied from year to year and monthly, (2) to determine whether litter production differed between standing and free-flowing hydrological regimes, and (3) to determine whether litter production differed between young and old cypress stands.

METHODS

Litter was collected at Caddo Lake, Texas, over a 36-month period (October 1998 through September 2001). Sites were selected from two hydrological scenarios of standing water and free-flowing, each with homogeneous stands of young or old trees. Sites were accessible by motor boat or canoes, and then by hip waders.

Study sites.–The Judd Hole and Mossy Break sites are located in the northwest quadrant of Caddo Lake in Texas (Fig. 1). These sites are approximately 3 km apart and were selected for diverse

water flow characteristics. Judd Hole is a natural backwater swamp, while Mossy break is in a flow-through area. Soils at both sites have large amounts of organic material and clay (Wilson 2003), but Mossy Break has sand that was observed to accumulate along the canal. The young and old stands at Mossy Break are separated by a constructed canal that frequently overflows and allows water to move freely and swiftly through this site. The baldcypress stands of Judd Hole are naturally separated by deep water. The young stand at Judd Hole is on a small island (< 2 ha) surrounded by deep water, while the young stand at Mossy Break is a contiguous area (~4 ha) which grades into a bottomland hardwood swamp. The Judd Hole and Mossy Break sites are also hydrologically connected so that water depths are assumed similar despite being physically separated by distance. The age stands within these sites are homogeneous without any apparent advanced regeneration.

Free-floating litter traps (0.5 m^2) were randomly placed in young and old stands at the two sites. Litter traps were square and constructed of a 2.5 by 15.2 cm pressure-treated wood frame mounted on a rectangular, 10.2 cm pvc pipe float. The inside of the trap was lined with aluminum screen and an additional layer of nylon screen to help reduce the tendency of an item to bounce out of the litter trap. Ten litter traps were placed in each of the young and old stands at each site for a total of 40 litter traps. The traps were tethered to live baldcypress trees which allowed them to be free-floating and track fluctuations in water level. Monthly water levels were obtained from U.S. Army Corps of Engineers water level records at the Caddo Lake weir (Fig. 2) and are concomitant with season and rainfall events occurring within the watershed.

Litter was collected from each trap mid-monthly. Litter was sorted by component (leaves, twigs, bark, Spanish moss, and reproductive parts) and dried at 60°C to a constant weight. Reproductive litter parts consisted of baldcypress male and female cones, as well as scales/seeds.

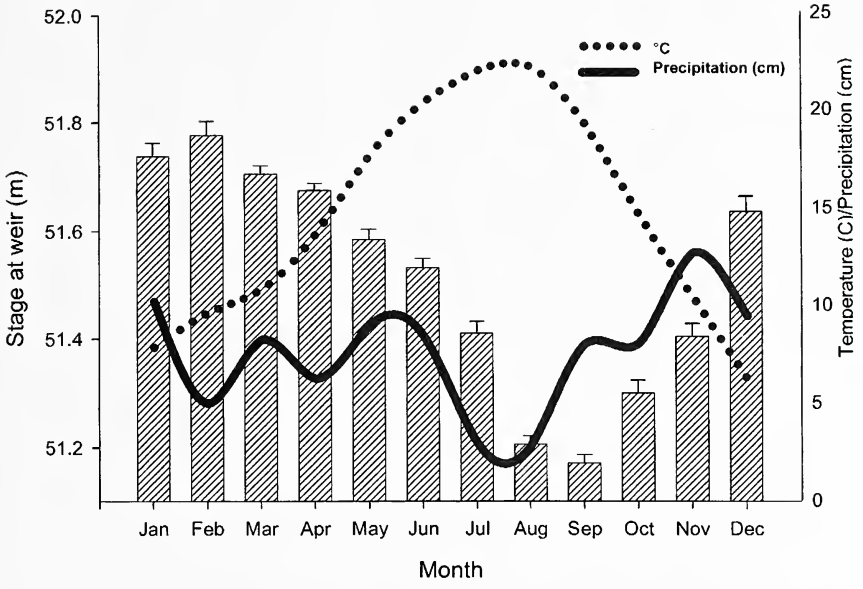

Figure 2. Monthly averages (with SE bars) of water level (m), temperature (°C), and precipitation (cm) from January 1998 to December 2000. Water levels are from unpublished U.S. Army Corps of Engineers data from the Caddo Lake weir. Precipitation and temperature are derived from ClimVis NOAA website East Texas data.

Each baldcypress stand was sampled once in 1998 to measure diameter at breast height (dbh) per tree at 1.37 m and above the buttress, in 200 m^2 circular plots centered at a tree with an attached litter trap. Basal area was calculated from the dbh.

Statistical analyses.– Monthly averages were calculated across all traps per age stand within each site, and were summed for three 12-month yearly totals beginning in October 1998. The use of a repeated measures analysis of variance was not justifiable and not applied because the likelihood ratio test was not significant for selecting repeated measure models and the three yearly totals as time periods were not correlated with each other. Instead, analysis of variance was applied to the yearly total litter production

Table 1. Stem density and basal area for live baldcypress trees in young and old stands at Mossy Break and Judd Hole sites of Caddo Lake, Texas.

Site	Density (stems/ha)		Basal Area (m^2/ha)	
	Young	Old	Young	Old
Mossy Break	2050	437	72.6	72.1
Judd Hole	2037	512	72.0	74.6
Combined	2043	475	72.3	73.3

responses (overall and per litter component separately), testing for differences among time periods, age stand types, and sites, as per Gomez & Day (1982). Monthly litter differences were compared between sites and stand types using *t* tests. The level of significance was $\alpha = 0.05$. All analyses were performed using SAS, version 8, SAS Institute Inc. (1999).

Results

Live stem densities for young stands (>2000 stems/ha) were four times greater than in old stands (~500 stems/ha) (Table 1). Basal areas, however, were alike (72.0–74.6 m^2/ha) in both age stands at the Mossy Break and Judd Hole sites (Table 1).

Leaves comprised about 64% of the total litter production, followed by reproductive parts (13%), twigs or Spanish moss (10%), and bark (3%). Monthly patterns followed natural leaf fall in the late autumn and winter months and reproductive parts in winter and spring, whereas patterns of other litter components were more variable and usually resulted from disturbances to the trees (Fig. 3).

Year was not significantly different for litter collection (Table 2). The amount that litter fluctuated yearly was not more than 10%. Despite the fact that the Judd Hole site is a backwater area and the Mossy Break site is a flow-through area, there were no significant differences between these hydrological sites for any litter component except Spanish moss (Table 2: $P = 0.0202$).

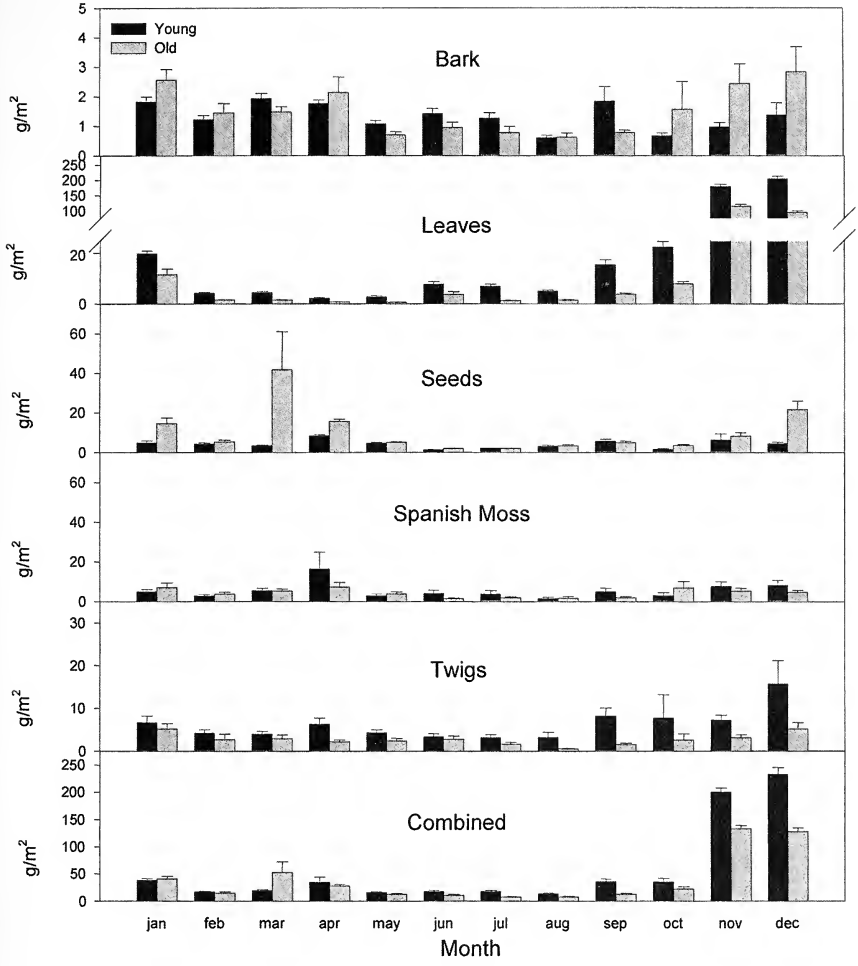

Figure 3. Monthly averages (g/m^2/month) over three years (October 1998 to September 2001) for each litter component and combined litter collected at Caddo Lake, TX. Note scaling: bark is 1/10th the weight of reproductive parts, twigs, and Spanish moss, whereas leaves are 10 times greater. Standard error bars are included.

There were significant differences between the young and old stands for most litter components (Table 2). Young stands produced 1.5 times more combined litter than old stands (P =

Table 2. Yearly sums of litter collected at Caddo Lake, Texas, are presented as g/m^2/year, where time period 1 is October 1998 - September 1999, time period 2 is October 1999 - September 2000, and time period 3 is October 2000 - September 2001. Mossy Break and Judd Hole have been abbreviated to the first word. Data are presented for the main effects of time period, site, and stand, followed by the combination of site with stand.

		Litter Component					
		Combined	Bark	Leaves	Reproductive	Spanish moss	Twigs
Time	1	605	20	375	99	71	40
	2	525	13	345	54	46	67
	3	568	18	357	74	56	63
Site	Mossy	579	18	380	78	40	62
	Judd	553	16	338	73	75	52
Stand	Young	673	14	468	47	63	80
	Old	460	19	249	104	53	34
Site-Stand	Mossy-Young	695	14	509	34	42	95
	Mossy-Old	462	22	251	121	39	29
	Judd-Young	650	15	428	59	83	65
	Judd-Old	457	17	248	87	67	38

0.0011), where there was significantly twice as much leaf and twig litter in young than old stands (P = 0.0002 and P = 0.0032, respectively). In contrast, the old stands produced twice the amount of reproductive litter than the young stands (P = 0.0077). Bark and Spanish moss litter did not statistically differ between stands.

Within both sites, young stands produced substantially more combined, leaf, and twig litter (P = 0.0133, P = 0.0011, and P = 0.0362, respectively) (Table 2). The young stands in Judd Hole had more Spanish moss (P = 0.0480). Old stands exceeded young stands in reproductive litter (P = 0.0328).

There were no significant monthly differences between the hydrological sites for the combined litter collection in young or old stands, and there were no significant site differences in any litter component, except for reproductive parts in young stands (P =

0.0012) and for Spanish moss in old stands ($P = 0.0162$). There were, however, significant differences in litter production between age stands, where young baldcypress stands produced higher monthly combined, leaf, and twig litter than old baldcypress stands ($P = 0.0213$, $P = 0.0020$, and $P = 0.0087$, respectively). Since there were no general statistical differences in sites, monthly data averaged over three years are presented as pooled over sites by age stand (Fig. 3).

Discussion

This study determined the relationship of baldcypress stand age with litter production at Caddo Lake. There were significant stand age differences for litter components except bark. Young baldcypress stands produced about twice the amount of litter than old baldcypress stands. Based on field observations, young stands of baldcypress trees had potentially a greater number of limbs that support more leaves affecting leaf litter production; whereas old baldcypress trees had relatively sparse number of limbs supporting fewer leaves. The amount of reproductive litter, however, was twice greater in the old stands than young stands, suggesting that the enhanced reproductive capability of baldcypress may be associated with maturity since there were 77% fewer baldcypress trees in the old stands than young stands.

Total annual litter production values at Caddo Lake were within the overall litter production range of baldcypress ecosystems between 300 and 600 g/m^2 (Deghi et al. 1980). The Caddo Lake study showed no significant differences in hydrological regimes for litter collection (579 g/m^2 in flow-through vs. 553 g/m^2 in backwater). Two other studies also showed no significant differences in hydrological regimes: 574 g/m^2 in better-drained vs. 620 g/m^2 in standing water (Conner & Day 1976), and 607 g/m^2 in flow-through vs. 650 g/m^2 in still water (Brown & Peterson 1983). Conner & Day (1992), however, reported significantly higher litter production for better-drained sites versus stagnant sites (579 g/m^2 in seasonally-flooded > 401–405 g/m^2 in slowly-flowing > 293 g/m^2

in impounded areas.) There are also examples of low total litter production values in still-water ecosystems, such as at Okefenokee Swamp, GA (328 g/m^2, Schlesinger [1978]) and a cypress swamp in Illinois (348 g/m^2, Mitsch et al. [1977]). Mitsch et al. (1991) reported low baldcypress litter production in slowly-flowing, permanently-flooded waters at Henderson Sloughs, KY (253 g/m^2).

Percentages of litter components are compared with other litter studies. At Caddo Lake, leaves represented 64% of yearly litter collected, with 70% in young stands and 54% in old stands. The reproductive parts comprised of 13% of the total litter, with higher production by old stands (23%) as compared to young stands (7%).

For baldcypress forest studies with comparable average age similar to young stands at Caddo Lake, the percentages of leaf production were often higher and reproductive parts lower. In young cypress ecosystems of Florida (~40 to 120 yrs of age), leaf litter of cypress species accounted for about 85%–95% of the total cypress litter production for most sites (Brown 1981). The Florida sewage-enriched pond cypress dome (~40 yrs old) in the Deghi (1977) study had a similar percentage of leaf litter (70%) as in the Caddo Lake study, but a higher proportion of cypress reproductive parts (15%) influenced by increased nutrient inputs (Brown 1981). Litter of baldcypress at the Prairie Creek floodplain forest (~90 yrs old) near Gainesville, Florida consisted of more leaves (82%) but similar percentages of reproductive parts (7%) as in Caddo Lake (Brown 1981). For the natural, non sewage-treated pond cypress dome (~120 yrs old) at Austin Cary Forest, Florida, leaves represented 85% of the litter but reproductive parts were lower at 2% (Deghi 1977). Similarly, 78% of the litter was leaves and only 3% was reproductive parts for baldcypress (70–95 yrs old) in the Great Dismal Swamp, Virginia (Gomez & Day 1982).

The still-water baldcypress swamps at Okefenokee Swamp, Georgia, had undisturbed remnant forest stands with canopy trees that were 120-200 yrs old (Schlesinger 1978). The yearly amount

of leaves (223 g/m^2) was similar to backwater old stands at Caddo Lake (248 g/m^2) (Table 2). Because of the lower amount of total litter production (328 g/m^2), leaves at Okefenokee Swamp represented a higher percentage (68%) of the total litter production but reproductive parts (10%) was lower than Caddo Lake.

The Caddo Lake study presents detailed monthly patterns of each litter component corresponding with the phenology of baldcypress (Burns & Honkala 1990a) (Fig. 3). Total litter production monthly patterns concurred with basic monthly patterns observed for baldcypress in the Dismal Swamp (Gomez & Day 1982) and in Lac des Allemands swamp in Louisiana (Conner et al. 1981). Leaf fall showed a distinctive pattern primarily from November to December, with gradual elevated amounts in September, October, and January. At Caddo Lake reproductive parts fell primarily in spring after female cones developed and matured during the autumn and male cones matured during the winter. Bark represents a very small fraction of litter and was relatively constant throughout the year with slightly lower quantities during the warmer months. Twig litter was lowest in the baldcypress active growing season and highest from autumn to winter.

Spanish moss comprised 10% of the total yearly litter produced at Caddo Lake and its yearly litter production of 58 g/m^2 was similar to that found by Schlesinger et al. (1975) at 65 g/m^2 collected at Okefenokee Swamp, Georiga. Because baldcypress is considered high in nutrients that leach from its leaves, the Spanish moss biomass is directly associated with nutrient availability (Schlesinger 1977). Furthermore, there was a positive correlation (r = 0.83) between the amount of bark and Spanish moss collected in litter traps. Spanish moss, an epiphyte, may anchor under bark or entwine in twigs of the baldcypress and fall with the shedding bark or breaking twigs.

In general, litter collection methods varied among other studies. The sampling designs were often for a single year with uneven sampling times (increased autumn collections to weekly or biweekly, then monthly or longer afterwards). Multiple yearly collections at Caddo Lake confirmed no statistical differences among typical years similarly as in the 2-year study of Megonigal et al. (1997) or in the 4–5 year study undertaken by Conner & Day (1992).

Traps were generally smaller and varied greatly in other studies, and usually numbered 5–15 per site/area, such as the use of stationary traps (0.25 m^2) (Brown 1981), wooden bushel baskets (0.11 m^2) at ~1.4 m above water level (Schlesinger 1978), stationary litterboxes (0.25 m^2) at 1.5 m above ground level (Mitsch et al. 1991), randomly-located 0.25 m^2 nylon-screen traps at 1 m above ground level (Megonigal et al. 1997), or stationary aluminum-screen baskets (0.25 m^2) elevated above maximum flood levels (Gomez & Day 1982). In addition, Gomez & Day (1982) have acknowledged that their sampling systems were better designed for leaves and not adequate for reproductive or other litter parts. Larger traps (1 m^2 baskets with mesh bottoms) that floated or were attached to fenceposts above floodwater were used in mixed species studies (Brown & Peterson 1983), and 1 m^2 boxes with mesh bottoms fitted on 1 m long legs above flood waters were used by Conner & Day (1992). At Caddo Lake, larger floating traps (with an improved design) tethered exclusively to baldcypress, along with consistent monthly measurements over several years, is believed to assure more accurate annual collections of baldcypress litter.

In conclusion, there were significant differences found in litter production between young and old baldcypress stands at Caddo Lake, Texas. Young stands produced about twice the amount of litter than old stands, with the majority as leaves, whereas old stands produced relatively more reproductive parts. Since age has been shown to be an important factor in litter production at Caddo

Lake, consideration should be given to determining stand age using methods such as tree-ring analysis, and selecting multiple stands with age diversity in litter production studies.

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HETEROBILHARZIASIS (TREMATODA: SCHISTOSOMATIDAE) IN RACCOONS (*PROCYON LOTOR*) OF NORTH-CENTRAL TEXAS

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Abstract.–The mammalian schistosome *Heterobilharzia americana* was collected from a sample of 36 raccoons (*Procyon lotor*) in Archer and Wichita counties of north-central Texas, providing new county records and a northern range extension for the fluke in the state. Overall prevalence of adult flukes in raccoons was 47.2 %, suggesting that the fluke is well established in the region. Fluke parasite load (abundance) and infection rates were compared among host subgroups by sex, age class, and season of capture. No significant differences were found by host sex. Seasonal discrepancies in fluke infection rates and parasite loads were insignificant, though sporadic cercarial exposure in young raccoons and small sample size may have obscured seasonal trends. Host age played a significant role in fluke parasite load and infection rate, with older age class raccoons having greater infection rates, prevalence, and parasite loads, but smaller, less variable mean intensities than younger age class raccoons. While the results of this study concur with previous studies of *H. americana* in mammalian hosts, the reduced mean intensity of flukes in older age class raccoons may indicate a degree of acquired immunity in infected adult hosts, as is commonly seen with other schistosome species.

Heterobilharzia americana (Trematoda: Schistosomatidae) is a common North American mammalian blood fluke. Naturally occurring *H. americana* have been found in a wide variety of mammalian hosts (Price 1929; Price 1943; Malek 1961; Kaplan 1964; Sponenberg 1976; Custer & Pence 1981; Goff & Ronald 1981; Schaffer et al. 1981; Shoop & Corkum 1982; Krotoski et al. 1984; Forrester et al. 1985; Fedynich et al. 1986; Yamini & Schillhorn van Veen 1988; McKown et al. 1991; Forrester et al. 1994; Flowers et al. 2002), and several additional mammalian species have developed patent infections experimentally (Lee 1962b; Malek 1970). However, the raccoon (*Procyon lotor*) appears to be the principle definitive host (Lee 1962a; Flowers et al. 2002).

Cercarial dermatitis (swamp itch) is a common result of human encounters with *H. americana*. However, risk of patent infections in humans appears to be very low, though limited interim development may be possible (Malek & Armstrong 1967). The enzootic region is primarily in the southeastern United States, although *H. americana* has been documented as far north as Kansas (McKown et al. 1991).

Heterobilharzia americana has a derived two-host pattern of transmission (Shoop 1988). All records of naturally infected hosts of *H. americana* indicate that adult flukes mature within the mesenteric veins (Goff & Ronald 1981). Known intermediate hosts of *H. americana* include the lymnaeid snails *Fossaria cubensis* and *Pseudosuccinea columella*, and their distributions exceed the known range of the fluke (Malek 1967; Malek et al. 1987). *Fossaria cubensis* is the predominate intermediate host in east-central Texas, with this region approximating the westernmost endemic range of the fluke (Goff & Ronald 1981).

Methods and Materials

Study site.–Heterobilharzia americana were collected and identified from captured raccoons (*P. lotor*, $n = 36$) in Archer (35° 30' N, 98° 30' W) and Wichita (34° 00' N, 98° 42' W) counties of north-central Texas over a nine-month period from December 2005 to August 2006.

Collection sites consisted primarily of mesquite-shrub savannah interspersed with deciduous trees along riparian zones. In Archer County, the primary collection site was located within the Little Wichita River drainage, 5.8 km N of Archer City, Texas, E of state highway 79. In Wichita County, raccoons were trapped along a 3 km reach of the Big Wichita River (navigated by canoe) just W of Wichita Falls, Texas, S of FM 367. A few raccoons donated from nearby hunters in both counties, and a small number of road-killed specimens from both areas were examined for schistosomes as well. Due to the ecological similarity and close proximity (~35 km)

between collection sites, data were pooled and the sites were treated as a single biotope. All raccoons in this study were taken from rural locations; however, human habitations may have been accessible, given the sizeable home ranges reported for Texas raccoons (Ghert & Fritzell 1997).

Parasite/Host treatment.–Adult *H. americana* were removed from the mesenteric venules following the method of Lee (1962a). No attempts were made to dissect the liver or lungs for immature schistosomules since readily identifiable adults were the focus of the study. Adult male and female *H. americana* specimens were deposited in the U.S. National Parasite Collection (USNPC), Beltsville, Maryland (100212).

Raccoons were aged following a condensed method of Grau et al. (1970) by denoting the sequence of tooth wear on the lower mandible. Their descriptions and diagrams were used as a guide to breakdown the specimens into four age classes: Class I: 0-14 months, Class II: 15-38 mos., Class III: 39-57 mos., and Class IV: $\geq$ 58 mos. Curatorial measurements were taken on captured raccoons (Hall 1962), and voucher specimens were deposited in the Midwestern State University Mammal Collection. The hypothesis that older age class raccoons would have higher fluke infection rates and parasite loads due to increased exposure to cercariae was statistically tested. Differences in fluke infection rates and parasite loads between host sexes and season of collection were also tested.

Data analysis.–The terms prevalence, mean intensity, and parasite load follow the standard reference definitions of Margolis et al. (1982), with prevalence equaling the number of hosts infected divided by number of hosts examined (expressed as a percentage), mean intensity being the total number of flukes in a sample of hosts divided by the number of infected hosts in the sample, and parasite load being the number of flukes among all examined hosts (infected & non-infected) in the sample. For the purposes of this study,

infection rate refers to the number of infected hosts within a sample.

Statistical analyses were conducted using the NCSS 2004 (NCSS, Kaysville, UT) statistical software package. Since count data of *H. americana* were overdispersed and failed to be normalized via transformation, a non-parametric Kruskal-Wallis one-way *ANOVA* on ranks (corrected for ties) was used to compare fluke parasite load and infection rates among host age classes and season of capture. Infection rate data of host subgroups (e.g., age class) were coded (i.e., infected = 1, non-infected = 2) prior to analyses. Where significant ($P < 0.05$) among group differences were indicated, a post-hoc Kruskal-Wallis multiple-comparison *Z*-value test was used to find specific pairwise differences. Mann-Whitney *U*-tests were used to compare fluke parasite load and infection rates between sexes.

Results

A total of 36 raccoons (17 males & 19 females) representing each of four age classes: I ($n = 9$), II ($n = 14$), III ($n = 7$), and IV ($n = 6$) were captured. *Heterobilharzia americana* were encountered frequently within host mesenteric venules, and this study provides new county records (Archer & Wichita), as well as a northern range extension for the fluke in Texas.

Prevalence of *H. americana* in raccoons was 17 of 36 or 47.2 percent (Table 1), and mean fluke intensity was 13.1 ± 5.2 *SE* (range: 2 – 90). Significant differences in infection rates of *H. americana* among raccoon host age classes were found ($H_c = 11.19$, $df = 3$, $P < 0.011$), with age classes III and IV having significantly higher infection rates than classes I ($Z = 2.49$, $P < 0.013$; $Z = 2.29$, $P < 0.023$, class III & IV, respectively) and II ($Z = 2.44$, $P < 0.015$; $Z = 2.22$, $P < 0.027$, class III & IV, respectively). Significant differences of *H. americana* parasite load among raccoon host age classes were also found ($H_c = 8.27$, $df = 3$, $P < 0.041$), with age class III having significantly greater fluke counts than classes I ($Z =$

Table 1. Summary data of *Heterobilharzia americana* infections by host variable (*Procyon lotor*, $n = 36$) from north-central Texas. A total of 223 specimens of *H. americana* were found during this study.

Variable	Prevalence		Intensity	
	n infected/ *n* examined	Percent (%)	Mean ± SE	Range
Age Class				
I (0-14 mos.)	2/9	22.2%	20 ± 18	2-38
II (15-38 mos.)	4/14	28.6%	28 ± 20.7	4-90
III (39-57 mos.)	6/7	85.7%	7.5 ± 2.4	3-19
IV (≥ 58mos.)	5/6	83.3%	5.2 ± 0.8	4-8
Sex				
Male	7/17	41.2%	17.7 ± 12.1	3-90
Female	10/19	52.6%	9.9 ± 3.4	2-38
Capture Season				
Winter (Dec. - Feb.)	10/19	52.6%	6.9 ± 1.6	2-19
Spring (Apr. - May)	3/6	50.0%	5.3 ± 0.6	4-6
Summer (Jun. - Aug.)	4/11	36.4%	34.5 ± 20.1	4-90
Total	17/36	47.2%	13.1± 5.2	$n = 223$

2.33, $P < 0.02$) and II ($Z = 2.11$, $P < 0.036$). Age class IV raccoons had higher parasite loads than classes I ($Z = 1.93$, $P < 0.055$) and II ($Z = 1.67$, $P < 0.095$), and slightly lower parasite loads than class III ($Z = 0.284$, $P < 0.78$); however, the differences were not significant. No significant differences were found regarding infection rates ($H_c = 0.74$, $df = 2$, $P < 0.70$) or parasite load ($H_c = 0.15$, $df = 2$, $P < 0.94$) among hosts by season of capture. Nor were significant differences found regarding infection rates ($U = 143$, $P < 0.51$) or parasite load ($U = 140$, $P < 0.48$) between host sexes.

Discussion

Prevalence of *H. americana* in raccoons from previous studies in Texas revealed fluke prevalence of 22 percent from Brown County (Schaffer et al. 1981) and 50 percent in Burleson County (Goff & Ronald 1981). A similar study involving wild canids in Jefferson, Chambers, Orange, and Galveston counties by Custer & Pence (1981) found *H. americana* prevalence to be 35 percent. Since fluke prevalence in this study (47.2%) exceeded or approached that

of previous reports in regions considered endemic for the fluke, it seems likely that *H. americana* is well established in north-central Texas and possibly much of the Red River Basin of Texas as well.

Significant differences in infection rates and parasite load of *H. americana* by host-age were found among raccoons in this study. Older age classes (III & IV) reflected positive exposure by having greater parasite loads and infection rates, exhibiting fluke prevalence upwards of 83 percent (Table 1). Similarly, Custer & Pence (1981) found a significant increase in prevalence of *H. americana* in aged wild canids. This age-related relationship seems logical due to increased opportunity for exposure to cercariae by mature host animals.

Surprisingly, no seasonal differences in adult *H. americana* infection rates or parasite loads were found among raccoon hosts. Transmission potential undoubtedly increases when snail intermediate hosts are available and ambient water temperatures are conducive for survival and motility of both miracidia and cercariae; however, age-related host responses, seasonal shifts in host foraging habits (Kelley & Horner 2008), and variable times of parasitic development and migration may obscure some seasonal parasite-host relationships.

Although greater fluke parasite loads were found among older raccoon age classes (III & IV), mean fluke intensities were larger and reflected greater variation among young raccoons (ages I & II) with lower and more stable mean intensities in older age classes III and IV (Table 1). Many schistosome spp. are long lived (Kindt et al. 2006) and a convex age-related pattern in mean intensity has been observed for schistosomiasis in humans, peaking in young adults, with acquired immunity increasing during adulthood (Kabatereine et al. 1999). Mean fluke intensities from raccoons in this study suggest that a similar relationship may exist for heterobilharziasis in infected raccoons (Table 1). However, this association should be interpreted cautiously due to small sample

size and the potentially sporadic nature of cercarial exposure among young raccoons.

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MARKER ASSISTED SELECTION IN THE TRANSFER OF ROOT-KNOT NEMATODE RESISTANCE IN THE COMMERCIAL PEANUT (*ARACHIS HYPOGAEA* L.)

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Abstract.–This project was designed to introgress root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood) resistance through backcross breeding into the commercially available peanut (*Arachis hypogea* L.) cultivar 'Tamrun 96' using Marker Assisted Selection (MAS) as the primary selection tool. Nematode-resistant lines were selected and crossed with Tamrun 96. Field and greenhouse screenings were conducted after completion of the RFLP analysis to study the reliability of MAS as a breeding tool. Resistant plants were identified correctly 85% of the time by MAS in the study. The high percentage of correct identification of resistant genotypes coupled with the savings of time resulting from RFLP based selection justifies its use as a selection tool, when coupled with a conventional backcross breeding program.

The root-knot nematode (*Meloidogyne* spp.) is found on the commercial peanut (*Arachis hypogea* L.) throughout the world (Argios 1997). In Georgia, at least one species of the root knot nematode is found in 25% of peanut fields (Motsinger et al. 1976). The most prevalent root-knot nematode species, *Meloidogyne arenaria*, is present in about 41% of Alabama peanut fields (Ingram & Rodriguez-Kabana 1980) and up to 26% of Texas peanut fields (Wheeler & Starr 1987). It has been noted that linear model estimates predict a 10% reduction in yield with an initial population of 44-83 *M. arenaria* eggs or J2 juveniles /500 cc soil (Wheeler & Starr 1987).

Resistance to *M. arenaria* was found in *Arachis* spp. germplasm (Nelson et al. 1989) and then transferred to advanced generation

breeding lines by Simpson et al. (2003). Molecular markers have been identified for *M. arenaria* resistance in *Arachis hypogaea* by Garcia et al. (1996) and Burow et al. (1996). This study reports the transfer of this resistance to *M. arenaria* into the cultivar 'Tamrun 96' (Smith et al. 1997) using RFLP screening methods developed by Burow et al. (1996). In addition, this method was evaluated for the accuracy and feasibility of its use as a viable gene introgression tool in the commercial peanut. Even though the RFLP technique may be outdated by PCR, RFLP remains a useful and repeatable technique for molecular genetic study.

Materials and Methods

Fifty-five component lines were combined to make up the cultivar Tamrun 96. Four of the component lines were selected to serve as recurrent parents in a backcross breeding program. M. C. Blacks' tests indicated an average tomato spotted wilt virus (TSWV) incidence of 34% for the cultivar as a whole (Simpson & Smith 1999) compared to a 50% incidence rate for 'Florunner' (Norden et al. 1969). Blacks' data also indicated that the four lines selected were more tolerant to TSWV when compared to the other component lines.

The nematode resistant lines for the project were chosen from the BC_7F_2 nematode resistant lines developed by Simpson et al. (2003). These were sister lines to NemaTAM, and the chosen lines all exhibited an average of one egg/gram of root or less during testing.

Eight crosses, where two plants of each recurrent parent were crossed with one of eight different nematode resistant male plants, were made at the Texas AgriLife Research and Extension Center at Stephenville under greenhouse culture during the spring of 1997. Ten pegs were produced from each cross. The crossing procedure used was a variation of a published method (Norden 1980), in which only the anthers are manually removed the evening before pollination. Following harvest and curing of hybrid seed, 24 F_1

seed were germinated and plants were grown to maturity under greenhouse culture. From these F_1 plants, 240 F_2 plants were grown and DNA was extracted.

DNA was extracted from the samples by a variation of the extraction protocol reported by Choi et al. (1999). Extractions were repeated if necessary until a minimum of two µg of undegraded DNA were extracted from all 240 F_2 plants. DNA was diluted to 32 µl per two µg with Tris EDTA pH 8.0 (TE). Upon dilution, DNA was digested with the restriction endonuclease *Eco*RI. Samples were then loaded into a 0.8% agarose gel and separated electrophoretically at 0.4 VDC/cm. The gels were run for 30 h. Southern blotting by capillary transfer in 0.4 M NaOH was used to transfer DNA fragments onto Hybond N+ nylon membrane filters (Amersham). The filters were then hybridized with the probe R2430E, which is derived from the root derived cDNA library of G. Kochert, University of Georgia, Athens. The tubes were radioactively labeled by Klenow fragment, using 25 µCi of [α-32P] dCTP, 6000 Ci/mmol according to the protocol used by Burow et al. (2001). Filters were then hybridized in hybridization solution for 36 h (Chittenden et al. 1994). The filters were placed against X-ray film and kept at -80°C until development. After development, samples were scored as susceptible, heterozygous resistant, and homozygous resistant by comparison to Florunner (Norden et al. 1969) or TxAG-6 (Simpson et al. 1993) as controls.

Field evaluations.–The accuracy of the Marker Assisted Selection (MAS) was then tested in two field and one greenhouse study. The field studies were grown in 1999 and 2000 but not replicated due to a limited amount of seed. Each plot was 0.91m by 1.5m and contained five $F_{2:3}$ seed and was grown in a sandy loam soil. The field tests were irrigated and standard management practices were used throughout the growing season. The tests consisted of 149 $F_{2:3}$ breeding lines and three control lines: Tamrun 96 (susceptible), Florunner (susceptible) and 'COAN' (resistant) (Simpson & Starr 2001), which were repeated at random

throughout the test area. In both years, soil samples were collected and nematode eggs extracted and counted on each plot. The results were subjected to chi-square analysis.

Resistance was defined as the reduction or inhibition of nematode reproduction (Holbrook et al. 1983; Starr et al. 1995). Mid-season and selected late season 500 cc soil samples were taken with a soil probe from eight, 30.5 cm deep cores evenly spaced around the outer edge of the root zone of each plot.

The eggs and/or J2 juveniles were extracted by the NaOCl extraction method (Hussey & Barker 1973). The extracted egg samples were agitated five min in a 20% Clorox® (NaOCl) solution diluted with tap water. Eggs were then sieved over a 250 mesh screen to remove debris and 500 mesh screen to capture the eggs. The eggs were rinsed from the 500 mesh screen into a beaker using tap water and made up to a total solution volume of approximately 50 mL. A one mL or five mL sample was then pipetted into a dish for counting. Nematode eggs were counted under a Bausch and Lomb dissecting microscope (Bausch and Lomb Inc., Rochester, New York 14602) with 30x magnification. For this project, any egg observed between a Stage I single cell egg to a Stage VI second stage juvenile was included in the counts. A 7.6 by 3.4 cm counting dish was used to make egg counts. A portion of the dish was counted and then the total population extrapolated from the count and expressed as the total number of eggs per 500 mL of solution. The 149 experimental lines were then evaluated as resistant or susceptible based on comparison with the susceptible controls. Any line with 12% or less of the average of the susceptible controls was considered resistant, while any plot above 12% was considered susceptible. The 12% level was selected as a modification of the 12.5% level as used by Starr et al. (1995).

Greenhouse evaluations.–A greenhouse study was conducted at the Texas AgriLife Research and Extension Center at Stephenville in 2000. The experimental design was randomized complete block

with three replications of 148 $F_{2:3}$ lines. Tamrun 96, Florunner, and COAN were used as controls in each replication, each occurring three times per replication. Plants were grown in sterilized silica sand in 10.2-cm pots. All samples were inoculated with nematode egg inoculant extracted from tomato (*Lycopersicon esculentum* (Mill)) roots obtained from J. L. Starr (Starr et al. 1995). The inoculant solution contained approximately 3,600 eggs/ml. Each pot was inoculated with 0.3 mL or approximately 1,200 eggs at 12 days after planting (DAP). At maturity, the root mass of each sample was separated from the vegetative growth and weighed. The eggs were then extracted using a 1% NaOCl solution, which allowed the eggs to be released into solution and captured using a 500 mesh sieve. The eggs were washed free of the sieve with 25-50 mL of tap water and counted using the method described above. Harvest, extraction, and counting of each replication occurred as one unit on consecutive days. Since the entire root system of each plant could be easily obtained, these egg counts were based on nematode eggs per gram of plant root. Data from the greenhouse samples were logarithmically (x+1) transformed prior to analysis in order to stabilize the variance. The greenhouse data were subjected to the general linear model (GLM) procedure (SAS, Cary, North Carolina).

Statistical analysis was conducted on the results of the molecular screening versus the field and greenhouse studies using chi-square analysis or *ANOVA* statistical analysis consisting of a general linear model test and a means separation using Fisher's protected least significant difference test (SAS, Cary, North Carolina).

Results

Molecular marker data were obtained successfully on 149 of the original 240 F_2 plant samples. $F_{2:3}$ progeny, were field screened the following season under natural infestation. Originally, this was to be the only year of field testing. However, following the 1999 harvest, during a routine species check it was discovered that the population in the field in which the test was conducted was a

mixture of two different species of root-knot nematodes, *M. arenaria and M. javanica*. Previous screenings only included populations of *M. arenaria.* Therefore, a subsequent year of testing using F_3 seed was conducted in 2000 at a different location known to have a pure population of *M. arenaria*.

Field screening will not differentiate between homozygous resistant and heterozygous resistant plants, therefore only two categories were used in classifying the field phenotype with respect to resistance, either resistant or susceptible. Using these parameters, field phenotype and marker score data were in good agreement. Using a resistance level threshold of 12% for the study (Starr et al. 1995), both years of field data did not depart significantly from the expected ratio based on the molecular analyses with a total chi square value of 0.68 for 1999 (Table 1) and 1.33 for 2000 (Table 2). In both years, these values corresponded to probabilities greater than 0.05.

Comparing the greenhouse inoculation trial with the molecular data, a total of 23 of 27 plants homozygous for the resistant marker allele were below the 12% (629 eggs/gram of root) average of the Florunner resistance rating. This represents an 85% correct identification of the homozygous resistant plants. In addition, plants homozygous for the susceptible marker allele had susceptible phenotypes identified in 75 of 84 plants, or 89% agreement between markers and phenotype. There was some ambiguity with regard to the identification of the heterozygous resistant plants. A total of 19 of 38 of the heterozygous resistant plants were below the 12% average of Florunner, for a total of 50% heterozygous plants identified correctly (Table 3). However, the heterozygous resistant plants had egg counts only slightly above the 12% resistance level of Florunner plot egg counts (approximately 629 eggs/gram of root). Assuming the phenotypes are scored correctly, the marker scores may give an incorrect estimate of the genotype due to recombination between markers and the resistance gene. Alternatively, there could be variation in phenotypic measurements

Table 1. Chi-Square analysis of phenotypic and RFLP scoring of 1999 field plots at the Bingham farm for nematode susceptibility. A line with ≤ 12% of the average of the susceptible controls is considered resistant.

Marker Reading	Marker Score	Field Phenotype	Partial χ^2
Resistant	84	89	29
Susceptible	65	60	38

Degrees of Freedom = 1
Critical value (.05) = 3.84
CHI-SQUARE = .68

Table 2. Chi-Square analysis of phenotypic and RFLP scoring of 2000 field plots at the Koonce farm for nematode susceptibility. A line with ≤ 12% of the average of the susceptible controls is considered resistant.

Marker Reading	Marker Score	Field Phenotype	Partial χ^2
Resistant	84	91	58
Susceptible	65	58	75

Degrees of Freedom = 1
Critical value (.05) = 3.84
CHI-SQUARE = 1.33

Table 3. A comparison of RFLP marker versus greenhouse inoculation readings for nematode resistant and susceptible plants.

Marker reading	Greenhouse Reading	Percentage in agreement
homozygous resistant =27	resistant = 23	85%
homozygous susceptible =84	susceptible = 75	89%
heterozygous resistant = 38	resistant = 19	50%

and occasional misclassification could cause some discrepancy. In addition, the possibility also exists that the allele does not act in complete dominance. However, it has been demonstrated that the trait is completely dominate (Choi et al. 1999).

DISCUSSION

A major goal of this study was to determine the feasibility of using MAS as a plant breeding tool. The process by which the

testing and selection of desirable lines was conducted had to be adjusted and refined, because no previous selection work had been conducted based on molecular marker data in peanuts. As more experience has been gained using the process, MAS has proven to be a valuable tool in the Texas A&M University, Texas AgriLife Research peanut breeding program. The high percentage of correct selections for homozygous lines, both resistance and susceptible using MAS, indicates that this method of selection is a viable option to use in any plant breeding program. For the purposes of this study only one resistance marker was used, reported to be 5.8 ± 2.1% cM from the gene of interest (Burow et al. 1996). It is expected that if flanking markers are used, then the accuracy of the molecular data will increase. Using flanking markers will reduce the chance of misidentification because only in the case of a double crossover will misidentification occur.

One obvious advantage associated with the use of RFLP markers is the ability to distinguish between homozygous and heterozygous resistant plants. For the purpose of this study, there was no way to quickly distinguish between homozygous and heterozygous resistant plants based on phenotype in the field and greenhouse studies. However, when selecting plants to utilize in a breeding program, the ability to know which are homozygous resistant before extensive testing of the plants or lines represents a substantial saving of time, labor, and expense.

One final goal of this study was to make the initial crosses required to begin the transfer of nematode resistance into four component lines of Tamrun 96. The original crosses did produce viable seed from which selections were made based on molecular marker data indicated homozygous resistant plants. These selections were then used to make the first backcross. As these lines were being carried forward in the breeding program, MAS was used as the initial tool from which all additional selections were made.

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GENERAL NOTES

NESTLINGS OF BAIRD'S POCKET GOPHER, *GEOMYS BREVICEPS* (RODENTIA: GEOMYIDAE), IN ARKANSAS

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Two species of pocket gophers occur in Arkansas, *Geomys breviceps* (Baird's pocket gopher) and *Geomys bursarius ozarkensis* (Ozark pocket gopher) (Elrod et al. 2000). Pocket gophers are fossorial rodents that spend almost their entire life in solitary burrows, exclusive of short durations during the breeding season and while the offspring are nursing (Chase et al. 1982). Due to these secretive habits, limited descriptions of young pocket gophers exist in the literature. Gestation is estimated at about 30 d, yet Sudman et al. (1986) reported a single case of a *Geomys bursarius* in Kansas giving birth 51 d after capture. Wood (1955) provided descriptions of three age classes of young Baird's pocket gophers in Texas.

Herein, descriptions are provided of different age classes of nestling Baird's pocket gophers in Arkansas. Nestling individuals were recovered by excavating the maternal female's nest and then subsequently removing the young individuals. External measurements (in mm) were recorded for total length, tail length, right hind foot, and right ear along with mass in grams. All individuals were deposited in the Mammal Collection of Arkansas State University Museum of Zoology (ASUMZ). The individuals collected in Johnson and White counties represent new county records supplementing the distribution reported by Elrod et al. (1996) and were verified as Baird's pocket gopher via sequencing of the mitochondrial cytochrome *b* gene as described by Sudman et al. (2006). Additionally, two adult voucher specimens (ASUMZ 28485, 28486) from Johnson County and one adult voucher

specimen (ASUMZ 28532) from White County were deposited after species identification via genetic analysis.

The ages of these litters are based on qualitative descriptions of external characteristics of a *G. bursarius* litter born and raised in captivity by Sudman et al. (1986). Although caution should be expressed when using measurements from captive individuals for extrapolation to non captive individuals, this method was chosen because no descriptions from known ages of non captive nestlings were available. Since adults of *G. breviceps* and *G. bursarius* are similar in morphological characteristics (Schmidly 1983), the juveniles are assumed to be similar as well.

Fifteen immature pocket gophers from nine nests were collected from Arkansas between 27 March 2009 and 25 April 2009. Three nests contained litters of two individuals, and six nests contained a single individual. Thus, average post gestation litter size for Baird's pocket gopher was 1.3 ($n = 9$). Table 1 provides morphological measurements of all the nestlings. The litters fall into three age groups: newborn (one d), 2 wk (10-14 days), and 3 wk (17-23 d). Individuals from the newborn age group were pink and hairless with undeveloped eyes, ears, and cheek pouches. Individuals from the 2 wk age group had undeveloped eyes and ears with the cheek pouches beginning to form and hair on the dorsal side and partially completed hair on the ventral side. Individuals from the 3 wk age group had complete pelage and developed unopened eyes.

The average litter size of *G. breviceps* of 1.3 is lower than average embryo counts of 2.51 and 2.6 reported from two studies in Texas (Pitts et al. 2005; Wood 1949). High postnatal mortality rates may explain the lower average litter size that this study reports versus those reported as embryo counts. Two possibilities may explain these differences. Some nestlings may have fled the nest while the burrow was being excavated and were not captured. On one occasion, a single nestling estimated to be about 21 d old was found hiding within a fecal chamber after the nest had been excavated. Thus, some nestlings may have not been captured, resulting in an underestimation of litter size. Another explanation

Table 1: Localities and biological attributes of nestling *Geomys breviceps* from central Arkansas.

Accession Number	Location	Nest Site	Mass (g)	Total Length (mm)	Tail (mm)	Foot (mm)	Ear (mm)	Age Estimate (Days)
ASUMZ 28514	Johnson Co.	1	36.5	116	30	18	1	21
ASUMZ 28515	Johnson Co.	2	12.5	74	13	10	0	10
ASUMZ 28516		2	12	74	14	9	0	10
ASUMZ 28517	Faulkner Co.	3	38	138	29	19	2	21
ASUMZ 28518	Faulkner Co.	4	12.5	75	13	9	0	10
ASUMZ 28519	Franklin Co.	5	19.5	95	17	11	0	14
ASUMZ 28521	Logan Co.	6	25.5	101	20	14	0	17
ASUMZ 28524	Logan Co.	7	15	85	15	11	0	10
ASUMZ 28525		7	15	86	16	11	0	10
ASUMZ 28531	Sebastian Co.	8	38	124	25	18	1	23
ASUMZ 28533	White Co.	9	4	46	7	5	0	1
ASUMZ 28534		9	5	50	8	5	0	1

may be that pocket gophers exhibit high postnatal mortality rates while in the nest for various reasons. Individuals within a litter may compete for food resources from the mother resulting in unequal survival. Furthermore, pocket gophers may exhibit infanticide in natural populations, subsequently increasing the chance of remaining individuals within litters to survive. Infanticide has been documented from captive *Geomys*, although the exact cause was unknown (Johnson 1926; Sudman et al. 1986).

The observed peak reproductive season seems to occur in Arkansas during March and April, similar to that observed in other studies of *Geomys*. *Geomys bursarius ozarkensis* juveniles have been collected during April, May, and June in Arkansas (Connior 2008), which reinforces the observed breeding peak of *Geomys* in Arkansas. Pitts et al. (2005) reported in Texas that approximately half of Baird's pocket gophers trapped in February and March were pregnant. Furthermore, approximately one-third of female plains pocket gophers collected in Missouri during March and April were pregnant (Pitts and Choate 1997). Vaughan (1962) reported that roughly 50% of *Geomys* females collected in March were either pregnant or ready to breed and 72% collected in April had bred. Although much further south, both *G. attwateri* and *G. personatus* females containing embryos were collected in February and March in southern Texas (Kennerly 1958).

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REPRODUCTION IN BROOK'S KEELED SKINK, *TROPIDOPHORUS BROOKEI* (SQUAMATA: SCINCIDAE), FROM BORNEO

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Brook's keeled skink, *Tropidophorus brookei,* a viviparous species, is endemic to Borneo where it inhabits rocky streams (Das 2004). Information on clutch size is in Inger & Greenberg (1966). The purpose of this paper is to present data on the reproductive cycle of *T. brookei* from a histological examination of museum specimens from Borneo as part of an ongoing study of the reproductive biology in tropical lizards. The first information on the testicular cycle is presented. Sizes for maturity of males and females are given. Evidence suggests that *T. brookei* produces multiple clutches in the same year.

A total of 116 *T. brookei* from Borneo including 48 males (mean snout-vent length, [SVL] = 95.7 mm ± 6.3 SD, range = 79-110 mm), 23 females (mean SVL = 90.8 mm ± 6.2 SD, range = 82-104 mm) and 45 juveniles (mean SVL = 61.13 ± 12.5 SD, range = 38-79 mm) were examined from the herpetology collection of the Field Museum of Natural History (FMNH), Chicago, Illinois. Skinks were collected 1959, 1961-1964, 1970, 1984, 1990. Lizards were from tropical rain forest localites in Borneo.

For histological examination, the left testis was removed from males and the left ovary was removed from females. Enlarged follicles (> 4 mm length) or oviductal eggs were counted (*in situ*). Tissues were embedded in paraffin and cut into sections of 5 µm. Slides were stained with Harris hematoxylin followed by eosin counterstain (Presnell & Schreibman 1997). Slides of testes were examined to determine the stage of the spermatogenic cycle. Slides of ovaries were examined for the presence of yolk deposition or corpora lutea. Histology slides were deposited in the Field Museum

of Natural History (FMNH) herpetology collection. An unpaired *t*-test was used to compare *T. brookei* male and female mean SVL and the relationship between female SVL and clutch/litter size was examined by linear regression analysis using Instat (vers. 3.0b, Graphpad Software, San Diego, CA).

Material examined.–The following specimens of *T. brookei* from Borneo were examined by state, division and district: SARAWAK STATE, Bintulu Division, Bintulu District: FMNH 120357, 120359, 120362, 120364, 120372, 149604, 149605, 149608-149611, 149614, 150937, 150940, 150942-150946, 150949, 150950, 150953, 150955, 150958, 150964, 150971, 150976, 150978, 150985, 150991, 150997, 151003, 151005, 151007, 151010, 151011, 158932, 158942, 158950, 158953, 158961, 158964, 158965, 158967, 158968, 158981, 158982, 158985, 158988, 158990, 188667, 188673, 188677, 188680, 188681, 188688, 221650, 221659, 221660, 221670; Kapit Division, Belaga District: FMNH 221648; Kapit Division, Kapit District: FMNH 145867, 145872-145874, 145876, 145891, 145923, 145926, 145943, 145954, 145959, 145960-145962, 145965, 145966, 145967, 145981, 145982, 145986, 145988, 145989, 145992, 145996, 146003, 146005, 146014, 146016, 146018, 146028-146030, 146032, 146033, 146037, 188668, 188669, 188682, 188683: SABAH STATE, Interior Division, Tenom District: FMNH 243848, 243849, 243860, 243863, 243865, 243866, 243868, 243869; Tawau Division, Lahad Datu District: FMNH 246307, 246311, 246312, 246316, 246317, 246324, 246328, 246333, 246337.

Males were significantly larger than females (unpaired *t*-test, $t = 3.1$, $df = 69$, $P = 0.003$). The only stage present in the male cycle was spermiogenesis (= sperm formation) in which the seminiferous tubules are lined by spermatozoa or groups of metamorphosing spermatids. Numbers of males undergoing spermiogenesis were January (5), February (3), March (3), April (3), May (5), June (4), July (5) August (3) September (5), October (4), November (3), December (5). The smallest reproductively active (spermiogenic) male measured 79 mm SVL (FMNH 146018) and was from August.

Monthly stages in the ovarian cycle are in Table 1. Four stages were present: (1) quiescent (no yolk deposition); (2) enlarged follicles > 5 mm diameter; (3) oviductal eggs or embryos; (4) oviductal eggs and yolk deposition for a subsequent clutch. The smallest reproductively active female (FMNH 150964) measured 83 mm SVL (2 follicles > 5 mm) and was from January. There was a significant positive correlation between female size (SVL) and clutch size ($n = 13$, $r = 0.58$, $P = 0.038$, $Y = -6.20 + 0.103X$). One March *T. brookei* (FMNH 221659) with oviductal eggs was undergoing concomitant yolk deposition for a subsequent clutch indicating multiple clutches are produced. There was no significant difference between clutch/litter sizes for 13 females reported herein and 16 in Inger & Greenberg (1966) (unpaired t-test, $df = 27$, $t = 1.6$, $P = 0.11$). Thus, values from Inger & Greenberg (1966) were combined with this study: mean clutch/litter size = 3.69 ± 1.17 SD, range 1-5.

Subadults were examined to ascertain the minimum sizes for reproductive activity in males and females. This heterogeneous group contained a wide range of sizes (38-79 mm SVL). All had reproductively inactive gonads.

The reproductive cycle of *T. brookei* appears similar to that of other tropical lizards as it exhbits an extended period of sperm formation and egg production (Fitch 1982). Inger & Greenberg (1966) reported spermiogenesis was continuous in *Cyrtodactylus malayanus, C. pubisulcus, Draco melanopogon* and *D. quinquefasciatus* from the same Bornean rain forest. They also reported *T. brookei* females were gravid in all months of the year except April, July and October. Findings from this study include gravid *T. brookei* from these months and these data on continuous spermiogenesis confirms that this species breeds throughout the year. Auffenberg & Auffenberg (1989) reported that females of the congeneric *T. grayi* from the Philippines exhibited a peak in numbers of gravid females prior to the start of the monsoon period. There is insufficient data on *T. brookei* females from Borneo to ascertain a peak in reproduction, if one exists.

Table 1. Monthly stages in ovarian cycle of *Tropidophorus brookei* from Borneo.

Month	*N*	Quiescent	Enlarged Follicles > 5mm	Oviductal eggs or embryos*	Oviductal eggs & yolk deposition
January	2	1	1	0	0
February	1	0	0	1	0
March	1	0	0	0	1
April	1	0	0	1*	0
May	3	0	0	3	0
June	2	1	1	0	0
July	2	0	0	1* 1	0
August	2	0	0	2	0
September	2	2	0	0	0
October	1	0	0	1	0
November	4	4	0	0	0
December	2	2	0	0	0

Continuous spermiogenesis and prolonged periods of reproduction are common in tropical skinks (see Goldberg et al. 2008a; 2008b; 2008c). Production of eggs appears correlated with day length in *Emoia cyanura* and *E. caeruleocauda* (as *E. werneri*) (Baker 1947) or rainfall for various species of skinks from the Philippines (Auffenberg & Auffenberg 1989). Subsequent studies on tropical skinks are needed before the diversity of reproductive patterns exhibited by these lizards can be known.

Acknowledgments

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A CASE OF TAIL MUTILATION IN A NORTH TEXAS SPECIMEN OF THE PLEISTOCENE *DASYPUS BELLUS* (XENARTHRA: DASYPODIDAE)

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The range of the beautiful armadillo, *Dasypus bellus*, extended over much of the southeastern United States that the nine-banded armadillo, *Dasypus novemcinctus*, has come to occupy during the past century (Taulman & Robbins 1996). The beautiful armadillo is known from the end of the Pliocene through the latest Pleistocene, exhibiting a temporal size increase that terminated with Rancholabrean armadillos twice the size of the modern *D. novemcinctus* (Kurten & Anderson 1980; Klippel & Parmalee 1984). As with other armadillos (Dasypodidae), this extinct taxon was protected by plates of tightly fitting osteoderms, and disintegration of this bony armor following each animal's demise provided the opportunity for many hundreds of these tiny diagnostic elements to be dispersed into the environment. Hence, isolated

osteoderms are the most commonly collected remains of fossil armadillos, regardless of context (e.g., alluvial terrace deposits, Slaughter 1959; cave sediments, Lundelius 1985; river gravel bars, Davis & Ball 1991; tar pits, Rincon et al. 2008). These include the several records for *D. bellus* from the north Texas region (Slaughter 1959; Klippel & Parmalee 1984; Dalquest & Schultz 1992).

On 10 Sept. 2009, one of us (RWS) recovered the intact, partially exposed tail tip of a large armadillo (MWSU-VP 14416) from a compact sand bank on the eastern shoreline of the Brazos River in Young County, Texas, approximately 3.0 mi E Elbert, Throckmorton County. This mineralized specimen retains the intact osteoderms of one complete tail ring and the damaged remnants of another (Fig. 1a, left), similar to comparable tail damage seen in a modern armadillo (Fig. 1a, right). The undamaged segment retains the enclosed caudal vertebra (Fig. 1b), and the stub terminates in a tapering boney cap (Figure 1c). This specimen of *D. bellus* is unusual because of the intact nature of the specimen and the preservation of damaged tissue sustained by the animal.

Species determination was determined on several grounds. First, *D. novemcinctus* did not occur locally until the 1950s (Dalquest & Horner, 1984). Some degree of mineralization was readily apparent, and the sound when tapped with a metallic object was reminiscent to that of tapping porcelain. To demonstrate the extent of replacement or mineralization, two small (ca. < 2 mm^3) fragments were removed from the interior of the fossil specimen and subjected to an energy-dispersive EDAX detector which was attached to a Philips XL 30 scanning electron microscope. The results were compared with two samples of bone (one endochondral, one dermal) from a modern animal. Representative component elements (and % weight) of the fossil were:

Al—7.24, 5.16; Si—14.22, 11.97; Fe—4.34, 3.88; Na—1.39, 1.21.

Elements (and % weight; dermal and endochondral, respectively) of a modern specimen were:

Al—0.50, 1.18; Si—1.03, 1.23, Fe—2.05, 0.44; Na—14.11, 5.72.

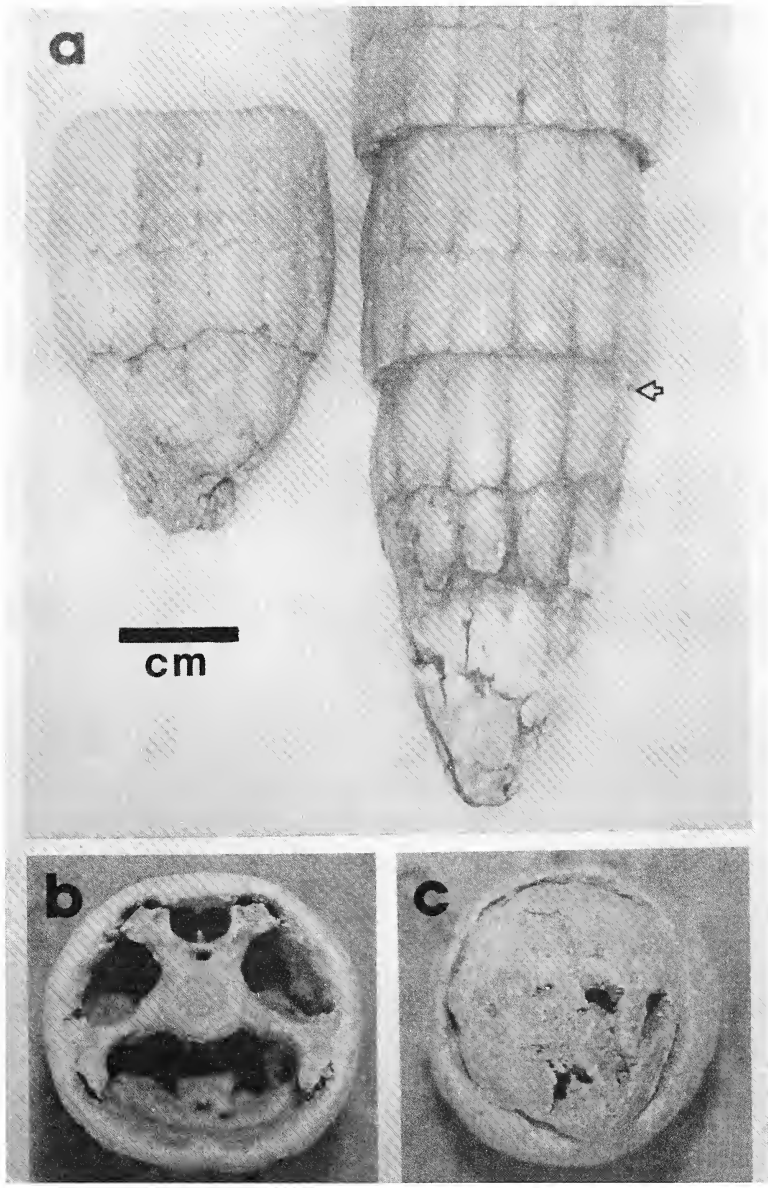

Figure 1. Perspectives of damaged tail nubs in armadillos (*Dasypus*): (a) ventral views of the Pleistocene *D. bellus* (left; MWSU-VP 14416) and a modern *D. novemcinctus* (MWSU 729); (b) proximal view of *D. bellus* tail section, with intact caudal vertebra; (c) distal view of *D. bellus* tail tip. Arrow (1a, right) indicates base of ninth tail ring. The complete fossil tail ring is aligned with the eighth segment of the modern specimen.

A series of 15 study skins of *D. novemcinctus* from the collections of Midwestern State University were assessed for comparative purposes, and included individuals ranging from subadult to old adult (for age criteria, see Stangl et al. 1995). Nearly half (n = 7) of the specimens provided examples of tail damage, ranging from superficial bite marks to the loss of the terminal two-thirds of the tail. Intact and undamaged tails were associated with subadults or young adults, whereas the tails of older animals provided examples of the most severe damage (e.g., Fig. 1a, right).

Initial side-by-side inspection suggested that the intact and undamaged fossil segment most closely corresponded to the 7^{th}-9^{th} tail segments of modern *D. novemcinctus*. Scute counts were taken from the distal-most row of the seventh, eight, and ninth tail segments, and the greatest width of each of the three segments were determined with digital calipers (to nearest 0.01 mm).

Dimensions of tail segments of modern armadillos decrease significantly from proximal to distal, and overlap of diameters between adjoining segments is minimal, even with inclusion of subadults (Table 1). Scute counts also decline significantly from proximal to distal, with minimal overlap (Table 1). This character is established *in utero*. For example, the 7^{th} ring scute count of a neonate, MWSU 19810, was 14 and corresponded precisely to the number of underlying osteoderms. The intact segment of the fossil specimen (width of tail ring, 26.6; osteoderm/scute count, 15) corresponds most closely with the 7^{th} segment in modern armadillos, and almost precisely matches one modern animal (MWSU 728; 7^{th} ring width, 26.9; scute count, 15).

Possible causative factors initiating the observed damage bony regeneration in fossil and extant armadillos is speculative, but would certainly include the bite or chewing of a persistent predator. However, it is noted that *D. novemcinctus* (and presumably also *D. bellus*) is a functional heterotherm with fluctuating body

Table 1. Greatest tail ring widths (in mm) and scute counts from distal-most row of each ring, taken from a series of the modern nine-banded armadillo (*Dasypus novemcinctus*) for comparisons with a specimen of the Pleistocene beautiful armadillo (*D. bellus*; MWSU-VP 14416, width of 26.6, scute count of 15). Numbering system of caudal rings is proximal to distal. Descriptive statistics are: sample size of specimens examined (*N*); mean, standard deviation (*SD*), range (minimum-maximum), and confidence intervals (C.I.).

Tail segment (*N*)	Mean ± *SD*	Range	95% C.I.	Tukey-Kramer Test
		Width of tail ring (mm) ***		
Ring 7 (15)	27.4 ± 1.6	23.4 - 29.4	26.5 - 28.2	|
Ring 8 (14)	24.3 ± 1.4	20.7 - 26.2	23.5 - 25.0	|
Ring 9 (14)	21.2 ± 1.2	18.5 - 22.8	20.6 - 21.9	|
		Distal scute count ***		
Ring 7 (15)	14.1 ± 0.8	13 - 15	13.6 - 14.5	|
Ring 8 (14)	13.1 ± 0.8	12 - 14	12.7 - 13.6	|
Ring 9 (14)	11.9 ± 0.7	11 - 13	11.5 - 12.4	|

*** One-way analysis of variance (*ANOVA*), *** = $P < 0.001$; Tukey-Kramer multiple comparison tests, bars indicate statistically significant subsets (at $P < 0.05$ level).

temperature values reported at 30-36 °C, depending on ambient conditions (McBee & Baker, 1982, and references therein). This physiological feature would be expected to rend extremities susceptible to tissue damage from frostbite, and subsequent loss of resulting gangrenous tissues. Comparable damage to other extremities of local *D. novemcinctus* (e.g., toes, ear pinnae) is not noted, although the tail may simply be more vulnerable.

The fossil specimen of *D. bellus* may well represent an older animal whose longevity was a measure of time of exposure to such potential threats as might cause the observed damage. If the fossil specimen represents the intact 7th and damaged 8th tail segments, then the living animal would have been comparable in size to modern animals. If this scenario is correct, then perhaps the specimen dates back to earlier Irvingtonian times corresponding with both the "smallish" armadillo scute from the Slaton Quarry

(see Dalquest & Schultz 1992), and reflecting the temporal size trend for *D. bellus* described by Klippel & Parmalee (1984).

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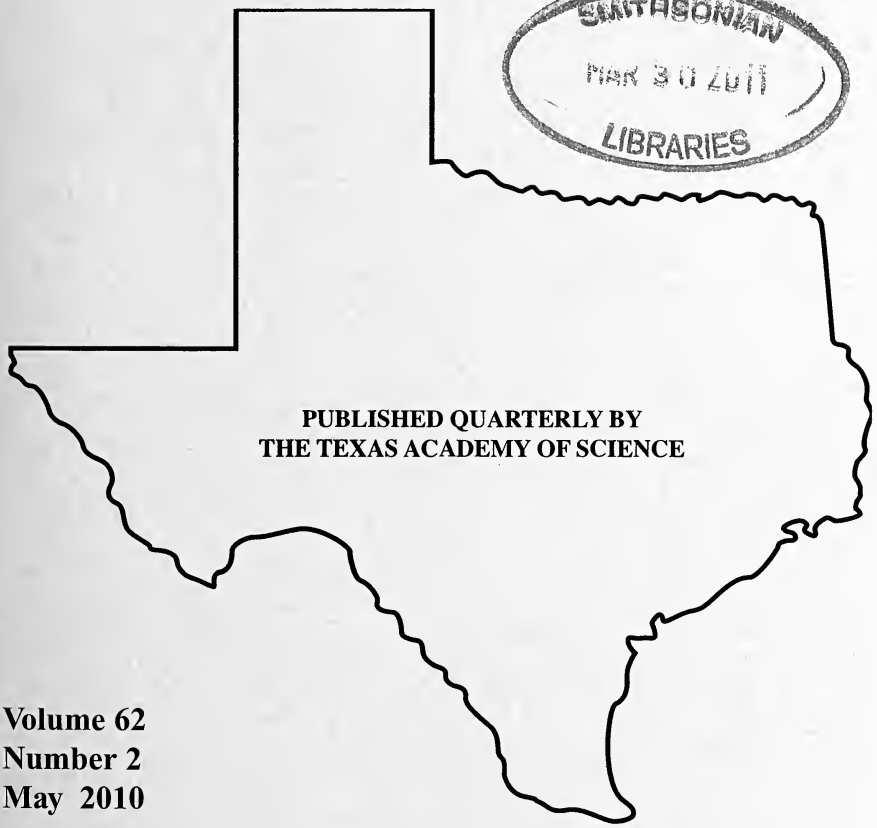

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INFLUENCE OF FEEDYARDS ON BIOAEROSOLS OF TWO SMALL TOWNS ON THE SOUTHERN HIGH PLAINS

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Abstract.–Aerosol particulates and bioaerosols were compared between two small cities located in the Southern High Plains. Aerosol particulate generators in rural communities have not been well studied. City A had more than 35 feedyards located in and near it, while City B had one feedyard located beyond the air sampling area. Two sites were located in each of the two cities, and one farm was located downwind of each city. The sites were monitored non-concurrently in the fall. Aerosol particulates were monitored by using $PM_{2.5}$ and PM_{10} gravimetric monitors; two cyclones air samplers, two laser aerosol monitors, six biological cascade impactors, and a weather station. There were significantly (P <0.0001) higher mean concentrations of $PM_{2.5}$ particulates for City A (16.48 ±1.3 $\mu g/m^3$ of air) compared to City B (7.22 ± 0.7 $\mu g/m^3$ of air). There were no significant differences in PM_{10} concentrations between the two cities (City A, 29.97 ± 2.7 $\mu g/m^3$ of air and City B, 31.63 ± 1.7 $\mu g/m^3$ of air). The cyclone monitor and laser aerosol monitor data indicated higher total concentration of particulates in City B than City A. City A had a significantly (P < 0.0001) higher concentration of total microbes 55.7 ± 3.9 $\mu g/m^3$ of air compared to City B, 33.9 ±2.2 $\mu g/m^3$ of air. The maximum windspeed was higher and lasted for a longer duration in City B than in City A. It was concluded that the feedyards probably increased the concentration of $PM_{2.5}$ particulates in and around City A. These data may have far reaching implications for cities considering having feedyards located in or around their vicinity.

Particulate aerosols and bioaerosols are currently intense areas of study which concern the public, government regulatory officials and public health officials. Various occupations may contribute to aerosol pollution. Heavy industry was the first to be recognized as contributing to urban air pollution, and this pollution was readily associated with detrimental health effects, especially under certain weather conditions such as occurred in London in 1952 (Logan 1953; Bell & Davis 2001) and Donora, PA in 1948 (Snyder 1994).

Initially, the total suspended particle (TSP) concentration was studied and regulated by the Environmental Protection Agency (EPA) Clean Air Act 1972. However, it soon became apparent that the size of the particles became the focus of the EPA, which set concentration standards in 1987 not to exceed (50 $\mu g/m^3$, annually nor 150 $\mu g/m^3$, 24 h). Then it was recognized that fine particles or respirable particles with a diameter of < 2.5 μm ($PM_{2.5}$) were associated with increased deaths due to heart attacks (Dominici et al. 2000; Samet et al. 2000). In 1996, the EPA published a proposal in the Federal Register to set 24-hour concentration standards for $PM_{2.5}$ particles at 50 $\mu g/m^3$ and they received more than 50,000 comments. A final decision was made to set the $PM_{2.5}$ aerosol concentration standard not to exceed 65 $\mu g/m^3$ per 24 h on 18 July, 1997. Again, concerns about the $PM_{2.5}$ standard resulted in proposals to get it lowered (Blodgett et al. 1997). After receiving more than 120,000 written comments, the $PM_{2.5}$ concentration standard was modified again on 21 September 2006 by reducing the 24-h standard to 35 $\mu g/m^3$ of air (EPA 2006). Now there is controversy over which is more important for pathogenicity, particle size or the chemistry of the particle (Seagrave et al. 2006). Many areas of the United States have been successful in reducing urban pollution and many are in compliance with the EPA and state regulations governing particulate pollution.

In the last 10 years, agriculture practices (Centner 2001; Horrigan et al. 2002) and concentrated animal feeding operations (CAFO's) have been recognized as major contributors to rural particulate aerosol pollution (Cole et al. 2000; Donham et al. 2002; Centner 2003; Mallin & Cahoon 2003). Rural aerosol pollution falls under the same EPA standards; however, little is known concerning rural particulate generators, concentration of particulates, and the transport of these pollutants (Lee et al. 2006). Even less is known about how to efficiently reduce this particulate pollution, other than the standard practice of wetting it down with water, which can be quite costly (Miller & Woodbury 2003; Miller & Berry 2005).

Most aerosol collection equipment was designed for collection of pollutants in urban settings and not for collecting excessive particulate pollutants from agriculture. Instrumentation for collecting data with tapered element oscillating microbalances (TEOM) may grossly over estimate PM_{10} particulate emissions compared to gravimetric samplers (Vega et al. 2003). There is also an inherent bias of the PM_{10} pre-collectors when sampling aerosols with mass median diameters (MMDs) greater than 10 μm in size, which is characteristic of agricultural dust (Busser et al. 2001). The collectors used in this study only sampled city industrial particulate pollution that was <10 μm in size.

The objective of this research was to study particulate air pollution and bioaerosols of two small towns, including one farm downwind of each town in order to determine the effects that a feedyard or feedyards might have on populated areas such as towns or small cities. The hypothesis was that associated feedyards may negatively influence air pollution.

Materials and Methods

Sample populations.–Two small towns (City A and City B), approximately 159 km apart with populations of approximately 15,000 were sampled non-concurrently during the fall. Each city was sampled in three locations at approximately the same distance from each site in both cities. Two sites sampled were within the city limits (2.6 km apart) in each city, and one site approximately 11.3 km downwind outside the city limits for each city. The major contrast between the two cities was that City A was near more than 35 feedyards and had an active railroad, while City B had one feedyard downwind, far from any collection sites and had a less active railroad. The distance of aerosol sampling sites from the wastewater plants were as follows: City A sites were 3.8 to 17.8 km from the water treatment sites and City B sites were 2 km to 9.8 km from the wastewater treatment sites. The PM_{10} generated particles do not travel far before they fall to the ground. However, $PM_{2.5}$

particles are capable of traveling long distances before they fall to the ground.

Experimental design.–Property owners granted us permission to set up and maintain monitoring instruments. Air particulate collecting equipment was placed side by side, six meters apart and orientated in a straight line parallel to the prevailing wind direction at each of the three sites in both cities. City A sites were identified as: Chamber of Commerce (C of C), site one, Independent School District (ISD), site two, and Farm, site three; and City B, Lumber Yard, site one, Eagles Lodge, site two, and Farm, site three. These sites were chosen to avoid being too close to buildings and for safety of personnel and equipment during monitoring, and also for their similar distance locations in the two cities.

Air monitoring instruments.–Aerosolized particulates were analyzed by use of high volume (1 m^3/h) sequential Andersen Reference Ambient Air Samplers (RAAS-300 series, Andersen Instruments, Smyrna, GA). PM_{10} (Code of Fed. Reg. 1997 appendix K) (two) and $PM_{2.5}$ (Code of Fed Reg. 1997 appendix L) (two) monitors are stand alone sampling systems that meet the Federal Reference Method (FRM). They provide for multi-filter PM_{10} and $PM_{2.5}$ sampling. These instruments collect dust on a filter (Whatman Filter Device 2µm PTFE, 46.2 mm, Cole Palmer, Vernon Hills, IL) over a 24-h period (Fed Reg. 1997) and then it rotates to another filter every day sequentially for a total of eight d. The $PM_{2.5}$ and PM_{10} filters were equilibrated for 24 h in a desiccator's chamber (Oakton Model #35890-00, Cole Palmer, Vernon Hills, IL) with a relative humidity [RH] of approximately 33%. Each filter was identified and its weight recorded after equilibration. This was done prior to the filters' use and again after the collection of ambient particulates. The weighing was done with an analytical balance accurate to 10 µg (Denver Instruments, Model #M-220D, Arvada, CO). To load, the filters were placed in a filter assembly (RASS-CASS, Thermo Electron Corp, Environmental Instruments). Then the filter assembly was placed in a metal

transport cassette (RAAS-TC2, Thermo Electron Corp, Environmental Instruments, Franklin, MA) that was taken to the site and placed in the appropriate instrument, and at the appropriate indicated day. The $PM_{2.5}$ WINS impactor (2.5 μm cut-off point) glass filter (Whatman 934-AH 37 mm, Cole Palmer, Vernon Hills, IL) was prepared with one drop of oil supplied by the company and it was replaced every eight days when the RAAS instrument was cleaned. The RAAS air flow rate was maintained at 16.5 L/min and the instrument was recalibrated (RAAS Operators's Manual, Section 8, 8-1-8-45, Andersen Instruments, Smyrna, GA) every three months.

Laser strategic aerosol monitor (SAM) (two).–The laser aerosol monitor (SAM, Model 2005, PPM, Inc., Knoxville, TN), is a real-time, microprocessor based, electro-optical instrument providing mass particulate concentrations (expressed in $\mu g/m^3$) and optionally, particle sizing distributions that are expressed in nine channels by size (1.25, 3.00, 4.25, 6.00, 8.50, 12, 17, 24, and > 24μm in diameter). The small particle component of sampled air is electro-optically weighed as mass concentration, and the large particle component is sized according to the projected area, and then converted to mass via an algorithm. The SAM flow rate was maintained at 1.5 L/min, and it utilized proprietary automatic calibration and zero methods covered under US Patents. The SAMs collected dust every three min. Total dust was calculated every hour for 24 h, and data were collected for eight days at each of three sites in City A and City B.

Cyclone air sampler.–Two cyclone air samplers (In-Tox Products, Albuquerque, NM) were made of brass piping with slip joints and specifically designed chambers that collected particulates based on their aerodynamic diameters (5.2 μm to 1 μm). The smallest particles (0.32 μm in diameter) were collected onto a filter. Vacuum pumps (Model 1531-320-G557X, Gast Mfg., Benton Harbor, MI) attached to the cyclone devices were calibrated to maintain a flow rate of 28.3 L/min for 24 h. The cyclone intake

orifice height was placed at 1 m. After collection of dust particles, the device was disassembled and the particulates weighed on an analytical balance.

Biological cascade impactors.–Two-stage and six-stage impactors (Andersen Instruments, Atlanta, GA) were used previously to determine the concentration of bacteria, fungi and endotoxin in the air of Southern High Plains feedyards (Purdy et al. 2004). Briefly, the impactors at one-m height were used to collect bacteria for 15 min at 28.3 L of vacuum per minute. An exception, aerobic mesophilic bacteria were collected on brain heart infusion (BHI) medium for 5 min only. This was done to prevent bacterial overgrowth. Culturable microbial concentrations were calculated as colony forming units per cubic meter (CFU /m^3 of air) after positive-hole correction (using conversion tables for 200 and 400 hole impactors) for possible multiple microbial particle impactions at the same hole in the multiple-hole sampling orifice plate (Macher 1989).

The cascade impactors were used at each site to determine the size of the viable respirable (< 3 μm in diameter) and non-respirable particles (> 5 to 10 μm in diameter) based on the stage of the device they impacted. Stage-zero of the two-stage impactor and stages 1, 2, 3 and 4 of the six-stage impactor were considered to collect culturable non-respirable bioparticles and stage-zero of the two-stage impactor and stages-five and six of the six-stage impactor were considered to collect the culturable respirable bioparticles. Biological cascade samplers were replicated twice in the AM and twice in the PM for each microbial medium at each City site.

This study cultured specifically for facultative anaerobic mesophilic bacteria, aerobic mesophilic bacteria, and thermophilic bacteria on BHI; enteric bacteria were cultured on brilliant green agar (BGA) and MacConkey agar (MAC). Gram-negative isolates were further identified by API-20E Enteric ID system (bioMerieux, Inc., Hazelwood, MO). *Enterococcus* spp. were cultured on

Enterococcosel agar (ECA). The BHI medium contained a fungal inhibitor (cyclohexamide, 100 mg/L). Fungi were cultured on 5% Malt extract agar (MEA) and Littman oxgall agar (LOA). The MEA medium contained antibiotic inhibitors (streptomycin, 100 mg/L and tetracycline, 5 mg/L). Air flow rates were 28.3 L/min, and collection times were 5 min for BHI, 15 min for BGA, MAC, MEA, LOA, and Enterococcosel agar. Both bacterial and fungal CFU were quantified and reported as CFU/m^3 of air. Details on these procedures and methods were previously reported (Purdy et al. 2004). Simultaneously, three two-stage and three six-stage impactors collected in duplicate on each type of medium in the AM and PM at each site. All bacteria were incubated at 37°C for 24 hours, except those on BHI which were incubated at 28°C (mesophilic) for two days. Thermophilic bacteria were incubated at 55°C for 24 hours.

The following parameters for analyzing the biological cascade impactors are reported: (1) various stages of the impactors (two-stage and six-stage), (2) City A and City B, (3) three city sites for each of two cities, and (4) time, AM and PM.

Identification of aerosolized fungi.–Air samples were collected by use of the biological impactors onto Petri plates containing LOA that were cultured to detect airborne mesophilic (28 °C) fungal colonies. Cultures were incubated five days. Fungal colonies were identified (Larone 1995; Watanabe 1994) to the genus level on the basis of gross morphology of a colony, color (top and bottom surfaces), and results of microscopic examination (320X magnification) of hyphae morphology (aseptate or septate), microconidia and macroconidia, and other fruiting structures. Clear 3-cm cellophane tape was bent into a loop with the sticky side on the exterior. The tape was lightly touched to the surface of a fungal colony, and the exposed tape was then placed into a drop of lactophenol cotton blue stain on a microscope slide.

Weather station.–Weather conditions were monitored for each city site by use of a portable weather station (Model Met Data1, Campbell Scientific, Logan, UT) equipped with a 3.5-m tower. The weather station measured wind speed, wind direction, relative humidity, precipitation, soil moisture, air temperature, solar radiation, barometric pressure, and time. The weather station was equipped with a memory storage module. The sampling time occurred at 30-sec intervals and the recording times were 15-min, 1-h, and 24-h intervals.

Statistical analysis.–The experiment was conducted as a completely randomized design with air sample as the experimental unit. $PM_{2.5}$ and PM_{10} gravity data were analyzed with an *ANOVA* by use of mixed linear model analysis for multi-location experimental designs (Littell et al. 1996). City and filter pore size were designated as a fixed effect, and locations were nested within each city. Dust concentrations ($\mu g/m^3$ of air) were used to compare $PM_{2.5}$ and PM_{10} levels at locations within each city.

All other data were analyzed by use of a general linear models procedure (SAS 1988). The nested model was used to examine the effect of city, site, and time on the total microbial populations, and on the respirable (2.5μm) and non-respirable (10μm) diameter size particulate populations. Significant differences between groups were further evaluated by use of the Bonferroni adjusted paired *t*-test. Differences were considered significant at $P \leq 0.05$. Standard error of the mean (± *SEM*) was used throughout the study.

The Pearson correlation coefficient was used to analyze the laser strategic aerosol monitors (SAM) hourly particulate ($\mu g/m^3$) data and the weather station meteorological parameters (hourly data). The analysis consisted of measuring mean total: particle concentrations, < $PM_{2.5}$ particles and < PM_{10} particles by combining the data of both cities (overall), and individually between City A and City B. The sample correlation coefficient is denoted by *r* and a probability that approaches $P < 0.00$. A similar

analysis was made between RAAS 300 (gravimetric) mean total $PM_{2.5}$ and PM_{10} particulates based on a 24 h gravimetric measurement and correlated with meteorological parameters collected for the same 24 h period.

Results

RAAS data.–The mixed model statement for the $PM_{2.5}$ concentration was significant at the $P < 0.0001$ level and there was a significantly higher $PM_{2.5}$ concentration (16.48 ± 1.3 $\mu g/m^3$) for City A compared to ($7.22 \pm 0.66 \mu g/m^3$) for City B. City A and City B $PM_{2.5}$ concentrations were not significantly different among the three collection sites (Figure 1). The only time that the EPA standard for $PM_{2.5}$ (35 $\mu g/m^3$/24 hours) was exceeded (35.46 $\mu g/m^3$) occurred in City A, site three for one day, and the PM_{10} standard was never exceeded. The mixed model statements for PM_{10} concentrations were not significantly different for City A, 29.97 ± 1.7 $\mu g/m^3$, and City B, 31.63 ± 2.66 $\mu g/m^3$. There was a significant ($P < 0.004$) difference in PM_{10} concentration among collection sites within each city: City A, site three was 25.87 ± 2.6 $\mu g/m^3$ and was less than site one, which was 29.86 ± 2.6 $\mu g/m^3$ and site two, which was 36.23 ± 2.8 $\mu g/m^3$. City B, site three was 43.61 ± 5.5 $\mu g/m^3$ which was greater than site one at 25.24 ± 3.3 $\mu g/m^3$, and site two at 24.77 ± 1.9 $\mu g/m^3$.

Biological cascade impactors.–There were significant differences in microbial concentrations between the cities for the following bioaerosols: aerobic mesophilic bacteria grown on BHI, Gram-negative bacteria grown on MAC, and mesophilic fungi grown on LOA and MEA. Significant differences were seen among city sites for: anaerobic mesophilic bacteria, aerobic mesophilic bacteria, aerobic thermophilic bacteria (all grown on BHI), Gram-negative bacteria grown on BGA, and fungi grown on LOA and MEA. A significant ($P < 0.0001$) increase (408 ± 69 CFU/m^3 of air) in fungal colonies grown on LOA was observed in the AM compared to the PM (232 ± 42).

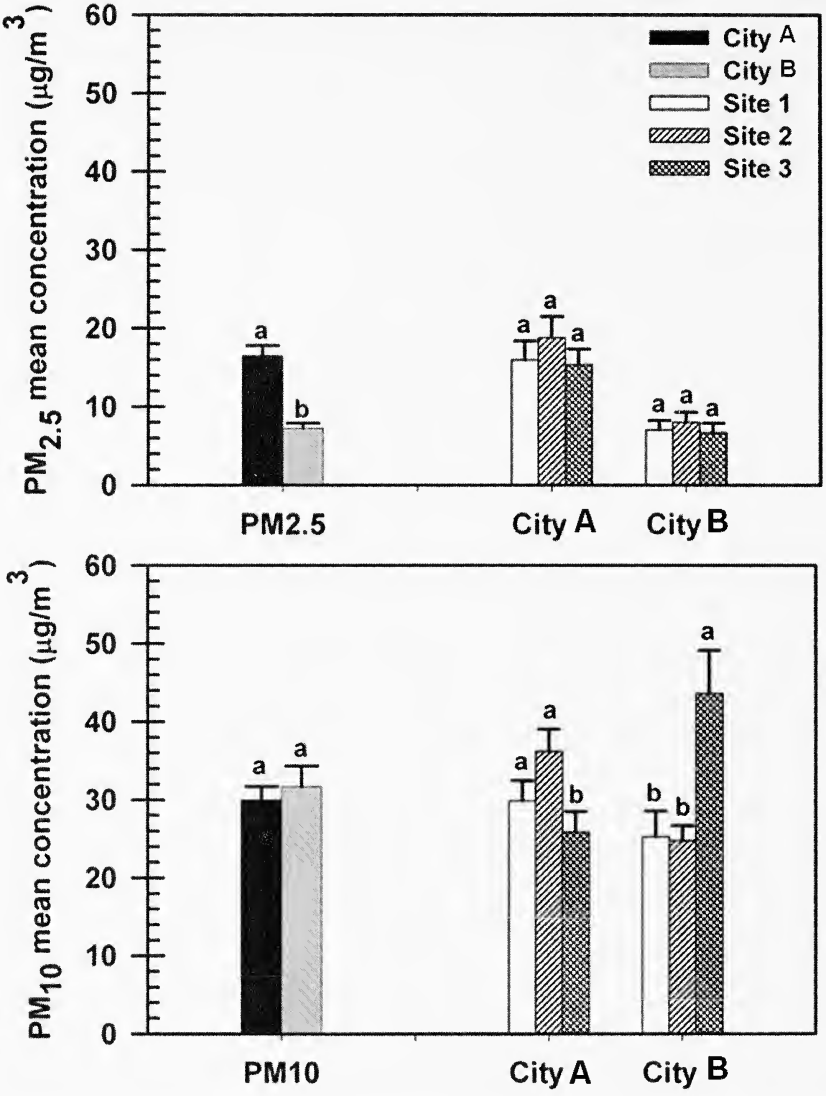

Figure 1. Mean ± *SEM* concentration of $PM_{2.5}$ and PM_{10} particulates are compared between City A and City B and among the three sampling sites in each city. Different under case letters represent significant ($P \leq 0.05$) differences in values.

The mean combined mean total bacterial aerosol (aerobic, facultative anaerobic, thermophilic, Gram-negative, and *Enterococcus* spp.) concentration is shown in Table 1 and the mean

Table 1. Mean bacterial bioaerosols detailed by stage, city, site, and time of day.

Six-Stage Impactor (mm)	Orifice diameter	(*P* <0.0001)	Replications	Mean ± *SEM* CFU/m^3
One	1.81	a	24	939 ±198
Two	0.91	ab	24	779 ± 200
Three	0.71	bc	24	480 ± 72
Four	0.53	bc	24	510 ± 90
Five	0.34	c	24	378 ± 74
Six	0.25	c	24	408 ± 77
Two-Stage Impactor		(*P* < 0.4827)		
Zero	1.55	a	24	1909 ± 518
Double zero	0.40	a	24	1419 ± 463
Cities		(*P* < 0.0630)		
City A		b	96	683 ± 120
City B		a	96	1022 ± 161
City sites		(*P* < 0.0001)		
City A- site one-Chamber of Commerce		b	32	527 ± 96
City A- site two-Independent School District		b	32	293 ± 46
City A- site three-Farm		b	32	1229 ± 325
City B- site one-Lumber Yard		b	32	1813 ± 417
City B- site two- Eagles Lodge		a	32	575 ± 166
City B- site three- Farm		ab	32	680 ± 90
Time		(*P* < 0.5655)		
AM		a	96	905 ± 153
PM		a	96	801 ± 133

Different small case letters represent significant difference (*P*< 0.05) by Bonferroni pairwise comparisons.

Table 2. Mean fungal bioaerosols grown on malt extract agar detailed by stage, city, site, and time of day.

Six-Stage Impactor (mm)	Orifice diameter	(*P* <0.0001)	Replications	Mean ± *SEM* CFU/m^3
One	1.81	b	24	121± 23
Two	0.91	b	24	95 ± 28
Three	0.71	b	24	130 ± 20
Four	0.53	a	24	277 ± 47
Five	0.34	a	24	286 ± 44
Six	0.25	b	24	54 ± 15
Two-Stage Impactor		(*P* < 0.2501)		
Zero	1.55	a	24	737 ± 197
Double zero	0.40	a	24	940 ± 174
Cities		(*P* < 0.0001)		
City A		b	96	169 ± 20
City B		a	96	491 ± 75
City sites		(*P* < 0.0001)		
City A- site one-Chamber of Commerce		c	32	214 ± 47
City A- site two-Independent School District		a	32	227 ± 28
City A- site three-Farm		c	32	66 ± 6
City B- site one-Lumber Yard		b	32	163 ± 46
City B- site two-Eagles Lodge		c	32	473 ± 124
City B- site three-Farm		c	32	837 ± 164
Time		(*P* < 0.8219)		
AM		a	96	335 ± 55
PM		a	96	325 ± 59

Different small case letters represent significant differences ($P<0.05$) by Bonferroni pair-wise comparisons.

Table 3. Mean total culturable microbial population compared between biological impactors and City A and City B.

Microbe growth parameter and medium cultured	Mean total ± *SEM* CFL/m^3 of air – 2-stage impactor (*n*=24)	Mean total ± *SEM* CFU/m^3 of air - 6-stage impactor (*n*-24)	Probability 2-stage different than 6-stage impactor	Mean ± *SEM* CFU/m^3 of air - City A (*n*-24)	Mean ± *SEM* CFU/m^3 of air - City B (*n*-24)	Probability that City A different than City B
Mesophilic facultative Anaerobic Bacteria (Brain Heart Infusion Agar)	2502 ± 729	2403 ± 377	$P < 0.8973$	1848 ± 443	3057 ± 668	$P < 0.1225$
Mesophilic aerobic bacteria (Brain Heart Infusion Agar)	633 ± 106	912 ± 218	$P < 0.0978$	756 ± 213	780 ± 123	$P < 0.8888$
Thermophilic aerobic bacteria (Brain Heart Infusion Agar)	197 ± 133	167 ± 29	$P < 0.8266$	122 ± 30	242 ± 132	$P < 0.3730$
Enterococcus (Enterococcosel Agar-aerobic)	1.67 ± 0.48	1.87 ± 0.60	$P < 0.7376$	1.28 ± 0.55	2.26 ± 0.52	$P < 0.1032$
Gram-negative bacteria (MacConkey Agar)	4.53 ± 0.89	7.57 ± 1.63	$P < 0.0857$	4.22 ± 0.74	7.87 ± 1.68	$P < 0.0419$
Gram-negative bacteria (Brilliant Green Agar)	3.45 ± 0.81	7.46 ± 2.00	$P < 0.0285$	6.19 ± 2.05	4.71 ± 0.85	$P < 0.3970$
Fungi (Littman Oxgall Agar)	1584 ± 310	977 ± 148	$P < 0.0004$	794 ± 96	1767 ± 324	$P < 0.0001$
Fungi (Malt Extract Agar)	1677 ± 336	962 ± 147	$P < 0.0008$	675 ± 80	1964 ± 321	$P < 0.0001$

total fungal aerosol concentration is shown (Table 2). The mean bacteria and fungi grown under different cultural conditions on different media are compared between the two-stage and six-stage impactors and City A and City B are compared (Table 3).

The efficiency of the two-stage and six-stage impactors was for the most part, not significantly different in collecting the various bacteria. However; the two-stage impactor was significantly ($P <$

Table 4. Mean cultural respirable and nonrespirable microbial populations compared among biological impactors and cities and probability City A differs from City B.

Microbe growth parameter and type of media used	Impactors	Mean total ± *SEM* Respirable CFU/m^3 of air n=12	Mean total ± *SEM* Nonrespirable CFU/m^3 of air n=12	Probability (P) that impactors respirable CFU different than nonrespirable CFU	Bioaerosol particle size; Respirable (R) or Nonrespirable (N)	City A mean total ± *SEM* CFU/m^3 of air n=24 Respirable	City B mean total ± *SEM* CFU/m^3 of air n=24 Nonrespirable	Probability city one CFU different than City B CFU
Mean anaerobic bacteria (Brain Heart Infusion agar)	2-stage	215 ± 30	408 ± 89	< 0.0748	R	160 ± 30	222 ± 29	P < 0.0727
	6-stage	166 ± 29	746 ± 199	< 0.0001	N	596 ± 193	558 ± 112	P < 0.7974
Mean total mesophilic bacteria (Brain Heart Infusion Agar)	2-stage	1038 ± 329	1328 ± 471	< 0.4952	R	451 ± 72	1163 ± 333	P < 0.1335
	6-stage	576 ± 119	1828 ± 309	< 9.0048	N	1397 ± 405	1758 ± 394	P < 0.1891
Mean total thermophilic bacteria (Brain Heart Infusion Agar)	2-stage	164 ± 132	82 ± 26	< 0.3839	R	39 ± 9	166 ± 131	P < 0.4438
	6-stage	41± 9	127 ± 24	< 0.3646	N	83 ± 25	125 ± 25	P < 0.1175

Table 4. Cont.

Mean total *Enterococcus* Spp. (Enterococcosel agar)	2-stage	0.59 ± 0.26	1.1 ± 0.45	<3110	R	0.2 ± 0.1	0.9 ± 0.3	$P < 0.2758$
	6-stage	0.49 ± 0.2	1.4 ± 0.53	< 0.717	N	1.1 0.5	1.4 ± 0.4	$P < 0.1558$
Mean total gram-neg. (Brilliant Green Agar)	2-stage	1.8 ± 0.58	1.7 ± 0.52	< 0.9359	R	2.5 ± 1.0	1.0 ± 0.3	$P < 0.5301$
	6-stage	1.7 ± 0.44	5.8 ± 1.49	< 0.0033	N	3.7 ± 1.5	3.7 ± 0.8	$P < 0.2410$
Mean total gram-neg. (MacConkey agar)	2-stage	1.4 ± 0.37	3.2 ± 0.74	< 0.1111	R	1.4 ± 0.4	1.7 ± 0.4	$P < 0.5274$
	6-stage	1.7 ± 0.44	5.9 ± 1.34	< 0.0003	N	2.8 ± 0.6	6.2 ± 1.4	$P < 0.3022$
Mean total fungi (Littman Oxgall agar)	2-stage	1113 ± 208	471 ± 131	< 0.0001	R	432 ± 81	1074 ± 219	$P < 0.0001$
	6-stage	393 ± 95	585 ± 91	< 0.0479	N	363 ± 53	693 ± 143	$P < 0.0056$
Mean total fungi (Malt Extract agar)	2-stage	940 ± 174	737 ± 197	< 0.1330	R	326 ± 58	953 ± 170	$P < 0.0001$
	6-stage	340 ± 51	622 ± 109	< 0.0381	N	349 ± 52	1010 ± 197	$P < 0.0002$

0.0008) more efficient at collecting the fungi cultured on MEA. Concentrations of various bacteria were not significantly different between the two cities, but the fungi were significantly ($P < 0.0001$) 2 to 3-fold higher in City B than in City A (Table 3). The Gram-negative bacterial concentrations were extremely low for both cities.

Respirable and non-respirable mean bioaerosol concentrations of various bacteria and fungi were compared between two-stage and six-stage impactors and between City A and City B (Table 4). The six-stage impactor collected significantly ($P < 0.0001$) more (746 ± 199) nonrespirable facultative anaerobic bacteria compared to respirable (166 ± 29) bacteria, while using the two-stage impactor there was no significant difference between the respirable and nonrespirable bioparticles. Both two-stage and six-stage impactors showed significant differences ($P < 0.05$) between respirable and nonrespirable fungal bioparticles, with the exception of the MEA two-stage impactor. There were no significant differences between bacterial respirable and nonrespirable bioparticles between the two cities. There were significant differences between fungal respirable and nonrespirable bioparticles, and City B had more of both two-stage and six-stage culturable fungal colonies compared to City A.

Fungal identification.–Fungal colonies were identified on all LOA Petri plates that were not too crowded for identification. Identification was completed on 11,432 fungal colonies from both cities; however 210 colonies had no micro- or macro-conidia to help in identification. Twenty-nine genera of fungi were identified from the two cities in descending order, and the top six genera from City A were: *Cladosporium*, 3028, *Alternaria*, 912, *Aspergillus*, 390, *Biospora*, 230, *Rhizopus*, 162, and *Sporothrix,* 37*;* and from City B were: *Cladosporium*, 3,790, *Alternaria*, 1531, *Chaetomium*, 298, *Penicillium*, 284, *Gliocladium*, 256, and *Aspergillus,* 172.

SAM data.–The nine sizes of particles measured by the SAM instruments were combined into three size categories which are as

follows: ≤ PM $_{2.5,}$ ≤ PM_{10}, and particles > PM_{10}. The mean total of the three configured particulate size concentrations were ≤ $PM_{2.5}$, 8.7 ± 0.5, ≤ PM_{10}, 11.6 ± 0.5, and > PM_{10}, 11.6-8.7 = 2.9 μg/m^3 for City A, and ≤ $PM_{2.5}$, 16.4 ± 3.3, ≤ PM_{10}, 20.2 ± 3.8, and > PM_{10}, 20.2–16.4=3.8 μg/m^3 for City B.

Cyclone data.–Mean total cyclone particulates were significantly higher ($P < 0.0049$) for City B, 25.1 (± 3.0) μg/m^3/day compared to City A, 18.5 (± 2.0) μg/m^3/day. The mean total particulate dust measured by Cyclone 1, 22.5 (± 2.6) μg /m^3 /day was comparable to Cyclone 2, 21.1 (± 2.6) μg /m^3 /day. The mean total dust was significantly different (P <0.0011) among the city sites (Table 5). The ambient air samples from each of the two City farm sites (site three) were considerably dustier than each of the intown sites of the two cities.

Meteorological data.–Meteorological data (air temperature, relative humidity, mean wind speed, wind direction, precipitation, and soil moisture) were collected and summarized at the three sites for each of the two cities (Table 6). The data were correlated with the hourly data measured by the SAM monitors and the hourly meteorological data collected by the weather station. There was great variability as expected, due to the many variables, and significant sample Pearson Correlation Coefficients (r) were very small.

Overall (City A and City B statistics combined) mean total ambient particulates (all different particle sizes combined) SAM concentrations were correlated with wind direction ($r = 0.07$, $P < 0.002$, $n = 2021$), and soil moisture (%v/v) (0.117, $P < 0.0001$, n =1441). City A, total particulate concentration correlated with wind direction ($r = 0.104$, $P < 0.0013$, $n = 963$), total solar radiation (W/m^2) ($r = 0.333$, P <0.0001, $n = 383$), and soil moisture (%v/v) ($r = 0.166$, $P < 0.0011$, $n = 383$). City B, total particulate concentrations correlated with wind direction ($r = 0.103$, $P <$

Table 5. Cyclone total mean particulate concentration compared between City A and City B /m^3 of air detailed by stage, city, and site.

5-Stage cyclone	50% cutoff point (μm)	(*P* <0.0050)	Replications	Mean ± *SEM* (μg/m^3/day)
One	5.2	ab	24	38.3 ±6.9
Two	2.1	b	24	17.6 ±2.4
Three	1.4	ab	24	24.9 ±5.9
Four	0.65	b	24	17.8 ±2.9
Five	0.32	b	24	15.5 ±2.3
Filter	<0.32	b	24	16.7 ±2.4
Cities		(*P* < 0.0450)		
City A		b	72	18.5 ±2.0
City B		a	72	25.1 ± 3.0
City sites		(*P* < 0.0011)		
City A- site one-Chamber of Commerce		b	24	14.0 ±2.6
City A- site two-Independent School District		b	24	13.8 ±2.2
City A- site three-Farm		ab	24	27.8 ±4.5
City B- site one-Lumber Yard		ab	24	22.7 ±4.3
City B- site two-Eagles Lodge		b	24	17.0 ±2.8
City B- site three-Farm		a	24	35.5 ±7.0
Cyclones		(*P* < 0.6778)		
One		a	72	22.5 ±2.6
Two		a	72	21.1 ±2.6

Different small case letters represent significant difference (P<0.05) by Bonferroni pair-wise comparisons.

0.0008, $n = 1058$) and soil moisture (%v/v) ($r = 0.117$, $P < 0.0001$, $n = 1058$).

Table 6. Meteorological data summarized for eight to 12 days at each of three sites for each of two cities. Values are means with ranges in parentheses.

City and Site	Air temp. °C	% Relative Humidity	Wind Speed m/second	Soil Moisture %v/v	Precipitation in mm
City A, Site one-Chamber of Commerce	18.8 (10.5 to 28.2)	63.2 (3.0 to 91.7)	1.2 (0.23 to 3.0)	0.67 (0.53 to 0.729)	0.18 (0 to 0.7)
City A, Site two-Independent School Dist.	20.2 (7.4 to 30.9)	44.9 (9.79 to 89.8)	1.6 (0.2 to 5.3)	0.070 (0.044 to 0.114)	1.8735 (0 to 13.2)
City A, Site three-Farm	22.3 (14.6 to 32.6)	ND	3.1 (0.3 to 6.7)	ND	0 (0 to 0)
City B, Site one-Lumber Yard	7,7 (-3.2 to 25.5)	65.3 (21.1 to 95.4)	2.6 (0.3 to 6.8)	0.94 (0.62 to 0.242)	0.1905 (0 to 2.3)
City B, Site two-Eagles Lodge	12.5 (-1.1 to 30.7)	60.3 (12.1 to 95.4)	2.5 (0.3 to 5.8)	0.465 (0.431 to 0.501)	0.03175 (0 to 0.254)
City B, Site three-Farm	13.4 (-5.0 to 32.2)	41.1 (9.4 to 84.1)	3.8 (0.3 to 11.9)	0.297 (0.245 to 0.5010)	0 (0 to 0)

ND = not done due to damaged probes.

City A, < $PM_{2.5}$ particulate concentration correlated with total solar radiation (W/m^2) (r = -0.348, $P < 0.0001$, n = 383) and soil moisture (%v/v) (r = -0.276, $P < 0.0001$, n = 383). City B, < $PM_{2.5}$ particulate concentration correlated with wind direction (r = -0.109, $P < 0.0004$, n = 1058), and soil moisture (%v/v) (r = -0.121, $P < 0.0001$, n = 1058). City A, < PM_{10} particulate concentration correlated with air temperature (°C) (r = -0.146, $P < 0.0001$, n = 963), total solar radiation (W/m^2) (r = -0.336, $P < 0.0001$), soil moisture (%v/v) (r = -0.204, $P < 0.0001$, n = 383), and

precipitation (mm) (r = 0.189, P < 0.0001, n = 963). City B, < PM_{10} particulate concentration correlated with wind direction (r = 0.117, P < 0.0001, n = 1058) and soil moisture (%v/v) (r = 0.204, P < 0.0001, n = 1058).

Overall (City A and City B statistics combined) RAAS gravimetric mean: $PM_{2.5}$ particulates and PM_{10} particulates collected over 24 h periods were correlated with meteorological parameters collected over the same 24 h period. Overall, mean $PM_{2.5}$ particles were correlated with mean air temperature (r = 0.404, P < 0.0001, n = 99), mean wind speed (m/s) (r = 0.287, P < 0.004, n = 99), and total maximum solar radiation (r = 0.345, P < 0.0021, n = 77). Overall, mean PM_{10} particles were correlated with mean % relative humidity (r = -0.349, P < 0.0019, n = 77), and mean wind speed (m/s) (r = 0.226, P < 0.0232, n = 101). City A mean $PM_{2.5}$ particles were not significantly correlated with any meteorological parameters and PM_{10} was only correlated with mean air temperature (°C) (r = -0.294, P < 0.0277, n = 56). City B mean $PM_{2.5}$ particles was only correlated with mean % relative humidity (r = 0.364, P < 0.013, n = 46) and PM_{10} was correlated with mean % relative humidity (r = 0.417, P < 0.0044, n = 45), mean wind speed (r = 0.459, P < 0.0015, n = 45), and total precipitation (mm) (r = -0.295, P < 0.0490, n = 45).

Discussion

The toxicity of rural dust compared to urban-generated $PM_{2.5}$ combustion pollution has not been well studied. Therefore, enforcement legislation for control of rural particulate pollution has been slow, due to the many unknowns about agricultural dust, and to the government's concentration on urban combustion pollution. Thus, there has been a rush to gather data on rural particulate pollution generated by many agricultural practices (Roy & Thorne 2003; Smit et al. 2006; Spaan et al. 2006) and CAFO's in general (Donham 1991; Duchaine et al. 1999; Purdy et al. 2004; Von Essen & Auvermann 2005; Rule et al. 2005).

This study serves as a model to better understand agriculture dust generators and their effect on particle size and transport. For example, it might be of great economic importance for a company deciding to build a multi-million dollar cheese or milk processing plant to know the $PM_{2.5}$ air quality of their desired site. Air handling equipment must be more sophisticated to handle fine particles compared to coarse particles. The integrity of the building must be much tighter to prevent the entrance of fine particles from ambient air. This includes keeping the inner building air under positive pressure and building sophisticated entrance and exit air locks to prevent the entrance of fine particles that may degrade their product. The best choice of location in the present study for an industry that needs better air quality, meaning less $PM_{2.5}$ particles, would be City B.

City B was determined to have the highest total particulate dust concentration during the two-city study, done non-concurrently over 24 d in the fall of the year. However, City A had significantly higher concentrations of $PM_{2.5}$ size particulates which were attributed to the $PM_{2.5}$ dust generated from many feedyards in and around City A compared to City B. This study analyzed the concentration of dust generated by feedyards (Purdy et al. 2007) by subtracting upwind $PM_{2.5}$ background dust from downwind $PM_{2.5}$ feedyard dust over a total of 8 d in the summer and 8 d in the winter in three feedyards. These data show that three feedyards contributed $PM_{2.5}$ dust in the mean amounts of 4.20, 12.18, and 18.18 $\mu g/m^3$ of air averaged over 16 d. This size dust does not settle out easily and may remain in the air for long periods of time. Seedorf (2004) reported that areas in Germany with the highest concentration of respirable particles were from three animal-dense areas, Grafschaft Bentheim, Cloppenburg, and Vechta.

Another possible contributor of $PM_{2.5}$ particles for City A, in contrast to City B would be diesel combustion from trains. This type of $PM_{2.5}$ particles would be expected to leave the gravimetric RAAS filters black. A black filter was collected on one occasion at

City A, site two which was attributed to diesel combustion particles from the trains. Several black filters from City B, site three were also collected. It was later determined that a carbon black plant was located some distance from the Farm site three of City B. It should be noted that there was no significant difference in $PM_{2.5}$ concentration between sites in either city; however, the concentration was uniformly high among sites in City A and uniformly low among sites in City B.

Feedyards also contribute PM_{10} particulates to the air (Purdy et al. 2007). For example, it was determined that immediately downwind from four feedyards the following mean PM_{10} concentrations were generated (272.24, 274.84, 139.00, and 29.63 $\mu g/m^3$ of air). However, these particles will settle out of the air very quickly and may not have any effect one km downwind. It was assumed that feedyard PM_{10} dust concentration had no effect on this two-city study. It is interesting to note that there was no significant difference in the concentrations of PM_{10} particulates between the two cities, although there were significant differences between the sites in both cities. The Farm site of City B had the highest PM_{10} concentration (43.61 ± 5.5 $\mu g/m^3$of air) and this farm had more pasture and prairie than the City A farm (PM_{10} concentration 25.87 ± 2.6 $\mu g/m^3$of air), which was surrounded by cultivated land. The prairie contributed much of the PM_{10} concentration and this difference can be seen (Table 2) in fungal CFUs (837 ± 164 CFU/$m^3$of air) of farm site 3 of City B, compared to farm site 3 of City A (66 ± 6 CFU/$m^3$of air). There is little difference in mean total bacteria between the two sites (Table 1). As long as residents have a healthy immune system and are not allergic to specific fungi, the fungal CFUs reported here are not harmful to humans.

It appears that the bioaerosol data generated in the two cities probably originated more from the PM_{10} particles and not the $PM_{2.5}$ particles. The bioaerosols collected were more concentrated in City B, therefore; bioaerosols do not appear to be associated with the

more concentrated $PM_{2.5}$ dust identified in City A. This is in agreement with Seino et al. (2005), as they reported bacterial aerosols were significantly associated with particles in the PM_5 range but not finer particles. A second report (Boreson et al. 2004) indicated that biological loading increases with an increase in coarse PM concentration. The higher wind speed (11.9 m/second) encountered at the City B farm may have influenced the higher fungal CFUs compared to those cultured at the City A farm where the wind speed was 6.7 m/second (Table 6).

For City B, the SAM recorded approximately twice the $PM_{2.5}$ and PM_{10} size particle concentrations compared to City A and the larger particle (PM_{10}) concentrations were very similar for both cities. The SAM appears to have underestimated the $PM_{2.5}$ particle concentration of 8.7 $\mu g/m^3$of air for City A, compared to the RAAS $PM_{2.5}$ gold standard of 16.48 $\mu g/m^3$of air. The particulate concentrations, as determined by the Cyclone air monitors, were higher (City A, 18.5 $\mu g/m^3$of air, and City B, 25.1 $\mu g/m^3$of air) than that generated by the SAM (<$PM_{2.5}$ for City A, 8.7 $\mu g/m^3$of air and City B, 16.4 $\mu g/m^3$of air).

The SAM data (total range of particulates, < $PM_{2.5}$ and < PM_{10}) were used to determine the sample Pearson Correlation coefficient (r). The SAM instrument had the capacity for collecting hourly concentrations whereas the RAAS 300 gravimetric instruments gave 24 h measurements which were paired with the hourly meteorological weather station data. The numbers of observations were fewer for City A (n = 963 at site 1, and 383 at site 2) compared to City B (n = 1058) because of missing weather observations due to instrument failure. The soil moisture parameter appeared to give the most significant consistent correlation with particulate dust based on the SAM data. City A had a negative correlation for the following dust parameters: total particulate concentration, ≤ $PM_{2.5}$ concentration, and for ≤ PM_{10} concentration; while City B was positive by correlation for the same three parameters. It is assumed this was in part because City A sites

received more rain and irrigation water compared to City B sites. However, there are many other factors such as soil type, vegetation cover, wind direction, and wind speed that can influence these correlations. The RAAS 300 gravimetric data for $PM_{2.5}$ and PM_{10} were also used in determining the sample Pearson Correlation coefficient (r) for 24-h periods. The r values, when significant, were larger than those generated by the laser particulate data correlated with the meteorological data. The only significant commonality between the overall $PM_{2.5}$ and PM_{10} data that correlated with the meteorological parameters was wind speed (m/s). Weather conditions, other than wind speed, are important when the formation of particulates are examined, but there was no significant difference ($P > 0.487$) in percent humidity between City A (64%) and City B (57%) (Table 6). There were significant differences ($P < 0.0001$) in temperature (City A, 20.8 degrees C and City B, 10.3 degrees C) and ($P < 0.001$) wind speed between City A (2.13m/s) and City B (2.95 m/s). The meteorological data were similar in some cases during the time of collection but not identical. There were also instances where the meteorological data were different during the time of collection. It is not believed that it was the weather on those particular days that affected the particulate counts. There were no significant differences in the % humidity between City A and City B (Table 6), and it is humidity that is important in particle formation.

Total mean bioaerosols (bacteria and fungi) were significantly ($P < 0.0009$) more concentrated in City B (1513 CFU/ $m^3$of air) compared to City A (852 CFU/ $m^3$of air) during the study. This held true for the total mean bacterial concentration for City B (1022 CFU/ $m^3$of air) compared to City A (683 CFU/ $m^3$of air), and for total mean fungal concentration in City B (491 CFU/ $m^3$of air) compared to City A (169 CFU/ $m^3$of air). The cyclone concentration data are similar to the RAAS $PM_{2.5}$ data for City A. However, cyclone monitor derived data for City B are 3-fold higher than the RAAS data. This difference may be due to the increased wind during sampling at City B.

In conclusion, the many associated cattle feedyards of City A appeared to have significantly increased $PM_{2.5}$ fine dust concentration. This fine dust would not be expected to settle out of the air. There were no significant differences between the two cities in concentration of PM_{10} particulates, but there were significant differences between sites. These site differences can be explained by the fact that PM_{10} particles settle out rapidly. The fungal CFUs were much higher on City B Farm than City Farm one. This could be due to the presence of prairie grasses compared to City A Farm which consisted mainly of cultivated soil; however there is nothing in the literature regarding this phenomenon. The prairie grasses, especially when wet, would supply a food source that would allow for the multiplication of fungal spores. The two most numerous genera of fungi isolated from both cities were *Cladosporium* and *Alternaria.* Both *Cladosporium* (with an average diameter of 3 μm) and *Alternaria* (average diameter of 5 μm) are respirable (Larone 1995). The most numerous aerosolized bacteria CFUs in both cities were aerobic mesophilic bacteria. There were more non-respirable bacterial and fungal CFUs in both cities compared to respirable CFUs. Microbes of the same genera were identified on all stages of the biological cascade impactors. This indicates that they were traveling on particles of varying sizes. There were usually more nonrespirable CFUs than respirable CFUs identified between the two-stage and six-stage impactors and between the two cities. There were no culturable Gram-negative enteric bacteria isolated from the ambient aerosols. This is an important finding because it decreases the chance of enteric diseases being transported by bioaerosols in ambient air. Gram-negative enteric bioaerosols are very susceptible to ultraviolet light (Chang et al. 1985) and desiccation (Marthi et al. 1990; Purdy et al. 2004), and these factors probably contribute to their absence in ambient air.

The summary of the main findings of this paper is that feedyards probably increase the concentration of $PM_{2.5}$ particulates in and

around cities where they are located. This is of considerable importance because $PM_{2.5}$ particulates are respirable.

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COUNTY RECORDS AND MAJOR RANGE EXTENSIONS FOR WEST CROSS TIMBERS' ANGIOSPERMS FROM TARLETON STATE UNIVERSITY'S HUNEWELL RANCH IN ERATH COUNTY, TEXAS

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Abstract.–Data from floras are critical in establishing species' ranges. This knowledge is essential to conserving plant species and monitoring the spread of introduced species. New plant records in Erath County as well as information on the number of native, endemic, introduced and rare species are reported for an ongoing flora of the vascular plants at Tarleton State University's Hunewell Ranch. Species are compared to those occurring on the state noxious weeds and threatened or endangered species lists. Plant specimens were collected over a twelve month period from September 2006 to August 2007. Ninety-eight taxa were new to Erath County and 35 of these are major range extensions of plants into the West Cross Timbers. Ninety-one taxa are native and seven are introduced species. Five of the native species are endemic to Texas. No plants occurred on the state noxious weeds list, are considered rare, nor were any threatened or endangered species discovered at Hunewell Ranch.

Comprehensive floristic knowledge of species composition and plant communities is fundamental to the preservation and management of plant species. The floristic knowledge of Erath County, located in the southwestern portion of the West Cross Timbers, is both dated and incomplete. The earliest known checklist of plants in Erath County was compiled by Lula C. Gough at Tarleton State University (TSU). Gough collected specimens from Erath County from 1921 to 1923, excluding Poaceae and Cyperaceae as part of her Master's degree from the University of Texas at Arlington (Gough 1923). In addition, a relatively recent atlas of state plants (Turner et al. 2003a; 2003b) has incomplete data for much of the West Cross Timbers including Erath County. The flora of Hunewell Ranch is part of a current and ongoing project to update and extend the floristic knowledge of Erath County.

Hunewell Ranch is currently owned and maintained by TSU in Stephenville, Texas, which is the Erath County seat. Few historical

records of Hunewell Ranch exist, but it is believed that farming operations included a haymaking enterprise, maintenance of cropland sites, and ranching from 1900 to 1935 (Vickery 1991). After World War II, a dairy operation was maintained until approximately 1970. Hunewell Ranch was willed to TSU by Mr. and Mrs. Davis G. Hunewell (Parker 2001). Portions of the ranch are allocated to natural grasses, hay production, and the observation of native biota by students studying field ecology and wildlife sciences at TSU.

Hunewell Ranch is situated in the West Cross Timbers (WCT) region of Texas (Figure 1), which is a belt of woodland interspersed by prairie habitat, ranging from northern Oklahoma to north central Texas (Dyksterhuis 1948; Diggs et al. 1999; Hoagland et al. 1999). Typically this woodland habitat is not continuous and is occasionally breached by the Fort Worth Prairie (Diggs et al. 1999). Historically, this area has not been exploited for its timber, and as a result, many old growth forests remain (Diggs et al. 1999). Farming, ranching, and the cultivation of crops occurred on prairies and the fringes of woodlands (Hoagland et al. 1999). Negligent agricultural practices and fire suppression exacerbated erosion and the overgrowth of weedy species resulting in the suppression of native mid- to tall-grasses and the loss of original climax understory vegetation (Hoagland et al. 1999; Van Auken 2000). Invasion of native prairies and some woody plant communities by plants such as juniper and mesquite has occurred over large areas (Van Auken et al. 1988; Van Auken 2000).

This investigation is part of an ongoing floristic study of Hunewell Ranch and Erath County. The purpose of this portion of the investigation is to document species that are new to Erath County and in some cases the West Cross Timbers.

Study Site

Hunewell Ranch is located in the east-central part of Erath County (coordinates at the ranch gate are 32°12.901 N, 98°06.147W). Elevation ranges from 376 m to 408 m. The total area of the ranch is 474.3 ha. Hunewell Ranch is dominated by post oak-blackjack oak savannah, midgrass prairie, and tall grass bottomland habitats (Parker

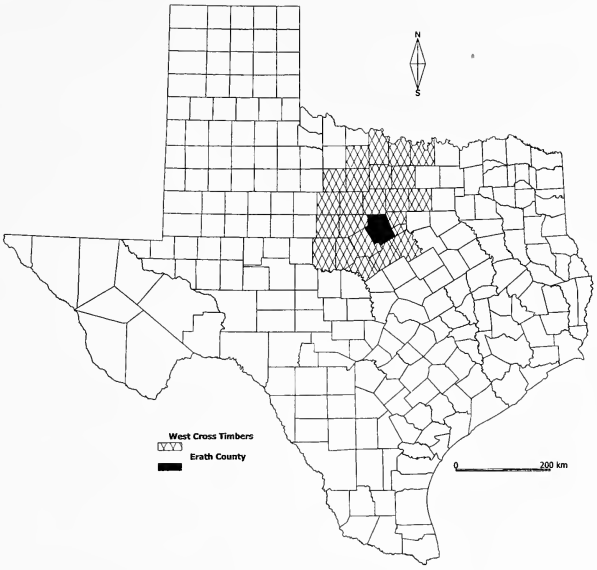

Figure 1. Map of Texas showing the location of Erath County and surrounding counties that contain portions of the West Cross Timbers.

2001). The ranch tends to be highly disturbed as a result of extensive livestock grazing. The incidence of juniper invasion is particularly problematic throughout the ranch (Vickery 1991). Periodic prescribed burning and juniper removal have been used to reduce brush invasion (Parker 2001). The area surrounding Hunewell ranch exhibits the gently rolling topographical relief typical of the WCT region.

Richardson Creek traverses the ranch in an east-west direction. Sycamore Creek is located in the southern half of the ranch and also runs in an east-west direction. There are several stock ponds distributed across the ranch, ephemeral drainage ditches, and gentle sloping terrain typified by occasional flooding is prevalent (Parker 2001). Windthorst-Duffau and Maloterre-Purves-Dugout (Wagner et al. 1973) are the two primary soil associations on the ranch. The Windthorst-Duffau association tends to be moderately deep to deep soil, and varies from sandy to sandy-loam overlying reddish loamy soils and clayey soils. This association ranges from gently sloping to sloping. The Maloterre-Purves-Dugout association consists of

shallow stony and gravelly, clayey soils overlying limestone bedrock (Wagner et al. 1973). This soil association primarily supports open prairie habitat, which is occasionally invaded by juniper and mesquite.

Materials and Methods

Field work was conducted at Hunewell Ranch over a twelve-month period from September 2006 to August 2007. Twelve localities within the ranch were visited monthly to collect plant specimens; however other sites were opportunistically sampled. All major plant communities and habitats occurring at Hunewell Ranch were sampled.

Specimens were processed at TSU Herbarium using standard herbarium procedures (Diggs et al. 1999). Each specimen from Hunewell Ranch was identified and classified as native, endemic, or introduced using *Shinners and Mahler's Illustrated Flora of North Central Texas* (Diggs et al. 1999). Plants from Hunewell Ranch were compared to those that occur on the Texas State-listed Noxious Weeds (USDA 2009), state threatened and endangered plant species list (TPWD 2009), and rare plants of Texas (Poole et al. 2007). Distributions of taxa were compared to those published in the *Atlas of the Vascular Plants of Texas* (Turner et al. 2003 a; 2003b). Taxa new to Erath County but occurring in bordering counties are listed in Table 1. Distributions new to Erath County and not reported from bordering counties are discussed as major range extensions for the WCT.

Results and Discussion

Ninety-one taxa are native and seven are introduced species. Five of the native species are endemic to Texas. No plants occurred on the state noxious weeds list, nor were any rare, threatened, or endangered species discovered at Hunewell Ranch.

Ninety-eight taxa are considered new records for Erath County (Table 1). Thirty-five of the taxa are major range extensions into the WCT and are discussed individually by family in the following paragraphs.

Table 1. Floral records for Erath County that have also been reported from bordering counties (Turner et al. 2003a; 2003b) including Bosque (B), Comanche (C), Eastland (E), Hamilton (Ha), Hood (Ho), Palo Pinto (P), and Somervell (S). For species that have common names, they are included in brackets. Plants that are native (N) to Texas, endemic to Texas (E), and introduced (I) are indicated. Tarleton State University herbarium (TAC) accession numbers are included.

Family/Species/Common name	Bordering Counties	N/E/I	TAC
Apiaceae			
Chaerophyllum tainturieri Hook. [Chervil]	C, E, Ha	N	4222
Cymopterus macrorhizus Buckley [Big-root wavewing]	E, Ha	N	3137
Eryngium leavenworthii Torr. & A. Gray [Leavenworth's eryngo]	B, C, E, Ha, P	N	4224
Sanicula canadensis L. [Canada sanicle]	P	N	4225
Torilis arvensis (Huds.) Link [Hedge-parsley]	P	I	3755
Aquifoliaceae			
Ilex decidua Walter [Deciduous holly]	C, S	N	4228
Asteraceae			
Ambrosia psilostachya DC. [Western ragweed]	B, C, E, Ha, P	N	4231
Baccharis texana (Torr. & A. Gray) A. Gray [Prairie baccharis]	P	N	4233
Chaetopappa asteroides Nutt. *ex* DC. [Common least daisy]	C, E, Ha, Ho, P	N	4235
Conyza canadensis (L.) Cronquist var. *glabrata* (A. Gray) Cronquist [Horseweed]	C, Ho, B	N	4237
Grindelia nuda A.W. Wood [Rayless gumweed]	B, C, P	N	4245
Helenium elegans D.C. [Sneezeweed]	P	N	4247
Helianthus annuus L. [Common sunflower]	E, Ha	N	4248
Hymenopappus scabiosaeus L'Her. var. *corymbosus* Torr. & A. Gray) Turner [Old-plainsman]	C, E, Ha, P	N	4252
Packera tampicana (DC.) C. Jeffrey [Yellowtop]	C, E	N	4253
Parthenium hysterophorus L. [False ragweed]	B, Ha	N	4254
Pluchea odorata (L.) Cass. [Canela]	C	N	4257
Pyrrhopappus grandiflorus (Nutt.) Nutt. [Tuber false dandelion]	C, E	N	4261

Table 1. Cont.

Family/Species/Common name	Bordering Counties	N/E/I	TAC
Senecio ampullaceus Hook. [Texas groundsel]	C	E	4262
Tetraneuris linearifolia (Hook.) Greene	P	N	4266
Verbesina encelioides (Cav.) Benth. & Hook. *ex* A. Gray [Cowpen daisy]	C	N	4267
Xanthium strumarium L. var. *canadense* (Mill.) Torr. & A. Gray [Cocklebur]	E, P, S	N	4268
Brassicaceae			
Lesquerella densiflora (A. Gray) S. Watson [Dense-flower bladderpod]	B, C, E, Ho, P, S	E	1374
L. recurvata (Engelm. *ex* A. Gray) S. Watson [Slender bladderpod]	B, Ha, Ho, S	E	4273
Campanulaceae			
Triodanis perfoliata (L.) Nieuwl. var. *biflora* (Ruiz & Pav.) T.R. Bradley [Clasping venus-looking-glass]	E, Ha	N	4280
Commelinaceae			
Tradescantia occidentalis (Britton) Smyth [Prairie Spiderwort]	C, E, Ha, P	N	4284
Convolvulaceae			
Convolvulus equitans Benth. [Gray bindweed]	C, E, Ha, P	I	4288
Cupressaceae			
Juniperus pinchotii Sudw. [Red-berry juniper]	B, S	N	4290
Cuscutaceae			
Cuscuta indecora Choisy var. *indecora* [Showy dodder]	C	N	4292
Euphorbiaceae			
Chamaesyce geyeri (Engelm.) Small [Geyer's euphorbia]	Ho	N	4293
Fabaceae			
Dalea aurea Nutt. *ex* Pursh [Golden dalea]	C, E, Ha, P	N	4295
D. compacta Spreng. var. *pubescens* (A. Gray) Barneby [Showy prairie-clover]	C, E	N	2395
D. multiflora (Nutt.) Shinners [Round-head dalea]	B, C, E, Ha, Ho	N	2408

Table 1. Cont.

Family/Species/Common name	Bordering Counties	N/E/I	TAC
D. tenuis (J.M. Coult.) Shinners [Prairie-clover]	B, C, Ha, Ho, P	E	4297
Desmodium paniculatum (L.) D.C. [Panicled tick-clover]	P	N	4299
Lespedeza texana Britton [Texas bush-clover]	Ho, S	N	2449
Prosopis glandulosa Torr. [Honey mesquite]	C	N	4305
Hydrophyllaceae			
Phacelia strictiflora (Engelm. & A. Gray) A. Gray var. *lundelliana* Constance	C, E, Ho, S	N	2080
Iridaceae			
Sisyrinchium angustifolium Mill. [Bermuda blue-eyed-grass]	Ha	N	2094
Juglandaceae			
Carya illinoinensis (Wangenh.) K. Koch [Pecan]	B	N	4316
Juncaceae			
Juncus torreyi Coville [Torrey's rush]	C	N	4319
Krameriaceae			
Krameria lanceolata Torr. [Trailing ratany]	B, E, Ha, P	N	4322
Lamiaceae			
Monarda fistulosa L. var. *mollis* (L.) Benth. [Wild bergamot]	P	N	4331
Lentibulariaceae			
Urticularia gibba L. [Cone-spur bladderwort]	P	N	4332
Liliaceae			
Nothoscordum bivalve (L.) Britton [Crow-poison]	C, E, Ha, S	N	4333
Nyctaginaceae			
Mirabilis linearis (Pursh) Heimerl [Linear-leaf four-o-clock]	E	N	4337
Oleaceae			
Forestiera pubescens Nutt. var. *pubescens* [Spring-herald]	B, Ho, P	N	4338
Onagraceae			
Gauara brachycarpa Small [Plains gauara]	Ho	N	4340

Table 1. Cont.

Family/Species/Common name	Bordering Counties	N/E/I	TAC
Papaveraceae			
Argemone albiflora Hornem. subsp. *texana* G.B. Ownbey [White prickly-poppy]	S	N	4343
Polygonaceae			
Eriogonum annuum Nutt. [Annual wild buckwheat]	C, E, P	N	4347
Polygonum amphibium L. var. *emersum* Michx. [Water smartweed]	C	N	4346
Ranunculaceae			
Anemone berlandieri Pritz. [Ten-petal anemone]	B, E, Ha, S	N	4350
Ranunculus sceleratus L. [Blister buttercup]	Ha	N	4351
Rosaceae			
Rubus oklahomus L.H. Bailey [Dewberry]	P	N	4355
Sapotaceae			
Sideroxylon lanugisonum Michx. subsp. *oblongifolium* (Nutt.) T.D. Penn. [Chittamwood]	B, C	N	4356
Scrophulariaceae			
Castilleja indivisa Engelm. [Texas paintbrush]	Ha	N	4359
Leucospora multifida (Michx.) Nutt. [Narrow-leaf conobea]	P, S	N	4360
Nuttallanthus texanus (Scheele) D.A. Sutton [Texas toad-flax]	B, C, P, S	N	4358
Solanaceae			
Datura wrightii Regel [Sacred datura]	Ha, Ho	N	4362
Urticaceae			
Parietaria pensylvanica Muhl. *ex* Willd. var. *pensylvanica* [Pennsylvania pellitory]	S	N	4366
Verbenaceae			
Verbena canescens Kunth [Gray vervain]	C, Ha, S	N	4367
Viscaceae			
Phorodendron tomentosum (DC.) Engelm. *ex* A. Gray [Mistletoe]	C	N	4370
Vitaceae			
Cissus incisa Des Moul. [Cowitch]	Ho, S	N	4372

FAMILY ACANTHACEAE

Ruellia humilis Nutt. (Prairie-petunia; TAC 4218) is a native herb found in prairies and open woods in southeastern and east Texas, west to the Panhandle and the Edwards Plateau (Diggs et al. 1999) but is considered rare in the western three-fourths of the state (Correll & Johnston 1970). The closest collection within the WCT is from Parker County (Turner et al. 2003a). However, more recently it has been found in Eastland County, which borders Erath County to the northwest (McPhail & Nelson 2005). The presence of prairie-petunia at Hunewell Ranch and in Eastland County extends the range of the species into the southwestern WCT.

FAMILY ALISMATACEAE

Sagittaria latifolia Willd. (Common arrowhead; TAC 370) is a native aquatic herb that is widespread in Texas occurring in lakes, ponds, or other wet areas (Diggs et al. 1999). The closest collection within the WCT is from Parker County (Turner et al. 2003a). Its collection in Erath County extends the known range southwestward in the WCT.

FAMILY AMARANTHACEAE

Amaranthus hybridus L. (Slender pigweed; TAC 4220) is a native plant of eastern North America, Mexico, and northern South America and has become a naturalized weed in the Mediterranean region (Diggs et al. 1999). It has been collected from Eastland County in the WCT and its presence at Hunewell Ranch represents a northeastward range extension into the WCT.

FAMILY ASCLEPIADACEAE

Asclepias viridis Walter (Green milkweed; TAC 4230) is a native forb that occurs in east and southeast Texas as well as west in the West Cross Timbers and Edwards Plateau where it occurs on disturbed ground, prairies, ditch banks, and pastures and can become abundant in overgrazed areas (Diggs et al. 1999). Based on Turner et al. (2003a), this specimen represents a range extension for this species into the southwestern WCT.

FAMILY ASTERACEAE

Bidens frondosa L. (Beggar-ticks; TAC 4234) is a native forb that occurs in moist areas in east Texas west to the Rolling Plains and Edwards Plateau (Diggs et al. 1999). The nearest locality reported from the WCT is Parker County (Turner et al. 2003a) and its presence at Hunewell Ranch extends the range of this species southwest into the WCT.

Eclipta prostrata (L.) L. (Pieplant; TAC 4239) is a native forb that occurs along ditches, shorelines, and stream banks throughout Texas (Diggs et al. 1999). The nearest locality reported from the West Cross Timbers is Parker County (Turner et al. 2003a) and its presence at Hunewell Ranch extends the range of this species southwest into the WCT.

Erigeron modestus A. Gray (Plains fleabane; TAC 4241) is a common native plant that often inhabits gravelly or rocky limestone and occurs mostly south and west of the WCT into extreme west Texas (Diggs et al. 1999; Turner et al. 2003a). The presence of this species at Hunewell Ranch extends the range of this plant into the southern portion of the WCT.

Gaillardia aestivalis (Walter) H. Rock var. *aestivalis* (Prairie gaillardia; TAC 4244) is a native forb that occurs in sandy open woods, prairies, and disturbed areas in East Texas, the WCT, and the Edwards Plateau (Diggs et al. 1999). The nearest locality reported from the West Cross Timbers is Parker County (Turner et al. 2003) and its presence at Hunewell Ranch extends the range of this species southwest into the WCT.

Pseudognaphalium obtusifolium (L.) Hill. & Burtt. (Fragrant cupweed; TAC 4259) is a native forb that occurs in southeast and east Texas extending into the Edwards Plateau in wooded areas and roadsides with sandy soils (Diggs et al. 1999; Turner et al. 2003a). The specimen collected from Hunewell Ranch would represent a range extension into the southwestern WCT.

FAMILY BORAGINACEAE

Buglossoides arvensis (L.) I.M. Johnst. (TAC 674), an introduced species originating in Europe, is often found in southeast and east

Texas, the WCT, and the Edwards Plateau (Diggs et al. 1999; Turner et al. 2003a). It often occurs on ditch banks, roadsides, and other disturbed areas (Diggs et al. 1999). Its closest locality to Erath County is a relatively recent report from Eastland County (McPhail & Nelson 2005). The presence of this species in Erath County extends its range northeastward from Eastland County in the WCT.

FAMILY BRASSICACEAE

Rapistrum rugosum (L.) All. (TAC 4275) is a native of the Mediterranean region of Europe. It is a recent introduction to the United States and has apparently been increasing in abundance (Diggs et al 1999). It typically ranges across the Blackland Prairie, southeast and east Texas, and the Edwards Plateau (Diggs et al. 1999; Turner et al. 2003). The collection at Hunewell Ranch represents a major range extension north and west into the southwestern WCT. It is often in great abundance along the roadsides and disturbed fields in Erath County.

Thalspi arvense L. (Field pennycress; TAC 4278) is a native of Europe and occurs in the Edwards Plateau and the northeastern portion of the WCT, often inhabiting various types of soils in disturbed areas (Diggs et al. 1999; Turner et al. 2003a). The presence of this species at Hunewell Ranch expands its range into the southwestern WCT.

FAMILY CACTACEAE

Mammillaria heyderi Muehlenpf. (Flattened mammillaria; TAC 4279) typically inhabits limestone soils in prairie habitat and its range extends across southwest Texas into the Trans Pecos region and from south Texas, north into the Edwards Plateau (Diggs et al. 1999; Turner et al. 2003a). The collection of this native cactus at Hunewell Ranch extends its range into the southwestern WCT.

FAMILY CAPRIFOLIACEAE

Lonicera japonica Thunb. (Japanese honeysuckle; TAC 4281), a native of Asia, is frequently cultivated and escapes becoming established in disturbed areas and woodland habitat across southeast and east Texas into the Rolling Plains and Edwards Plateau regions of Texas (Diggs et al. 1999; Turner et al. 2003a). The presence of this

species at Hunewell Ranch extends its range into the southwestern WCT. This species could be problematic as it may become invasive in some ecosystems (Diggs et al. 1999).

FAMILY CHENOPODIACEAE

Chenopodium leptophyllum (Moq.) Nutt. *ex* S. Watson (slim-leaf goosefoot; TAC 4283) is a native forb that occurs mostly across the western portion of Texas (Diggs et al. 1999; Turner et al. 2003a). The presence of this species at Hunewell Ranch would represent a major range extension into the southwestern WCT.

FAMILY CUSCUTACEAE

Cuscuta cuspidata Engelm. (Cusp dodder; TAC 1406) is a native parasitic plant that occurs in the Edwards Plateau, East Cross Timbers, Panhandle, and the Rolling Plains regions of Texas (Diggs et al. 1999; Turner et al. 2003a). The collection of this species at Hunewell Ranch extends the range of this species into the southwestern WCT.

FAMILY EUPHORBIACEAE

Chamaesyce prostrata (Aiton) Small (Prostrate euphorbia; TAC 4294) occurs along stream banks, prairies, and disturbed sites in clay soils throughout most of Texas (Diggs et al. 1999; Turner et al. 2003a). The collection of this native species at Hunewell Ranch extends its range into the southwestern WCT.

FAMILY FABACEAE

Cercis canadensis L. var. *canadensis* (Eastern redbud; TAC 2375) is a native shrub or small tree that occurs on sandy or silty soils predominantly in northeastern Texas (Diggs et al. 1999; Turner et al. 2003a). Its collection from Hunewell Ranch serves as a major range extension into the southwestern WCT.

Lathyrus pusillus Elliott (Low peavine; TAC 4301) is a native legume that occurs in sandy and silty soils mostly in the southern one-half of Texas and the Edwards Plateau (Diggs et al. 1999; Turner et al. 2003a). The presence of this species at Hunewell Ranch extends the range of this species into the southwestern WCT.

Mimosa strigillosa Torr. & A. Gray (Powderpuff; TAC 4304) is a native legume that occurs in southeast and east Texas to the coast, including the sourthern Edwards Plateau (Diggs et al. 1999; Turner et

al. 2003a). The specimen collected from at Hunewell Ranch extends its range into the southwestern WCT.

Senna occidentalis (L.) Link (Coffee senna; TAC 4308), a native legume, occurs from the Edwards Plateau into southeast and east Texas in disturbed sites (Diggs et al. 1999; Turner et al. 2003a). Its collection at Hunewell Ranch extends its range into the WCT.

Vicia sativa L. (Narrow-leaf vetch; TAC 4310; 4312), introduced from the Mediterranean region of Europe, occurs in disturbed areas and its range extends from southeast and east Texas into the Edwards Plateau (Diggs et al. 1999; Turner et al. 2003a). The presence of this species at Hunewell Ranch and a relatively recent report from Eastland County (McPhail & Nelson 2005) extends its range into the southwestern WCT.

FAMILY HYDROPHYLLACEAE

Phacelia patuliflora (Engelm. & A. Gray) A. Gray var. *teucrifolia* (I.M. Johnst.) Constance (TAC 4313), a native forb, occurs in open woodlands of southeast Texas extending west and north into the Edwards Plateau (Diggs et al. 1999; Turner et al. 2003a). The collection of this species from Hunewell Ranch would represent the first incidence of this variety in the WCT.

FAMILY IRIDACEAE

Sisyrinchium pruinosum E.P. Bicknell (Dotted blue-eyed grass; TAC 4314) is a common native plant occurring in prairies and disturbed areas across southeast and east Texas, the WCT, and the eastern Edwards Plateau (Diggs et al. 1999; Turner et al. 2003b). The presence of this species at Hunewell Ranch extends the range of this plant into the southwestern WCT.

FAMILY JUNCACEAE

Juncus bufonius L. (Toad rush; TAC 4317) is widespread, native rush found in damp soils throughout much of Texas including the Trans-Pecos, Edwards Plateau, and eastern Texas (Diggs et al. 1999; Turner et al. 2003b). The collection of this species from Hunewell Ranch extends the range of this species in the southwestern WCT.

FAMILY LAMIACEAE

Calamintha arkansana (Nutt.) Shinners (Ozark savory; TAC 2172) occurs on calcareous outcrops from east Texas, the Lampasas Cut Plain, and the Edwards Plateau (Diggs et al. 1999; Turner et al. 2003a). Its collection at Hunewell Ranch would represent the first incidence of this native species in the WCT.

Lycopus americanus Muhl. *ex* Barton (Virginia bugleweed; TAC 4324) is a native mint that occurs in low, wet, moist areas in the Panhandle, WCT, Lampasas Cut Plain, and the Edwards Plateau (Diggs et al. 1999; Turner et al. 2003a). The nearest locality to Erath County reported from within the WCT is Parker County and its presence at Hunewell Ranch extends the range of this species southwest into the WCT.

Monarda citriodora Cerv. *ex* Lag. var. *citriodora* (Lemon bee-balm; TAC 4328) is a native mint that occurs in savannahs, prairies, and roadsides throughout Texas (Diggs et al. 1999). Turner et al. (2003a) reports only on a coastal variety, omitting the widespread typical variety. The specimen collected from Hunewell Ranch confirms the presence of the typical variety in the southwestern WCT.

FAMILY MALVACEAE

Sida abutifolia Mill. (Spreading sida; TAC 4336) is a widespread native occurring on rocky prairies, limestone outcrops, and roadsides across Texas, including the Trans-Pecos, Blackland Prairie, Edwards Plateau, WCT, and the East Cross Timbers (Diggs et al. 1999; Turner et al. 2003a). Our collection of this species at Hunewell Ranch extends its range into the southwestern WCT.

FAMILY ONAGRACEAE

Gauara longiflora Spach. (Tall guara; TAC 2879), a native forb, occurs mostly in the eastern one-half of Texas in open, disturbed habitats (Diggs et al. 1999; Turner et al. 2003a). Its presence at Hunewell Ranch would extend its range into the southwestern WCT.

Oenothera triloba W.L. Wagner (Evening-primrose; TAC 4342) is an endemic preferring open grasslands and disturbed habitat throughout much of Texas (Diggs et al. 1999; Turner et al. 2003a).

The presence of this species at Hunewell Ranch expands the range of this species in the southwestern WCT.

FAMILY RHAMNACEAE

Berchemia scandens (Hill) K. Koch (Supplejack; TAC 4352) inhabits woodland habitat in the eastern one-half of Texas (Diggs et al. 1999; Turner et al. 2003a). The presence of this native species at Hunewell Ranch extends its range into the southwestern WCT.

FAMILY SCROPHULARIACEAE

Agalinus heterophylla (Nutt.) Small *ex* Britton (Prairie agalinis; TAC 4357) is a native forb occurring in prairies and open woodlands in the eastern one-half of Texas (Diggs et al. 1999; Turner et al. 2003a). The nearest locality to Erath County reported from within the WCT is Parker County and its presence at Hunewell Ranch extends the range of this species into the southwestern WCT.

FAMILY TYPHACEAE

Typha latifolia (Cat-tail; TAC 370) ranges across most of Texas, usually inhabiting low, wet areas (Diggs et al. 1999; Turner et al. 2003b). Its collection at Hunewell Ranch confirms the presence of this native species in the southwestern WCT.

FAMILY ULMACEAE

Ulmus americana L. (American elm; TAC 4364) is a native tree that occurs in stream bottoms across most of Texas with the exclusion of the southern one-fourth of the state (Diggs et al. 1999; Turner et al. 2003a). The presence of this species in Erath County extends its range into the southwestern WCT.

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METAZOAN PARASITES OF *PEROMYSCUS PECTORALIS* (RODENTIA: MURIDAE) IN CENTRAL TEXAS

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Abstract.–New records for *Peromyscus pectoralis* as a host species were confirmed for nine arthropods: three fleas (*Jellisonia bullisi*, *J. ironsi*, and *Atyphloceras echis echis*), one tick (*Ornithodoros* sp.), two mites (*Androlaelaps fahrenholzi* (=*Haemolaelaps glasgowi*) and *Ornithonyssus* sp.), two chiggers (*Euschoengastia fronteriza* and *Euschoengastia criceticola*) and one sucking louse (*Hoplopleura hesperomydis*), and six helminths: one nematode (*Carolinensis tuffi*), two trematodes (*Scaphiostomum pancreaticum* and *Zonorchis komareki*), two cestodes (*Catenotaenia dendritica* and a larval cestode, *Strobilocercus* sp.), and one acanthocephalan (*Moniliformis clarki*). *Carolinensis tuffi* and *Zonorchis komareki* had the highest prevalence and total abundance.

Metazoan parasites have been reported for 19 of the approximately 60 species of *Peromyscus* (Hall 1981); however, except for *P. boylei*, *P. floridanus*, *P. leucopus*, *P. maniculatus*, and *P. truei*, few comprehensive parasite surveys on individual species of white-footed mice (*Peromyscus*) have been completed (Layne 1963; Whitaker 1968). Few records exist for metazoan parasites of the white-ankled mouse, *Peromyscus pectoralis* (cf. Schmidly 1974). The first parasite reported for this species was a flea, *Pleochaetoides* (= *Jellisonia*, Traub 1950) *bullisi* Auguston, from Camp Bullis, Bexar County, Texas (Auguston 1944). Barnes et al. (1977) reported another flea, *Anomiopsyllus nudatus hiemalis* Eades & Menzies (= *A. hiemalis*), parasitizing *P. pectoralis* in Big Bend National Park, Brewster County, Texas. The first chigger, *Microtrombicula welbourni* Loomis & Webb, was described from Val Verde County, Texas (Loomis & Webb 1972). Another chigger, *Euschoengastia chisosensis* Wrenn, Baccus & Loomis was recorded from Boulder Meadow, Big Bend National Park, Brewster County, Texas (Wrenn et al. 1976). Gingrich & Barrett (1976) conducted laboratory infestations of *P. pectoralis* with the dipteran, *Cuterebra fontinella* Clark. Sabrosky (1986) identified a new

species, *Cuterebra clarki* Sabrosky, from *P. pectoralis* collected at Bustamente, Nuevo Leon, Mexico. Durette-Desset & Santos (2000) identified a new species of trichostrongylid nematode, *Carolinensis tuffi* Durette-Desset & Santos from the small intestine of *Peromyscus pectoralis* at Colorado Bend State Park in Lampasas County, Texas. No tick, louse, or mite species have been previously reported for the white-ankled mouse (Schmidly 1974). The objectives of this study were to: 1) survey the metazoan parasites of *P. pectoralis* in its natural environment, 2) conduct an ecological survey of the prevalence and intensity of metazoan parasites, and 3) examine variation in prevalence and intensity of metazoan parasites with respect to host age, sex, or month of collection.

Materials and Methods

Study area.–Colorado Bend State Park (CBSP), a 2,160-ha facility (31°02'35.9"N, 98°28'12.3"W) owned and operated by the Texas Parks and Wildlife Department, is located 25.6 km south and 24.8 km east of San Saba, Texas in San Saba and Lampasas counties, Texas. The park is replete with rolling hills, caves, and steep bluffs bordering the Colorado River which bisects the park. The elevation ranges from 305 m to 440 m (Schwausch 1997). Average rainfall is 64.75 cm (Data for 1897 to 1997, NOAA 1998). A drought occurred during most of the study, and rainfall for some months in 1996 was 3.6 cm below the annual mean.

Parasites were collected from *P. pectoralis* inhabiting four distinct habitat types (riparian, grassland, rocky ridge, and Ashe juniper (*Juniperus ashei*) woodland) (Anonymous 1989; Schwausch 1997). Riparian habitat occurs on steep limestone bluffs along the Colorado River and is dominated by tall trees of Pecan (*Carya illinoensis*), American elm (*Ulmus americana*), sugarberry (*Celtis laevigata*), and sycamore (*Platanus occidentalis*) (Schwausch 1997). The grassland habitat has Texas wintergrass, little bluestem (*Schizachyrium scoparium*), King Ranch bluestem (*Bothriochloa ischaemum*), buffalograss (*Buchloe dactyloides*), and

sideoats grama (*Bouteloua curtipendula*) as dominants with patches of prickly pear (*Opuntia lindheimeri*) and shrubs and trees of Ashe juniper and Texas persimmon (*Diospyros texana*) (Schwausch 1997). The rocky ridge habitat is predominantly a shrubland of prickly pear, Texas persimmon, evergreen sumac (*Rhus virens*), Ashe juniper and plateau live oak (*Quercus fusiformis*) (Schwausch 1997). The composition of the Ashe juniper woodland is primarily Ashe juniper shrubs and trees (Schwausch 1997).

Collecting methods.–Mice were live-trapped using Sherman live traps (H. B. Sherman Traps, Tallahassee, FL) baited with oats or birdseed. All mice were euthanized according to Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research (*Ad Hoc* Committee on Acceptable Field Methods in Mammalogy 1987), Texas State University Animal Care and Use Committee Permit Number 1015, and Texas Parks and Wildlife Scientific Collecting Permit Number SPR-0890-234. Total length, tail length, hind foot, ear length, weight, age, and sex were recorded; if a female, the number of fetuses was noted. Age was assigned based on distinct pelage colors for juvenile (gray), subadult (mixture of gray and dull brown), and adult (rich brown) mice (Brown 1963; Schmidly 1974). In addition to *P. pectoralis*, the rodent community was composed of *Reithrodontomys fulvescens*, *P. maniculatus*, *Baiomys taylori*, and *Sigmodon hispidus*. *Peromyscus pectoralis* accounted for 91.7% of rodents collected.

Ectoparasites were preserved in 70% glycerated ethanol. The subcutaneous region was examined for the presence of cuterebrids and filarial nematodes. Commonly parasitized organs (lungs, liver, gall bladder, stomach, small intestine, caecum, and large intestine) were removed and examined for presence of parasites (see Schmidt 1992). All adult and larval stages of cestodes, trematodes, nematodes, and acanthocephalans were removed and washed in deionized water. After this wash, all parasites (with a few exceptions noted later) were stored in 70% ethanol.

Microscopic examination.–Preparation of trematode, cestode, and nematode specimens for microscopic examination followed Schmidt (1992). In addition, nematodes were cleared in lactophenol and stored in 70% ethanol. Specimens were stained in aceto-carmine, destained in 70% acidulated alcohol until light pink, and then dehydrated in a stepwise manner with 1 h each in the following concentrations of ethanol: 85%, 95%, 100%, and 100% again. After these ethanol dehydrations, worms were cleared in xylene by placing them first in a 50% xylene/absolute ethanol solution for 1 h and then 100% xylene twice for 1 h each. After clearing in xylene, worms were permanently mounted in Canada balsam and dried.

After washing in deionized water, cestodes and trematodes were immediately placed in 10% formalin for overnight fixation. Prior to staining, worms were removed from formalin and placed in distilled water to remove all fixative. Specimens were then stained in a 1:15 stock dilution of Wright's hematoxylin for 24 h, rinsed in distilled water to remove excess stain, and dehydrated in 35%, then 50% ethanol for 1 h each. Worms were then destained in 70% acidulated ethanol until a light pink color. Destained worms were then dehydrated, cleared, mounted, and dried. The single acanthocephalan was stained in aceto-carmine, destained in 70% acidulated alcohol, and stored in 70% ethanol.

Identification.–All parasites were identified to species when possible. If parasites could not be matched with species currently reported in the literature, an attempt was made to determine whether the organism was new to science, or its apparent uniqueness could be attributed to geographical or host-induced differences. Specimens were sent to recognized authorities for conformation of identifications and in some cases, identification. Fleas were sent to R. E. Lewis (retired), Iowa State University. The single specimen of a tick and all adult mites were sent to F. J. Radovsky, Oregon State University, for identification after mounting in CMC-S 10. Chiggers were also mounted in CMC-S

10. Chiggers were sent to William J. Wrenn (retired), University of North Dakota for identification. Lice were sent to L. A. Durden, Institute of Arthropodology and Parasitology, Georgia Southern University. Helminths, excluding the acanthocephalan, were sent to J. M. Kinsella (retired), University of Montana, for confirmation. B. B. Nickol, University of Nebraska at Lincoln, identified the single specimen of acanthocephalan.

Voucher specimens.–Voucher specimens are deposited in various collections. R. E. Lewis assigned voucher numbers 61978 through 62006 to fleas identified and retained in his personal collection. Lice are in the ectoparasitic collection of the Institute of Arthropodology & Parasitology at Georgia Southern University and have the following accession number: RML 122,473. The tick, all adult mites, and all helminths, including the acanthocephalan are in the invertebrate collection at Texas State University.

Analysis of data.–The following ecological parameters were examined: 1) variations in endoparasites prevalence and intensity with respect to season of collection, 2) variations in endoparasites abundance due to the host sex, 3) variations in endoparasites ecology due to host age, 4) variations in endoparasites ecology due to host locality, and 5) variations between endoparasites prevalence and intensity as they relate to host body length. Ecological terminology was based on Bush et al. (1997). Prevalence, expressed as a percent, was defined as number of hosts parasitized by 1 or more individuals of a particular parasite species divided by number of hosts examined multiplied by 100. Intensity of infection was the number of individuals of a particular parasite species on/in a single infected host. Locality referred to the geographical locale of the external environment where the parasite occurred and a host captured; whereas, location of a parasite was the topological or spatial location in/on the host where a particular sample of parasites was collected. Mean abundance referred to the total number of individuals of a particular parasite species in a sample of a

particular host divided by the total number of hosts of that species examined, both infected and uninfected.

The Significance Test for Comparing Two Proportions (Moore & McCabe 1993) was used to test prevalence of parasites between populations of mice. There was no difference in helminthes infecting males (n = 98) and females (n = 91) in prevalence, so males and females were pooled in further analyses. Intensity between populations was compared by the Mann-Whitney U Test (Zar 1984). The notations for one- or two-tail tests and degrees of freedom in reporting the results of statistical tests followed the subscript format of Zar (1984).

Results

Trematoda.–Twenty-three specimens of the pancreatic fluke, *Scaphiostomum pancreaticum* McIntosh, occurred exclusively in the pancreatic duct of 15 mice. Flukes were assigned to the family Brachylamidae based on long and filiform bodies with the genital pore and associated reproductive structures in the posterior-most region. *Peromyscus pectoralis* is a new host record.

A total of 589 specimens of the liver fluke, *Zonorchis komareki* McIntosh were collected from bile ducts or gall bladders (McIntosh 1939) and occasionally in the small intestine proximate to the bile duct opening. The largest of these flukes was approximately 3 mm in length and about 0.75 mm in width at its widest point. *Peromyscus pectoralis* is a new host record.

Cestoda.–Thirty-six mice contained the adult cestode, *Catenotaenia dendritica* (Goeze). This craspedate cestode has four suckers on an unarmed scolex. Mature proglottids contained between 100 and 120 testes. The cestodes recovered had fewer testes than reported for *C. dendritica*, but too many to meet the description of the alternative species, *C. peromysci* Smith (Yamaguti 1971). In addition to this tapeworm, a single mouse had one larval cestode on the surface of the liver. It was a

strobilocercus type and incomplete organogenesis of the specimen prohibited any further identification. *Peromyscus pectoralis* is a new host record.

Acanthocephala.–A single male specimen of the acanthocephalan *Moniliformis clarki* (Ward) was recovered from the small intestine of one mouse. *Peromyscus pectoralis* is a new host record.

Nematoda.–One trichostrongylid nematode species, *Carolinensis tuffi* Durette-Desset & Santos, was recovered from the small intestine (Durette-Desset & Santos 2000). *Peromyscus pectoralis* is currently the only known host for this nematode.

Another nematode was found in one mouse but did not have enough visible internal morphology for identification. This nematode, also found in the small intestine, may be a juvenile form of *C. tuffi*, or some other species.

Arthropoda.–Three species of fleas were recovered from *P. pectoralis*: *Jellisonia bullisi* (Augustson), *J. ironsi* (Eads), and *Atyphloceras echis echis* (Jordan & Rothschild). Most host individuals had no fleas. *Peromyscus pectoralis* is a new host for *J. ironsi* and *A. echis echis*.

One damaged larval tick, *Ornithodoros* sp. was recovered from one host. *Peromyscus pectoralis* is a new host record, since no tick has been previously reported for *P. pectoralis*.

Two mites, *Androlaelaps fahrenholzi* (Berlese) (= *Haemolaelaps glasgowi*) and *Ornithonyssus* sp., were recovered from two host individuals. *Peromyscus pectoralis* is a new host record for both mites.

The chiggers, *Euschoengastia fronteriza* Wrenn, Baccus & Loomis and *E. criceticola* Brennan were found on 10 hosts.

Peromyscus pectoralis is a new host record for both chigger species.

Ecology.–Peromyscus pectoralis infected with parasites inhabited the riparian, grassland, rocky ridge, and Ashe juniper woodland habitats. The prevalence of parasites was not skewed toward a specific habitat type. Prevalence, intensity, mean abundance, and total number of individual parasites associated with *P. pectoralis* were skewed by the abundance of two species, *C. tuffi* and *Z. komareki* (Table 1). There was no difference in prevalence [$P\ (Z \geq 0.43) > 0.05$] or intensity [$P\ (U_{a\ (2)\ 53,54} > 1511.5) > 0.05$] for helminths infecting males ($n = 98$) and females ($n = 91$). Adult mice were more likely to be infected than subadults [$P\ (Z \geq -1.55) < 0.10$]. Also, adult mice were more likely to be infected than juvenile mice [$P\ (Z \geq -2.19) < 0.05$]. No difference [$P\ (Z \geq -1.18) > 0.10$] was found in prevalence between juveniles and subadults, but the sample size for juveniles was small ($n = 15$). Adult mice were infected at a higher rate (prevalence) and with an increased intensity compared to juvenile mice. Although prevalence varied somewhat by age group, there was no difference in intensity between juveniles and subadults [$P\ (U_{a(1)\ 7,38} \geq 144) > 0.05$] and between juveniles and adults [$P\ (U_{a(1)\ 7,62} \geq 297) > 0.05$]. Prevalence varied by month with most helminths less prevalent in late summer and fall. Overall, there was a sharp decrease in the prevalence of helminthes in fall.

Discussion

Whitaker (1968) suggested the predominantly vegetarian diet of rodents in the genus *Peromyscus* explains the relatively small number of parasitic helminths. Kennedy (1968) proposed differences in infection patterns of helminths in fish could be related to feeding behavior. Watve & Sukumar (1995) identified host diet as an ecological factor influencing the parasite community by carnivores having higher parasite loads and species richness compared to herbivores, as the diet of the former contained intermediate hosts of a variety of parasites. Diet may in part

Table 1. Prevalence, intensity, mean abundance and total number of helminths collected from 189 *Peromyscus pectoralis* at Colorado Bend State Park, San Saba County, Texas from February 1996 through March 1997.

Helminth taxon	Prevalence[a]		Intensity[b]			
	n	%	$\bar{X}$ ±SE	Range	Mean abundance[c]	Total
Trematoda						
Scaphiostomum pancreaticum	15	8	1.5 ± 0.3	1-4	0.12	23
Zonorchis komareki	53	28	11.1 ± 1.5	1-61	3.12	589
Cestoda						
Catenotaenia dendritica	36	19	1.8 ± 0.2	1-5	0.35	
Acanthocephala						
Moniliformis clarki	1	1	1.0 ± 0.0		0.01	1
Nematoda						
Carolinensis tuffi	57	30	11.2 ± 3.3	1-175	3.38	640

[a] Prevalence = [(# Infected Hosts) / (# Hosts Examined)] * 100

[b] Intensity = (Total # Parasites) / (# Hosts Infected) ± Standard Error

[c] Mean abundance = (Total # Parasites) / (# Hosts Examined)

explain the low prevalence and diversity of trematodes and other endoparasitic species parasitizing the *P. pectoralis* population sampled at CBSP. Baccus et al. (2009) found substantial amounts of plant matter (range = 62.5%-73.3%) in the seasonal diet of *P. pectoralis* in central Texas. Pfaffenberger et al. (1985) and Grundmann (1957) discovered low prevalence and intensity of infections by helminthes in primarily herbivorous rodents similar to findings for this study on *P. pectoralis*. The results of this study suggest the vegetarian diet of *P. pectoralis* is a prime ecological factor influencing a lower parasitic species richness and prevalence.

Zonorchis komareki had low prevalence (28%) across all age groups, sex, and season variables. Although it had the second highest prevalence and abundance of all helminth species (Table 1), it still rarely or infrequently occurred in the host sample. The intermediate host for *Z. komareki* is the terrestrial snail, *Polygyra*

texasiana (Moricand). However, gastropod matter formed a miniscule portion of the diet of *P. pectoralis* in central Texas (Baccus et al. 2009). The presence of this snail is the limiting factor for the incidence or newly acquired infections of *Z. komareki* by *P. pectoralis*. A study of snails in the park could help in understanding the function of snails in the parasitism of *P. pectoralis*. *Zonorchis komareki* also parasitizes *Peromyscus gossypinus* (McIntosh 1939, Kinsella 1991) and *Peromyscus polionotus* (Kinsella 1991).

Scaphiostomum pancreaticum was not as successful a parasite of *P. pectoralis* as *Z. komareki*. The terrestrial snail, *Anguispora alternata* (Say) is an intermediate host for this trematode, although two other snails, *Triodopsis albolabris* (Say) and *Haplotrema concavum* (Say), may serve as secondary intermediate hosts to the microcercous cercaria as well as *A. alternata*; but only *A. alternata* can function as the primary intermediate host for the miracidia (Jenson 1972). Thus, the population of *A. alternata* may be the limiting factor in *S. pancreaticum* prevalence and intensity. *Scaphiostomum pancreaticum* spends about nine months of its life cycle in a snail host, and Jenson (1972) postulated this phase may be an overwintering adaptation. Although this assumption may have application in northern North America where Jenson's work was done, *P. pectoralis* at CBSP were active every month and parasitized by *S. pancreaticum* in winter. Snails in the park should be examined to expand on Jenson's work and to determine their function in the pancreatic fluke's life cycle. *Scaphiostomum pancreaticum* also parasitizes *P. gossypinus* (McIntosh 1935).

The low prevalence of the tapeworm, *C. dendritica*, in summer and fall may be attributed to fluctuations in the population of the tyroglyphid mite, which serves as the intermediate host for the merocercoid larvae. The best method for confirming the cause of the low prevalence would be surveys of mites for the presence of the larvae. *Catenotaenia dendritica* also parasitizes *P. maniculatus* (Leiby 1962).

The single male acanthocephalan recovered, *Moniliformis clarki*, may occur in the park in low numbers due to low populations of necessary intermediate hosts or the mice are not eating substantial quantities of insects. Baccus et al. (2009) determined insects composed as much as 36.2% of the diet of *P. pectoralis* in Hays County, Texas depending on season. If this also applies to the diet of mice at CBSP, more acanthocephalans should have been recovered. *Moniliformis clarki* also parasitizes *P. maniculatus* (Grundmann & Frandsen 1960).

Why the nematode *C. tuffi* has not been previously reported is puzzling. Perhaps, it affects only *P. pectoralis* and no helminth data existed for this species prior to this study, or similarity of *C. tuffi* with the closely related *Longistriata carolinensis* Dikmans (Dikmans 1935) or *Nippostrongylus brasiliensis* (Travassos 1914) caused misidentification. It is suggested specimens previously reported as *L. carolinensis* or *N. brasiliensis* from *Peromyscus* be reexamined to verify the authenticity of their identification.

The absence of *C. tuffi* from mice collected in October through December was also puzzling. Since this nematode, as others, is probably spread through direct contact with ova, it should be found in the population year round. Possibly the trichostrongylid exists in another vertebrate host also found in the park, although this conjecture has not been examined. Two mice had an over-dispersion of the parasite load (90 and 175) of this nematode, but they did not appear malnourished.

The overall pattern of endoparasitism for *P. pectoralis* was a low diversity of species with a limited number of other species of *Peromyscus* being infected by the endoparasites infecting *P. pectoralis*.

The distribution of ectoparasites was characterized by their aggregation or overdispersion (Krasnov et al. 2002) on a few host

individuals; whereas, most mice had few ectoparasites or none at all (Anderson & May 1978; Poulin 1993).

The genus *Jellisonia* is a group of ceratophyllid fleas essentially restricted to peromyscine rodents (*Peromyscus*, *Baiomys*, *Reithrodontomys*, *Scotinomys*) from the foothills of southern Texas to montane Mexico and Central America (Traub et al. 1983). In addition to an association with *P. pectoralis*, *J. bullisi* only parasitizes *P. eremicus* (Traub 1950). *Jellisonia ironsi* is closely associated with species of the genus *Baiomys* (Traub et al. 1983, Morrone & Gutiérrez 2005). This record from *P. pectoralis* at CBSP is the first and only host record of this flea from a species of *Peromyscus* (Whitaker 1968, Morales 1990). *Atyphloceras echis* is also an ectoparasite of *P. boylii* (cf. Morlan 1955), *P. maniculatus* (cf. Stark 1958), *P. nasutus* (cf. Morlan 1955), and *P. truei* (cf. Morlan 1955) in addition to *P. pectoralis*.

Ticks of the genus *Ornithodoros* also occur on *P. maniculatus* (cf. Cooley & Kohls 1941; Kohls & Clifford 1963), and *P. crinitus* (cf. Egoscue 1964) in addition to *P. pectoralis*.

The two mites associated with *P. pectoralis* inhabit several other species of *Peromyscus*. *Androlaelaps fahrenholzi* parasitizes *P. boylii* (cf. Allred 1957; Allred & Beck 1966; Whitaker & Wilson 1974), *P. californicus* (cf. Strandtmann & Wharton 1958; Whitaker & Wilson 1974), *P. crinitus* (cf. Allred 1957; Allred & Beck 1966; Keegan 1953; Whitaker & Wilson 1974), *P. eremicus* (cf. Allred 1957; Allred & Beck 1966; Whitaker & Wilson 1974), *P.* (= *Podomys*) *floridanus* (cf. Layne 1963; Whitaker & Wilson 1974), *P. gossypinus* (cf. Worth 1950a, 1950b; Morlan 1952; Hays & Guyton 1958; Whitaker & Wilson 1974; Clark & Durden 2002), *P. leucopus* (cf. Drummond 1957; Hays & Guyton 1958; Florschutz & Darsie 1960; Tindale & Darsie 1961; Whitaker & Wilson 1968; Whitaker & Wilson 1974; Clark & Durden 2002; Ritzi & Whitaker 2003), *P. maniculatus* (cf. Keegan 1953; Allred 1957; Rapp 1962; Scholten et al. 1962; Allred & Goates 1964; Elzinga & Rees 1964;

Hansen 1964; Lawrence et al. 1965; Allred & Beck 1966; Whitaker & Wilson 1968; Whitaker & Wilson 1974; Durden & Wilson 1991; Ritzi & Whitaker 2003), *P. melanotis* (cf. Álvarez-Castañeda 2005), *P. polionotus* (cf. Morlan 1952; Hays & Guyton 1958; Whitaker & Wilson 1974), *P. truei* (cf. Keegan 1953; Holdenried & Morlan 1955; Allred 1957; Allred & Goates 1964; Allred & Beck 1966; Whitaker & Wilson 1974), *P. zarhynchus* (cf. McClellan & Rogers 1997), and *P.* spp. (cf. Judd 1950). The genus *Ornithonyssus* has been associated with *P. boylii* (cf. Allred 1957; Allred & Beck 1966; Whitaker & Wilson 1974), *P. crinitus* (cf. Allred 1957; Allred & Beck 1966; Whitaker & Wilson 1974), *P. eremicus* (cf. Allred 1957; Allred & Beck 1966; Whitaker & Wilson 1974), *P. floridanus* (cf. Layne 1963; Whitaker & Wilson 1974), *P. gossypinus* (cf. Worth 1950a, 1950b; Morlan 1952; Whitaker & Wilson 1974; Clark & Durden 2002), *P. leucopus* (cf. Drummond 1957; Whitaker & Wilson 1968; Whitaker & Wilson 1974; Clark & Durden 2002; Durden & Wilson 1991), *P. maniculatus* (cf. Allred 1957; Allred & Beck 1966; Whitaker & Wilson 1968; Whitaker & Wilson 1974), *P. polionotus* (cf. Morlan 1952; Whitaker & Wilson 1974), *P. truei* (cf. Holdenried & Morlan 1955; Allred 1957; Allred & Beck 1966; Whitaker & Wilson 1974), and *P.* spp. (cf. Ellis 1955).

The sucking louse *Hoplopleura hesperomydis* infects *P. californicus* (cf. Ferris, 1951; Kim 1965), *P. gossypinus* (cf. Morlan 1952; Kim 1965), *P. leucopus* (cf. Osborn 1891; Kellogg & Ferris 1915; Bell & Chalgren 1943; Morlan & Hoff 1957; Wilson 1957; Cook & Beer 1959; Scanlon 1960; Tindale & Darsie 1961; Wilson 1961; Mathewson & Hyland 1962; Parsons 1962; Kim 1965; Kim et al. 1966), *P. maniculatus* (cf. Kellogg & Ferris 1915; Ferris 1916; Augustson 1941; Morlan & Hoff 1957; Cook & Beer 1959; Scanlon 1960; Wilson 1961; Scholten et al. 1962; Elzinga & Rees 1964; Hansen 1964; Kim 1965; Lawrence et al. 1965; Kim et al. 1966), *P. nasutus* (cf. Kim 1965), *P. polionotus* (cf. Morlan 1952),and *P. truei* (cf. Morlan & Hoff 1957; Kim 1965) in addition to *P. pectoralis*.

Other than *P. pectoralis*, the chigger *Euschoengastia fronteriza* parasitizes *P. difficilis* (= *P. nasutus*) (cf. Hoffmann 1990). *Euschoengastia criceticola* infests *P. boylii* (cf. Allred 1952; Brennan & Jones 1954; Brennan & Beck 1955; Gould 1956; Jameson & Brennan 1957; Allred & Beck 1966; Hoffmann 1990), *P. californicus* (cf. Brennan & Jones 1954; Gould 1956; Loomis & Bunnell 1962), *P. eremicus* (cf. Brennan & Beck 1955; Gould 1956; Allred & Beck 1966; Hoffmann 1990), *P. leucopus* (cf. Loomis 1956), *P. maniculatus* (cf. Brennan 1948; Brown & Brennan 1952; Brennan & Jones 1954; Brennan & Beck 1955; Gould 1956; Allred 1957; Jameson & Brennan 1957; Allred & Goates 1964; Elzinga & Rees 1964; Allred & Beck 1966; Hoffmann 1990), *P. sitkensis* (= *P. keeni*) (cf. Hogan et al. 1993; Kim et al. 1986), *P. melanotis* (cf. Loomis & Somerby 1966), *P. truei* (cf. Brennan & Jones 1954; Gould 1956; Allred & Beck 1966; Hoffmann 1990), and *P.* spp. (cf. Farrell 1956) in addition to *P. pectoralis*. Although *E. chisosensis* was not collected from *P. pectoralis* in this study, this chigger was collected from *P. pectoralis*, *P. boylei*, and *P. difficilis* (= *P. nasutus*) (cf. Wrenn et al. 1976).

The prevalence and intensity of parasites fluctuates seasonally on the basis of such factors as sex, month of collection, parasite specificity to a host, predator pressure, or host age, size, body size, gregariousness, or population density (Stock & Holmes 1988, Watve & Sukumar 1995, Little et al. 2006). This study did not reveal a sex bias in the prevalence of helminth infections in *P. pectoralis* at CBSP (Snyder & Fitzgerald 1987; Pistole 1988; Perez et al. 1995). Older, adult white-ankled mice were parasitized with higher intensities than subadults or juveniles (Anderson & Gordon 1982, Rousset et al. 1996, Soliman et al. 2001), yet larger adult mice (< 95 mm body length) appeared to be infected at a higher rate and with increased intensity, but most noteworthy was the overdispersion in a few host individuals with extraordinarily large total lengths (< 180 mm), while most host individuals had only a few parasites or none at all (Anderson & May 1978, Poulin 1993,

Krasnov et al. 2002). Krasnov et al. (2006) found the relationships between prevalence and host size in flea infestations of larger size, older-age class *Apodemus agrarius* and *Microtis arvalis* had either a concave or positive linear relationship. In host-parasite systems, a parasite's selection of habitat, host species or host size, may influence parasite-host dynamics (Mangel & Roithberg 1992), which can produce cascading effects on populations that shape the structure of communities (Petchey et al. 2008). A parasite's host selection can be described using a variation of the optimal diet breadth models (Charnov 1976, Stephens & Krebs 1986) where the diet breadth exhibited by parasites is the number of size classes used and intensity of infection (Henry et al. 2006). Larger, older individuals eat more food and therefore have a higher probability of consuming the infective life stage of a parasite. Larger mice can probably accommodate and tolerate more worms. *Peromyscus pectoralis* with high intensity infections may simply have been older adults with a broader diet, which were at greater risk or had more exposure for infection (Soliman et al. 2001; Hawlena et al. 2005, Krasnov et al. 2006) or suffered from immunoscenescence (Møller & de Lope 1999; Cattadori et al. 2005). However, the herbivorous diet of *P. pectoralis* may have protected smaller, younger individuals in that many plant parts, ingested by animals as dietary supplements, act as prophylactics or remedials for parasite infection (Dogiel 1964). Another explanation for the higher intensity of infection of larger, older mice may be host quality because large hosts (older mice) contain more resources; therefore, they are supposed to be higher quality and represent a greater resource than small hosts (Charnov et al. 1981; Charnov 1982; Jones 1982; Liu 1985; Mackauer 1986; Opp & Luck 1986; King 1988).

Most parasites became less prevalent in late summer and fall. Intermediate hosts populations may have influenced the prevalence of trematodes and cestodes but failed to explain fluctuations in nematode populations in *P. pectoralis* where no intermediate host was involved in transmission of the parasite. Overall, there was a

sharp decrease in the prevalence of helminthes in October shortly after the first frost of the season.

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GENERAL NOTES

A NEW RECORD OF THE PARASITIC BEAVER BEETLE (*PLATYPSYLLUS CASTORIS*) FROM TEXAS

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Platypsyllus castoris Ritsema (Coleoptera: Leiodidae) is an ectoparasite of the American beaver (*Castor canadensis* Kuhl) and Eurasian beaver (*C. fiber* L.) that feeds on host epidermal cells, skin secretions, and possibly blood (Wood 1965). Adult *P. castoris* beetles are acutely dorso-ventrally flattened (Fig. 1) and can move rapidly through the beaver's dense underfur; this louse or flea-like appearance led to its initial description as a new species of flea (Ritsema 1869). The beetle is somewhat resistant to freezing temperatures and responds quickly to warmth, but is vulnerable to desiccation (Janzen 1963). The ectoparasitic habit of *P. castoris* is likely derived from a cholevid beetle ancestor which was a scavenger in small mammal nests or burrow systems (Wood 1965; Waage 1979; Peck 2006).

Both larval and adult *P. castoris* parasitize the host, and the life cycle is unique in that it is completed in its entirety upon the host, save for three brief periods (Wood 1965): gravid females briefly abandon their hosts to oviposit on debris within beaver lodges or burrows where their eggs hatch after ~32 days; emergent larvae subsequently migrate to an available host and undergo three instar stages of development, with mature third-instars leaving the host to pupate in elevated soil of lodges and burrows, and adults emerging after 11-22 days, depending on ambient temperature.

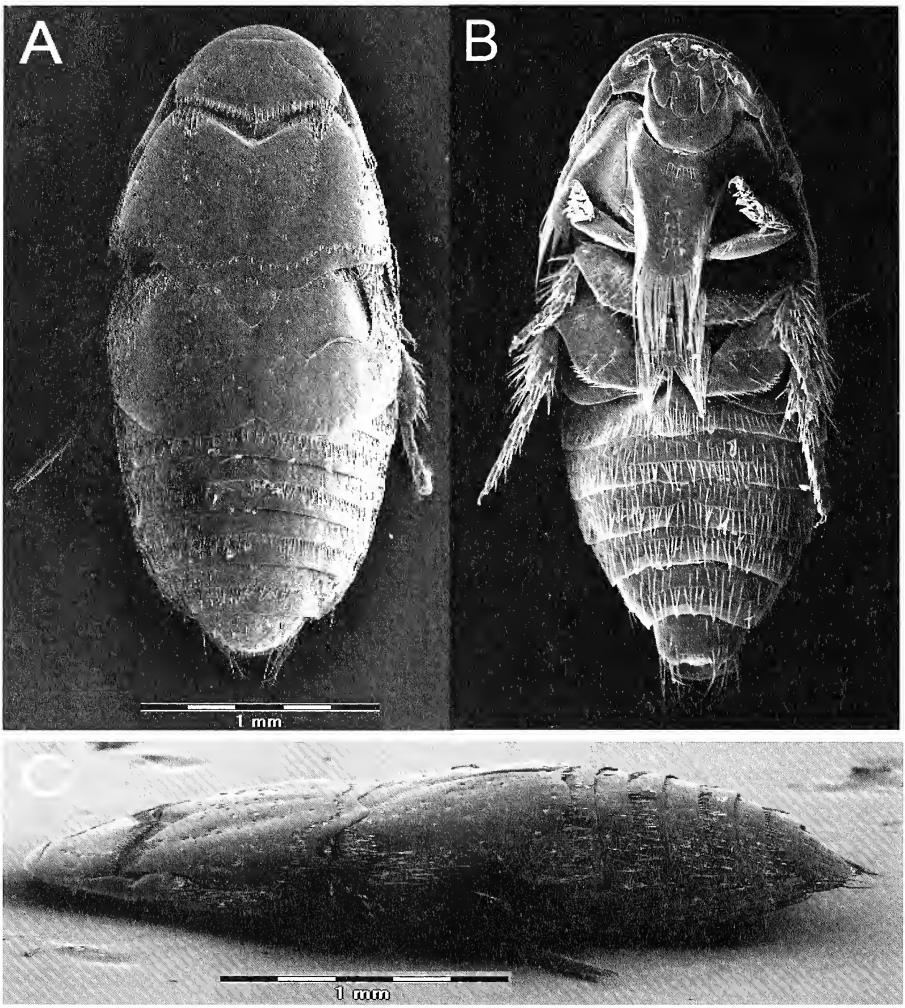

Fig. 1. Scanning electron micrographs from dorsal (A), ventral (B), and lateral (C) perspectives of an adult *Platypsyllus castoris* originating on a beaver (*Castor canadensis* Kuhl) from Clay County, Texas.

In December 2008, an adult male beaver was trapped by the senior author on the Wichita River, 4.7 km SSE Thornberry, Clay Co., Texas U.S.A. (34° 01' 42" N; 98° 22' 04" W). While skinning the beaver, a large number of *P. castoris* (ca. 75) were noticed moving about on the facial area. After freezing the pelt, specimens were removed using a knit comb and forceps, and

were stored in 80 percent ethanol or slide mounted. This discovery led to the examination of other beaver pelts collected by the senior author. Six additional *P. castoris* specimens were combed from another male beaver collected on the same excursion (stored separately) ca. 12 km downstream of the aforementioned beaver, while none were found on three beavers trapped along the Wichita River in Wichita County. Vouchers were deposited at Midwestern State University, Texas A&M University, Carleton University (Ottawa, Canada), and the entomological collection at the National Museum of Natural History, Smithsonian Institution (assignment of insect accession numbers not practiced by above collections).

In North America, *P. castoris* is thought to occur on beavers throughout their distributional range, although beetle abundance appears to be higher in the Midwest and northern regions of the United States and lower in the south (Peck 2006). Only two other existing specimens of *P. castoris* from Texas are known, and both reside in the Texas A&M University (TAMU) insect collection. The TAMU specimens are labeled "Tex" but unfortunately have no additional data, and it is clear from the style of mounting and general appearance of their labels that they are quite old (E. Riley, pers. comm.). In addition, Leng (1920) also cited "Tex." as a locale for *P. castoris*, but no further data were given. Other specimens of *P. castoris* from Major, Payne and Latimer counties of Oklahoma (R. Grantham & E. Riley, pers. comm.) lend additional support for established populations in southern climes. In Texas however, the beetle appears to be either uncommon or unobserved, and it has never been previously seen by the senior author despite many years of trapping beaver within the Wichita River drainage, although admittedly, it would be easy to overlook. This report represents the first record of *P. castoris* from Texas with both known host and location data.

Although *P. castoris* is thought to be an obligate parasite of beaver, Belfiore (2006) recently reported a specimen from a river otter (*Lontra canadensis* Schreber) in California. In Texas, the muskrat (*Ondatra zibethicus* L.) and the introduced nutria (*Myocastor coypus* Molina) also seem likely alternate hosts for the beetle; however, no known records of *P. castoris* from either species exist, and Lawrence et al. (1961) report that *P. castoris* did not survive on experimentally infected muskrats.

The high-saline and low-gradient character of the Wichita River precludes many aquatic macrophytes and seems to be generally poor muskrat habitat with negligible evidence of their presence. In north-central Texas, there is only one known record of the river otter (Johnson County), and nutria in the northern Rolling Plains are rare (Schmidly, 2004), with the senior author having observed only three nutria (one on Beaver Creek in Wichita County, and two on the Clear Fork of the Brazos River in Stephens County) in 17 years of canoeing and trapping local riverine areas. Although beaver are common in the area, potential for aberrant *P. castoris* infestations is probably higher in regions of the state with greater numbers of diverse aquatic mammals. In the northern Rolling Plains of Texas, it appears that *P. castoris* will likely remain an obligate parasite of the beaver.

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FOOD HABITS OF THE SOUTHERN SHORT-TAILED SHREW (*BLARINA CAROLINENSIS*) IN EAST TEXAS

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Generally, an urban habitat is characterized as being fragmented and heterogenous (Schmid-Holmes & Drickamer 2001). Because of the heterogeneity, urban settings can place multiple pressures that may affect the foods present for a species. Little is known of the food habits of the southern short-tailed shrew (*Blarina carolinensis*) throughout its range (see McCay

2001). No published reports were found on the food habits of the species in the extreme western part of its range, or any information concerning the species in an urban ecosystem. The purpose of this paper was to investigate the food habits of *B. carolinensis* in an urban ecosystem in east Texas.

The study site was located on the East Texas Baptist University campus (Marshall; 32°33' N; 94°22' W) in a mixed pine-hardwood forested area, and is surrounded on three sides by forested areas and on the fourth by an athletic field. Within 150 m on two wooded sides there are residential areas. Several locations within the study site contain discarded trash.

Dominant canopy trees found on the site are oaks (*Quercus sp.*), sweet gums (*Liquidamber styraciflua*), hickories (*Carya sp.*), elms (*Ulmus sp.*), and loblolly pines (*Pinus taeda*). Poison ivy (*Toxicodendron radicans*) and grape (*Vitis sp.*) are found in the canopy with poison ivy being extensive in the herbaceous layer. Understory vegetation is dominated by saplings of the canopy trees, flowering dogwood (*Cornus florida*), poison ivy (*Toxicodendron radicans*) and green briar (*Smilax sp.*). Ground layer vegetation is sporadic and is primarily poison ivy and green briar.

Twenty-two *Blarina carolinensis* taken from 5 October to 21 December 2004 were the result of trap mortality from a larger study investigating habitat use. Traps (7.6 cm by 8.9 cm by 22.9 cm folding Sherman Traps; H. B. Sherman Traps, Tallahassee, FL) were baited with oatmeal rolled in peanut butter. Shrews were placed in sealed plastic bags and placed in a freezer for later investigation of the stomachs.

Presence or absence of selected groups of food items was assessed in each of the stomachs. Foods were placed into the

following groups: grasshoppers and crickets, earthworms, spiders, snails and slugs, fungi, and unidentified items. To analyze the importance of each food group, Cochran's Q (Tate & Brown 1970) was used. Because Zar (1999) suggested Cochran's Q can be affected by individuals with no food items, only 20 individuals were included in the analysis.

Twenty of 22 individuals examined contained food in the five selected categories. Individuals with at least three different food categories ($n = 8$) and four different food categories ($n = 7$) were the most common. One individual contained five categories. Two individuals contained one and two food categories, each.

The most common food item was snails and slugs (42.7% of all food items). Grasshoppers and crickets comprised 22.2% and earthworms 14.9% of food items identified in the stomachs. Spiders comprised 1.2% of food items. A single shrew contained fungal spores in the stomach. Unidentified food items comprised 18.9% of food items and were found in 13 individuals. Most unidentified food items appeared to be remnants of bait used in the traps.

The food habits of *B. carolinensis* in an urban ecosystem on the western part of its range are similar to those in the eastern part of the species range (Whitaker et al. 1994) with noticeable differences. No beetle remains (adults or larvae) or centipedes were recorded in this current study. Individuals in a bottomland hardwood forest were found to have consumed beetles in Tennessee in May (Calhoun 1941), and Georgia from June to September (Whitaker et al. 1994) and those in a xeric pine forest in Georgia, centipedes (McCay 2001). The habitat examined during this study is a mixed pine-deciduous forest in which both of these groups of arthropods are found. The lack of beetles and centipedes may simply reflect seasonal differences in timing of

the previous studies and this study.

This is the first study, to the authors' knowledge, to investigate the food habits of *B. carolinensis* in the western part of its range and, also, in an urban ecosystem. It appears that the food habits of *B. carolinensis* in this habitat are similar to that in other parts of its range. *Blarina carolinensis* is known to exhibit population irruptions that are potentially controlled by extrinsic factors (Gentry et al. 1971; Smith et al. 1974). Therefore, urban habitats may provide unique situations related to abundance of food resources that could have a greater influence on the population size and structure of a species exhibiting population irruptions.

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NEW DISTRIBUTIONAL RECORDS FOR THE CENTIPEDE, *SCOLOPENDRA HEROS* (CHILOPODA: SCOLOPENDROMORPHA: SCOLOPENDRIDAE), IN ARKANSAS

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The largest North American centipede, *Scolopendra heros* Girard is a widely ranging scolopendromorph found from western Arkansas and western Louisiana, southwestern Missouri, central and southern Kansas, and west through Oklahoma, Texas and the southeastern corner of Colorado to New Mexico and western Arizona in the United States and southward to Nayarit, Mexico (Shelley 2002; McAllister et al. 2003; Guarisco et al. 2007). Although the original type specimen of *S. heros* reported by Girard (1853) is lost, a neotype is available from Mt. Scott, Wichita Mountains Wildlife Refuge, Comanche County, Oklahoma (Chamberlin 1931; Shelley 2002).

In Arkansas, *S. heros* has been previously reported from the western half of the state (Shelley 2002); however, the species has been documented from only 7 of 75 (9.3%) counties, including Crawford, Garland, Hot Spring, Perry, Pulaski, Sebastian, and Washington (Shelley 2002; McAllister et al. 2006). A significant range extension for *S. heros* in Arkansas, as well as eight new county records for the state are reported within this paper.

Between May 1990 and June 2009, giant red-headed centipedes were collected from various counties in central and northern Arkansas. Collecting techniques involved turning decaying logs and moving leaf litter and rocks with a potato rake and walking trails and road cruising. Following preliminary

identification, specimens were placed in vials containing 70% ethanol and shipped to Rowland M. Shelley at the North Carolina State Museum of Natural Sciences, Raleigh, North Carolina (NCSM) for verification of identifications. Voucher specimens were subsequently deposited in the NCSM.

Thirteen specimens of *S. heros* were collected from Arkansas as follows: BENTON CO.: Rogers at Prairie Creek (36.343752°N, 94.091892°W), 28 May 2005. CARROLL CO.: Eureka Springs (36.401183°N, 93.737971°W), 8 June 2002. CONWAY CO.: Petit Jean State Park, Seven Hollows Trail (35.107563°N, 92.952563°W), 9 September 2006. IZARD CO.: 10 km NE of Sylamore off St. Hwy 9 (35.975094°N, 92.041897°W), 1 May 2007. JOHNSON CO.: Clarksville (35.471412°N, 93.466573°W), 21 October 2008. LOGAN CO.: Subiaco Abbey, 0.8 km NW of Subiaco (35.201050°N, 91.831833), 28 Sept. 2007. POPE CO.: Russellville, 293 Channel Circle (35.297611°N, 93.065888°W), 18 August 2005 & 21 July 2008. SEBASTIAN CO.: 3.2 km NE of Greenwood (35.218697°N, 94.216003°W), 22 May 1990; Greenwood, NE corner of Backbone Mountain (35.242254°N, 94.273682°W), 20 April 2006. SCOTT CO.: vicinity of Y-City (34.735147°N, 94.044630°W), 28 April 2006. SEARCY CO.: vicinity of Mull, 3 km S on Ramblewood Trail from junction of Ramblewood Trail and St. Hwy 14 (36.052048°N, 92.594601°W), 11 June 2009. WASHINGTON CO.: no specific locality, 25 September 1967.

In Arkansas, the easternmost record for *S. heros* is Little Rock, Pulaski County (Shelley 2002). In addition, Shelley (2002; fig. 42) mapped the distribution of *S. heros* from north to south arcing across the western part of the state. However, the current record from Izard County extends the range further eastward (Fig. 1) and well beyond the eastern edge of the range shown by Shelley (2002) in the United States. To date, *S. heros* has been reported

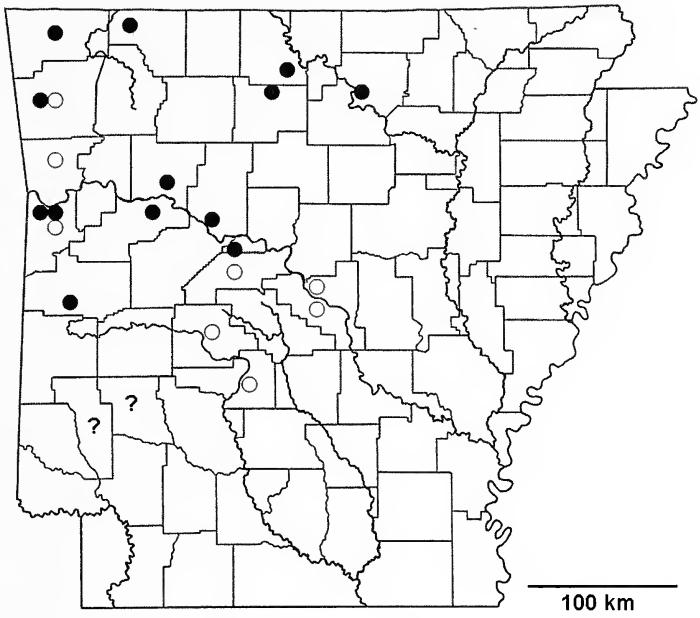

Figure 1. Distribution of *Scolopendra heros* in Arkansas (open dots = literature records; closed dots = new records; question marks = need verification with vouchers).

from 17 counties in Arkansas. Additional efforts at collecting along the edge of the Interior Highlands may reveal a more extensive eastern range in the state. Interestingly, Barnes (2002) notes that there are reliable reports of this species from Howard and Pike counties (see Fig. 1, question marks). However, these reports await verification through deposition of authentic *S. heros* in natural history collections.

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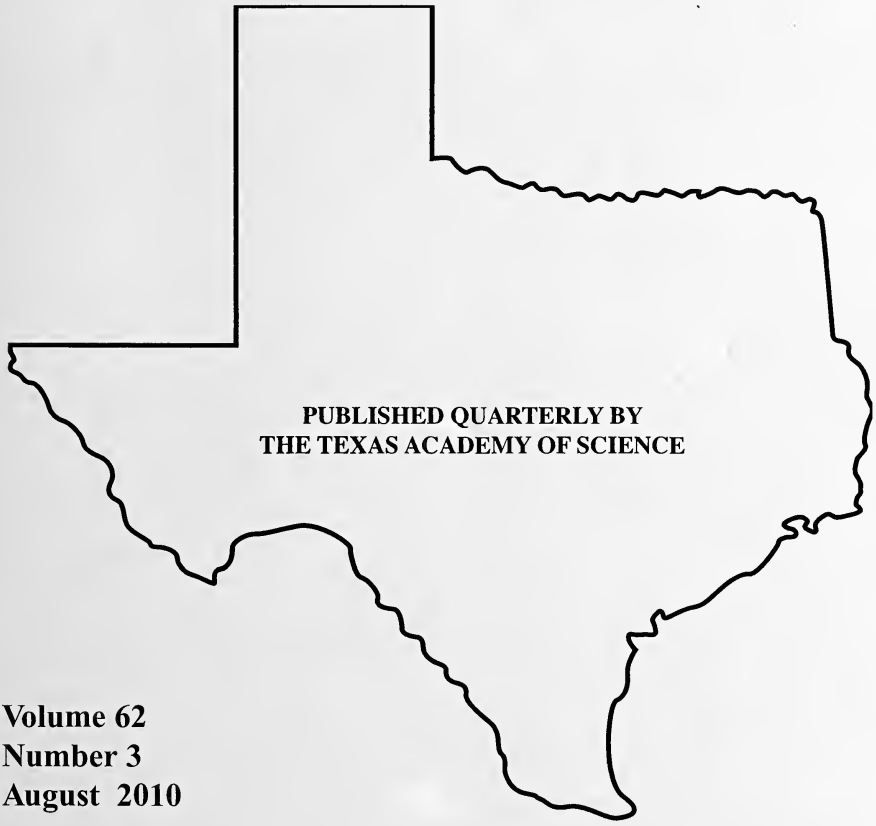

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DISTRIBUTION OF *VERBESINA VIRGINICA* (ASTERACEAE, FROST WEED) IN CENTRAL TEXAS AND POSSIBLE CAUSES

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Abstract.–*Verbesina virginica* (Asteraceae, Frost Weed) is a tall, perennial, herbaceous plant mostly found in the eastern United States. In central Texas, it occurs mainly under the canopy of *Quercus virginiana* (live oak) or *Ulmus crassifolia* (cedar elm). Its ecological niche and factors affecting its distribution are not well recognized. To better understand conditions limiting its distribution, a combination of transects and leaf level gas exchange measurements were completed. Transects were from below a woodland canopy into an adjacent grassland. Mean density below the canopy was 700 ± 120 (mean ± one SD) plants/100 m^2 decreasing to zero in the associated grassland. Ninety-nine percent of the plants were below the canopy. Soil depth along the transect was highly variable but not significantly different. A light gradient from 207 ± 53 $\mu mol/m^2/s$ below the canopy to 2126 ± 71 $\mu mol/m^2/s$ in the grassland was found. Maximum photosynthetic rates (A_{max}) were 12.68 ± 1.40 $\mu molCO_2/m^2/s$ at the highest light level and changed very little as light levels increased from 400 to 2000 $\mu mol/m^2/s$. Dark respiration (R_d) was 0.76 ± 0.07 $\mu molCO_2/m^2/s$. Stomatal conductance was 0.311 ± 0.110 $molH_2O/m^2/s$ at the A_{max} and did not change significantly over the light levels tested. Transpiration was 6.10 ± 1.40 $mmolH_2O/m^2/s$ at the A_{max} and decreased with light levels, but not significantly until 100 $\mu mol/m^2/s$. Light saturation (L_{sat}) was 288 $\mu mol/m^2/s$ and light compensation (L_{cp}) was 16 $\mu mol/m^2/s$. Results suggest that *V. virginica* is a shade adapted plant that can carry out gas exchange below the woodland canopy. Its A_{max} is higher than typical understory plants and results do not explain why this species is not found in associated grasslands.

When a species is found in a given location, it is because that species can tolerate or requires the environmental conditions present in that area. Measuring population density of terrestrial plants is relatively easy to do (Van Auken et al. 2005), but sorting out the characteristics or factors that determine why a species is present or dominant where it is found is much more challenging (Begon et al. 2006). It is just as taxing to ascertain why similar species fit together in communities. Certain species are limited to open habitats, some to woodlands or forests, others seem to occur at the edge of communities, while others do not seem constrained.

Limiting factors could be biotic or abiotic, but are not always easy to define. Certainly light levels, soil depth, soil moisture, nutrient levels, competition, biotic characteristics, or combinations of these factors are possibilities (Valladares & Niinemets 2008).

In the central Texas Edwards Plateau region, savannas are associated with upland or riparian woodlands or forests in many places (Van Auken et al. 1981; Van Auken & McKinley 2008; Van Auken & Smeins 2008). A species found in some of these communities is *Verbesina virginica* L. (Asteraceae, Frost Weed) (Correll & Johnston 1979). It seems to be an understory species, forming almost mono-specific communities in some understory habitats especially on deeper soils, but isolated plants are found below the canopy in some communities (Enquist 1987). It can establish below some trees, but no studies were found concerning its light requirements, needs for establishment or successional status.

Comparison of other species growing in shady habitats have been done, and physiological differences between plants found in shade compared to those found in full sun are fairly well known (Begon et al. 2006; Valladares & Niinemets 2008). Plants growing in low light usually have reduced photosynthetic rates when exposed to high light levels, light saturation occurs at lower light levels, light compensation points are lower (photosynthetic rate equals respiration rate) and dark respiration is lower (Boardman 1977; Larcher 2003; Valladares & Niinemets 2008). Sun plants on the other hand have higher photosynthetic rates at high light levels, and also have higher transpiration and stomatal conductance rates (Young & Smith 1980). Adaptive crossover is displayed by some species allowing them to acclimate to high or low light environments, consequently they could have a broader ecological niche (Givnish 1988; Givnish et al. 2004).

In the present study, linear transects, perpendicular to the canopy edge, were carried out from below the canopy into the adjacent

grassland to determine where the highest density of *V. virginica* plants were located. In addition, light levels and soil depth were measured along each transect. Based on most information about this species, it was hypothesized that it was a shade plant and would have characteristics of a shade plant. Consequently, photosynthetic rates, light saturation point, light compensation point, respiration, conductance, and transpiration were expected to be low when compared to sun adapted plants.

METHODS

Study species.–Verbesina virginica (Asteraceae, Frost Weed) is a 0.9 to 1.8 m tall, erect, unbranched, perennial, herbaceous plant mostly found in the eastern United States with Kansas, Oklahoma and Texas as its western limit of distribution (Correll & Johnston 1979; USDA 2009). In central Texas, it occurs mainly under the canopy of *Quercus virginiana* (live oak) or *Ulmus crassifolia* (cedar elm) and on deeper soils in some of these communities. It sometimes forms almost mono-specific communities in understory habitats especially on deeper soils including some riparian soils. In addition, isolated plants are found below the canopy in some upland communities (Enquist 1987). Its main stem has four to five prominent wings, which are usually absent from the highly branched head region. It has large ovate to oblong-lanceolate, pubescent leaves and it flowers from late summer through fall. The flower heads usually have three to four white to greenish white ray flowers and up to 15 disk flowers. It seems to tolerate high temperatures but not dry or compacted soil. The type of rooting system of the plants is unreported.

Study area.–This field study was carried out on the southern edge of the Edwards Plateau region of central Texas just south of the Balcones Escarpment in northern Bexar County (Correll & Johnston 1979; Van Auken et al. 1981; Van Auken & McKinley 2008). The Balcones Escarpment consists of a rough, well-drained area, with elevations increasing from approximately 250 m above mean sea level (AMSL) at the southern edge to between

approximately 500 and 700 m AMSL near the center, but in most places the increase in elevation is abrupt. This study area was about 350 m AMSL near the low end of the escarpment and at the upper edge of the Cibolo Creek floodplain. Most of the subsurface of the area is Cretaceous limestone, and soils are usually shallow, rocky or gravelly on slopes, and deeper in broad valleys and flats (Taylor et al. 1962; NRCS 2006). Soils are dark colored and calcareous with usually neutral or slightly basic pH.

Mean annual temperature of the area is 20.0°C with monthly means ranging from 9.6°C in January to 29.4°C in July (NOAA 2004). Mean annual precipitation is 78.7 cm and bimodal, with peaks occurring in May and September (10.7 cm and 8.7 cm, respectively), with little summer rainfall and high evaporation (Thornthwaite 1931; NOAA 2004). However, rainfall is highly variable and rarely average.

Area vegetation.–Juniperus-Quercus savanna or woodland is the community type in the study area and is representative of savannas and woodlands found throughout this region, but higher in woody plant density than savanna communities farther to the west (Van Auken et al. 1979; 1980; Van Auken et al. 1981; Smeins & Merrill 1988). The high density woody species are *Juniperus ashei* (Ashe juniper) and *Quercus virginiana* (=*Q. fusiformis*, Live oak) followed by *Diospyros texana* (Texas persimmon) and *Sophora secundiflora* (Texas mountain laurel). *Ulmus crassifolia* (cedar elm) is found in these communities, but usually at lower density and on the deeper soils. Associated with these woodlands are relatively small grasslands and sparsely vegetated intercanopy patches or gaps (openings in the woodlands) (Van Auken 2000). The major herbaceous species below the canopy is *Carex planostachys* (cedar sedge) (Wayne & Van Auken 2008) or in the current study sites it was *V. virginica*. In the grasslands and gaps *Aristida longiseta* (red three-awn), *Bouteloua curtipendula* (side-oats grama), *Bothriochloa* (=*Andropogon*) *laguroides* (silver bluestem), *B. ischaemum* (KR bluestem), various other C_4 grasses,

and a variety of herbaceous annuals are common (Van Auken 2000).

Transect measurements.–Each study site consisted of a stand of *V. virginica* plants located under a canopy of *Q. virginiana* or *U. crassifolia* and an adjacent grassland. There were five *V. virginica* stands sampled. A transect was established through the approximate center of each stand. Stands ranged in size from approximately 400 m^2 to over 10,000 m^2. Transects were 10 m in length, perpendicular to the canopy edge and centered on the canopy edge or drip line. Contiguous 0.5 m^2 quadrats were sampled along each transect. Each quadrat was 0.25 m wide and 2.00 m long with the long axes parallel to the canopy edge. All *V. virginica* plants within each quadrat were counted and standardized to plants/100 m^2.

Light levels and soil depth were also measured along each transect. Light levels were measured at 0.50 m intervals along each transect using a LI-COR® LI-190 SA integrating quantum sensor. A total of 105 measurements were made, 21/transect, and values were averaged for each location along the transects. Soil depth was measured at the same points along the transects. A 1.5 cm diameter iron bar was driven into the ground until it wouldn't penetrate any deeper, removed, depth was measured, and values were averaged (Van Auken 2000).

Gas exchange measurements.–Gas exchange rates were measured as a function of light level or photosynthetic flux density (PFD) and plotted for leaves of *V. virginica* plants growing in shade (Hamerlynck & Knapp 1994; Furuya & Van Auken 2009; Wayne & Van Auken 2009). There were five separate plants or replications and one leaf was measured per plant. Plants sampled were approximately 1.5 m tall. All plants were in the field and below the canopy of either *Q. verginiana* or *U. crassifolia* trees. The fifth leaf from the newest fully expanded leaf from the plant apex was measured. Ambient PFD was measured with the Li-Cor®

portable photosynthetic meter with an integrating quantum sensor at the approximate surface of each leaf at the time the light response curves were initiated (LI-COR, Inc, Lincoln, NE).

Measurements were made within ± three hr of solar noon with a Li-Cor® 6400 portable photosynthetic meter. Irradiances were generated by the Li-Cor LED red-blue light source using a modified light curve program with the Li-Cor® 6400. A gas flow rate of 400 μmol/s and a CO_2 concentration of 390 μmol/mol was used. One mature, undamaged, fully expanded leaf per replication was used with the two x three cm chamber. The Li-Cor® 6400 was operated at approximate ambient summer, midday, daytime temperature (34°C) and relative humidity (50%), and was calibrated daily. Response data were recorded after at least two minutes when a stable total coefficient of variation was reached (<0.3%), usually less than five minutes. Light response curves were started at a PFD of 2000 μmol/m^2/s and then reduced stepwise to 1800, 1600, 1400, 1200, 1000, 800, 600, 500, 400, 300, 200, 150, 100, 75, 50, 25, 10, and 0 μmol/m^2/s (19 total measurements per leaf).

Measurements included net photosynthesis, stomatal conductance, and transpiration. Separate one way *ANOVAs* were used to determine if net photosynthesis, stomatal conductance, and transpiration were significantly different over the PFD's tested (Sall et al. 2001). A repeated measures *ANOVA* was not used because only one leaf type was examined. If experiment wide differences were found, Tukey's HSD was used to detect differences between PFD's examined. Shapiro-Wilks tests were used to test for normal distributions and the Bartlett's Test was used to test for homogeneity of variances. If data were not normal or the variances were not homogenous, and could not be corrected with transformations, non-parametric Kruskal-Wallis *ANOVA* and Dunn's multiple range test were used.

Maximum photosynthesis (A_{max}), PFD at A_{max}, transpiration at A_{max}, conductance at A_{max}, light saturation point, dark respiration,

light compensation point, and the quantum yield efficiency (initial slope) were determined for each replicate, and means were calculated (Wayne & Van Auken 2009). The A_{max} was the highest net photosynthesis rate. Light saturating photosynthesis was the PFD when the slope of the initial rate line reached the A_{max}. Dark respiration was the gas exchange rate at a PFD of 0 μmol/m^2/s (y-intercept of the line for the initial slope or rate). The light compensation point was calculated as the PFD when the photosynthetic rate = 0 μmol CO_2/m^2/s (x-intercept of the line for the initial slope or rate). The quantum yield efficiency or initial slope was calculated using the dark value and increasing PFDs until the regression coefficient of the slope decreased (150 μmol/m^2/s PFD). Significance level for all tests was 0.05.

Results

The mean density of *V. virginica* varied significantly by position along the transects (Fig. 1, Kruskal-Wallis *ANOVA*, P=0.0001). Plants were mostly distributed under the canopy as opposed to being in the associated open grassland. The density of *V. virginica* plants decreased from about 700 plants/100 m^2 below the canopy to zero at the edge of the grassland (Fig. 1). No *V. virginica* plants were found in the open grassland beyond 0.75 m from the dripline outward into the grassland in any of the 5 transects sampled. The highest density of *V. virginica* was 850 plants/100 m^2 which occurred 3.5 m from the drip line, under the canopy. A total of 283 plants were counted, with 99% below the canopy and only three in the grassland or at the drip line.

The mean light level (PAR, μmol/m^2/s) varied significantly by position along the transects (Fig. 2, Kruskal-Wallis *ANOVA*, P=0.0001). Measurements from the open grassland positions were higher than those below the canopy. The lowest mean light level was 207 ± 53 μmol/m^2/s and was found five meters from the dripline, below the canopy. The highest mean light level was 2126 ± 71 μmol/m^2/s and was found four meters from the dripline into the associated grassland. The mean light level at the canopy edge

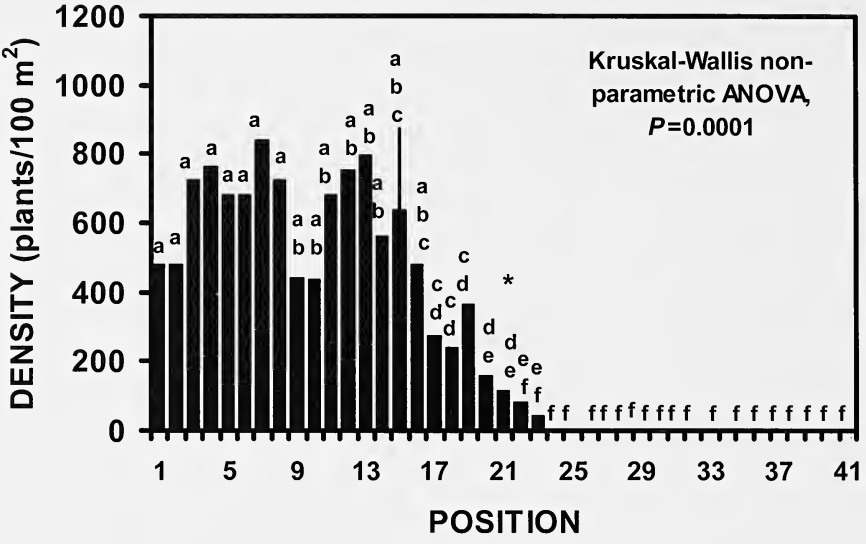

Figure 1. Mean *Verbesina virginica* plant density/100 m^2 along a transect from below the canopy into an associated grassland in the southern part of the Edwards Plateau of central Texas. There were significant differences in plant density along the transect (Kruskal-Wallis non-parametric *ANOVA*, P=0.0001). Positions 1-20 are below the canopy, position 21 (*) is the drip line and positions 22-41 are in the grassland. Means with the same letter are not significantly different (Dunn's multiple range test, P<0.05). The error bar is representative and is one standard deviation.

was 721 ± 325 μmol/m^2/s and was significantly different from the light levels in the grassland and most of the positions below the canopy (Fig. 2, Dunn's multiple range test). There were few significant differences in light levels in the open grassland.

Mean soil depth along the five transects from under the canopy into the open grassland was patchy but did not vary significantly by position (Fig. 3, *ANOVA*, F=1.4936, P=0.1054). The deepest mean soil depth was 42.4 ± 18.2 cm and was 2.5 m from the drip line below the canopy. The shallowest mean soil depth was 20.7 ± 14.4 cm and was found 0.5 m from the drip line below the canopy.

The mean ambient light level under the canopy at the level of the leaves of *V. virginica* was 110 ± 17 μmol/m^2/s (Table 1). This light

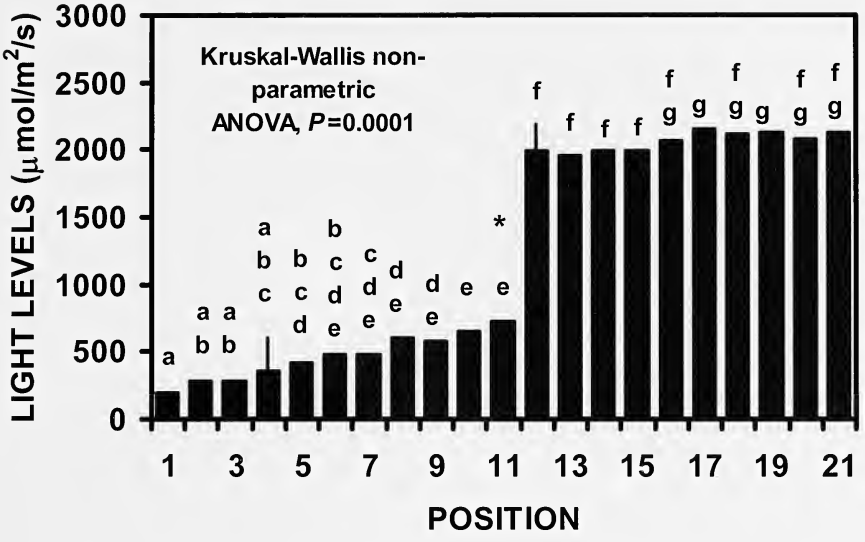

Figure 2. Mean light levels (µmol/m^2/s) along a transect from below the canopy into an associated grassland in the southern part of the Edwards Plateau of central Texas. There were significant differences in light levels along the transect (Kruskal-Wallis non-parametric *ANOVA*, P=0.0001). Positions 1-10 are below the canopy, position 11 (*) is the drip line and positions 12-21 are in the grassland. Means with the same letter are not significantly different (Dunn's multiple range test, P<0.05). The error bars are representative and equal one standard deviation.

level was lower than the light saturation point but above the light compensation point and the carbon assimilation rate was 35% of the A_{max}. The photosynthetic response of the *V. virginica* leaves was significantly different over the light levels tested (one-way *ANOVA*, F=178.9, P<0.0001, Fig. 4). At PFD's above approximately 300 µmol/m^2/s, rates were fairly constant and there were few differences (Tukey's HSD; $P \geq 0.05$), while at PFD's lower than 300 µmol/m^2/s, leaves generally had lower and significantly different rates at most of the light levels tested (Tukey's HSD; $P \leq 0.05$). *Verbesina virginica* had a fairly high maximum photosynthetic rate (A_{max}) at 12.7 ± 1.4 µmol CO_2/m^2/s at the highest light level tested, 2000 µmol/m^2/s (Table 1). Light saturation (L_{sat}) was 288 µmol/m^2/s and the light compensation point L_{cp} was 16 µmol/m^2/s (Table 1). The dark respiration (R_d)

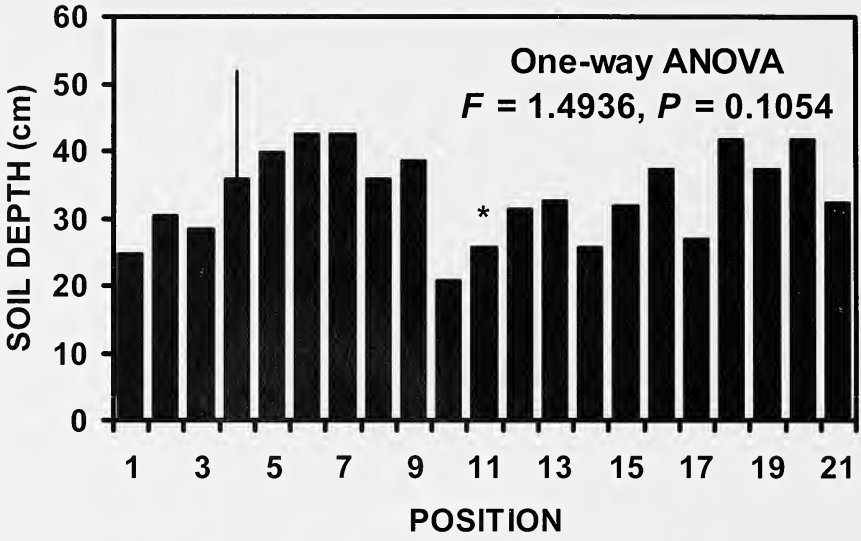

Figure 3. Mean soil depth (cm) along a transect from below the canopy into an associated grassland in the southern part of the Edwards Plateau of central Texas. Positions 1-10 are below the canopy, position 11 (*) is the drip line and positions 12-21 are in the grassland. Soil depth did not vary significantly by position (one-way *ANOVA*, P=0.1054). The error bar is representative and is one standard deviation.

was low at 0.76 μmol $CO_2/m^2/s$. The quantum yield efficiency or the initial slope (slope of the line from 0 -150 μmol/m^2/s) was 0.0375 ± 0.0019 μmol CO_2/μmol quanta. Mean stomatal conductance (g_{leaf}) of *V. virginica* plants below the canopy was not significantly different over the light levels examined (*ANOVA, F=1.16, P=0.3126,* Fig. 5; Table 1). The lowest stomatal conductance was 0.190 ± 0.080 mol $H_2O/m^2/s$ at the lowest light level tested (zero μmol/m^2/s), while the highest mean stomatal conductance was 0.311 ± 0.110 mol $H_2O/m^2/s$ at the highest light level tested (2000 μmol/m^2/s). Transpiration rates (E) varied significantly over the light levels tested (*ANOVA*; F=5.09, P<0.0001, Fig. 6). The lowest mean transpiration rate was 3.46 ± 0.96 mmol $H_2O/m^2/s$ and was found at the lowest light level tested (zero μmol/m^2/s, Table 1). The highest mean transpiration rate was 6.10 ± 0.99 mmol $H_2O/m^2/s$ at 2000 μmol/m^2/s or full sunlight.

Table 1. Means and standard deviations for the ambient canopy light levels (PFD) and ecophysiological characteristics for shade leaves of *Verbesina virginica* plants growing in shade.

Parameter	Sun
Ambient canopy Light - PFD ($\mu mol/m^2/s$)	110 ± 17
A_{can} - Canopy CO_2 Uptake ($\mu molCO_2/m^2/s$)	4.5 ± 0.5
A_{max} - Maximum CO_2 Uptake ($\mu molCO_2/m^2/s$)	12.7 ± 1.4
PFD - Light Level at A_{max}	2000 ± 0
L_{sat} - Light saturation ($\mu mol/m^2/s$)	288 ± 32
L_{cp} - Compensation point ($\mu mol/m^2/s$)	16 ± 2
R_d - Dark Respiration ($\mu molCO_2/m^2/s$)	0.76 ± 0.07
IS or QY - Initial slope ($\mu molCO_2/(\mu mol$ quanta)	0.0375 ± 0.0019
g_{leaf} - Conductance ($molH_2O/m^2/s$) at A_{max}	0.311 ± 0.110
g_{leaf} - Conductance ($molH_2O/m^2/s$) at L_{cp}	0.190 ± 0.080
E - Transpiration ($mmolH_2O/m^2/s$) at A_{max}	6.10 ± 0.99
E - Transpiration ($mmolH_2O/m^2/s$) at L_{cp}	3.46 ± 0.96

Discussion

Verbesina virginica was found below or at the edge of the canopy of *Q. virginiana* or *U. crassifolia* (Fig 1) but not in associated grasslands. Transects were short and focused on the canopy edge of these woodland communities. No *V. virginica* plants were seen in the grassland beyond the end of the transects. In addition, density below the canopy, where the transects ended, did not appear to decrease. Light levels measured appeared to be important, with almost no *V. virginica* plants found in the high light open grassland habitat (Fig. 2). Soil depth did not seem to be a factor determining the presence or density of *V. virginica* (Fig. 3), but they were not expected in the shallow soils of the more arid woodland communities (Van Auken et al. 1981).

Shade leaves of *V. virginica* plants growing in the low light environments of various canopy trees (Enquist 1987), had a high

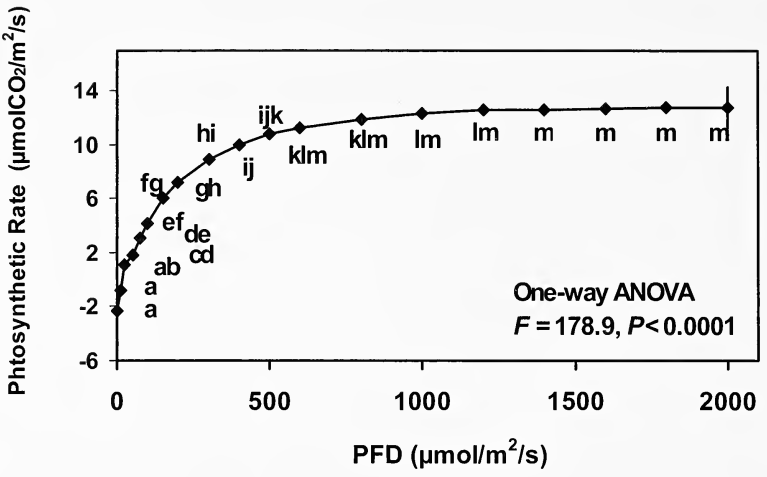

Figure 4. Mean photosynthetic light response curve for leaves (♦) of five *Verbesina virginica* plants growing in canopy shade. There were significant differences in photosynthetic rates between light levels (one-way *ANOVA*, F=178.9, P<0.0001). The error bar is representative and is ± one standard deviation of the mean. Means between light levels with the same letters are not significantly different (Tukey's HSD, $P \geq 0.05$).

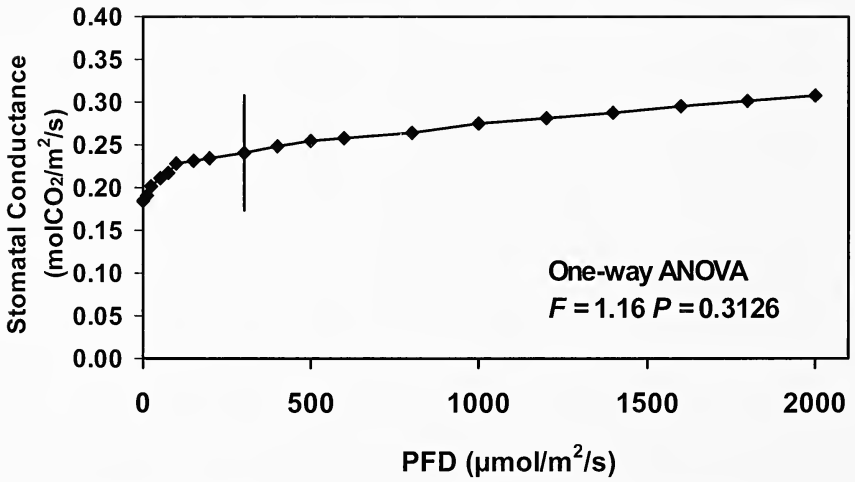

Figure 5. Mean stomatal conductance curve for leaves (♦) of five *Verbesina virginica* plants growing in canopy shade. There were no significant differences in stomatal conductance rates between light levels (one-way *ANOVA*, F=1.1600, P=0.3126). Error bar is representative and is ± one standard deviation of the mean.

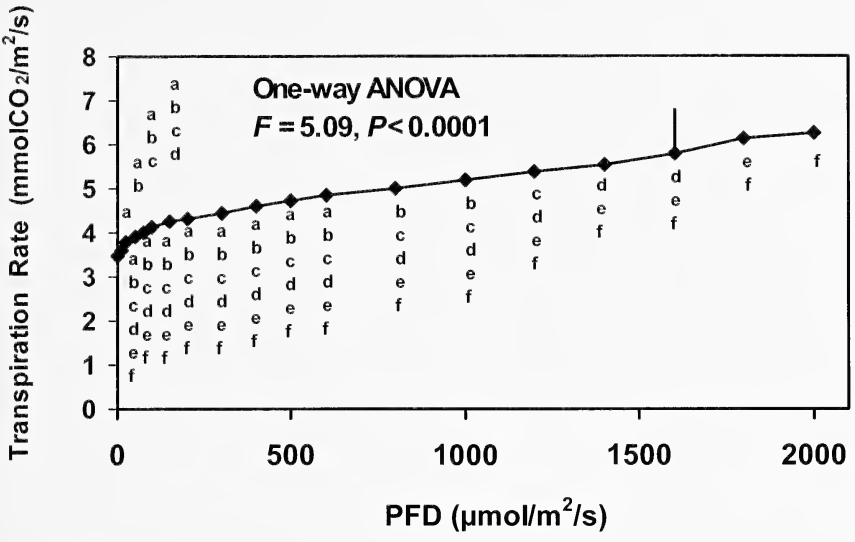

Figure 6. Mean transpiration curve for leaves (♦) of five *Verbesina virginica* plants growing in canopy shade. Transpiration rates varied significantly between light treatments (one-way *ANOVA*, F=5.09, P<0.0001). Error bar is representative and is one standard deviation of the mean. Means between light levels with the same letters are not significantly different (Tukey's HSD, P=0.05). The letters above the line represent the first four light levels (0-50 µmol/m^2/s) but the points can not be distinguished separately in the figure.

maximum photosynthetic rate (A_{max}), which is atypical of many species found growing below a woodland or forest canopy habitat (Begon et al. 2006). Shade adapted leaves of one spring green eastern deciduous forest understory species was 1.16 times higher than rates for *V. virginica,* but a second spring green species was 52.4% lower (Hull 2002). Summer green eastern deciduous forest understory species had lower A_{max} rates at 44.4% and 30.9% of the *V. virginica* A_{max} rates (Hull 2002).

Other photosynthetic parameters, including light saturation, light compensation, and dark respiration were in the range expected for shade adapted plants (Boardman 1977; Hull 2002; Larcher 2003; Givnish et al. 2004; Begon et al. 2006; Valladares & Niinemets 2008). Conductance and transpiration were relatively high for

shade adapted leaves. These responses are not completely consistent with findings for shade plants, but are suggestive that *V. virginica* is a shade species (Boardman 1977; Hull 2002; Larcher 2003; Givnish et al. 2004; Begon et al. 2006; Valladares & Niinemets 2008). In addition, conductance and transpiration rates suggest that *V. virginica* was not water limited and that photosynthetic rates reported were light dependent and typical for this species (Matzner et al. 2003; Vilagrosa et al. 2003).

Verbesina virginica is a native species with a fairly broad distribution, especially in the eastern United States. However, very little is known about its photosynthetic capability. No studies have been identified which evaluate the physiological responses or growth responses of this species to light levels or other environmental factors. Most of the parameters measured for shade leaves suggest that this species is a shade adapted species, and its growth is best in full or at least partial shade. *Verbesina encelioides,* a related species, had an A_{max} of 12.3 μmol $CO_2/m^2/s$, which is within the range reported here for *V. virginica*, but *V. encelioides* is a disturbance species and not expected to do well at low light levels below a canopy (Gleason et al. 2007).

Usually, true understory species like summer green species (Hull 2002) have much lower photosynthetic rates than the rates reported for *V. virginica* in the current study. Photosynthetic A_{max} rates of three European montane understory forests species were 3.4 – 5.5 μmol $CO_2/m^2/s$) (Hättenschwiler & Körner 1996). These forest understory species reached light saturation at 210 ± 20 μmol/m^2/s compared to 288 ± 32 μmol/m^2/s for *V. virginica*. *Arnica cordifolia*, an herbaceous perennial which grows in the understory of lodgepole pine forests in southeastern Wyoming, also had maximum photosynthetic rates lower than *V. virginica*. However, light saturation was higher at 350 μmol $CO_2/m^2/s$ (Young & Smith 1980). *Polygonum virginianum*, an herbaceous perennial found in the forest understory and at the forest's edge in the eastern United States, had an A_{max} of ~ 3 μmol$CO_2/m^2/s$ at a light saturation of ~

500 μmol/m^2/s (Zangerl & Bazzaz 1983). *Carex planostachys* from the central Texas Edwards Plateau *Juniperus* woodland understory had an A_{max} value of 4.9 ± 0.3 μmolCO_2/m^2/s which was lower than the shade leaves of *V. virginica* and reached light saturation at 151 ± 43 μmol/m^2/s (Wayne & Van Auken 2009). While *V. virginica* in central Texas is typically found growing in shaded habitats or the edge of woodlands, its high A_{max} for shade adapted leaves compared to other herbaceous shade plants would suggest it could grow in a variety of light environments including open habitats, but it was not found there.

True sun plants are adapted to high light conditions and have high rates of gas exchange. For example, *Abutilon theophrasti* an early successional herbaceous perennial, had A_{max} rates between 15-25 μmolCO_2/m^2/s (Wieland & Bazzaz 1975; Bazzaz 1979; Munger et al. 1987a; Munger et al. 1987b; Hirose et al. 1997; Lindquist & Mortensen 1999). Two oaks of gallery forest in tall grass prairies of northeastern Kansas, *Quercus muehlenbergii* and *Q. macrocarpa* had A_{max} rates of 11-13 μmolCO_2/m^2/s for shade leaves (Hamerlynck & Knapp 1994) which is comparable to 12.7 ± 1.4 μmolCO_2/m^2/s reported for *V. virginica* in the present study.

Plants can acclimate to the variability of the light environment where they are found, including some early successional species or plants from disturbed (open) communities (Bazzaz & Carlson 1982). For example, *Polygonum pensylvanicum*, a colonizing annual of open fields, had an A_{max} of ~ 12 μmolCO_2/m^2/s at ~ 1500 μmol/m^2/s when plants from a shaded-habitat (200 μmol/m^2/s) were measured (Bazzaz & Carlson 1982; Zangerl & Bazzaz 1983); however the rate was ~ 24 μmol/m^2/s at ~ 1800 μmol/m^2/s when plants from a full sun habitat were examined (Bazzaz & Carlson 1982). Species like *V. virginica* that have a relatively high A_{max} and one that does not change significantly over a wide range of light levels would do well in canopy shade especially in the presence of various sunflecks (Hull 2002). Further studies would be needed to

determine if *V. virginica* does acclimate to variability in the light environment as reported for other understory species.

The dark respiration of shade leaves of *V. virginica* (0.76 ± 0.07 $\mu molCO_2/m^2/s$) is also similar to other shade-adapted plants (Hamerlynck & Knapp 1994). This rate is 33% of the R_d of shade adapted leaves of *S. secundiflora* found in the same area (Furuya & Van Auken 2009). The R_d for shade adapted leaves of one spring green eastern deciduous forest understory species was about 1.58 times higher than rates for *V. virginica.* The rate for another spring green species studied was the same as the rate for *V. virginica* (Hull 2002). The R_d for shade adapted leaves of summer green eastern deciduous forest understory species was about 57% and 38% of the rates for *V. virginica* (Hull 2002). Dark respiration for shade-adapted species is typically lower than sun-adapted species, due to the lower metabolism of shade-adapted species (Bjorkman 1968; Bazzaz & Carlson 1982). *Polygonum pensylvanicum* grown at 200 $\mu mol/m^2/s$ had a respiration rate of ~ 0.5 $\mu molCO_2/m^2/s$, although the rate was twice as high when plants from full sun were measured (Bazzaz & Carlson 1982). Some suggest that low dark respiration is the best predictor of a species ability to exist in shaded environments (Valladares & Niinemets 2008).

Other photosynthetic parameters reported in this study for *V. virginica* are similar to those values reported in the literature. Quantum yield efficiency reported here (0.038 $\mu molCO_2/\mu mol$ quanta) for shade leaves is within the range of values (0.035 – 0.052 $\mu molCO_2/\mu mol$ quanta) reported for other shade adapted species (Hirose et al. 1997; Hull 2002). Stomatal conductance and transpiration reported in the current study were similar to other studies, however many factors affect these parameters (Wieland & Bazzaz 1975; Zangerl & Bazzaz 1984; Yun & Taylor 1986; Munger et al. 1987a; Munger et al. 1987b; Stafford 1989).

The shade leaves of *V. virginica* showed interesting photosynthetic responses. These physiological responses to various

light levels more than likely are contributors to the apparent niche observed for this species in the field. In general, resource utilization is spatially partitioned among species along complex environmental gradients, such as changes in light from open areas to woodland or forest edges (Wayne & Van Auken 2009). The ability of *V. virginica* to reach high photosynthetic rates at lower light level, its light saturation, and light compensation point allow it to exist in shaded environments. At light levels below 300 $\mu molCO_2/m^2/s$, data suggests that other more shade tolerant species such as *C. planostachys* would probably be able to out-compete *V. virginica* (Wayne & Van Auken 2009), but not after *V. virginica* was established. At light levels above 300 $\mu molCO_2/m^2/s$, *V. virginica* could dominate, in part because it has photosynthetic rates as high as or higher than most co-occurring species and its large leaves would reduce light levels to very low values below its canopy (Grunstra 2008; Furuya & Van Auken 2009; Wayne & Van Auken 2009). However, its absence in associated grasslands is not explained. The established C_4 grasses would have equal or higher photosynthetic rates and perhaps be more tolerant of higher light levels and lower soil water levels than *V. virginica*.

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AN UPDATE ON THE BENTHIC ALGAE OF MANSFIELD PASS, TEXAS

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Abstract - The macroalgal flora of the jetties at Mansfield Pass were examined from July 2002 to June 2003 in order to observe seasonal variations, to assess the effect of wave energy on macroalgal populations, and to compile a comprehensive species checklist. Nine sites, six on the north jetty and three on the south, were established to observe differences in the macroalgae flora with respect to time and season. Thirty-six species were collected during this study, twenty-two of which were Rhodophyta. Sixteen species are reported for the first time at Mansfield Pass. A Cheney ratio of 5.13 was calculated from this study, which indicates that the Mansfield Pass area is sub-tropical. Species richness appears to be somewhat seasonal; only sixteen of the thirty-seven benthic species were harvested year-round. Seasonality, with respect to biomass, varied monthly with a high of 21.9kg m^{-2} in April 2004 and a low of 3.63kg m^{-2} in October 2003. Wave energy affected the macroalgal flora as several species appeared only at those sites where wave energy was low.

The macroalgae, a critical component of ecological communities in the estuaries and gulf waters of Texas, are among the least studied biological groups in the U.S. (Lehman 1999). Because of its isolation, the Mansfield Pass area has been particularly understudied, especially with regard to macroalgae. Kaldy et al. (1995) reported little or no information available regarding the Mansfield Pass jetties, their investigation being the first to report exclusively on them. They suggested that the number of macroalgal species recorded from Mansfield Pass was low in comparison to other Texas coastal jetties and a more diverse flora would be likely revealed with continued investigation.

This study provides an update to the macroalgal community of Mansfield Pass. Through intensive sampling this study was able to assess a broader coverage of macroalgae than previous studies. In addition, over a prolonged period (multiple seasons) this study was able to account for seasonal periodicity in the macroalgal

community that previous studies did not include. As a result 16 additional species of macroalgae were documented and catalogued that had not been previously reported for the area, increasing the total species richness for the site from thirty-two to forty-eight.

Study Site

Mansfield Pass is a relatively remote, man-made, tidal inlet linking the Port of Mansfield to the northwestern Gulf of Mexico. It is accessible only by a nine-mile boat ride from Port Mansfield, a 63-mile four-wheel drive south down Padre Island National Seashore from Corpus Christi, or a 34-mile four-wheel drive north from South Padre Island. The Mansfield Pass jetties are located at approximately 26° 34' N and 97° 16' W. Finished in 1958, Mansfield Pass connects the Gulf of Mexico to the lower Laguna Madre and is lined with granite jetties. Mansfield Pass and the Laguna Madre are considered subtropical. Both air and water temperatures are highest during July, and coolest during January and February, respectively.

Materials and Methods

This study was conducted from July 2002 to June 2003 to assess the macroalgal community along the rocky jetties of Mansfield Pass (26° 34' N and 97° 16' W). Nine sites along both sides of the north and south jetties were identified to ensure that both the protected and exposed sides were sampled bimonthly using destructive sampling techniques. At each site transects were set along jetty rocks extending vertically from the high water line to the submerged sandy substratum and a 20 by 20cm ($0.04m^2$) quadrat was sampled at 10cm intervals. Transects extended from the supralittoral to the sublittoral zones to allow all macroalgae present to be sampled. Transect length varied by location due to the depth variation from near to offshore.

For this study a total of 168 quadrats were examined for their algal content. Identification of specimens to the level of species (or

to the lowest possible taxon) took place at the Center for Coastal Studies Graduate Laboratory at Texas A&M University-Corpus Christi. Species identification followed Littler & Littler (2000) and Taylor (1960). Taxonomic classification was later updated following Wynne (2005; 2009). Herbarium voucher specimens were prepared according to Tsuda & Abbott (1985), and are stored with the Phycological Collections in the Ruth O'Brien Herbarium at Texas A&M University-Corpus Christi. Microscope slides of structures aiding in species identification, such as reproductive and identifying structures (e.g., tetraspores and utricles), were also prepared by washing specimens with seawater and 45% isopropyl alcohol followed by preservation with Polymount™

.

In addition to species richness, Cheney's floristic ratio was calculated (Cheney 1977; Mathieson & Penniman 1986; Kaldy et al. 1995), where a value < 3.0 indicates a temperate flora and a value > 6.0 indicates a tropical flora; intermediate values represent mixed flora. The values obtained from this ratio were used as an index of the geographical and climatic nature of algal flora.

Results

This study reports 36 species of benthic macroalgae in three divisions from the Mansfield Pass jetties (Table 1). There were 22 species of Rhodophyta recorded (56.4% of the total), followed by the Chlorophyta with 11 species (28.2% of the total), and three species of Ochrophyta (12.8% of the total). Species richness was greatest during July (29 species) and lowest during June (23 species). In December and February (the two coolest months) species richness was 24 and 28 species, respectively.

It should be noted that of the 36 species of benthic macroalgae reported from Mansfield Pass, 16 species (43%) were consistently reported during each sampling period (Table 2). Those species collected were considered common algae, whereas those species not

Table 1. Taxonomic list showing species confirmed for Mansfield Pass, Texas. An asterisk (*) indicates a new record for Mansfield Pass. Species marked by a dagger (†) were found by Kaldy et al. (1995) but were not recorded during this study, July, 2002 – June, 2003. Taxonomic arrangement follows Wynne (2009).

DIVISION: RHODOPHYTA
Subdivision Eurhodophytina
Class Bangiophyceae

ORDER: BANGIALES
Family: Bangiaceae
Porphyra rosengurttii Coll et J. Cox [*P. leucosticta*]

Class Florideophyceae
Subclass Nemaliophycidae

ORDER: ACROCHAETIALES
Family: Acrochaetiaceae
Acrochaetium spp. Nägeli in Nägeli & Cramer, 1858 [*Liagorophila*] †

ORDER: CORALLINALES
Family: Corallinaceae
Subfamily: Corallinoideae
Jania capillacea Harv. †
Jania cubensis Mont. ex Kütz. [*Haliptilon cubense**, *Corallina cubensis*]*
Jania subulata (J. Ellis et Sol.) Sond. [*Haliptilon subulatum**, *Corallina subulata*]*

ORDER: CERAMIALES
Family: Callithamniaceae
Aglaothamnion halliae (Collins) N. Aponte, D. L. Ballant & J.N. Norris [*byssoides sensu auct.*; *pseudobyssoides sensu auct.*; *westbrookiae*; *Callithamnion byssoides sensu auct. pro parte*; *C. byssoides* ["*byssoideum*"] var. jamaicensis; *C. halliae*; *C. pseudobyssoides sensu auct.*]*
Family: Ceramiaceae
Centroceras clavulatum (C. Agardh in Kunth) Mont. in Durieu de Maisonneuve
Ceramium cimbricum H.E. Petersen in Rosenv. [*fastigiatum* Harv. In Hook., non Roth; *fastigiramosum*]*
Family: Spyridiaceae
Spyridia hypnoides (Bory in Belanger) Papenf. [*aculeata*]*
Family: Rhodomelaceae
Bryocladia cuspidata (J. Agardh) De Toni
Bryocladia thrysigera (J. Agardh) F. Schmitz in Falkenb.
Chondria spp. C. Agardh, nom. cons. †
Digenia simplex (Wulfen) C. Agardh †
Palisada poiteaui (J.V. Lamour.) K.W. Nam [*Chondrophycus poitei, Laurencia poiteaui; L. poitei*]
Polysiphonia denudata (Dillwyn) Grev. ex Harv. In Hook.
Polysiphonia subtilissma Mont.

Table 1. Cont.

ORDER: GELIDIALES
Family: Gelidiaceae
Gelidium crinale (Turner) Gaillon †
Gelidium pusillum (Stackh.) Le Jolis *
Pterocladiella bartlettii (W.R. Taylor) Santel. [*Pterocladia bartlettii*]

ORDER: GIGARTINALES
Family: Cystocloniaceae [Hypneaceae]
Hypnea musciformis (Wulfen in Jacqu.) J.V. Lamour. [*arborescens*]
Hypnea valentiae (Turner) Mont. [*gracilarioides*]
Family: Solieriaceae [Wurdemanniaceae]
Agardhiella subulata (C. Agardh) Kraft & M.J. Wynne [*tenera; Neoagardhiella baileyi*]*

ORDER: GRACILARIALES
Family: Gracilariaceae
Gracilaria isabellana Gurgel, Fredericq & J.N. Norris [*lacinulata* (Vahl) M. Howe nom. illeg.; non *G. lacinulata* (P. Crouan & H. Crouan) Piccone; *foliifera sensu* Taylor *pro parte*; *multipartita sensu auct.*] †
Gracilaria tikvahiae McLachlan [*foliifera var. Angustissima*] *
Hydropuntia caudata (J. Agardh) Gurgel & Fredericq [*Gracilaria caudata*]*

ORDER: HALYMENIALES
Family: Halymeniaceae
Grateloupia filicina (J.V. Lamour.) C. Agardh [*concatennata*]
Grateloupia pterocladina (M.J. Wynne) S. Kawaguchi & H.W. Wang in Wang et al. [*Prionitis pterocladina*]

ORDER: RHODYMENIALES
Family: Rhodymeniaceae
Rhodymenia pseudopalmata (J.V. Lamour.) P.C. Silva

DIVISION: OCHROPHYTA
Class Phaeophyceae

ORDER: DICTYOTALES
Family : Dictyotaceae
Dictyota ciliolata Sond. ex Kütz. [*ciliata*] †
Dictyota menstrualis (Hoyt) Schnetter, Hörning, & Weber-Peukert [*dichotoma* var. *menstrualis*; *dichotoma sensu auct.*, non (Huds.) J. V. Lamour.] †
Padina gymnospora (Kütz.) Sond. [vickersiae]

Table 1. Cont.

ORDER: ECTOCARPALES
Family: Ectocarpaceae
Ectocarpus siliculosus (Dillwyn) Lyngb. [arctus; confervoides; dasycarpus]
Family: Scytosiphonaceae [Chnoosporaceae]
Petalonia fascia (O. F. Müll.) Kuntze *

ORDER: FUCALES
Family: Sargassaceae
Sargassum filipendula C. Agardh

DIVISION: CHLOROPHYTA
Class Ulvophyceae

ORDER: BRYOPSIDALES
Family: Bryopsidaceae
Bryopsis pennata J.V. Lamour.
Family: Codiaceae
Codium taylorii P.C. Silva *

ORDER: CLADOPHORALES
Family: Cladophoraceae
Chaetomorpha aerea (Dillwyn) Kütz. *
Chaetomorpha antennina (Bory) Kütz. [media] †
Chaetomorpha linum (O.F. Müll.) Kütz *
Cladophora albida (Nees) Kütz. [glaucescens; scitula] *
Cladophora prolifera (Roth) Kütz. *
Cladophora vagabunda (L.) C. Hoek [crucigera; expanda; fascicularis; sertularina; mauritiana; brachyclona]

ORDER: DASYCLADALES
Family: Polyphysaceae
Acetabularia crenulata J. V. Lamour. †

ORDER: ULVALES
Family: Ulvaceae
Ulva fasciata Delile
Ulva flexuosa Wulfen [*Enteromorpha flexuosa; E. lingulata; E. prolifera var. flexuosa; E. tubulosa*] *
Ulva lactuca L. *
Ulva prolifera O.F. Müll. [*Enteromorpha prolifera; E. salina; E. salina* var. p*olyclados; E. torta*]*

found every sampling period were considered unique (seasonal) algae. Of the 22 species for division Rhodophyta, only half of them were consistently found at every sampling event; however, of the Chlorophyta only three species were consistently present and only two of the Ochrophyta.

Three species of macroalgae were common to all nine sites: *Grateloupia filicina, Cladophora vagabunda*, and *Ulva fasciata*. An additional six species were common to eight of the nine sites, five belonging to the Rhodophyta and the other to Chlorophyta. An additional fifteen species were common to at least five of the nine sites.

Discussion

Of all the species observed during this study, seven were limited to one side of a jetty: five on the north sides and two on the south sides. Five of the seven unique algae were located on the north jetty of Mansfield Pass, and four of those five were located on the north side of the north jetty (beach side). This phenomenon of species of macroalgae living at only one wave energy level has been documented. Whorff et al. (1995) found that delicate algae (e.g., *Polysiphonia denudata*) occur only in locations with low mean wave height. Begin & Scheilbling (2003) reported that survival of an individual *Codium fragile* ssp. *tomentosoides* plant was negatively related to the ratio of its circumference-to-length. This suggests that large bushy plants are more likely to be dislodged by wave forces. Thus, it is probable that the location of these macroalgae at a particular site is limited by wave energy. An additional factor affecting distribution, not sampled in this study, was the presence or absence of algal grazers.

The Cheney ratio for the Mansfield Pass jetties was found to be 6.83, indicating a tropical flora. This value is higher than the ratio of 5.0 reported by Kaldy et al. in 1995. Other recent floristic surveys have found lower ratios, for example Kapraun (1974) on

Table 2. Seasonal periodicity of macroalgal species confirmed for Mansfield Pass, Texas from July 2002 to June 2003. An "x" indicates that a species was collected and confirmed for the indicated sampling period.

Species	July	Oct.	Dec.	Feb.	April	June
Agardhiella subulata	x	x	x	x		
Aglaothamnion halliae					x	
Bryocladia cuspidata	x	x	x	x	x	x
Bryocladia thrysigera	x	x	x	x	x	x
Centroceras clavulatum	x	x	x	x	x	x
Ceramium cimbricum	x	x		x	x	x
Gelidium pusillum	x	x	x	x	x	x
Hydropuntia caudata					x	x
Gracilaria tikvahiae	x	x		x	x	
Grateloupia filicina	x	x	x	x	x	x
Jania cubensis	x					
Jania subulata	x	x	x	x	x	x
Hypnea musciformis	x	x	x	x	x	x
Hypnea valentiae	x	x	x	x		
Palisada poiteaui					x	
Polysiphonia denudata						x
Polysiphonia subtilissma	x	x	x	x	x	x
Porphyra rosengurttii				x	x	
Grateloupia pterocladina	x	x		x	x	x
Pterocladiella bartletii	x	x				x
Rhodymenia pseudopalmata	x	x	x	x	x	x
Spyridia hypnoides	x	x	x	x	x	x
Bryopsis pennata	x	x	x	x	x	x
Chaetomorpha aerea	x	x	x	x		x
Chaetomorpha linum		x			x	
Cladophora albida	x	x	x	x	x	
Cladophora prolifera	x			x	x	x
Cladophora vagabunda	x	x	x	x	x	x
Codium taylorii	x					x
Ulva flexuosa	x		x	x	x	
Ulva prolifera	x	x	x	x	x	
Ulva fasciata	x	x	x	x	x	x
Ulva lactuca	x	x	x	x	x	x
Ectocarpus siliculosus			x	x		
Padina gymnospora	x	x	x	x	x	x
Sargassum filipendula	x	x	x	x	x	x

the Louisiana coast obtained a ratio of 2.7, and López-Bautista et al. (2002) calculated a ratio of 4.7 using the macroalgae of offshore oil platforms off the coast of Louisiana. More recently a study examining the jetties of Packery Channel indicated a ratio of 9.0, which suggests a highly tropical nature (Fikes & Lehman 2008; Fikes & Lehman 2010). In contrast, Cheney ratios of 6.2 and 5.2 were reported by Edwards & Kapraun (1973) and by Edwards (1976), respectively, in the Port Aransas, Texas area. In 1999, Lehman reported on the macroalgal species of the Corpus Christi Bay area, and determined a Cheney ratio of 6.4.

The number of species of benthic macroalgae found at the Mansfield Pass was expanded during this study from 32 species listed by Kaldy et al. (1995) to 48 species. In their study of Mansfield Pass, Kaldy et al. (1995) found ten species of algae that were not collected in this study (six in division Rhodophyta, two in division Ochrophyta, and two in division Chlorophyta). Seventeen additional species were reported during this study previously unrecorded (eight in division Rhodophyta, one in division Ochrophyta, and eight in division Chlorophyta).

Using the Cheney ratio for all confirmed species at Mansfield Pass for both studies results in a ratio of 8.2. This ratio indicates tropical affinity, but one that is probably too high for the area. Other areas considered more tropical have a lower ratio, for example, a Cheney ratio of 7.2 was obtained for Enmedio Reef, Veracruz, Mexico (Lehman & Tunnell 1992). Cheney's Floristic Ratio was designed to characterize established communities that are not in a state of transition. Depending on the rate of disturbance in a habitat the Cheney method may not be the best approach to characterize the community. Similar findings have also been shown for Packery Channel, where a community in early successional stages yielded a higher than expected ratio (Fikes & Lehman 2010).

Ecological Considerations

Temperature is the major factor controlling geographical distribution of marine algae (Edwards & Kapraun 1973). Macroalgal seasonality in warm-water regions such as the western Gulf of Mexico is often related to temperature and desiccation (Mathieson et al. 1981; Mathieson & Penniman 1986). Seasonal variation in the macroalgal flora was found at this location; however, species richness changed only slightly between seasons. Philips (1960) suggested that mild winters allow for the growth of subtropical algae in the colder months. This trend is supported by the research of others (Edwards & Kapraun 1973; Kapraun 1974), and has been explained by some as being the result of increased tolerance to reduced salinity in colder water (Conover 1958;, Wood & Palmatier 1954).

Wave exposure appeared to be a major factor affecting species richness along the jetties. Wave energy was distinguished by making visual observations in wave amplitude, turbidity, and current. Due to the southeasterly nature of the winds in this region, sites on the south side of a jetty received more energy than sites on the north side. This has been shown by a number of studies, several occurring locally (Kaldy et al. 1995; Agan & Lehman 2001; Fikes & Lehman 2010). For this study, as wave energy increased so did the species richness, which is important for understanding spatial differences in the algal community of the jetty system. Also, benthic macroalgal communities most often exhibit spatial patchiness in species composition (Chapman & Underwood 1998), making them difficult to characterize at a microhabitat scale.

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NOTES ON HABITAT AND BURROWING BEHAVIOR OF *OBOVARIA JACKSONIANA* (BIVALVIA: UNIONIDAE) IN THE UPPER NECHES RIVER OF EAST TEXAS

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Abstract.–North American freshwater mussels are diverse and ecologically important, but are highly imperiled as a result of alterations to river habitat. The southern hickorynut (*Obovaria jacksoniana*) occurs in the southeastern United States and has recently been listed as threatened in Texas; however, minimal information exists on its habitat associations and behavior. Here we (1) describe, in detail, habitat conditions at two collection sites in the upper Neches River in east Texas and (2) present the results of a laboratory experiment on burrowing behavior in gravel and sand substrates. We collected a total of twelve live mussels at two sites in the upper Neches River. Mussels occurred in reaches with highly connected floodplain. Mussels occurred in shallow water (< 1.0 m) and substrate ranging from silt to gravel (10 mm diameter). In the burrowing experiment, horizontal movement was significantly greater in gravel compared to sand substrates. Vertical movement did not differ significantly between sand and gravel treatments. All movements occurred within the first 24 h. These results suggest that horizontal movements in southern hickorynuts can differ among substrates and horizontal and vertical movement can occur relatively quickly. Rapid horizontal and vertical movement may be important in avoiding displacement and mortality during unpredictable floods of regulated rivers. Moreover, sediment pollution has altered substrates which may influence burrowing behavior of this species.

North American freshwater mussels in the family Unionidae represent a diverse and ecologically important group (Parmalee & Bogan 1998). They often occur in dense beds where biomass may be an order of magnitude greater than all other benthic organisms (Strayer et al. 1994). Filter feeding and burrowing behavior results in a variety of water column and sediment related ecosystem functions (Vaughn et al. 2001). Unionids are also sensitive to environmental degradation, making them important indicators of ecological integrity (Parmalee & Bogan 1998). A large number of

species in the United States hold endangered or threatened status as a result of flow modification of rivers, pollution, and invasive species introductions (Lydeard et al. 2004). However, a lack of fundamental knowledge on life history, behavior and habitat associations of freshwater mussels hinders conservation efforts (Bogan 1993).

The southern hickorynut (*Obovaria jacksoniana* Frierson 1912) occurs from Alabama, west to eastern Texas and in the Mississippi River drainage north to southern Missouri (Williams et al. 2008). In Texas, historically, the species occurred in the Neches, Sabine, and Red River drainages (Howells et al. 1996). Little information exists on the ecology and behavior of the southern hickorynut. Hoggarth & Gaunt (1988) reported glochidia in specimens in October; however, fish host preference has not been established (Williams et al. 2008). Habitat association data for the species has been very general. Oesch (1995) reported that southern hickorynuts showed a preference for creeks and rivers with moderate flow and gravel substrate. Williams et al. (2008) gave similar habitat preference for the species in Alabama. The few specimens that were collected in Tennessee occurred in slow water in silt and fine gravel (Manning 1989; Kesler et al. 2001). Data on southern hickorynut behavior are absent.

In November 2009, Texas Parks and Wildlife Department listed the southern hickorynut as threatened in Texas and has proposed listing this species as legally endangered. In Tennessee, fewer than six have been recorded (Parmalee & Bogan 1998), and in both Arkansas and Alabama, it is designated of special concern (Harris et al. 1997; Garner et al. 2004). The Natural Heritage Database lists it as critically imperiled in Missouri, Tennessee and Louisiana; and imperiled in Oklahoma, Arkansas and Mississippi (mdc.mo.gov/nathis 2009). In Texas, it has rarely been recorded and recent statewide surveys have not found them (Howells, pers. comm.). A small population known in Village Creek, Hardin County (Bordelon & Harrel 2004) has subsequently disappeared

(Howells et al. 2007). Improving knowledge of life history of rare species, including the southern hickorynut, serves as a key step toward their conservation. This report includes (1) detailed habitat conditions at two collection sites in the upper Neches River, Cherokee/Anderson County, Texas, and (2) burrowing behavior in sand and gravel substrates.

Study Site

In August 2009, 12 live southern hickorynuts were collected using timed surveys in wadeable (i.e., <1m deep) reaches of the upper Neches River (Fig. 1). Tactile and visual perception were used to locate living mussels on the surface of the sediment and extending eight cm into the sediment. Six live mussels were collected 1.5 km upstream of the U.S. Highway 84 bridge (U.S. 84, Latitude 31.78763 N, Longitude 95.39433 W) and six live mussels 1.3 km downstream of U.S. 84 (Latitude 31.77128 N, Longitude 95.39632 W) between Cherokee and Anderson counties in east Texas. In addition to the living mussels, the valves of two deceased individuals were collected during the timed surveys. From GIS maps, it was determined that the river at these sites drains 3234 km^2 of primarily grazed and forested land. A wide, low lying floodplain characterizes this stream section (~20 km) and the river is not as entrenched as stream sections upstream (i.e., immediately below Lake Palestine Dam) and downstream (i.e., near State 294) but is more sinuous. Standard habitat assessment protocols were employed to assess physical/chemical conditions at the reach scale (i.e., 400m) at four equally spaced transects on 27 July 2009 (Texas Commission on Environmental Quality 2007).

All physical/chemical variables appeared comparable in both upstream and downstream reaches with the exception of temperature, turbidity and mean current velocity (Table 1). Although there was a significant difference in temperature between the two reaches, the difference of less than one degree Celsius is less than diel and seasonal variation and, likely is not biologically relevant. On a stream section scale (e.g., 5-20 km), channel width

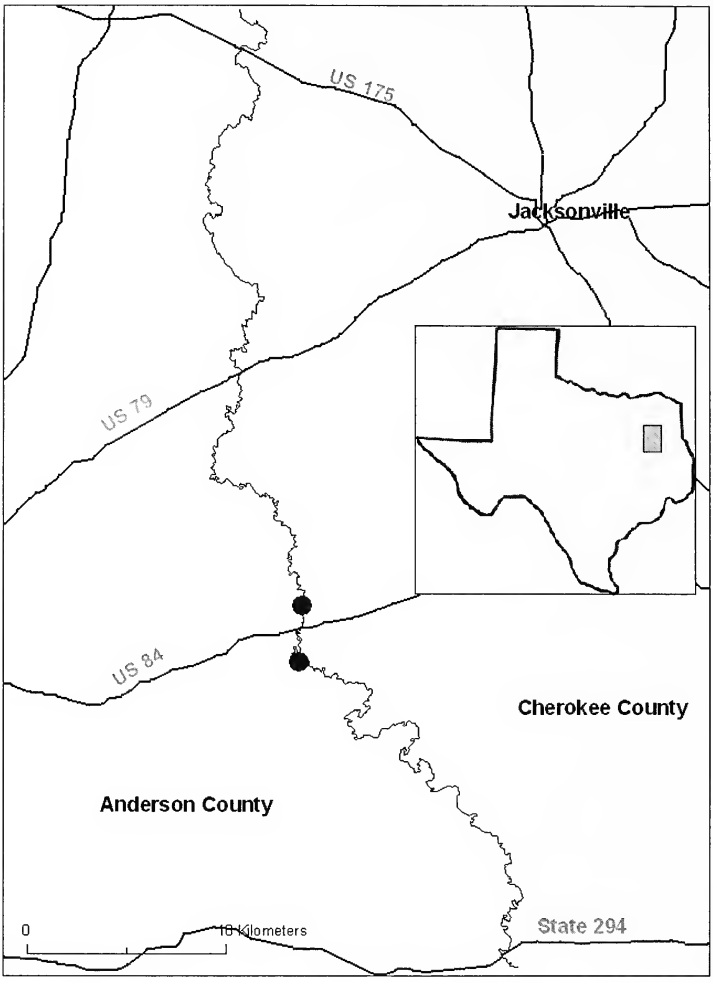

Figure 1. Map of sampling locations of two southern hickorynut (*Obovaria jacksoniana*) collection sites in the Neches River between Cherokee and Anderson counties in east Texas.

and bank angle were substantially less when compared to stream sections upstream (i.e., immediately below Lake Palestine Dam) and downstream (i.e., near State 294). The remaining physical/ chemical variables (Table 1) did not differ significantly from stream sections upstream and downstream of the two collection sites.

Table 1. Mean physical/chemical conditions (n=4) for two reaches on the upper Neches River near U.S. 84 between Anderson and Cherokee counties, Texas. Values within parentheses indicate standard error of the mean. Asterisks indicate level of significant differences between reaches (Kruskal-Wallis test, * P<0.05, ** P<0.01)

Variable	Above U.S. 84	Below U.S. 84
Temperature (°C)	28.64 (0.025)**	28.47 (<0.00)**
pH	7.72 (0.02)	7.69 (0.03)
Conductivity (mS/cm)	0.21 (0.00)	0.21 (0.00)
Turbidity (FAU)	27.55 (0.29)*	29.98 (0.63)*
Dissolved oxygen (mg/L)	6.3 (0.35)	6.25 (0.05)
Wetted channel width (m)	12.88 (0.77)	13.13 (0.31)
West bank angle (°)	3.25 (1.18)	1.25 (0.75)
East bank angle (°)	9.75 (5.09)	5 (5.00)
Mean depth (m)	0.91 (0.08)	1.13 (0.23)
Mean current velocity (m/s	0.22 (0.03)*	0.12 (0.02)*
Canopy cover (%)	28.75 (18.86)	33.75 (2.39)

Habitat Preference

On the micro-habitat scale, southern hickorynuts occurred in a variety of physical conditions. In the reach downstream of U.S. 84, southern hickorynuts occurred in areas with depths of 0.4 to 1.0 m, moderate to fast current velocities, and gravel substrates (mean particle diameter 10 mm). In the reach upstream of U.S. 84, southern hickorynuts occurred in areas with 0.4 to 1.0 m depths with slow to moderate current velocities. Fine sand and silt (mean diameter <0.13 mm) containing a substantial amount of detritus comprised mostly of twigs and broken down pieces of large wood composed the substrate in this reach. Generally, wood and detritus in or on the substrate indicate poor mussel habitat, but this stream section is highly connected to its floodplain and it is likely that this section supports these mussels because channel scouring during floods is reduced (Zigler et al. 2008; Galbraith & Vaughn 2010). These findings agree with those in Tennessee, where the southern hickorynut also occurred in silty and sandy substrate (Manning 1989). Because the specimens appeared to be both light-sensitive and tactilely sensitive, they might be able to burrow quickly in such substrate in response to rising waters as suggested for species without pimples and pustules (Watters 1994; Perles et al. 2003;

Allen & Vaughn 2009). Therefore, a laboratory behavior experiment was prepared to test their burrowing behavior.

Methods and Materials

To investigate burrowing behavior, mussels were observed in sand (mean diameter <0.5 mm) and gravel (mean diameter 8 mm) in laboratory aquaria. Eight individuals from the upper Neches River (near U.S. 84 on the Cherokee and Anderson County border) were collected on 18 August 2009, 72 hours prior to the beginning of the experiment. Prior to the experiment, mussels were housed in a 38 liter aquarium lined with sand substrate. Each mussel was placed flat and in the center of a 38 L aquarium (25 cm by 50 cm by 30 cm) lined with either 10 cm of sand (n = 4) or gravel (n = 4) for 72 h. After 72 h, mussels were switched to an aquarium with the other substrate and the experiment was repeated. Sand and gravel were obtained from a home improvement store and washed prior to the experiment. These substrates were mined from naturally occurring deposits rather than being manufactured by crushing, so surfaces were similar in shape to the substrates that occur in the Neches River. Prior to the experiment, observations of captive mussels indicated that most movement occurred within approximately one hour of being placed on the surface of a substrate. Once these initial movements occurred, minimal movement occurred over the next 24 to 72 h. Accordingly for the experiment, mussel movements were recorded every 0.25 h for the first 1.25 h and every 24 h after. Horizontal movement was measured as the distance between starting location and final location (after 72 h) using a ruler to measure distance moved on an X–Y axis. Vertical movements were measured as percent buried. Orientation (e.g., siphon up, angled posteriorly, etc.) was also noted. Kruskal-Wallis tests were used to test for significant differences in burrowing behavior among sand and gravel treatments.

Results

A significant difference in horizontal movement between mussels in gravel and sand substrates was not observed (df=1, U=43.5, P=0.224). However, horizontal movement was significantly lower in the second round of experiments compared to the first round (df=1,

U=57.5, P=0.007), indicating that horizontal movement may have been affected by the repeated experimental protocol. Consequently, the first experiment was analyzed separately. Following removal of the second round of experiments, a significant increase in horizontal movement was observed in gravel substrates (5.45 cm) relative to sand substrates (2.92 cm) (df=1, U=16.0, P=0.021). All horizontal movement occurred between 1 h and 48 h after the start of the experiment (Fig. 2a). Total horizontal movement always occurred in a single incident lasting less than 24 h with the exception of one individual in a gravel treatment that moved in two separate 24 h periods (Fig. 2a).

Percent buried was not significantly different between the first and second rounds of experiments (df=1, U=42, P=0.283), indicating that vertical movement was not influenced by this experimental design. Percent buried did not differ significantly between sand and gravel substrates with both rounds included (df=1, U=22.8, P=0.308) or with the second round removed (df=1, U=4.0, P=0.234). However, visually it did appear that mussels tended to bury themselves more thoroughly in sand compared to gravel (Fig. 2b). Within 48 h, six of the eight mussels buried themselves 100% in sand. By contrast, none of the mussels in gravel buried themselves completely (i.e., 100%).

Mussels oriented themselves differently in sand versus gravel substrates. With the exception of the two mussels that did not burrow, all mussels in sand oriented themselves with their siphon facing up. This probably occurred because they buried themselves completely (i.e., 100%). In gravel, mussels oriented their siphons upward but remained at an angle; not directly upward as was the case with the mussels in the sand.

Discussion

Understanding burrowing behavior is important because mussels may burrow to avoid displacement during high flows and associated bed movement (Watters 1994; Allen & Vaughn 2009). This behavior may be particularly important immediately downstream of dams where sudden and high magnitude flows cause bed scouring

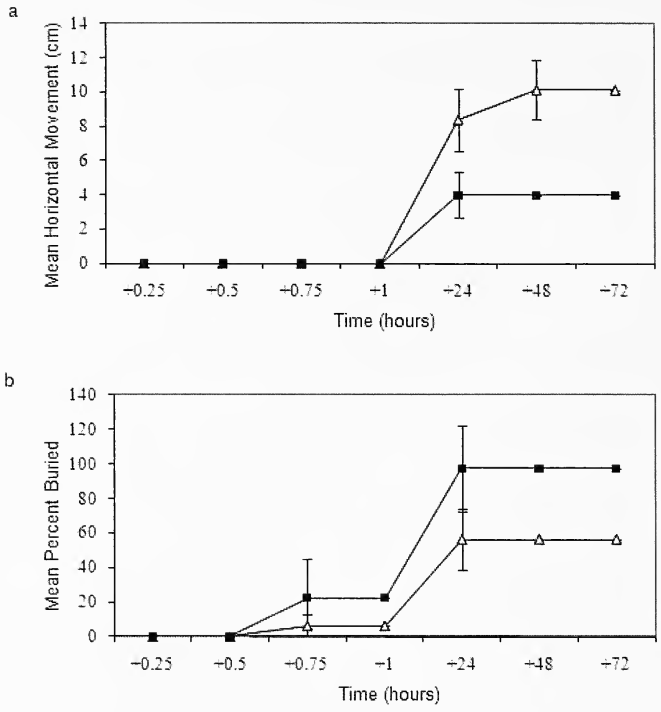

Figure 2. Mean cumulative (n=4) (a) horizontal movement and (b) vertical movement of southern hickorynuts (*Obovaria jacksoniana*) over 72 h in sand (black squares) and gravel (white triangles) substrates. Error bars represent standard error of the mean.

(Galbraith & Vaughn 2010). Thus, rapid burrowing may prevent downstream displacement. The results of this behavioral study suggest that this species of mussel is able to burrow relatively quickly. Results of this study also suggest that this species responded to movement with any disturbance such as a person touching the aquarium or even nearby movements. In the upper Neches River, 24 h or more are required to increase discharge from baseflow to bankfull flow. In this study, southern hickorynuts were able to bury themselves completely in less than 24 h, indicating that they could respond to rising water and subsequent dislodgement by burying deeper in the substrate.

Understanding variation in burrowing behavior among substrate types (e.g., sand vs. gravel) is relevant to conservation because lotic habitats are increasingly polluted with fine sediment, thus potentially altering a mussel's ability to burrow and avoid downstream displacement during high flows (Watters 1994). The results of the burrowing experiment indicate that these mussels exhibit differences in horizontal (and possibly vertical) movement between the two substrates. It cannot be concluded whether these differences are the result of a reduced ability to burrow in the gravel substrate or a behavioral decision; however, results of this study demonstrate that horizontal movement varies among substrate types and this has implications for potential displacement during high flows.

The U.S. Fish and Wildlife Service (2005) has recently established the North Neches River Wildlife Refuge upstream of the collection sites and has proposed a more extensive refuge that would encompass both collection sites. The occurrence of this state threatened species within this proposed wildlife refuge validates the suggestion of the USFWS that preservation of this river corridor is important for biological conservation.

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NUTRIA (*MYOCASTOR COYPUS*) IN BIG BEND NATIONAL PARK; A NON-NATIVE SPECIES IN DESERT WETLANDS

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Abstract.–Nutria are large, semi-aquatic rodents first introduced into the United States from South America as a fur resource during the 1890s. Nutria first were reported at Rio Grande Village, Big Bend National Park, in 1993. During 2004 and 2005, more than 30 locations of nutria activity were documented along a 16 km section of the Rio Grande River from 7.6 km upstream of Rio Grande Village to Boquillas Canyon including the Rio Grande Village beaver pond. Seventeen nutria were captured, marked, and released. Using the Schnabel and Chapman methods, 38-74 nutria were estimated to inhabit the RGV area. Stomach contents (n = 14) contained common reed (*Phragmites australis*), water pennywort (*Hydroctyle umbellata*), giant reed (*Arundo donax*), spikerush (*Eleocharis caribaea*), bermudagrass (*Cynodon dactylon*), water hyssop (*Bacopa monnieri*), foxtail (*Alopecurus sp.*), and flatsedge (*Cyperus sp.*). Seven adult nutria were radio-collared and released between May 2004 and June 2005. The mean home-range size was estimated to be 10.0 ha (14.8 ha for males, 2.9 for females), and the mean maximum daily distance moved was estimated to 637.4 m (738.3 m for males, 486 m for females).

Nutria (*Myocastor coypus*) are large hystricomorph South American rodents adapted to semi-aquatic environments (Gosling 1981). This monotypic species is native to Brazil, Bolivia, Paraguay, Uruguay, Argentina, and Chile (Carter & Leonard 2002) where it occupies wetland habitats such as ponds, streams, rivers, and marshes. Nutria occupy similar habitats throughout their range in North America, including marshes and swamps in both freshwater and brackish water communities (Borgnia et al. 2000).

Nutria were introduced or migrated into 30 states of the United States beginning in California in 1899 (Carter & Leonard 2002) and are considered an aggressively invasive species. High nutria densities are found in the south-central, southeastern, and Atlantic

coastal areas of the United States with largest populations occurring in Louisiana and Maryland (Carter & Leonard 2002). The nutria continues to expand its range in some areas of the United States and elsewhere (Bounds et al. 2001).

Nutria were introduced into rivers of arid eastern New Mexico as early as 1938 and now are year-around residents along the Pecos and Rio Grande rivers in Texas and New Mexico (Findley et al. 1975; Schmidly 2004). In Texas, nutria have spread westward to the Trans-Pecos ecological region since their entry into east Texas in the early 1940s (Dozier 1952; Swank & Petrides 1954; Evans 1983; Schmidly 1983). They first were reported from Big Bend National Park (BBNP) along the eastern park boundary in 1993 (R. Skiles, pers. comm.). Restricted riparian habitat along waterways in arid regions limits nutria population size, although colonies of these rodents are capable of living in high densities in small areas (Brown 1975).

The primary foods of nutria are emergent plant species in wetland habitats (Borgnia et al. 2000). Intensive foraging by nutria in wild areas has severely damaged wetlands (Jenkins 2002). They are an increasing concern in areas with limited wetland habitat. Desert wetlands, such as springs, seeps, and riparian corridors are fragile and small in size (Hubbs 1977; Schmidly 1977; Wauer 1977). Nutria activity in these areas could lead to irreparable damage of complex ecological communities and associated indigenous taxa such as the endangered Big Bend gambusia (*Gambusia gaigei*) (Williams et al. 1989; Reeder 2001; National Park Service 2006) and the endangered Mexican beaver (*Castor canadensis mexicanus*). Disruption of foraging sites, shade, and sheltering vegetation may place the Big Bend gambusia population at critical risk (Rio Grande Fishes Recovery Team 1984). The Rio Grande Village (RGV) beaver population maintains a beaver dam which forms the largest pond containing Big Bend gambusia (Reeder 2001). Should the presence of nutria disrupt the maintenance of the beaver dam and pond, the gambusia population

would be further jeopardized (Rio Grande Fishes Recovery Team 1984; National Park Service Water Resources Division 1992).

The objectives of this study were to: 1) determine distribution of nutria along the Rio Grande River from the gravel pit to the mouth of Boquillas Canyon, including Hot Springs, RGV, and Beaver Pond; 2) describe and quantify centers of nutria activity in the research area; 3) quantify the food habits of nutria; 4) estimate nutria population size in the study area; and 5) use radio telemetry to estimate home range size and movements of nutria living in the limited wetland habitat of the Chihuahuan Desert.

Study Site

Big Bend National Park consists of 324,219 ha located in the Trans-Pecos ecological region of Texas. This represents the largest area of protected Chihuahuan Desert habitat in the United States. The Rio Grande River is the 190 km southern border of the park and creates a productive riparian corridor that is refuge to numerous species of concern. Approximately 10,000 ha of wetlands and 315 water sources exist within BBNP, many found near or along the Rio Grande (Shaw & Finch 1996).

This research project was conducted along a 16 km stretch of the Rio Grande River from a gravel pit located 7.6 km upstream of Rio Grande Village (29°9.1′N, 103°0.2′W) to the mouth of Boquillas Canyon (29°12.2′N, 102°54.8′W), including the RGV campground (29°10.8′N, 102°57.7′W) and adjacent areas, and the Beaver Pond (29°10.7′N, 102°57.2′W) (Fig. 1).

The Beaver Pond is bisected by a boardwalk used for visitor access to hiking trails. Farms and villages are located across the Rio Grande River in Mexico. Boquillas del Carmen, Coahuila, Mexico, is the largest village in the area bordering approximately 1.5 km of the Rio Grande.

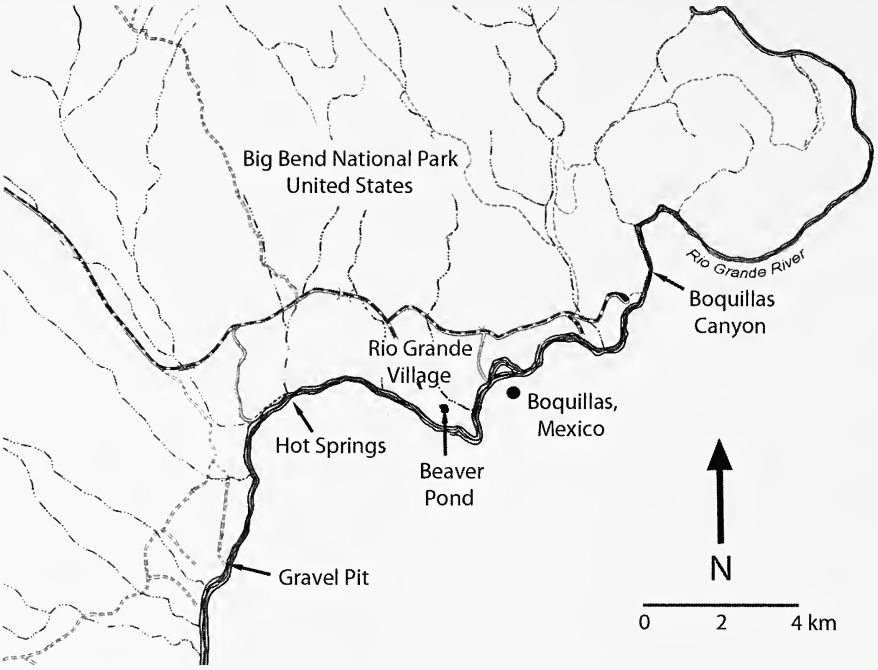

Figure 1. Big Bend National Park nutria study sites along the Rio Grande River from the gravel pit campground to Boquillas Canyon.

Dense stands of native common reed (*Phragmites australis*) and the introduced giant reed (*Arundo donax*) were found on the United States side of the river, whereas willow baccharis (*Baccharis glutinosa*), salt cedar (*Tamarix sp.*), and bermudagrass (*Cynodon dactylon*) comprised the majority of vegetation on the Mexican side. Common reed was the primary plant occupying the shoreline and interior of the Beaver Pond. Giant reed was more common closer to the river with only a few remaining stands of common cane found along the river bank. Other emergent and submerged vegetation in the Beaver pond included: cattail (*Typha latifolia*), water hyssop (*Bacopa monnieri*), spikerush (*Eleocharis caribaea*), and water pennywort (*Hydrocotyle umbellata*). Woody vegetation near the Beaver Pond included cottonwoods (*Populus acuminata*) and huisache (*Acacia smallii*). Within a short distance of the riparian zone, the vegetation was primarily a thorn-shrub desert

characterized by ocotillo (*Fouquieria splendens*), a variety of yuccas (*Yucca sp.*), chollas and cactuses (*Opuntia sp.* and *Echinocereus sp.*, respectively), cenizas (*Leucophyllum sp.*), Texas persimmon (*Diospyros texana*), agarito (*Berberis trifoliolata*), and many other xeric adapted plant species (Wauer 1992; Evans 1998).

Materials and Methods

Nutria were captured using Tomahawk live traps (90 by 33 by 30 cm, Tomahawk Live Trap Company), baited with sweet potatoes and Victor 2 ½ cushioned leg-hold traps (Texas State University IACUC #HOASJQ 02, NPS Permit # BIBE-2003-SCI-0003, and BIBE-2004-SCI-0043). Captured nutria were weighed using a spring scale and then sedated with ketamine hydrochloride at 0.25 cc/kg (Jalanka & Roeken 1990; Bó et al. 1994). Gender and standard external measurements of nutria along with field data were recorded. Age was estimated using the hind foot measurement (Adams 1956; Towns et al. 2003). Tags were attached to the hind-foot webbing (right hind-foot, male; left hind-foot, female). Commercial hair bleach and developer (Clairol Company®) was applied to the top of the nutria's head and shoulders for improved visibility during visual recaptures (Johnson 1992). Global Positioning System (GPS) data were taken at the site of capture and release. Diurnal and nocturnal surveys were conducted to assess nutria numbers and activity patterns throughout the study.

The nutria population was estimated from capture data using the Schnabel formula (Schnabel 1938) and a modified Chapman formula (Schneider 1998). These estimates were based on number of trap nights, trap success, and number of recaptures. Though the Schnabel method typically is used for closed systems, it allows for multiple trapping efforts where accumulation of captured and marked animals is allowed (Krebs 1989). The Chapman variation of the Petersen estimate also was used because it allows for population estimates to be calculated months after recapture (Schneider 1998).

Fourteen nutria were euthanized to obtain stomach samples for dietary analysis. Stomach contents were placed in 10% formalin for later laboratory analysis. Voucher material (10 skulls, TTU-M 100646 to TTU-M 100655) were deposited in the mammal collection at the Natural Science Research Laboratory, The Museum, Texas Tech University.

Stomach contents were cleaned following methods used by Towns et al. (2003). Approximately 10% of each sample was removed for analysis. Samples were cleared with 6% sodium hypochlorite according to Holechek & Valdez (1985). A ten-point frame (Chamrad & Box 1964) was used to select samples for mounting on slides for identification. Fifty slides were prepared from each stomach sample using Mount-Quick® mounting medium and 22 by 22 mm cover slip. Slides were allowed to dry for at least five hours before inspection. A National microscope (MFG# 163-ASC) was used to view each slide. Two fields of view per slide were randomly selected for comparative analysis with reference slides. Plant fragments closest to the pointer within the microscope field of view were identified. One hundred fields of view were examined for each stomach.

For identification, reference slides were prepared according to Green et al. (1985) from roots, stems, and leaves of wetland plants of the RGV area for use in identifying foods eaten by nutria. Epidermal layers from both sides of leaves were used (Korschgen 1980; Towns et al. 2003). Reference material was cleared with bleach as discussed above, mounted to slides using Permount® and a 22 by 22 mm coverslip, and allowed to dry for at least two days (Baumgartner & Martin 1939; Green et al. 1985; Litvaitis et al. 1996; Towns et al. 2003). Photographs, using a Nikon Cool Pix 995 camera mounted on the National microscope, were taken and cataloged for comparison.

Percent composition and frequency of occurrence of each plant species per stomach were calculated following Fracker & Brischle

(1944) formulae. Data were pooled and an overall percentile calculated based on 1400 fields of view.

Radio collars (Model # HLPM-3180 or Model # HLPM-3210, Wildlife Materials, Murphysboro, IL) were placed on seven nutria. Location data were gathered using a portable receiver (model TRX 100S, Wildlife Materials International, Murphysboro, IL) and Yagi antenna (Y-4FL 151-153 MHz, Televilt TVP Positioning AB, Lindesberg, Sweden). Locations of collared nutria were determined by triangulation. A Garmin GPS 12XL unit (Olathe, KS) was used to record positions. Location data were gathered as close as possible to 300 h, 900 h, 1500 h, and 2100 h each day of tracking.

A digital orthophoto using GIS software (ArcMap 8.3 ESRI®, Redlands, CA) was used to plot tracking data. Minimum convex polygons (MCP) were constructed to represent the home range for each animal (Mohr 1947). Unsuitable habitat outside wetlands was removed from each MCP using GIS software. Home range area was determined using the GIS software's field calculations function (August et al. 1996; Ostro et al. 1999). Home ranges were calculated only for individuals with >20 locations. Also, daily linear movement was calculated for each nutria.

Results

A total of 17 nutria were captured, marked, and released during 234 trap nights from March through November 2003. There were five recaptures. The Schnabel population estimate was 38 nutria and the Chapman variation suggested a population of 74 nutria in the study area.

Percent composition in the diet for each food species was 59.9% common reed, 12.7% water pennywort, 6.3% giant reed, 6.1% spikerush, 4.8% bermudagrass, 2.0% water hyssop, 0.9% foxtail (*Alopecurus sp.*), 0.7% flatsedge (*Cyperus sp.*), and 6.6% unidentified fragments (Table 1). Frequency of occurrence of food

plants among stomach samples was 100 % common reed, 92.9% bermudagrass, 85.7% giant reed, 50.0% spikerush, 50.0% water hyssop, 50% water pennywort, 28.6% foxtail, 14.3% flatsedge, and 100% unidentified fragments (Table 1).

Five adult nutria (three males and two females) were captured and fitted with radio transmitter collars between 13 May 2004 and 24 August 2004. Trapping occurred on 47 nights for a total of 530 trap nights (488 live trap nights and 42 leg-hold trap nights). Data on tracking time period for each animal, MCP home ranges, and mean and maximum daily linear movement are presented in Table 2.

Home range was calculated for five animals using an average of 27 locations per animal. Duration of tracking ranged from 14 to 53 days. The mean size of nutria home ranges in BBNP was 10.1 ha (SE = 5.55). The mean home range of male nutria was 14.8 ha (SE = 8.60) and the mean home range of females was 2.9 ha (SE = 0.67).

The mean maximum distance traveled per day by nutria near RGV was 637.4 m (SE = 177.44 m) (Table 2). The mean maximum distance traveled was 738 m (SE = 262.97) for males, and 486 m (SE = 263.04) for females. The mean distance traveled by males was 260.3 m (SE = 74.18) per day, while females traveled 179.4 m (SE = 106.46) per day. Two nutria, male N2 and female N3, rarely left the beaver pond. Their movement distances were less than nutria inhabiting riparian areas. The maximum distance traveled by nutria in the beaver pond was < 250 m.

Approximately 30 locations of nutria activity as indicated by tracks and scat were found along the 16 km study area of the Rio Grande. At nutria activity sites, the riparian zone width averaged approximately 25 m. At shoreline areas with nutria sign, vegetation was composed of bermudagrass, salt cedar, willow baccharis, and giant reed. Very little nutria sign was noted in fast moving portions

Table 1. Percent composition in the diet and frequency of occurrence of food plants in nutria stomachs from Big Bend National Park.

Species	Percent Composition ± *SE*	Frequency of Occurrence (%)
Common reed	59.9 ± 7.6	100.0
Water pennywort	12.7 ± 5.1	50.0
Giant reed	6.3 ± 1.5	85.7
Canada spikesedge	6.1 ± 3.5	50.0
Bermudagrass	4.8 ± 0.8	92.9
Water hyssop	2.0 ± 0.7	50.0
Flatsedge	0.7 ± 0.6	14.3
Foxtail	0.9 ± 0.5	28.6
Unidentified	6.6 ± 0.6	100.0

Table 2. Home range sizes and distances moved daily for nutria inhabiting the Rio Grande River near Rio Grande Village, Big Bend National Park. MCP (Minimum convex polygons).

Nutria	Days Tracked	MCP (ha)	Mean daily movement (m) ± *SD*	Maximum daily movement (m)
N1	53	30.6	362.3 ± 240.4	830
N2	42	1.0	116.0 ± 72.9	244
N3	18	2.2	72.9 ± 69.9	223
N4	14	3.6	285.8 ± 246.8	749
N5	48	12.8	302.8 ± 354.0	1141

of the river, shallow areas, or within canyons. Nutria activity typically occurred in deep, slow-moving pools with emergent shoreline vegetation and a low or moderate shoreline slope. The majority of nutria activity was recorded in or near the Beaver Pond at Rio Grande Village.

Discussion

Sample sizes in this study were low because nutria populations are limited to small and dispersed wetlands in this arid environment. After an initial capture, adult nutria avoided traps, thus affecting population estimates. Although nutria appeared abundant in the study area, population estimates may be inflated due to "trap-shy" behavior after handling and marking (Simpson & Swank 1979).

Although nutria sign was noted throughout the study area, nutria activity was greatest in riparian areas with abundant food sources and deeper waters, such as near Hot Springs, the boat ramp at RGV, Boquillas crossing, and in the Beaver Pond. Results from radio telemetry data suggest nutria move freely between the river and adjacent wetland areas.

Dietary components of nutria in BBNP generally conformed to items reported in nutria diets elsewhere. Towns et al. (2003) reported nutria eating water hyssop in the Hill Country of Texas, while Borgnia et al. (2000) found bermudagrass, spikerush, and water pennywort in diets of nutria from the Argentinean pampas. Shirley et al. (1981) and Willner et al. (1979) reported nutria feeding on spikerush, water pennywort, and common reed in Louisiana and Maryland. These plant taxa comprised 78.7% of the nutria's diet from collected individuals in RGV, with common reed contributing 59.9%. Non-native, invasive plants such as bermudagrass and giant reed also were consumed, but in small amounts.

Activity of the Mexican beaver was observed alongside that of nutria in the same regions of the Rio Grande River and the Beaver Pond. These semi-aquatic mammals have similar habitat requirements (Retzer et al. 1956; Novak 1987). Whereas nutria do not typically consume woody vegetation, beaver depend on

herbaceous vegetation in habitats with limited woody vegetation (Schmidly 2004) as found in the Beaver Pond and along the Rio Grande River. If nutria foraging activities disrupt the maintenance of the beaver dam and pond by beaver, this could jeopardize the endangered Big Bend gambusia population (Rio Grande Fishes Recovery Team 1984, National Park Service Water Resources Division 1992).

Large stands of giant reed currently exist along the Rio Grande River and adjacent wetland areas. This non-native, invasive plant may dramatically alter the riparian habitat (Bell 1993). Rivers and ponds with a high density of giant reed typically show decreased water oxygen concentrations and increased pH resulting in lower diversity in the fish community of these systems (Dunne & Leopold 1978; Chadwick & Associates 1992). The giant reed also requires large amounts of water to support its rapid growth rate (Perdue 1958; Iverson 1994).

Nutria diets in the RGV area are composed of greater amounts of common reed than the more abundant giant reed. This might be due to toxic and unpalatable chemicals in the giant reed leaves which protect the plant (Bell 1993). If this disproportionate use of common reed by nutria continues, it might hasten the replacement of common reed stands by stands of giant reed, leaving little food resources for beaver in times of stress (Strong 1982; Bell 1993). Further research is needed on Mexican beaver populations and their response to nutria.

Home range sizes varied greatly between individual nutria. However, males had larger home ranges than females. Nutria living on or near the river had larger home ranges than those living in or near the pond. Doncaster & Micol (1989) documented the home ranges of nutria as 5.68 ha for males and 2.47 ha for females in France, and the difference between genders

was significant. Denena et al. (2003) found home range size also varied by gender in Central Texas. In Mississippi, Lohmeier (1981) found male nutria to have smaller home ranges than females. This is similar to results observed for nutria that inhabited the beaver pond (male = 1.01 ha and female = 2.23 ha).

Variations in home range size might be directly associated with habitat. In limited habitats such as small ponds, home ranges are smaller, and in large marshes home ranges are larger. In BBNP, most of the habitat is a narrow riparian area bounding the Rio Grande River. This makes home ranges linear, following the river corridor. Food and adequate feeding and nesting platforms may be spread out over a greater distance causing increased movements. The cane marsh surrounding the Beaver Pond contains large amounts of food in a small area, thus producing smaller home ranges.

Movement data suggest that nutria are capable of traveling long distances along the riparian corridor. Distance traveled in one day suggests that nutria can travel significant distances up and down river. However, less suitable habitat (e.g., canyons) of several kilometers may be a significant barrier to movement.

An IPM (Integrated Pest Management) for nutria management within BBNP is essential to preserving the limited riparian wetland habitats found along the Rio Grande. The small size of the habitat may help to limit nutria densities in BBNP, however areas such as the Beaver Pond could serve as refugia for larger populations. Careful monitoring and management of nutria populations should to be implemented before irreparable damage is made to this sensitive desert wetland area. More data are needed for other locations along the Rio Grande River within the park.

Results reported in this study indicate that controlling nutria within the RGV area might be timely and imperative before their population size becomes too large to control effectively. Bertolino et al. (2005) suggest that nutria removal accelerates native species restoration. Nutria removal campaigns began in Britain (Baker & Clarke 1988) in April of 1981 because of nutria's destructive influence on native habitats. In the United States, Congress has approved, under the Coastal Wetlands Planning Protection and Restoration Act, spending $12.5 million to pay a $4 bounty in Louisiana and Maryland (Schmidly 2004). Management of this invasive species is necessary due to its potential impact to native species; specifically, the Mexican beaver, Big Bend gambusia, and the limited remaining stands of *Phragmites* within the RGV area.

A nutria management program under the Invasive Species Management Program (Clinton 1999) may be essential to preserving the Big Bend gambusia and Mexican beaver populations. Under NPS policies (National Park Service United States Department of the Interior 2006), an appropriate program includes Integrated Pest Management strategies for the nutria population, impact monitoring, establishment of thresholds for control, and a science-based control plan that accommodates local ecological conditions, best available methods, and social constraints as influenced by human use patterns.

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LONGITUDINAL DISTRIBUTION OF HEAVY METALS IN FLUVIAL SEDIMENTS OF THE TRINITY RIVER, TEXAS

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Abstract.–A survey of fluvial sediment heavy metal concentrations (HMC) within the Trinity River was conducted to determine HMC distribution patterns and elution potential. HMC peaks associated with large population centers have been observed in Japanese rivers, most often in the downstream coastal areas where cities are concentrated. It was hypothesized that similar peaks would occur in the upper third of the Trinity River Watershed due to the influence of the Dallas/Fort Worth area. Samples were collected at 22 positions along the Trinity River during December of 2005 and analyzed for cadmium (Cd), lead (Pb), chromium (Cr), arsenic (As), mercury (Hg), nickel (Ni), zinc (Zn), and copper (Cu) content. Longitudinal HMC patterns attributable to watershed geology, hydrology, and human activity were present. The Dallas / Fort Worth metroplex was associated with increases in HMC. Regardless of presence, all HMC in Trinity River sediments, except As, were below environmental regulatory values. Elution experiments indicated minimal leaching potential.

Trinity River sediments were surveyed from the rivers' headwaters to the Gulf coast for heavy metal concentrations (HMC) in order to determine distribution patterns and leaching potential. The position of the Dallas/Fort Worth metroplex (DFW) within the Trinity River Basin provided an opportunity to document the effect of anthropogenic sources upon background HMC distribution patterns.

As parent rock erodes, it may contain a source of heavy metal elements, which are transported downstream by surface flow and deposited within river sediments (Shimokawa et al. 1983). River sediment HMCs exhibit two general background patterns driven by

watershed geology and hydrology; 1) high upstream concentrations near the source descending to lower concentrations downstream from the source or 2) a homogeneous concentration throughout the watershed. Decreasing HMC over distance can be attributed to dilution through downstream movement and sediment additions from sources lacking heavy metal content. Homogeneous HMC results from a lack of heavy metal containing parent material or a steady input throughout the watershed (i.e., homogeneous geology).

Many variables affect HMC in river sediments but human activity is a major cause (de Groot et al. 1971; Maejima & Kawasaki 2006; Wakida et al. 2008). Human effect upon HMC sediment distribution patterns is well illustrated in Japanese rivers. Peaks commonly occur near the coast where population densities are highest. Several patterns have been observed which provide a base line for quantifying human activity (Tada et al. 1984). They may be used to determine historical conditions and predict potential environmental problems (Ito & Matsumoto 2008). Mining operations, manufacturing, and urban development affect river sediment HMC (Asami et al. 1981; Giusti & Taylor 2007). Sediments from the Tama River and Tsurmi rivers in Kanagawa and Tokyo prefectures, the Shonai River in Yamagata Prefecture, and the Yahagi River in Aichi Prefecture, Japan were sampled from their headwaters to the coast and analyzed for HMC. Dilution effects were observed in the Tama River for both Ni and Cd where geological deposits in the headwaters provide heavy metal input which is then diluted through downstream transport. Japan's mountainous geography forces the majority of the population to live near the coast and, as a result, most rivers experience little HMC human influence near their source and high influence near their outflow. The Yahagi River exhibits a HMC near the outflow, a result of little or no natural heavy metal sources in the upper portions of the watersheds and a large human population near the outflow (Tada et al. 1984). As a contrast, the Tama River exhibits a dilution effect followed by a peak in the lower watershed. Peaks were observed for Ni and Cd. A flat line with a peak in the lower

watershed, for NI, Cu, Zn, Cr, Pb, and Cd were seen in the Tsurumi and Shonai rivers (Tada et al. 1984). Matsumoto (2009) recently observed similar population-associated HCM peaks within Hii and Iinashi river sediments in Shimane Prefecture, Japan. Human-associated HMC peaks have also been observed in the Rhine and Ems rivers in Germany (de Groot 1971), the lower Mississippi River in the United States (Garbarino et al.1995), the Fratta-Gorzone River in Italy (Giusti & Taylor 2007), and the Tecate River in Mexico (Wakida et al. 2009).

Several studies have documented the occurrence and levels of HMC in U.S. Gulf Coast estuary and bay sediments (Windom et al. 1989; Hanson et al. 1993; Morse et al. 1993; Sharama et al. 1999a; 1999b; Santschi et al. 2001) but none have focused on HMC distribution over a river course or watershed. This report describes longitudinal HMC distribution in Trinity River sediments from its headwaters to the coast and the influence of the DFW metroplex. Based on observations of HMC distributions in Japanese rivers, it was predicted that in the Trinity River system; 1) parent materials in the headwaters would produce both high and low HMCs, 2) HMC peaks would occur near the DFW population centers, and 3) a dilution effect would be evident above and below the urban centers. Elution experiments were conducted to determine HMC leaching potential

Materials and Methods

Study area description.–As the Trinity River flows southeasterly through Texas toward its confluence with Galveston Bay and the Gulf of Mexico, the upper-stream portion is divided into four forks: the Clear, Elm, East, and West forks. The river's length is approximately 681km from the West Fork to Galveston Bay and has a drainage area of 47,606 km^2 (USSC 1962).

The Trinity River basin geological sequence progression begins

Figure 1. Map of Texas with the Trinity River watershed, major geologic divisions, and sampling locations

with older materials in the upper portion of the watershed and moves downstream towards newer material at the coast. The upper portion of the basin (Figure 1; Area I) is the source of eroded material that is deposited as sedimentary material as it is carried to the coast (Judy et al. 2004). Paleozoic formations of Wolfcampian, Virgilian, Missourian and Desmoinesian series consisting mainly of shale, sandstone, and limestone are present in the most upper portions of the basin (USSC 1962; Wermund 1996; 1999). Mesozoic groups of Trinity, Fredricsburg and L.Washita are also present (Figure 1; Area I). These parent materials provide natural sources of several heavy metals including; lead (Pb), chromium (Cr), arsenic (As), nickel (Ni), zinc (Zn), and copper (Cu). The upper middle portion (Figure 1; Area II) of the basin contains Mesozoic groups of Austin, Eagle Ford, Woodbine, Washita, Navarro and Taylor as well as Paleocene groups of Wilcox and

Midway, and Eocene groups of Claiborne and Jackson (USSC 1962; Wermund 1996; 1999). These materials consist mainly of shales and limestones and contribute only small amounts of heavy metals, relative to the materials in the upper portion of the basin. The middle and lower portions of the basin (Figure 1; Area III-V) contains Oligocene formations of Catahoula, Miocene formations of Fleming and Oakville, Pliocene formation of Willis, and Quaternary formations of Lissie and Beaumont (USSC 1962; Wermund 1996; 1999). These formations consist of chalk, marl, unconsolidated sands, deltic sands, and muds that contribute little or no natural heavy metal elements.

Field sampling.–Twenty-two (22) sampling locations were chosen beginning in the West Fork of the Trinity River and continuing along the main segment from upstream at Jacksboro to the confluence with Galveston Bay, just south of Anahuac (Figure 1 & Table 1). Sediment sampling took place between 15 and 18 December 2005.

Sediment samples were collected within the river course and analyzed for cadmium (Cd), mercury (Hg), Pb, Cr, As, Ni, Zn, and Cu concentration. A single, representative, composite sample (~500 g dry weight) was collected from each location by combining sediments from three separate points of fluvial deposition within the reach. Samples were collected between 2 and 15 cm of the sediment-water surface interface using a 7.5 diameter by 15 cm long coring tool, homogenized by hand in a plastic bucket, placed in polyethylene sample bags, labeled, and stored on ice until delivery to Blackland Research Center in Temple, Texas. Samples were dried at 105°C for 72 hours before being repackaged and sent to Japan for chemical analysis.

Temperature, pH, conductivity, and dissolved oxygen were measured concurrently with each sediment sampling event using a portable water probe (Model Quanta, HACH Co. Loveland, CO). Results are presented in Table 1.

Table 1. Sample ID number, location description, latitude and longitude coordinates, water quality parameters (pH, temperature, conductivity) and concentration of cadmium (Cd), lead (Pb), chromium (Cr), arsenic (As), mercury (Hg), nickel (Ni), zinc (Zn), and copper (Cu) from Trinity River sediments. All metal concentrations are reported as ppm. N/A: Not Available.

ID	Location description	Latitude	Longitude	pH	Temp (°C)	Cond (mS/cm)	Cd (mg/Kg)	Pb (mg/Kg)	Cr (mg/Kg)	As (mg/Kg)	Hg (mg/Kg)	Ni (mg/Kg)	Zn (mg/Kg)	Cu (mg/Kg)
1	W Trinity at Jacksboro	33.29	-98.08	7.60	13.0	0.208	< 1	34	41	18	< 0.05	30	51	18
2	W Trinity at Boyd	33.09	-97.56	7.76	16.0	0.343	< 1	6	29	1	< 0.05	6	17	8
3	W Trinity at CR 4757	33.03	-97.53	7.91	15.4	0.347	< 1	6	25	1	< 0.05	7	21	9
4	W Trinity at 10 Mi Bridge	32.86	-97.47	8.03	18.5	0.374	< 1	30	25	1	< 0.05	4	26	12
5	W Trinity at Lake Worth	32.83	-97.45	8.21	17.1	0.382	< 1	16	19	< 1	< 0.05	3	17	8
6	W Trinity at Hwy 183	32.77	-97.41	8.14	18.0	0.411	< 1	5	< 10	3	< 0.05	3	8	4
7	W Trinity at Ft Worth	32.76	-97.33	7.56	16.2	0.439	< 1	36	35	4	< 0.05	11	49	13
8	W Trinity at Ft Worth	32.75	-97.29	7.65	16.1	0.490	2	138	46	5	0.16	13	86	23
9	W Trinity at Grand Prairie	32.79	-97.03	7.61	18.4	0.723	< 1	8	22	5	< 0.05	14	25	6
10	Trinity at Dallas	32.77	-96.82	7.63	19.8	0.641	< 1	21	44	3	< 0.05	16	61	14
11	Trinity near Dallas	32.71	-96.74	7.56	19.0	0.590	1	61	44	4	0.20	17	86	20
12	Trinity at Belt Line Rd	32.62	-96.62	7.53	19.5	0.059	< 1	10	41	3	< 0.05	15	35	10
13	Trinity near Rosser	32.43	-96.46	7.77	18.5	0.563	< 1	23	43	5	0.07	18	55	14
14	Trinity at Trinidad	32.15	-96.10	7.63	18.5	0.556	< 1	12	44	2	< 0.05	16	45	12
15	Trinity near Oakwood	31.65	-95.79	7.80	17.1	0.820	< 1	9	43	3	< 0.05	10	31	6
16	Trinity near Crockett	31.34	-95.66	7.98	16.7	0.786	< 1	< 5	12	2	< 0.05	3	11	3
17	Trinity at Riverside	30.86	-95.40	7.86	20.0	0.714	< 1	9	39	3	< 0.05	11	34	10
18	Trinity near Goodrich	30.57	-94.95	8.30	19.9	0.401	< 1	< 5	10	< 1	< 0.05	< 1	6	2
19	Trinity at Romayor	30.43	-94.85	8.27	19.9	0.382	< 1	< 5	< 10	1	< 0.05	< 1	4	2
20	Trinity at Liberty	30.06	-94.82	7.85	19.7	0.392	< 1	< 5	< 10	1	< 0.05	< 1	3	2
21	Trinity at Anahuac	29.76	-94.69	8.14	23.1	19.00	< 1	8	26	2	< 0.05	5	19	5
22	Trinity at Galveston Bay	29.37	-94.78	8.01	21.2	37.70	< 1	8	< 10	2	< 0.05	2	74	4
QSVSJ: Quality Standard Value for Soil of Japan (MEGJ 2002)							150	150	250	150	15	N/A	N/A	N/A
SQL: Sediment Quality Limits for US soils (Ingersoll et al. 2000)							3.53	91.3	90	N/A	N/A	36	315	197
TRRP: Texas Risk Reduction Program - Tire 1 sediment Protection Concentration Limits (TCEQ 2006)							1,100	500	36,000	110	34	1,400	76,000	21,000

Laboratory methods.–All analyses were performed by DOWA Techno-Research co., ltd., Akita Prefecture, Japan using Japanese standard methods (MEJG 2002) analogous to United States Environmental Protection Agency (EPA) methods.

Heavy metal concentration in river sediment samples was determined using batch extractions followed by spectrometry. Dried sediment samples were sieved through a 2 mm mesh screen to remove large fractions and homogenize the sample. A 3:100 sample to solution ratio was prepared by placing a 6 gram sub-sample in a disposable polyethylene centrifuge tube along with 200 mL of extractant solution. Cadmium, Pb, As, Ni, Zn and Cu were extracted using a 1 mol HCl solution. Chromium was extracted with a 0.005 mol Na_2CO_3 / 0.01 mol HCl solution. Samples were mixed on a linear reciprocating agitator operating at 200 cycles per minute (5 cm travel distance) for 6 hours. Following extraction, samples were centrifuged at 3000 relative gravitational fields (RGF) for 20 minutes. The supernatant was removed and filtered through a 0.45 μm membrane filter before analytical quantification. Quantification of Cd, Pb, Ni, Zn, and Cu was done by flame atomic absorption spectrometry (EPA Method 7000B). Chromium was determined using colorimetric spectrophotometry (EPA Method 7196A), and As was quantified using hydrite generation atomic adsorption spectrometry (EPA Method 7061A) Mercury analysis required an additional digestion. A 0.5 gram sub-sample was added to a mixture of deionized water (1 mL), concentrated nitric and perchloric acids (1:1 mixture, 2 mL), and concentrated sulfuric acid (5 mL), and heated at 200-230°C for 30 minutes. The resulting sample was analyzed using reduction vapor atomic adsorption (EPA Method 7470A).

Potential heavy metal leaching from river sediments to the water column was determined using batch water elution experiments. Nickel, Zn, and Cu were not included in the analysis. Dried sediment samples were sieved through a 2 mm mesh screen to remove large fractions and homogenize the sample. A 1:10 sample

to water ratio was prepared by placing a 50 gram sub-sample in a 1 liter disposable polyethylene centrifuge tube along with 500 mL of de-ionized water. The solution was adjusted to pH 5.8-6.3 using 1M HCl and placed on a linear reciprocating agitator operating at 200 cycles per minute (5 cm travel distance) for 6 hours. Following elution, samples were centrifuged at 3000 RGF for 20 minutes. The supernatant was removed and filtered through a 0.45 μm membrane filter before analytical quantification. Cadmium, Pb and As elution concentrations were determined by inductively coupled plasma mass spectrometry (EPA Method 200.7). Chromium was quantified using colorimetric spectrophotometry (EPA Method 7196A), and Hg was quantified with reduction vapor atomic absorption (EPA Method 7470A).

Results

As expected, HMC peaks were observed near the DFW population centers. A dilution effect was also evident above and below DFW. Lead, Cr, As, Hg, Ni, Zn and Cu concentrations generally decreased from upper portion of the watershed, increased as the Trinity River passes through the DFW metropolitan area, and then decreased again, toward the coast (Fig. 2). Cadmium showed little or no presence in Trinity River sediments (Table 1) and was above detection limit at only two points. Lead showed lower concentrations above DFW followed by a 60% increase in mean concentration (18 to 45 ppm). Immediately below the urban area, Pb concentrations decreased as sediments moved down stream and were diluted (Table 1 & Fig. 2a). Chromium concentrations were high in the upper portion of the watershed and rapidly decreased until the DFW area, where they increased again. Mean value above DFW was 28 ppm. This increased to 34 ppm within DFW. Downstream of the DFW area, mean Cr concentration decreased to 26 ppm (Table 1 & Fig. 2b). Mean As concentrations above and within DFW were the same (4 ppm), however, at the first sampling point above DFW, the value was very high (18 ppm) and the next three points were very low ($\leq$ 1 ppm). Concentrations below DFW

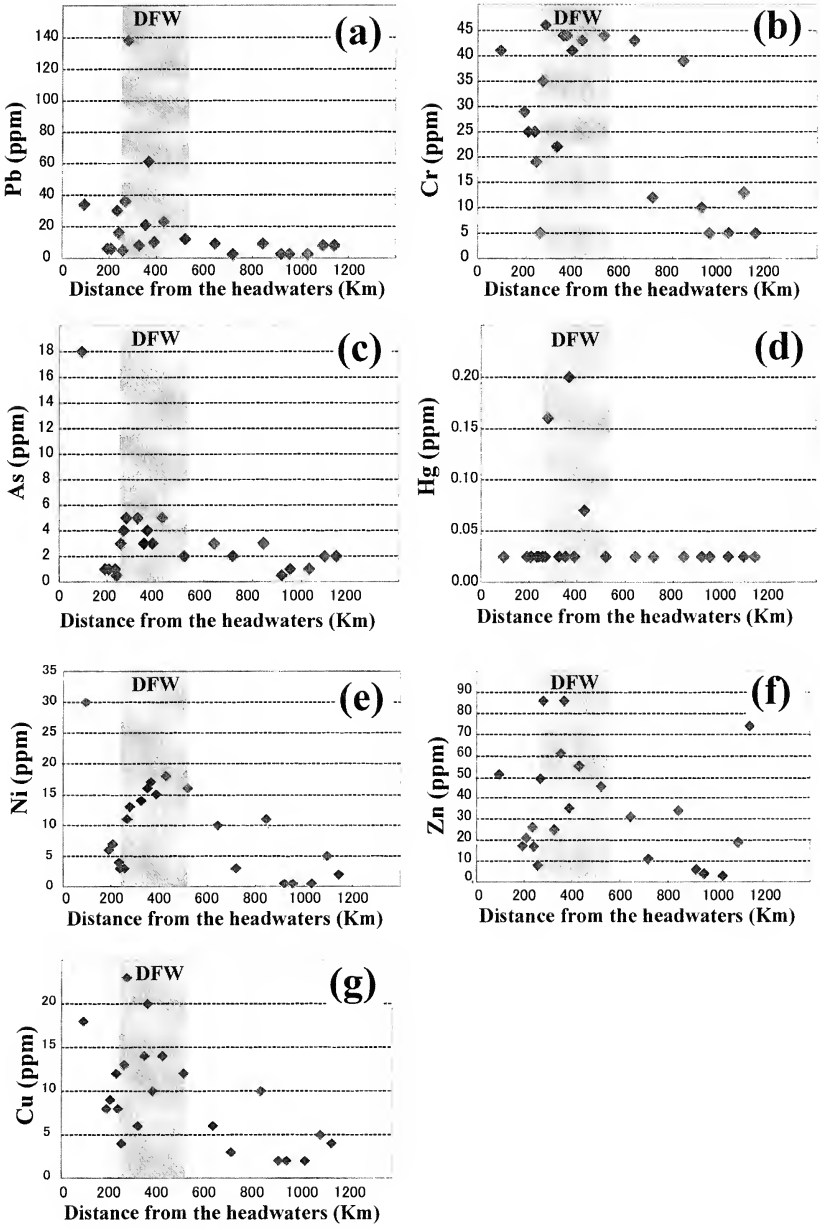

Figure 2. Relationships between distance (Km) and heavy metal sediment concentation (ppm) for Pb, As, Cr Hg, Ni, Zn, and Cu. Shading indicates location of Dallas - Fort Worth (DFW) metroplex.

showed a slight decrease with a mean of 2 ppm (Table 1 & Fig. 2c). Mercury was not detected in river sediments above DFW. Concentrations within DFW averaged 0.1 ppm. Downstream concentrations rapidly returned to below detection limits (Table 1 & Fig. 2d). Mean concentrations for Ni concentration above and within DFW were the same (12 ppm), however, above DFW, at the first sampling point, the value was very high (30 ppm) and the next four points were relatively low (≤ 7 ppm). Concentrations below DFW gradually decreased (Table 1 & Fig. 2e). Average concentrations for Zn and Cu above DFW (26 and 11 ppm, respectively) were lower than within DFW (53 and 13 ppm, respectively). Downstream dilution occurred for both metals reaching an average concentration of 29 ppm for Zn and 6 ppm for Cu. There was a large increase in Zn concentration (74 ppm) at the final sampling point (Table 1 and Figs. 2f and 2g).

All metals quantified, except As, remained bound to sediments in elution experiments. Following the 6 hour water-based elution As concentrations increased from below detection limit of <0.001 ppm and ranged from 0.001 to 0.005 ppm, well below regulatory standards.

Discussion

Naturally occurring HMC sediment sources can determined from the composition of parent material (Taylor & McLenman 1985; Condie 1993). Traversing upstream to downstream, the Trinity River exhibits HMC sediment distribution patterns which can be described as homogenous, decreasing, increasing, or both. These patterns are attributable to local geology above DFW and dilution effects below DFW. Cadmium is frequently a part of the geologic base material and can usually be detected in river sediments, but was not present in any of the samples. In Japan Cd has been documented in river sediments as results of mining operations, but recent environmental laws have reduced the problem (Asami et al. 1981; Kito et al. 1984). Relatively high values for Cr, As, Ni, and Cu were measured at the first sampling point in the Trinity River headwaters. Immediately downstream at the second sampling point, and before reaching the DFW urban area, the mean concentrations of these metals suddenly decreased by 50 to 95%

(Figs. 2b, 2c, 2e & 2g). This result is explained by the presence of a reservoir directly between sampling point one and two which effectively creates a large sediment trap. Any future sampling efforts will include the reservoir in the survey.

A number of HMC peaks occur within the DFW area including; Pb, Cr, As, Hg, Ni, Zn, and Cu (Figs. 2a-g). In Japan, previous studies have made similar observations of HMC peaks associated with human population; however, they are located in the lower portion of the watershed near the coast where population centers coincide (Tada et al. 1984; Taki et al. 2001; Matsumoto 2003; Watanabe et al. 2004; Matsumoto 2005; 2009). This shift from the lower portion of the watershed, as in Japan, to the center, in Texas, is similar to results seen in Japan. These HMC peaks can be attributed to human activities such industrial or manufacturing processes.

The rise of Zn concentration near the mouth of the Trinity River may be explained by the location's close proximity to Houston, just west of its confluence with Galveston Bay. Galveston Bay itself is home to large complexes of petrochemical refining, and other industrial activity, possible sources of Zn within the system.

All but one point in this survey were below Japanese, United States Environmental Protection Agency (EPA), and Texas standards for soil HMC. The Soil Environmental Standard Values of Japan (MEGJ 2002), EPA Sediment Quality Limits (Ingersoll et al. 2000), and the Texas Risk Reduction Program - Tire 1 Sediment Protective Concentration Levels (TCEQ 2006) are shown in Table 1. One Pb measurement exceeded the EPA's sediment quality limit by 51% but it did not exceed standards set by the Japanese Government and the State of Texas. The low concentrations of these metals present no significant threat to the Trinity River ecosystem.

Elution analysis indicated that heavy metals sorbed to Trinity River sediments were not susceptible to leaching under laboratory conditions with the exception of As, which exhibited some slight desorption. At higher pH values, such as those found within the

waters of the Trinity River, metals tend to remain bound to sediments (Zerbe et al. 1999). Standardized testing procedures dictated the extracting water solution to be adjusted to pH ~6.0, potentially increasing metal solubility and subsequent leaching. This was not observed, and while these results are surprising, it is beneficial, as this indicates that heavy metals should remain bound within Trinity River sediments due to the higher pH conditions (>7.5).

Conclusions

This study is the first to report HMC occurring in fluvial sediments from the Trinity River headwaters to the coast. As expected, HMC along the river course showed a variety of HMC patterns including; homogenous distributions, peaks, and peak dilutions, which were most pronounced downstream from the DFW metroplex. Dilution occurred above DFW but more rapidly than expected, and may be due to sedimentation within a reservoir between the first and subsequent sampling points. Also as expected, HMC peaks were present near the DFW metroplex and are probably due to industrial activity. All but one HMC were below environmental standards for both Texas and Japan. Elution analyses indicated that potential heavy metal leaching from Trinity River alluvial sediments is minimal.

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GENERAL NOTES

TWO NOTEWORTHY GEOGRAPHIC DISTRIBUTION RECORDS FOR THE WHITE SUCKER, *CATOSTOMUS COMMERSONII* (CYPRINIFORMES: CATOSTOMIDAE), FROM NORTHERN ARKANSAS

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The white sucker, *Catostomus commersonii* (Lacépède) is a slender, terete fish with heavily papillose lips and very small scales that reaches a maximum length and weight of 635 mm and 3.3 kg, respectively (McPhail & Lindsey 1970: Lee & Kucas 1980). It has a vast range in a wide variety of streams and lakes east of the Rocky Mountains including northern Canada south to the Tennessee River drainage, northern Alabama, Mississippi, and Georgia, and the Arkansas River drainage, New Mexico; it has been introduced in the Colorado River drainage, Wyoming, Colorado, and Utah (Page & Burr 2011). In Arkansas, *C. commersonii* is scarcely found along the northern boundary in the Arkansas and upper White River systems (Robison & Buchanan 1988). In addition, the Nature Conservancy (NatureServe 2009) lists populations as vulnerable (S3) in the state. To the author's knowledge, there have been no recent published noteworthy records of this fish from Arkansas since Robison & Buchanan (1988). The purpose of this note is to document two significant geographic distribution records for *C. commersonii* in the state.

On 17 October 2006 while shocking brown trout (*Salmo trutta*), a female (159 mm TL) *C. commersonii* was collected using a boat-mounted electrofisher, 200 m below the Norfork Dam, North Fork of the White River, Baxter County, Arkansas (36.14°N, 92.14°W). In addition, while collecting white crappie (*Pomoxis annularis*)

brood stock, an adult male (331 mm TL) *C. commersonii* was taken on 17 March 2009 using a boat-mounted electrofisher from Simpson Slough, 0.4 km S of the White River bridge off US 167 in Batesville, Independence County, Arkansas (35.45°N, 91.39°W). Specimens were preserved in 10% formalin and later transferred to 45% isopropanol. The specimen from Baxter County was deposited in the University of Arkansas at Fort Smith fish collection as UA-FS 1899; the specimen from Independence County was deposited in the fish collection at Henderson State University, Arkadelphia, Arkansas as HSU 3331.

In Arkansas, *C. commersonii* is an uncommon schooling fish of small streams where it prefers spring pools and spring-fed feeder creeks with sizeable amounts of aquatic vegetation and gravelly bottoms (Robison & Buchanan 1988). In adjacent Oklahoma, it is found in clear Ozark streams of the extreme northeastern part of the state (Miller & Robison 2004). The new site in Independence County (Simpson Slough) can be characterized as an old White River channel and muddy lowland slough with high turbidity that drains cleared row crop bottomlands, which is atypical of white sucker habitat.

There are seven pre-1960 records of *C. commersonii* from the state, most from the northwestern corner of the Illinois River drainage but including a disjunct record from a tributary of the Spring River to the east (Robison & Buchanan 1988) (Fig. 1). Nine additional records are available for the period 1960-1987, all from Illinois River drainage streams (Robison & Buchanan 1988) (Fig. 1). In addition, there is a record of the white sucker from the upper White River system, a small feeder stream south of Bull Shoals Reservoir (Robison & Buchanan 1988) (Fig. 1).

Robison & Buchanan (1988) suggested that the scattered populations of *C. commersonii* in the Illinois River drainage are threatened by progressive deterioration of that system's aquatic environment. However, Trauthman (1957) suggested the white

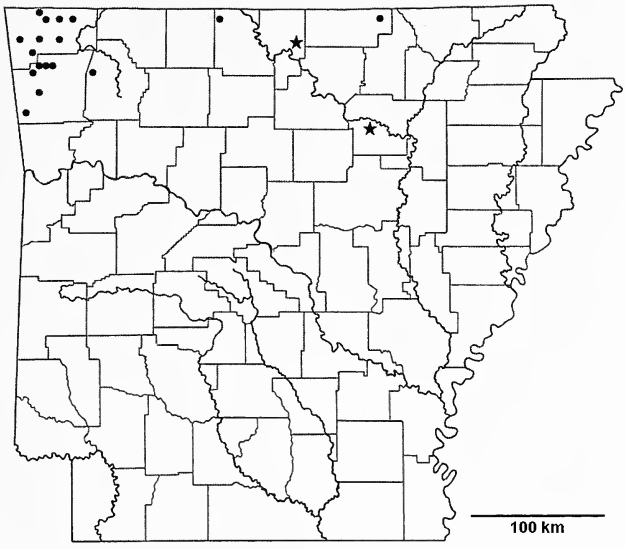

Figure 1. Previous records of the white sucker in Arkansas (closed circles); new records (stars).

sucker is tolerant of siltation and other pollutants. Interestingly, as recent as July 2007, ichthyologists from the North Carolina State Museum of Natural Sciences-Raleigh (Wayne Starnes, pers. comm.) collected several other catostomids using backpack electroshockers and conventional seining, but not any *C. commersonii* from white sucker historical sites (Benton and Washington counties). In Oklahoma, Miller & Robison (2004) noted the critical factor in maintaining populations of white suckers may be absence of appropriate spawning habitat throughout most of the state.

In summary, this study documents two significant geographic distribution records for the rarely collected white sucker, including the southernmost range extension in the state. The species has now been reported from watersheds in seven counties of Arkansas, including Baxter, Benton, Fulton, Independence, Madison, Marion, and Washington. Further studies into determining the overall distribution of this fish in the northeastern part of the state are suggested, particularly in the Black and White rivers and their tributaries using boat stream electroshocking as this species and

other suckers (McAllister et al. 2009) are not often collected by seine or shocking a riffle with backpack equipment.

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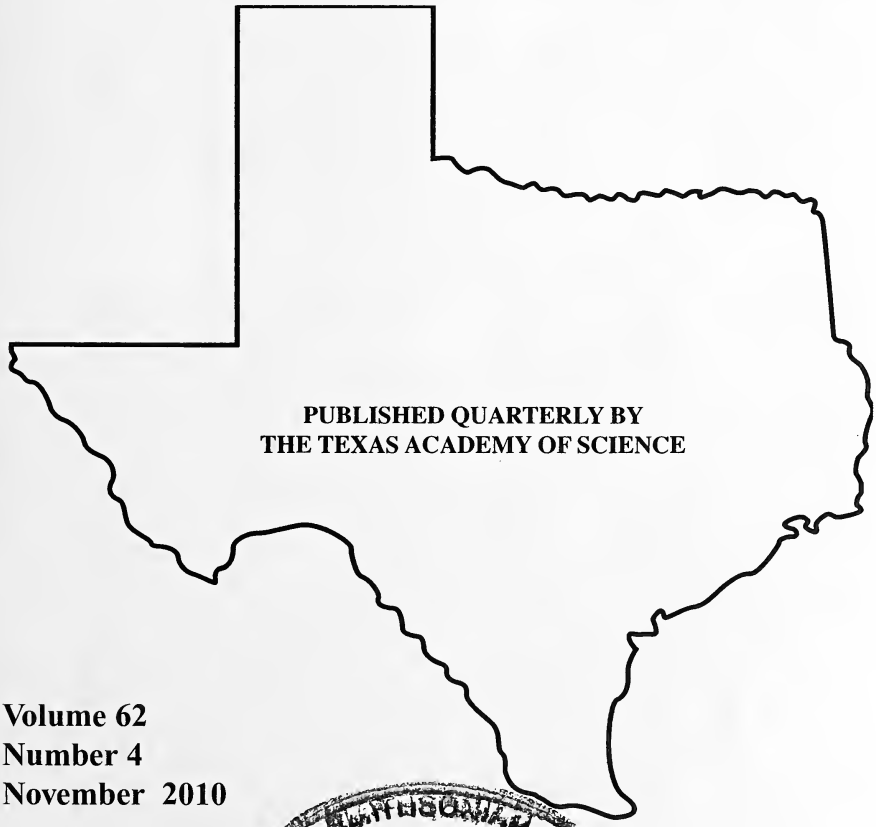

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ESTIMATING BLACK-TAILED PRAIRIE DOG (*CYNOMYS LUDOVICIANUS*) DISTRIBUTION IN TEXAS

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Abstract–In response to petitions to list the black-tailed prairie dog (BTPD, *Cynomys ludovicianus* Ord) as threatened under the Endangered Species Act, an inventory of the BTPD in Texas was undertaken. The historical and current distributions of the species were estimated and compared, current complexes were identified, vegetative systems colonized by the species were characterized, and the effect of improved aerial imagery on current population estimates was estimated. Historical records of BTPDs were found in 114 Texas counties. Remote sensing and roadside ground-truthing were used to find current colonies in 73 counties. An estimate of 3,180 colonies of BTPDs in Texas occupying 59,300 ha was developed. The mean area occupied by a colony was 21.7 ha, and the mean rate of occupancy of a colony was 77.8%. Two to six complexes of colonies >2,023 ha were found, and 40–80% of the BTPDs in Texas were found living in complexes >404 ha. Current BTPD populations were concentrated on the Great Plains Shortgrass Prairies ecosystem, but colonies were also found on four other ecosystems and three anthropogenic systems. The population of BTPDs had receded from the southern and eastern boundaries of the historical range in Texas.

In 1998, under provisions of the Endangered Species Act, the National Wildlife Federation, the Biodiversity Legal Foundation, the Predator Project, and Jon C. Sharps petitioned the U. S. Fish and Wildlife Service (USFWS) to list the black-tailed prairie dog (*Cynomys ludovicianus*; BTPD) as threatened throughout its range (USFWS 1999, Van Putten & Miller 1999). Citing the effects of plague, habitat loss, poisoning, recreational shooting, and a lack of regulations to conserve the species, the USFWS classified the BTPD as a candidate for listing (Gober 2000). While the USFWS evaluated the petition, stakeholders from nine of 11 states within the historical range of the BTPD signed an interstate agreement establishing guidelines for the management and conservation of the species (Miller & Cully 2001). They recommended

performing an inventory of the current BTPD population in each participating state (Van Pelt 1999, Luce 2003).

Methods used in the Texas inventory were developed from efforts to study BTPDs by remote sensing. Beginning more than 30 years ago, biologists used aerial photographs produced by government agencies to locate BTPDs on the landscape (Ernst 2001). Later, they used small aircraft to monitor BTPD towns and to produce new aerial imagery (Sidle 1999). Landsat satellite imagery allowed researchers to detect BTPD grazing patterns, or halos, on the landscape (Johnson et al. 2000). Digital Orthophoto Quadrangles allowed them to detect BTPD mounds and burrows, or pucks. A puck (circular) and halo (grazing) signature was ideal for remote sensing (Johnson et al. 2003).

Between 1999 and 2004, a GIS application was developed to conduct an inventory of BTPD in Texas. The objectives were to: 1) estimate the historical (pre-2000) distribution of the species in Texas, 2) estimate the current (2002-04) distribution using serial estimations and error analysis in Texas, 3) compare the historical and current distributions, and 4) identify metapopulations living in complexes >2,023 ha and complexes >404 ha.

Study Area

The study area was the High Plains and Rolling Plains Ecoregions and portions of the Edwards Plateau, Trans-Pecos, Blackland Prairies, and Cross Timbers and Prairies Ecoregions in Texas (Gould 1975, Lyndon B. Johnson School of Public Affairs 1978).

Methods

Defining prairie dog colonies.–The spatial definition of a colony was developed from diverse definitions and concepts. Two definitions of a colony were encountered. One was descriptive, defining a colony in terms of the presence of mounds, burrows,

and modified vegetation (King 1955). The other was quantitative, defining a colony as a grouping of animals having a minimum density of 25 BTPD/ha (Luce 2003). A ward within a colony was used to designate disjunct subpopulations in a colony existing close enough to one another to communicate vocally (King 1955, Hoogland 1995), and an element occurrence used to describe disjunct populations of BTPDs occurring within 1,000 m of one another (NatureServe 2006).

Estimating historical distribution.–A historical record was defined as a record of a BTPD population that was recorded before 2000, when this inventory began. Mammalogy texts, journal articles, published and unpublished inventories, personal communications, and theses were used to accumulate historical records from 114 Texas counties (Bailey 1905; Hall & Kelson 1959; Cottam & Caroline 1965; U. S. Department of Agriculture Soil Conservation Service 1973; Pizzimenti 1975; Cheatheam 1977; Schmidly 1977; Flores 1985; Normand 1993; Davis & Schmidly 1994; Ernst 2001; Schmidly 2002; J. Wood pers. comm.). An exhaustive search of specimen collections, historical writings, and government records of BTPD poisoning programs was also considered, but those sources proved beyond the scope of the inventory.

Historical records of BTPDs in Tarrant, Smith, Fayette, and Bexar Counties were classified as relocations (Cottam & Caroline 1965: Fig. 3) and were not used in this study. Existing range maps for BTPDs in Texas were examined (Bailey 1905; Hall & Kelson 1959; Cheatheam 1977; Schmidly 1977; Davis & Schmidly 1994; Schmidly 2002). Maps delineating the North American range of the species were not utilized because of imprecision. Historical records in Bell and Lamar counties were treated as outliers rather than as part of the contiguous range (Fig. 3).

Estimating current distribution.–Current distribution was defined as the BTPD population in Texas between 2002 and 2004,

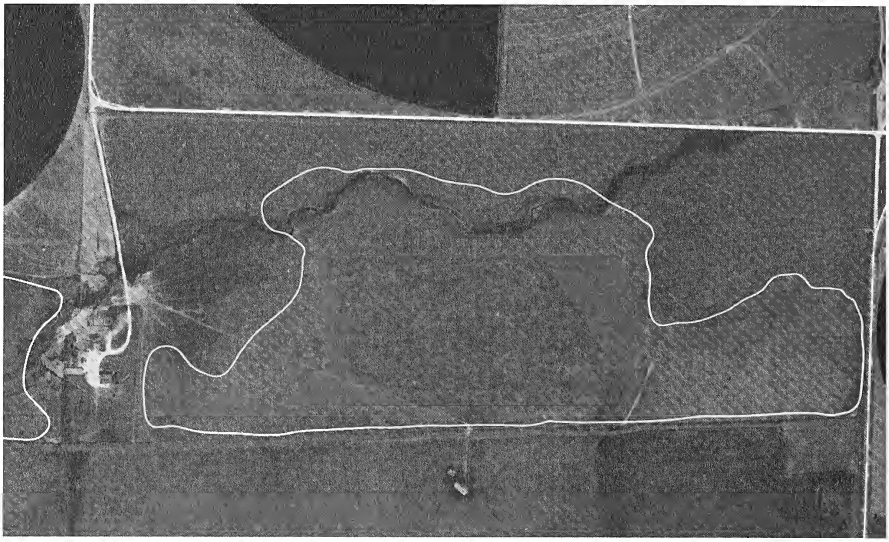

Fig. 1. The open polygons represent the prairie dog edit layer ground-truthed in the field using public roads (white lines).

when the data was ground-truthed (directly verified remotely sensed information). DOQs from 93 counties were remotely sensed to build that distribution. The DOQs came from aerial photography taken between 1994 and 1997. Leica Systems' ERDAS Imagine 8.x was used to search DOQs for BTPD puck and halo signatures and to digitize polygons around the signatures at a scale of 1:5000 with the county as the organizational unit.

After digitization, the set of polygons was saved as the raw layer for the county. Before ground-truthing, the raw layer atop of the DOQs was re-examined, and polygons representing the lowest probability signature were removed. The result was designated the edit layer (Fig. 1).

Using ESRI's ArcGIS 8.x, field maps were created for ground-truthing. The maps showed the edit layer (Fig. 1) and the roads atop a DOQ. Most ground-truthing was performed from roadsides, collecting data at sites with BTPDs and at sites with abandoned mounds. The ground-truth data was improved by

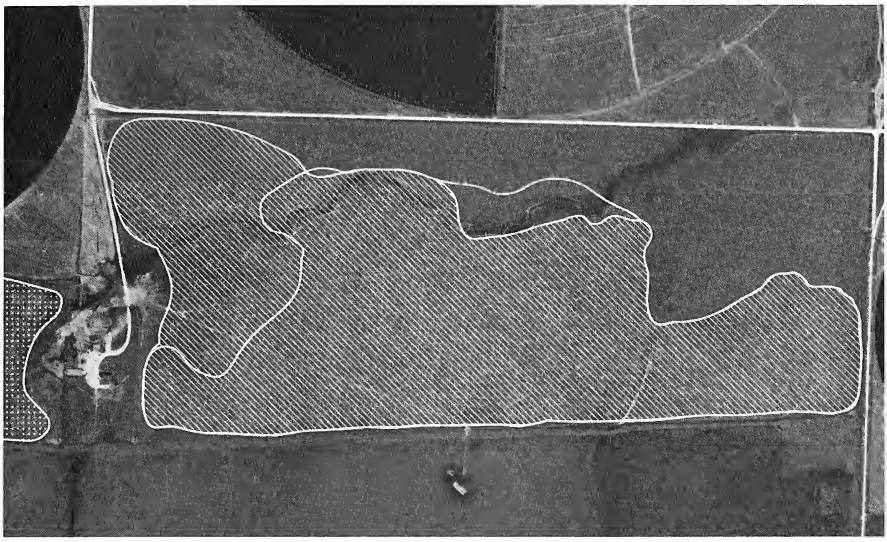

Fig. 2. The NA (not accessed) prairie dog colony polygon on the left is represented with stipple. The prairie dog colony polygon on the right with the diagonal hatch polygon represents the truthed layer with expansion to the northwest. The open polygons represent inactive areas within the truth layer in the northeast corner.

roadside searches between remotely sensed sites and via access to sites granted by landowners. Local expertise from biologists and residents was also considered. A Global Positioning System (GPS) point was taken at each site using either a Trimble or a Garmin GPS unit, and the date, presence or absence of BTPDs, and vegetation associated (visual dominance) with the site was recorded. The extent of edit layer (Fig. 1) polygons on the field map was compared to the extent of occupied areas on the landscape, and boundaries on the field maps were adjusted to match the actual colony on the landscape.

The truth layer (Fig. 2) was created by incorporating boundary adjustments made on field maps as a result of ground-truthing. County layers were merged into statewide edit and truth layers, and overlapping polygons along county boundaries were reconciled. The edit layer (Fig. 1) atop the DOQs was reviewed a final time, and polygons representing classes of signature variants that had not yielded BTPDs were removed.

The edit layer was split into two layers. The first, the calculation layer, contained edit layer polygons accessed during ground-truthing. Polygons in the calculation layer represented inactive sites with no field evidence of BTPDs, inactive sites with abandoned mounds, and active sites without boundary adjustments noted on the field maps. The second layer, the NA (not accessed) layer (Fig. 2), contained edit layer polygons not accessed during ground-truthing.

The current population of BTPDs in Texas was estimated using the following formula:

$$\text{best estimate} = \text{truth} + [(\text{truth} / \text{calculation}) \times (\text{NA})],$$

where truth, calculation, and NA represented the total area of polygons in the corresponding layers. The minimum aerial estimate was defined as the area of the truth layer, and the maximum aerial estimate was the sum of the areas of the truth and NA layers.

Polygons within 200 m of one another were defined as belonging to the same colony, using the formula above, where truth, calculation, and NA represented the total numbers of colonies in the corresponding layers. The minimum estimate was the number of colonies in the truth layer, and the maximum estimate was the sum of the number of colonies in the truth and NA layers.

The occupied areas of ground-truthed polygons were removed from corresponding edit-layer polygons to create a layer representing unoccupied areas of ground-truthed colonies adjacent to occupied areas. If a colony on the landscape had shifted outside of the edit-layer polygon, the polygon was re-examined atop the corresponding DOQ to determine whether the additional area should have been included in the edit layer (i.e. interpretation error). The edit layer was adjusted as needed, using the improving knowledge of BTPD signature variants as a guide. The resulting

layer was used to estimate the mean rate of occupancy of colonies. The best and maximum aerial estimates were adjusted to reflect occupancy.

Colonies in the northern High Plains suffered an outbreak of sylvatic plague in 2003 and had lost an estimated 1,050 ha of BTPDs by May 2004. This loss was accounted for in the estimates, but no assumptions were made about losses beyond May.

Comparing historical and current distributions.–A layer of points was created from historical BTPD records, digitizing versions of historical range maps of the BTPD in Texas (Bailey 1905; Cottam & Caroline 1965; Cheatheam 1977) and merging the versions into one polygon. This historical range polygon encompassed all but two historical records, which were classified as outliers. Additionally, a description of the historical Rolling Plains megatown between Clarendon and San Angelo (Merriam 1902) was translated into a digital approximation of the town perimeter. This description was compared to the current population within this perimeter estimated in this study.

The current truth layer was converted into a layer of points representing the geographic centers, or centroids, of current BTPD colonies. This layer of points was translated into a polygon representing the current range of the BTPD in Texas, and the current point and polygon layers were compared to the historical layers.

Evaluating BTPD complexes.–Following Luce (2003), a complex of BTPDs was defined to be a group of disjunct colonies with perimeters $\leq$7 km apart. The truth layer was buffered by 3.5 km to create a minimum version of BTPD complexes, and the area of occupied colonies contained was calculated within each complex. The truth and NA layers were jointly buffered by 3.5 km to create a maximum version of BTPD complexes, and the

area of occupied colonies contained was calculated within each complex. Following recommendations of the multi-state BTPD management plan (Luce 2003), the number of BTPD complexes >2,023 ha and the percentage of the current population occupying complexes >404 ha were estimated.

Characterizing vegetation.–Definitions of vegetative alliances and associations (NatureServe 2006) were defined to characterize the ecosystems colonized by the BTPD in Texas. Ecosystems included the Great Plains Shortgrass Prairies Ecosystem, the Great Plains Playa Lakes Ecosystem, the Southern Great Plains Mesquite Woodlands and Shrublands Ecosystem, the Southern Great Plains Deep Sand Shrublands Ecosystem, and the Great Plains Mixed Grass Prairies Ecosystem (NatureServe 2006). The following three anthropogenic systems were included: croplands, old fields, and conservation reserve program fields.

RESULTS

Estimating historical distribution.–Historical records of BTPDs were found in 114 Texas counties, four of which were classified as relocations (Fig. 3). Records were widely distributed throughout the High Plains, Rolling Plains, Edwards Plateau, and Trans-Pecos Ecoregions. Historical records also reached into the western Cross Timbers and Prairies Ecoregion. One outlying record was found in the northern Blackland Prairies Ecoregion.

Estimating current distribution.–Between 2000 and 2004, occupied BTPD colonies in 73 Texas counties were ground-truthed, and reports of occupied colonies in Reeves, Irion, and Tarrant Counties were received (Fig. 4). The edit layer was created from 6,408 digitized polygons around remotely sensed BTPD signatures; 56.7% (n = 3,632 of 6,408) of the polygons were ground-truthed to create the truth layer.

Polygons within 200 m of one another were defined as

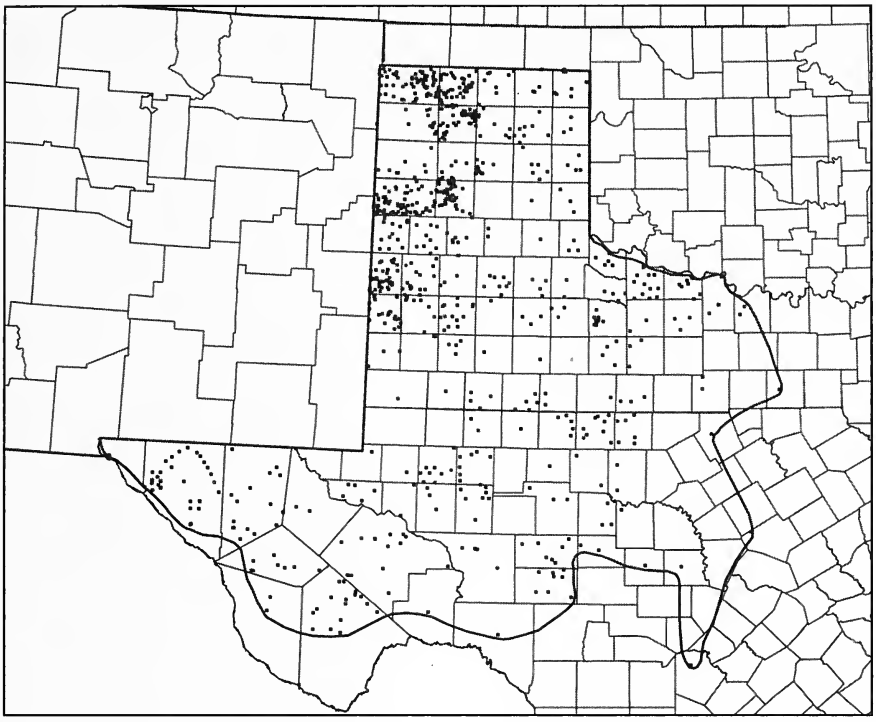

Fig. 3. Estimated historical (pre-2000) distribution of the black-tailed prairie dog in Texas.

belonging to the same colony, and 58.5% (n = 2,695 of 4,608) of the remotely sensed colony signatures were ground-truthed, finding 62.2% (n = 1,676 of 2,695) to represent occupied colonies. Between 1,676 - 3,590 colonies in Texas were found, with an estimate of 2,870 colonies. Of the total area of signatures remotely sensed, 62.7% (50,300 ha) were ground-truthed, with 72.4% (36,400 ha) occupied. Between 36,400–66,300 ha of occupied areas in colonies were found, with an estimate of 58,100 ha (Table 1, Raw Data).

Occupied portions of ground-truthed colonies varied from 0.03-1,420 ha with a mean occupied area of 21.7 ha. Seventeen colonies (1.34%, n = 1,676) that were >200 ha were ground-truthed, 43 colonies (2.68%) from 100-200 ha, 98 colonies (6.57%) from 50-100 ha, 550 colonies (35.2%) from 10-50 ha, 859 colonies (48.6%) from 1-10 ha, and 109 colonies (5.63%) <1 ha.

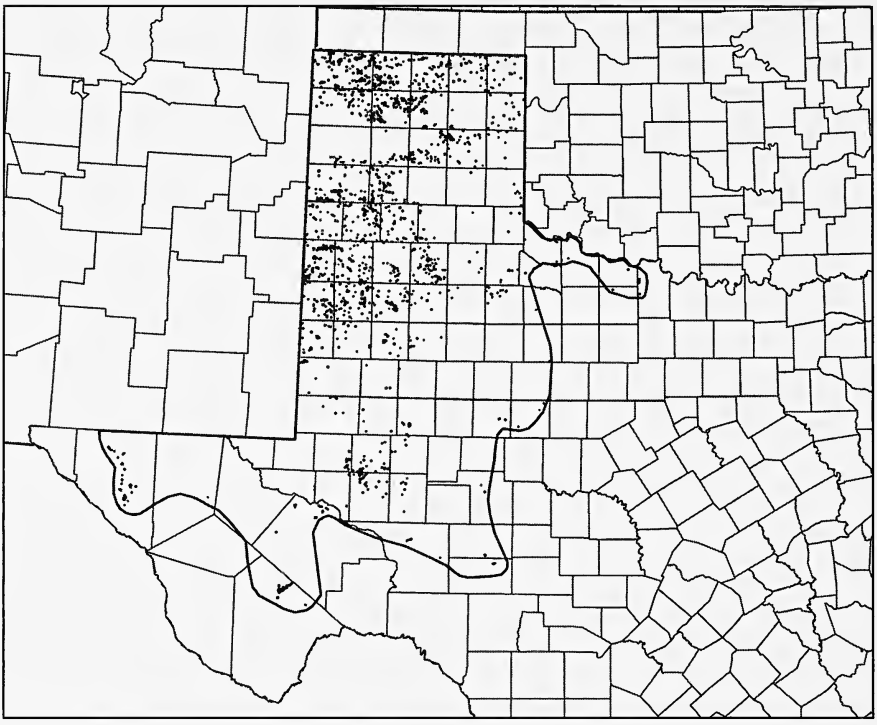

Fig. 4. Estimated current (2002-04) distribution of the black-tailed prairie dog in Texas.

The mean rate of occupancy of ground-truthed colonies was 77.8%, so the maximum and best aerial estimates were adjusted to assume 22.2% of NA layer colonies were unoccupied. With the adjustment, the maximum aerial estimate became 59,700 ha and the best aerial estimate became 53,300 ha (Table 1, Adjusted for Occupancy).

Comparing historical and current distributions.–Of the historical records in the High Plains Ecoregion, 59.7% were found, whereas 84.4% of the current colonies were in the High Plains. For both historical and current distributions, colonies were found to be more densely distributed in the northern and central High Plains than in the southern High Plains (Fig. 3 and Fig. 4).

Throughout the Rolling Plains Ecoregion, 18.3% of the historical records were found, whereas 7.66% of current colonies

Table 1. Summary of black-tailed prairie dog population estimates. Data adjusted for occupancy assume inaccessible colonies were 77.8% occupied. Data adjusted for imagery assume 10.8% more colonies and 11.3% more area.

Prairie dog population estimates		
	Colonies	Area (ha)
Raw data		
min.	1676	36,400
best	2870	58,100
max.	3590	66,300
Adjusted for occupancy		
min.	1676	36,400
best	2870	53,300
max.	3590	59,700
Adjusted for imagery		
min.	1860	40,500
best	3180	59,300
max.	3980	66,400

were found scattered throughout the Rolling Plains. Most current colonies were found in the northern quarter of the ecoregion. Both historical records and current colonies were virtually absent from the Canadian Breaks (Fig. 3 and Fig. 4). Within the perimeter of the historical Rolling Plains megatown between Clarendon and San Angelo, 183 widely scattered colonies totaling 1,840 ha were located, whereas early researchers reported the megatown as covering nearly 6.5 million ha between the two Texas towns (Merriam 1902; Bailey 1905).

Throughout the northern half of the Edwards Plateau Ecoregion, 6.82% of historical records were found. Historical records were much more sparse on the eastern plateau. Scattered throughout the northwestern Edwards Plateau 3.43% of current colonies were found, but they were concentrated near where the plateau meets the High Plains. Both historical records and current colonies were absent from the Balcones Canyonlands (Fig. 3 and Fig. 4).

Throughout the Trans-Pecos Ecoregion, 13.5% of historical

records were found and were sparse in the eastern Trans-Pecos. Only 3.22% of current colonies were found in scattered clusters in the ecoregion (see Discussion: Estimating current distribution). Both historical records and current colonies were absent from Big Bend National Park (Fig. 3 and Fig. 4).

Scattered throughout the northwestern quarter of the Cross Timbers and Prairies Ecoregion, 1.69% of historical records were found. Historical records were most dense in the area between the Red River and the Rolling Plains. One historical outlier was found near the border of Bell and Coryell counties (Normand 1993). In the Cross Timbers and Prairies, 1.29% of current colonies were concentrated near the northernmost border with the Rolling Plains. A current report of a colony was received in Tarrant County; the colony might be the descendant of relocation (Fig. 3 and Fig. 4).

One historical outlier in the Blackland Prairies Ecoregion (Flores 1985) was found, and no reports of current colonies were received in the ecoregion (Fig. 3 and Fig. 4). For this reason, no remote sensing work was performed in the region.

Based on these results, the range of the BTPD in Texas had receded from the southern and eastern historical boundaries and from the western historical boundary in the Trans-Pecos (Fig. 3 and Fig. 4). The distribution of the BTPD had declined more in the Rolling Plains Ecoregion than elsewhere, and the distribution was more stable in the High Plains than in other regions.

Evaluating BTPD complexes.–Depending on the data layers used, 2–6 BTPD complexes >2,023 ha in Texas were found. Buffering only the truth layer, only two complexes >2,023 ha were found, occurring in the northern High Plains. The largest of these contained >10,000 ha of occupied colonies. When the truth and NA layers were jointly buffered, six complexes >2,023 ha were found, occurring on the High Plains (n = 4), on the western edge

of the Edwards Plateau (n = 1), and in the Trans-Pecos (n = 1). The largest complex from the jointly buffered layers contained >20,000 ha of occupied colonies. Of the BTPDs in Texas, 40-80% were living in complexes >404 ha. The 2004 NAIP imagery showed 10.8% more colony signatures and 11.3% more area of colony signatures than on the 1994-97 DOQs. Adjusting colony estimates to reflect improvement in imagery, between 1,860–3,980 colonies in Texas were found with a best estimate of 3,180 colonies. After adjustments, 40,500–66,400 ha of BTPD colonies were found with a best estimate of 59,300 ha (Table 1).

Discussion

Estimating historical distribution.–BTPD advocates have used an historical baseline of 800 million animals to compare to the current population of BTPDs in Texas. Bailey (1905) calculated the number by doubling Merriam's (1902) estimation of animals in the megatown between Clarendon and San Angelo. The megatown reportedly cut a continuous, 161-km-wide swath between the two Texas towns. Merriam used a mean density of 62 BTPD/ha to estimate the number of animals in the 64,750 km^2 megatown. Merriam's mean density figure was high relative to the observations in this study of the counties encompassing the megatown. King (1955) reported mean densities >21 BTPD/ha from three years of measurements and an anecdotal density >86 BTPD/ha.

The assumption that BTPDs occupied the entire 64,750 km^2 of the megatown is probably incorrect for three reasons. First, BTPD colonies shift on the landscape when the animals exhaust food resources in an area and then move towards fresh vegetation (King 1955, Hoogland 1995). Second, though BTPDs will dig test holes in many types of soil, they normally abandon holes in rocky soils to colonize tight, clayey soils (King 1955, Buseck et al. 2005). Third, BTPDs avoid colonizing slopes >10% (Buseck et al. 2005) and prefer slopes of 2–5% with well-drained soil (Vermeire et al. 2004). Sloping drainages, rivers basins, and rocky outcrops

interrupt grasslands within the perimeter of the Rolling Plains megatown, all features which are not normally suitable for BTPD colonization. Historical observers were probably not describing a continuous town but were reporting that they were rarely away from the sight and sound of BTPDs between Clarendon and San Angelo.

Even if the assumptions made to estimate an historical population of 800 million BTPDs in Texas were correct, the population might have been increasing at the time for both climatic and anthropogenic reasons. Because the assumptions and conditions integral to the historical estimate were not justifiable by the authors of this study, the historical estimate was not used as a baseline to which to compare the current Texas population.

The historical distribution in this study was conservative for three reasons. First, although the Tarrant, Smith, Fayette, and Bexar county historical records were classified as relocations, the Tarrant and Bexar county records might have been classified as natural occurrences south and east of the historical range. Second, classifying the Bell and Lamar county historical records as outliers, rather than as part of the contiguous range, confined the historical range to areas where clusters of historical records were found. The methodology in this study for accumulating BTPD records was not exhaustive, so clusters of records may exist in areas not included in the historical distribution. Third, the degree to which the BTPD occupied mixed–grass prairies remains unresolved. Mixed–grass prairies existed throughout the Cross Timbers and Prairies and Blackland Prairies ecoregions, but some researchers believe that the prairies were suitable for the BTPDs only after disturbance reduced the height of the vegetation (Vermeire et al. 2004). Droughts in the last quarter of the 19th century (Bailey 1905; Haley 1953), coupled with increased grazing pressure and predator control resulting from settlement, may have facilitated the expansion of the BTPD into formerly marginal habitats (Bailey 1905; Haley 1953; Vermeire et al.

2004). A BTPD reintroduction attempt in Callahan County may corroborate this hypothesis. Reintroduction failed twice during years of average and above average rainfall only to succeed after a drought had reduced the height of the vegetation on the site. The landowner and project manager posited reduced cover for predators as the reason for eventual success (J. Wood, U. S. Department of Agriculture, pers. comm.).

Classifying the Bell and Lamar county historical records as part of the contiguous range, classifying the Tarrant and Bexar county records as natural occurrences, and assuming that undisturbed mixed–grass prairies were more than marginal habitat for the BTPD could have placed the species in >130 Texas counties.

Estimating current distribution.–Equating colonies with polygons overstated the number of colonies, since disjunct populations of BTPDs were found separated by a road, a draw, or unoccupied mounds. An extreme example occurred where five small patches of occupied mounds were found remaining within a poisoned colony. Clearly, the five polygons did not represent five separate colonies but rather the disjunct remnants of a single colony.

In defining polygons within 200 m of one other to be part of the same colony, the average maximum distance at which we could hear a BTPD alarm call was estimated. The assumption was that disjunct populations derived colonial benefits from each other when alarm calls were audible among populations, with BTPDs hearing an alarm call at least as well as human beings. A generic 200 m buffer, however, mistakenly implied a BTPD might hear an alarm call from one mesquite opening to another as well as across open grasslands.

In the search for a definition of a BTPD colony, the historical megatown between Clarendon and San Angelo was considered. The historical perimeter of the town encompassed 183 current

colonies by the definition. The colonies might be classified either as subpopulations of a fragmented historical colony or as individual current colonies. For this reason, a colony might require definition on the landscape on an individual basis. Although a mean rate of occupancy of a colony of 77.8% was found, occupancy rates >90% were observed on shortgrass prairies in the High Plains and <20% in desert habitats in the Trans-Pecos.

The current estimates in this study were conservative for two reasons. First, the entire potential historical range of the BTPD in Texas was not remotely sensed. The Trans-Pecos ecoregion contained vast tracts of land that were out of sight of roadsides, creating more uncertainty about BTPD populations there than in other ecoregions. Reported colonies and historical records were utilized more than direct remote sensing work in the Trans-Pecos more so than in other ecoregions because the arid Trans-Pecos habitat resulted in high reflectance in the DOQs. The reflectance made large areas of the Trans-Pecos look like BTPD signature variants. In this ecosystem, 60% of the historical range of the BTPD was remotely sensed, and 2,300 ha of occupied colonies were ground-truthed. The colonies might represent only a fraction of the population in the Trans-Pecos. Another area included the southernmost and easternmost portions of the historical range. Less remote sensing work was performed there because fewer recent reports of BTPDs were received in those areas. Isolated colonies not represented in the data might exist in the area and would affect the current range. The potential colonies would not significantly affect aerial and colonial estimates.

The second reason the current estimates of this study were conservative was that the methodology contained two procedures that restricted the magnitude of the estimates. The first procedure occurred before ground-truthing, when polygons from the raw layer representing the lowest probability signature variants were removed. The second procedure occurred after ground-truthing, when polygons from the edit layer representing classes of

signature variants that had not yielded BTPDs were removed. The examination of the results on top of 2004 NAIP imagery confirmed that some polygons removed would have remained in the dataset had the NAIP imagery been used instead of the 1994-97 DOQs. An underlying assumption was that BTPD colonies are relatively stable over a 3-5 year period. This assumption was supported by post-inventory observations from 2005-2007.

General conclusions about trends in the Texas BTPD population were drawn, but historical data could not be precisely compared to current data for three reasons. First, no well-defined study area from a previous inventory was found to compare to this study area. Second, no well-defined historical data was found to compare to the current population data in this study. Third, no well-defined methods from previous studies were found to validate comparisons to estimates generated by methods in this study (see Bailey 1905, Cottam and Caroline 1965, U. S. Department of Agriculture Soil Conservation Service 1973, and Cheatheam 1977).

Estimating effects of imagery.–Some BTPD signatures appearing as variants on 1994-97 DOQs appeared as puck and halo signatures on 2004 NAIP imagery. Similarly, signatures not interpretable on DOQs were interpretable on NAIP imagery. This was true even though the DOQs were in *.tif* and *.img* formats and NAIP imagery was in compressed *.sid* format. Future researchers will need to be aware of the formats and technical specifications of future imagery used to generate comparisons to the population data from this study. Resolution and type of color used will be especially relevant, since NAIP imagery subsequent to 2004 has varied from 1-2 m resolution and has varied from false color with infrared to true color.

Management implications.–The diverse emotional responses triggered by the BTPD will continue to be a factor in management efforts affecting the species, but with more landowners managing lands for wildlife, a BTPD recovery program may generate more

interest. The dependence of the black-footed ferret on BTPD metapopulations may increase interest in recovery programs for both species on lands located within complexes >2,023 ha.

In areas with frequent sylvatic plague outbreaks, fragmentation of BTPD complexes may benefit the BTPD, since plague is more devastating in areas with dense concentrations of BTPDs than in areas with isolated colonies (Luce 2003). Since the 2003 outbreak in the northern High Plains occurred in the largest BTPD complex in Texas, black-footed ferret recovery programs there may be affected. The threat posed by plague justifies the goal in BTPD management plans (Luce 2003, Texas BTPD Working Group 2005) to maintain colonies in 75% of the historical range of the species. Distance from an epidemic is the only defense against plague (Luce 2003), so colonies far from an epidemic represent potential recovery populations.

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HYDROBALLOCHORY IN TWO TEXAS SPECIES OF SKULLCAP (*SCUTELLARIA DRUMMODII*, *S. WRIGHTII*; LAMIACEAE)

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Abstract.–*Scutellaria drummondii* var. *edwardsiana* (Drummond's skullcap) and *S. wrightii* (Wright's skullcap) use hydroballochory to disperse nutlets following rains. The nutlets form in a fruiting calyx called a scutellum that expands in size and changes in color from green to yellow and finally brown. The scutellum has a cup-shaped upper portion and a scale-like lower portion. When a drop of rain hits the cup-shaped top of a yellow or brown scutellum, it dehisces and falls off the plant and the resulting mechanical energy from this event causes the scales to throw the nutlets away from the plant. Based on field and greenhouse experiments scutella were observed to disperse following precipitation events.

Although widespread among fungi, lichens, and bryophytes, fruit and/or seed dispersal mechanisms operated by rain (ombrohydrochory) are not common among angiosperms as a whole (Brodie 1952; Van der Pijl 1982; Pizo & Morellato 2002) but have been found in several angiosperm genera (Parolin 2006). Two general mechanisms are involved in fruit and/or seed dispersal by rains: the splash-cup or rain ballists (hydroballochory, Brodie 1951; Pizo & Morelanto 2002; Parolin 2006) and springboard or catapult (Brodie 1955). In splash-cup seed dispersal, raindrops are caught by cup-shaped capsules, and the seeds in the capsule are dispersed by the splashing water. In the springboard or catapult mechanism, the fruit or persistent calyx tube (or gemmae in the case of *Kalanchöe tubiflora*; Brodie 1955) is attached to the stem by a resilient pedicel, which is bent downward by falling raindrops and, as it recoils upward, seeds are dispersed.

The genus *Scutellaria* (skullcap) is named from the Latin *scutella*, a small dish or shield, in allusion to a dish-like protrusion of the calyx (Diggs et al. 1999). In this paper, hydroballochory utilizing the specialized calyx structure (the scutellum and under lip) is discussed. This dispersal mechanism, found in the genus *Scutellaria* (Lamiaceae) and described for *S. altissima* from Europe (Nordhagen

1936; Van Der Pijl 1982; Leins 2000), is examined in two southwestern species of North American skullcap (*S. drummondii* Benth. var. *edwardsiana* B. L. Turner and *S. wrightii* A. Gray) that are widespread in north central Texas (Diggs et al. 1999).

Nordhagen (1936) observed that the scutellum received a shock from rain drops causing it to be thrown off the under lip releasing the fruits called nutlets. The structures involved were photographed and explained by Leins (2000). Collins (1976) described calyx development in eastern species of *Scutellaria.* In these species, the calyx body expands to about 3-5 times its size at anthesis while the scutellum expands disproportionately more, becoming 4-6 times its original size at anthesis. Typically four nutlets develop per calyx and time of nutlet maturation varies from 3-6 weeks in spring-blooming species to 2-4 weeks for fall-bloomers. Dehiscence of the fruiting calyx allows the release of the mature nutlets from the persistent scale (Collins 1976).

Van Der Pijl (1982) used the Nordhagen (1936) description and illustrations in his book on fruit and seed dispersal but termed the underlip a kettle. In addition to the term "kettle", Miller (2001), when describing *S. integrifolia*, introduced the term "spoon" for the underlip. Correll & Johnston (1970) describe dehiscence of the scutellum and refer to the underlip, kettle, or spoon as a scale. This terminology is preferable because scale is commonly used in botanical descriptions.

The objectives of this study were to test hypotheses of hydroballochory in two common species of *Scutellaria* using quantitative data and qualitatively examine fruit maturation. Misconceptions regarding dispersal and anatomy of the scutellum are discussed.

Materials and Methods

Scutellaria drummondii var. *edwardsiana* (Drummond's skullcap), an annual species, was observed in fruit before and after a heavy rain on 24 May 2009 and again on 22 May 2010. On 22 May 2010,

40 plants were flagged and mature scutella counted before and after a heavy rain (Table 1).

In addition to these field observations, two live specimens of *S. wrightii* (Wright's skullcap), a perennial, were collected on 25 May 2009. Upon examination of the fruiting calyces, only green scutella and scales from scutella with fruits that had likely dispersed after the aforementioned rain were observed on these collected plants. The two plants were placed in pots and monitored daily for scutellar development. During precipitation events, pots were taken from the growing area and placed outside, directly in the rain (Table 2). After the precipitation event, the plants were placed back in the greenhouse and carefully examined for dispersed scutella.

To test the hypothesis that rainfall played a significant role in fruit dispersal, statistical analyses were conducted using SigmaPlot 11 (Systat Software, Inc.). Numbers of scutella were counted on individual plants and these data analyzed for significant differences between pre- and post-precipitation events in the field and greenhouse study. A Mann Whitney Rank Sum test was conducted on the field data and a paired *t*-test was used for the greenhouse data.

In addition to testing hypotheses regarding fruit dispersal, scutella were classified as green, yellow, brown, or aborted based on size and color (Table 3). Aborted scutella could be recognized by their small size, which is about one fourth that of a mature scutellum. Mature scutella were large and began as a green color, turned yellow, and eventually became brown in color. Greenhouse plants were observed from 30 June to 3 August, 2009. Scutellar classifications began on 21 July and ended on 3 August, 2009.

Information presented in Texas floras and field guides was evaluated for accuracy regarding fruit dispersal mechanisms in the two species. Discrepancies of terminology, morphology, and function were noted in some of these works and are discussed within this manuscript.

Table 1. Mature scutella of 40 Drummond's skullcap prior to and after a precipitation event during the field experiment.

Scutella prior to event	Scutella after event	Scutella prior to event	Scutella after event	Scutella prior to event	Scutella after event
9	3	3	0	40	11
12	4	11	0	3	0
5	3	9	0	6	0
8	0	13	2	5	1
41	18	6	4	25	3
6	1	5	2	5	0
7	2	5	0	6	0
4	1	11	1	4	0
14	2	5	0	22	2
26	8	5	1	5	1
10	6	6	4	5	1
17	0	6	0	3	0
8	1	7	0	20	3
37	6				

Table 2. Precipitation events and number of mature scutella prior to and after each rain during the greenhouse experiment using Wright's skullcap.

Precipitation event	Scutella prior to event	Scutella after event
7/21/09	3	0
7/26/09	21	14
7/27/09	15	12
7/28/09	11	8
7/29/09	8	7
7/30/09	7	6
7/31/09	6	5
8/1/09	5	5
8/2/09	5	3

Table 3. Classification of Wright's skullcap scutella during precipitation events of the Greenhouse experiment.

Precipitation Event	Scutellar Classification Categories			
	Aborted	Green	Yellow	Brown
7/21/09	3	23	0	3
7/26/09	7	1	17	4
7/27/09	7	0	11	4
7/28/09	11	0	7	1
7/29/09	11	0	6	1
7/30/09	11	0	5	1
7/31/09	11	0	4	1
8/1/09	11	0	4	1
8/2/09	11	0	3	0

Voucher specimens for *S. drummondii* var. *edwardsiana* (TAC 4373) and *S. wrightii* (TAC 4374) are deposited at the Tarleton State University Herbarium (TAC). Localities of collections are the Goetze property in Mills County (31.56080N, 98.68190W) for Drummond's skullcap and the Tarleton State University Agricultural Center (32.24820N, 98.20990W) for Wright's skullcap.

Results

Prior to a heavy rain that occurred on 24 May 2009, specimens of Drummond's skullcap were being collected for a floristic study. While collecting specimens, numerous mature scutella were observed and when pressed, fruits were expelled into specimen papers. About 75-100 scutella were observed in the population of plants. Following the heavy rain, the plants were observed to have only scales present, indicating that fruit dispersal had occurred. During the following field season on 22 May 2010, the 40 flagged plants were observed to have dispersed 60 scutella (Table 1) and the numbers of scutella prior to and after the precipitation event were significantly different ($P = <0.001$).

In the greenhouse study using Wright's skullcap, 34 scutella were found to have dehisced leaving scales on the plants and scutella on the surface of the potting soil following nine precipitation events (Table 2). There was a significant difference prior to and after the precipitation events ($P = 0.009$).

Aborted scutella numbers remained the same after 28 July 2009. Scutella classified as aborted remained immature, were about one-fourth the size of mature scutella, and contained no nutlets. Other scutella quickly turned from green to yellow and more slowly turned brown in color (Table 3) and only yellow or brown scutella were observed dehiscing. Similar patterns of scutellar development were observed in the field study of Wright's skullcap.

Discussion

Prior to these observations, dispersal by hydroballochory had only been described for *Scutellaria altissima*, which is only known from

Massachusetts in North America. The observations from this investigation indicate that hydroballochory also occurs in Wright's and Drummond's skullcap. Although there are several qualitative descriptions of rain drops causing scutellar dispersal in *S. altissima* (Nordhagen 1936; Van Der Pijl 1982; Leins 2000), this analysis represents the only quantitative data available that indicates that scutella are dispersed after rain.

Following pollination and subsequent fertilization, scutella are green and enlarge in size, turn yellow, and if are not dispersed, eventually turn brown. Yellow scutella will disperse but, based on mechanical manipulation with a pencil, require more force to dehisce than brown scutella. Green scutella turn yellow relatively quickly, but the brown coloring, likely resulting from drying and dehydration, found in the more commonly dispersed scutella occurs more slowly. Immature scutella likely formed from flowers that were unpollinated. At the end of the experiment, no fruits were found in the 11 immature scutella that persisted and none had dehisced. All 11 of the immature scutella turned brown and five of the 11 had been bent downward from the force of the rain but did not dehisce. Fruits were observed in all the yellow and brown scutella during the course of the experiment.

After observing the plants, it appears that the scutella swell after fertilization and turn from green to yellow to brown. Once the scutella are yellow or brown, they may dehisce. When a raindrop hits the cup-shaped surface of the scutellum it inverts and falls off the scale and the mechanical force of this event causes the scale to throw the mature fruits. The mature fruits or nutlets are then likely carried by water currents some distance from the plant depending on the amount of run-off occurring during the precipitation event. There were significant differences between the numbers of scutella prior to and after precipitation events.

Even though this dispersal mechanism was described over 70 years ago (Nordhagen 1936), there is confusion regarding dispersal mechanisms in these widespread Texas plants. Some floras and field guides only mention the scutellum or crest as an identification

character without any indication of its function (Loughmiller & Loughmiller 1992; Diggs et al. 1999; Tull & Miller 1999; Loughmiller et al. 2006; Nieland & Finley 2009). Correll and Johnston (1970) describe dehiscence accurately but do not mention rainfall as a mechanism for fruit dispersal.

Others have not described the morphology accurately. Niehaus et al. (1984) stated that the seeds of *Scutellaria* resemble skullcaps. The seeds are within oval-shaped nutlets enclosed by the scutellum and scale and the skullcap-shaped structure would correctly be identified as the scutellum.

Ajilvsgi (1991) and Tveten & Tveten (1993) stated that the vernacular and scientific names of the genus *Scutellaria* are derived from the small cap-like structure which covers the seed during the fruiting period and remains on the plant long after the seed is gone. The scutellum covers the fruit and is not persistent once dispersal occurs. Only the scale is persistent, not the cap-like scutellum.

Bowers et al. (2009) indicate that each tube-shaped flower has a fuzzy bulbous upper petal (lip) that has a cap shape that resembles a monk's headgear. This description is erroneous because the petal does not form the scutellum, it is an outgrowth of the calyx.

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GEOGRAPHIC DISTRIBUTION RECORDS FOR FISHES OF CENTRAL AND NORTHERN ARKANSAS

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Abstract.–New geographic distribution records are documented for 17 taxa of Arkansas fishes within seven families (Atherinopsidae, Catostomidae, Cyprinidae, Fundulidae, Lepisosteidae, Percidae, Petromyzontidae) from 15 counties of the central and northern portions of the state. Several species are reported from the Eleven Point (*Notropis sabinae*, *Percina evides, Percina sciera* and *Percina vigil*), Saline (*Opsopoeodus emiliae* and *Fundulus chrysotus*), Spring (*Moxostoma carinatum*), Strawberry (*Ichthyomyzon castaneus*) and Petit Jean (*Ichthyomyzon gagei*) river watersheds for the first time. In addition, a new locality in Stone County is added for the endemic and federal candidate species yellowcheek darter (*Etheostoma moorei*).

McAllister et al. (2009a; 2009b; 2009c; 2010a; 2010b) recently provided geographic distribution records for various fishes of Arkansas to update those of Robison & Buchanan (1988). In addition, McAllister et al. (2010c) reported new distributional records for 10 species of fishes from five major rivers of the state. Since comprehensive regional faunal works and their updates are typically published only after long intervals, it is important to periodically update occurrence data, especially for rarer species, such that researchers and resource managers are made aware of additional habitats that may warrant further targeted monitoring, as well as be included in future conservation planning that may benefit the species as a whole. Therefore, the purpose of the present report is to update the status of additional fishes of the central and northern portions of the state.

Materials and Methods

Fishes were collected between June 1978 and June 2002, and again during July 2007 with standard nylon seines (1.8 by 0.5 m and 2.7 by 0.5 m of 3.2 mm mesh, all dates) or backpack electroshocker (July 2007 collections only) from streams throughout various localities in central and northern Arkansas. These included watersheds in 15 counties (Benton, Boone, Crawford, Franklin, Grant, Independence, Johnson, Lawrence, Marion, Randolph, Sharp, Stone, Van Buren, Washington and Yell). Fish were identified, preserved in 10% formalin and later transferred to 50% isopropyl alcohol or 70% ethanol. Additional specimens from the July 2007 collections were preserved in 95% ethanol to provide tissues for DNA investigations. Voucher specimens in 70% and 95% ethanol were deposited in the fish collection and genetic resources collection at the North Carolina State Museum of Natural Sciences (NCSM), Raleigh, North Carolina; those in 50% isopropyl deposited in the Southern Arkansas University Fish Collection (SAU), Magnolia, Arkansas.

This study reports the collection of 96 fishes within seven families, selected from among 35 noteworthy collections. Detailed data provided on the new collection sites are as follows: (total number of specimens in parentheses, county, specific locality [section, range and township or latitude and longitude, WGS 84 geodetic datum], collection date, NCSM accession number, and comments). The nomenclature of Nelson et al. (2004) is followed.

List of Families and Species

Material examined.–The following is a listing of fish families and species collected and their collection localities in central and northern Arkansas.

PETROMYZONTIDAE

Ichthyomyzon castaneus Girard (n = 1). Lawrence Co.: Strawberry River at St. Hwy. 115, 6.4 km SW of Smithville (Sec.

17, R3W, T16N). 2 May 1989. SAU. The chestnut lamprey, the most widely distributed petromyzontid in the state, is reported for the first time from the Strawberry River; Robison & Buchanan (1988) show proximate records in the Current and Spring rivers. More recently, Robison et al. (2006) reported 22 additional localities in six counties of Arkansas for *I. castaneus*.

Ichthyomyzon gagei Hubbs & Trautman (*n* = 1). Yell Co.: Petit Jean River at co. rd., 3.2 km S of Havana (Sec. 16, R24W, T5N). 19 April 2001. SAU. This record documents the first specimen of the southern brook lamprey from the Petit Jean River system. Robison et al. (2006) reported additional *I. gagei* from six counties of the state, including a specimen each from more northern proximate locations in Little Piney Creek (Johnson County) and the Mulberry River (Franklin County).

LEPISOSTEIDAE

Lepisosteus osseus (Linnaeus) (*n* = 1). Randolph Co.: Eleven Point River at U.S. 62, 8.0 km NE of Imboden (Sec. 33, R1W, T19N). 18 June 1990. SAU. The longnose gar is expected statewide (Robison & Buchanan 1988); this is only the second record of *L. osseus* from the Eleven Point River system.

CYPRINIDAE

Opsopoeodus emiliae (Hay) (*n* = 1). Grant Co.: Saline River at U.S. 270, 21.4 km W of Sheridan (34.3192°N, 92.5876°W). 20 July 2007. NCSM 47162. This is a new county record and first report of *O. emiliae* from the mainstem Saline River; there are six previous records in tributaries of the Saline River (Robison & Buchanan 1988).

Erimystax x-punctatus (Hubbs & Crowe) (*n* = 3). Randolph Co.: Eleven Point River at U.S. 62, 8.0 km NE of Imboden (Sec. 33, R1W, T19N). 20 June 1985. SAU. This represents only the

second report of *E. x-punctatus* from the Eleven Point River system (Robison & Buchanan 1988).

Notropis sabinae Jordan & Gilbert (*n* = 2). Lawrence Co.: Spring River at Imboden off U.S. 62 (Sec. 15, R2W, T18N). 15 August 1992. SAU. Randolph Co.: Eleven Point River at U.S. 62, 8.0 km NE of Imboden (Sec. 33, R1W, T19N). 20 June 1985. SAU. Robison & Buchanan (1988) mapped 15 sites for *N. sabinae* in the Black, Current, Strawberry and White river systems for the period 1960-1987. This study documents the first and second records for the Sabine shiner, respectively, from the Eleven Point and Spring River systems of the state. McAllister et al. (2009c) recently reported three *N. sabinae* (NCSM 47139) from the Strawberry River, Lawrence County. Since 1988, however, other attempts by HWR and WCS at collecting specimens of *N. sabinae* from other historical sites where populations were previously numerous have not been productive. The species is considered imperiled (S2) in Arkansas (NatureServe 2009).

Cyprinella whipplei (Girard) (*n* = 1). Randolph Co.: Eleven Point River at St. Hwy. 93 at Dalton (Sec. 36, R2W, T21N). 18 June 1990. SAU. The steelcolor shiner is widely distributed in the uplands of Arkansas (Robison & Buchanan 1988), and this locality documents a new geographic record for the Eleven Point River.

CATOSTOMIDAE

Moxostoma carinatum (Cope) (*n* = 1). Sharp Co.: Spring River at Hardy, public beach area (Sec. 12, R5W, T19N). 20 July 1987. SAU. This study documents the first record of the river redhorse from the Spring River system; there is a pre-1960 record of *M. carinatum* from the proximate Black River (Robison & Buchanan 1988). The Arkansas range of *M. carinatum* has been reduced due to construction of reservoirs (Robison & Buchanan 1988).

Moxostoma duquesnii (Lesueur) (*n* = 4). Johnson Co.: McKinney Creek at co. rd. 2020, S of I-40, 19.2 km W of

Clarksville (35.4982°N, 93.6728°W). 19 July 2007. NCSM 47062. Lawrence Co.: Strawberry River at St. Hwy. 25 (Sec. 33, R3W, T16N). 22 July 1987. SAU. Marion Co.: Crooked Creek at St. Hwy. 101 (Sec. 35, R15W, T19N). 14 June 1978. SAU. This common sucker is restricted to Ozark-Ouachita upland streams in Arkansas (Robison & Buchanan 1988). This study provides a new county record and first report from the extensive McKinney Creek watershed (Arkansas River drainage); the third and most downstream record from the Strawberry River system; and the first record from the Crooked Creek system (White River drainage).

FUNDULIDAE

Fundulus chrysotus ($n = 2$). Grant Co.: Saline River at U.S. 270, 21.4 km W of Sheridan (34.3192°N, 92.5876°W). NCSM 47163. 20 July 2007. McAllister et al. (2006) provided a recent summary of the distribution of *F. chrysotus* in Arkansas. This fish may be expanding its range in response to increasing removal of submergent vegetation in which *F. chrysotus* is typically associated (Keck & Etnier 2005). This study documents a new county record and the first time the golden topminnow has been reported from the Saline River.

ATHERINOPSIDAE

Labidesthes sicculus (Cope) ($n = 20$). Boone Co.: Crooked Creek at gravel road, 2.4 km N of Harman (Sec. 7, R18W, T18N). 24 May 1993. SAU. Independence Co.: Curia Creek at St. Hwy. 25, 2.4 km N of Dowdy (Sec. 9, R3W, T14N). 19 June 1996. SAU. Data Creek at Charlotte (Sec. 32, R4W, T14N). 19 June 1996. SAU. Randolph Co.: Eleven Point River at St. Hwy. 93 at Dalton (Sec. 36, R2W, T21N). 20 June 1985. SAU. The brook silverside is expected statewide (Robison & Buchanan 1988); these are the first records from the Curia and Data Creek systems and the second records from the Crooked Creek and Eleven Point systems.

PERCIDAE

Etheostoma sp. (cf. *blennioides*) Rafinesque (n = 47). Benton Co.: Osage Creek at co. rd. 12, 18.2 km E of Siloam Springs (36.1962°N, 94.3377°W). 18 July 2007. NCSM 47477. War Eagle Creek at War Eagle community, 17.5 km ESE of Rogers (36.2676°N, 93.9428°W). 19 July 2007. NCSM 47367. Crawford/Franklin cos.: Mulberry River at co. rd. 67, 3.2 km N Mulberry (35.5302°N, 94.0412°W). 19 July 2007. NCSM 47524. Grant Co.: Saline River at U.S. 270, 21.4 km W of Sheridan (34.3192°N, 92.5876°W). NCSM 47352. 20 July 2007. Independence Co.: Curia Creek at St. Hwy. 25, 12.4 km N of Dowdy (Sec. 9, R3W, T14N). 19 June 1996. SAU. Data Creek at Charlotte (Sec. 32, R4W, T14N). 19 June 1996. SAU. Johnson Co.: McKinney Creek at co. rd. 2020, S of I-40, 19.2 km W of Clarksville (35.4982°N, 93.6728°W). 19 July 2007. NCSM 47063. Stone Co.: Middle Fork Little Red River at Arlberg at low water bridge, E off St. Hwy. 110, 28.5 km SW of Mountain View (35.7348°N, 92.3898°W). 20 July 2007. NCSM 47073. Van Buren Co.: Archey Fork of Little Red River (Sec. 31, R14W, T12N). 27 July 1986. SAU. Archey Creek at U.S. 65 in Clinton (Sec. 10, R14W, T11N). 28 July 1986. SAU. Archey Creek at St. Hwy. 254 (Sec. 14, R16W, T12N). 28 July 1986. SAU. Washington Co.: War Eagle Creek at co. rd. 526, 22.0 km ENE of Springdale (36.2278°N, 93.9014°W). 19 July 2007. NCSM 47354. A common darter of the Ozark Upland drainages of the state, these 13 localities supplement previous sites in central and northern Arkansas (Robison & Buchanan 1988). Recent genetic studies of the greenside darter (*Etheostoma blennioides*) complex by Haponski & Stepien (2008) and Piller et al. (2008) suggested that the White-Ouachita river populations were quite distinct; these populations with high lateral scale counts (66-78) warranted taxonomic recognition as *E. blennioides newmanii* (see Page & Burr 2011).

Etheostoma moorei Raney & Suttkus (n = 5). Stone Co.: Middle Fork Little Red River at Arlberg at low water bridge, E off St. Hwy. 110, 28.5 km SW of Mountain View (35.7348°N, 92.3898°W). 20

July 2007. NCSM 47072. Specimens were collected at 0.3 m depth with a backpack electroshocker. There are few records of the endemic yellowcheek darter in the state; it is known historically from only six sites in four headwater tributaries of the upper Little Red River drainage above Greer's Ferry Lake in Cleburne, Searcy, Stone and Van Buren counties (Raney & Suttkus, 1964; Robison 1980; Robison & Buchanan 1988). Indeed, much of the shallow stream habitat was inundated by impoundment of Greer's Ferry (which began filling in 1962) and the remaining isolated populations are in peril from habitat loss and degradation (Buchanan 1974; Mitchell et al., 2002). This species is considered critically imperiled (S1) in the state (NatureServe, 2009) and it is listed by the Arkansas Game and Fish Commission as a species of special concern (Anonymous 2004a). It is also listed by the U.S. Fish and Wildlife Service as a federal candidate species (Anonymous 2004b). This new site record supplements previous localities in Stone County.

Etheostoma zonale (Cope) (n = 4). Independence Co.: Curia Creek at St. Hwy. 25, 2.4 km N of Dowdy (Sec. 9, R3W, T14N). 7 August 1984. SAU. Data Creek at Charlotte (Sec. 32, R4W, T14N). 7 August 1984. SAU. According to Robison & Buchanan (1988), the banded darter is an upland species in all major drainages of the Ozarks and in all except the Red River drainage of the Ouachitas. These are the first records of *E. zonale* in those two creek systems.

Percina evides (Jordan & Copeland) (n = 1). Randolph Co.: Eleven Point River at U.S. 62, 8.0 km NE of Imboden (Sec. 33, R1W, T19N). 20 August 1999. SAU. This represents the first record of the gilt darter from the Eleven Point River system. Records of *P. evides* are common further downstream in the Black River system (Robison & Buchanan 1988).

Percina sciera (Swain) (n = 1). Randolph Co.: Eleven Point River at U.S. 62, 8.0 km NE of Imboden (Sec. 33, R1W, T19N). 23

June 2002. SAU. This represents the first record of the dusky darter from the Eleven Point River system; Robison & Buchanan (1988) report proximate records for *P. sciera* from the Spring and Strawberry rivers.

Percina vigil (Hay) (n = 1). Randolph Co.: Eleven Point River at U.S. 62, 8.0 km NE of Imboden (Sec. 33, R1W, T19N). 23 June 2002. SAU. This study documents the first report of the saddleback darter from the Eleven Point River system; there are proximate records in the Current and Spring rivers (Robison & Buchanan 1988).

In summary, this study provides some noteworthy geographic distribution records for 17 taxa of Arkansas fishes within seven families (Atherinopsidae, Catostomidae, Cyprinidae, Fundulidae, Lepisosteidae, Percidae, Petromyzontidae) from 15 counties of the state. Most importantly, it documents the extension of the range of several species into the Eleven Point, Saline, Spring, Strawberry and Petit Jean river watersheds. As noted by McAllister et al. (2009c) and reiterated here, additional collections are warranted using boat-mounted electrofishing devices to help further provide an additional understanding of the distribution of fishes of the state of Arkansas.

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REPRODUCTION OF THE ELEGANT EARLESS LIZARD, *HOLBROOKIA ELEGANS* (SQUAMATA: PHRYNOSOMATIDAE) FROM ARIZONA, NEW MEXICO, SINALOA AND SONORA

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Abstract.–Reproduction in *Holbrookia elegans* was studied by a histological analysis of gonadal material from museum specimens. Spermiogenesis began in April and continued into September. Females commenced yolk deposition in June and continued into September. The mean clutch size for 23 female *H. elegans* was 8.22 ± 2.3 *SD*, range = 3-11. Multiple egg clutches were produced. The smallest reproductively male measured 45 mm SVL. The smallest reproductively active female measured 51 mm SVL

Holbrookia elegans ranges from coastal southern Sinaloa, Mexico, north through central and eastern Sonora, to south central Arizona, and to extreme southwestern New Mexico where it inhabits mesquite grasslands and grassy oak and juniper woodlands (Axtell 2009). Information on its reproduction is limited. Axtell (2009) speculated 6 to 10 eggs were deposited from late June to late August or early September, Brennan & Holycross (2006) reported, that mating occurs in spring and one or two clutches were laid in spring and summer. The purpose of this paper is to present information on reproduction of *H. elegans*. Information on the reproductive cycle is important for formulating conservation policies to protect and maintain animal populations.

MATERIALS AND METHODS

A sample of 98 *Holbrookia elegans* from Santa Cruz County, Arizona, Hidalgo County, New Mexico, Sinaloa and Sonora, Mexico consisting of 55 males (mean snout-vent length, [SVL] = 57.5 mm ± 7.7 *SD*, range = 43-74 mm) and 43 females (SVL = 57.7 mm ± 3.6 *SD*, range = 51-65 mm) were examined from the herpetology collection of the Natural History Museum of Los

Angeles County (LACM), Los Angeles, California. Lizards were collected 1945-1974.

Material examined.–The following specimens of *H. elegans* were examined: Arizona, Santa Cruz County: LACM 15540, 53617, 53619, 76392-76394, 95199, 112472-112473, 123333, 123334: New Mexico, Hidalgo County: LACM 4199, 4200, 4202, 76433, 76434, 113391-113394; Mexico, Sinaloa: 4197, 4199, 4200, 6600-6605, 6608, 8624, 13680, 25712-25714, 41298, 95209-95211,113391-113393; Sonora: 6609, 6610, 13670, 25150-25152, 25154-25156, 52796, 59946, 61480, 95215, 95216, 95218, 95221, 95222, 95227-95232, 95234-95238, 95241, 95243-95245, 95347-95253, 95256-95259, 95261, 95264, 95265, 95267, 95268, 95272, 95274, 95275, 99422, 108846, 136869.

For histological examination, the left testis was removed from males and the left ovary was removed from females. Enlarged follicles (> 4 mm length) or oviductal eggs were counted (*in situ*). Tissues were embedded in paraffin and cut into sections of 5 µm. Slides were stained with Harris hematoxylin followed by eosin counterstain (Presnell & Schreibman 1997). Slides of testes were examined to determine the stage of the spermatogenic cycle. Slides of ovaries were examined for the presence of yolk deposition or corpora lutea. Histology slides were deposited in the Herpetology Collection at LACM. An unpaired *t*-test was used to compare *H. elegans* male and female mean SVL. The relationship between female SVL and clutch size was examined by linear regression analysis using Instat (vers. 3.0b, Graphpad Software, San Diego, CA).

Results

Monthly changes in the testicular cycle are in Table 1. There was no significant difference between male and female SVL (P = 0.84) (unpaired *t*- test). Three stages were observed: (1) Regressed, the germinal epithelium of the seminiferous tubules is reduced to one or two layers of spermatogonia and Sertoli cells; (2) Recrudescence, there is a proliferation of primary and secondary

spermatocytes for the upcoming period of spermiiogenesis; (3) Spermiogenesis, seminiferous tubules are lined by sperm and/or clusters of metamorphosing spermatids. The first males to exhibit spermiogenesis were observed in April (2/15) = 13%. Spermiogenesis continued into September. The smallest reproductively active male was from April, and measured 45 mm SVL (LACM 4197).

Monthly changes in the ovarian cycle are in Table 2. Four stages were noted: (1) Quiescent, no yolk deposition; (2) Early yolk deposition, basophilic yolk granules are in the ooplasm; (3) Enlarged ovarian follicles > 4 mm length; (4) Oviductal eggs are present. The mean clutch size for 23 female *H. elegans* was 8.22 ± 2.3 *SD*, range = 3-11. The smallest reproductively active female measured 51 mm SVL (LACM 95264), contained oviductal eggs and was from July. Linear regression analysis revealed a significant positive correlation (r = 0.47) between *H. elegans* female size (SVL) and clutch size (n = 23, P = 0.023, Y = -9.47 + 0.31X). One female from July (LACM 95258) and two from August (LACM 95209, 95211) contained oviductal eggs and concomitant yolk deposition for a subsequent clutch indicating *H. elegans* produces multiple clutches in the same reproductive season.

Discussion

Droge et al. (1982) reported a mean clutch size of 3.5 for the congener *H. maculata* from Western Nebraska. This contrasts with the larger mean clutch size reported herein for *H. elegans* (8.22 ± 2.3 *SD*, range = 3-11) and is in accordance with Fitch (1970; 1985) who reported an increase in clutch sizes from north to south for *H. maculata* and *H. elegans*, respectively. As was the case for *H. elegans,* regression analysis revealed a significant positive correlation between clutch size and female body size for *H. maculata* and *H. propinqua* (Droge et al. 1982; Selcer & Judd 1982). Some females of *H. elegans, H. lacerta, H. maculata*, and *H. propinqua* may produce multiple egg clutches (Axtell 1956; Droge et al. 1982; Selcer & Judd 1982).

Table 1. Monthly stages in the testicular cycle of 55 *Holbrookia elegans* from Santa Cruz County, Arizona (n = 9), Hidalgo County, New Mexico (n = 5), Sinaloa (n = 11) and Sonora (n = 30), Mexico.

Month	n	Regressed	Recrudescent	Spermiogenesis
March	3	3	0	0
April	14	8	5	1
May	4	0	0	4
June	9	0	0	9
July	19	2	0	17
August	5	0	0	5
September	1	0	0	1

Table 2. Monthly stages in the ovarian cycle of 43 *Holbrookia elegans* from Santa Cruz County, Arizona (n = 4), Hidalgo County, New Mexico (n = 4), Sinaloa (n = 11) and Sonora (n = 24), Mexico.

Month	n	Quiescent	Early yolk Deposition	Enlarged follicles > 4 mm	Oviductal eggs
April	5	5	0	0	0
May	1	1	0	0	0
June	6	2	3	1	0
July	23	1	4	14	4*
August	7	2	0	3	2**
September	1	1	0	0	0

* = One female from July

** = Two females from August contained oviductal eggs and concomitant yolk deposition for a subsequent egg clutch indicating *H. elegans* produces multiple clutches in the same reproductive season.

Females of *H. maculata* from Nebraska began vitellogenesis in early May (Droge et al. 1982) as did *H. maculata* from the Zuni Mountains, New Mexico (Gelbach 1965). In contrast, *H. elegans* females reported herein did not commence yolk deposition until

June (Table 2). Delayed onset of yolk deposition until June may allow *H. elegans* to oviposit at the onset of the summer monsoon which occurs in their range. Similar timing of oviposition was noted for other lizards subject to southwestern summer monsoons: *Phrynosoma solare (*Ivanyi 2009), *Sceloporus clarkii* (Schwalbe & Rosen 2009), and *Sceloporus magister* (Jones & Schwalbe 2009). Parturition in live-bearing *Sceloporus jarrovii*, in southern Arizona, occurred in late June just prior to start of the summer monsoon (Goldberg 1971).

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MICROHABITAT USE OF *BLARINA CAROLINENSIS* (SOUTHERN SHORT-TAILED SHREW) IN EAST TEXAS

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Abstract.–A mark-recapture study from 8 October 2002 through 21 December 2002 was conducted to assess the microhabitat use of *Blarina carolinensis* (southern short-tailed shrew). Animals were captured during three intervals of 21 days with two 10-day intervening periods when the trapping grid was closed. The study site was located in a mixed pine-hardwood forest in an urban ecosystem near the campus of East Texas Baptist University, Marshall, Texas. Using a bootstrap estimation, density of *B. carolinensis* was estimated to be 0.58 ± 0.56 shrews per hectare. Habitat was assessed at three scales (1 m^2, 5 m^2, and 10 m^2) centered on each trap site. Variation of understory cover and total understory cover were found to be important at all three scales. However, stepwise analysis at each scale indicated different habitat variables to be important. Predictive power of the variables determining sites where *B. carolinensis* were captured for each of the selected scales was 1m^2 (r^2 = 0.3198, P = 0.1253), 5 m^2 (r^2 = 0.3249, P = 0.0084), and 10 m^2 (r^2 = 0.3177, P = 0.0203). Principal components analysis showed sites where *B. carolinensis* were not captured to be outliers at the 1 m^2 scale with no discernable patterns or limited grouping in status of captures at the other two scales. The first three components at the 1m^2, 5m^2, and 10 m^2 accounted for 83.6%, 84.0%, and 81.1% of the variation, for each scale, respectively.

Generally, an urban habitat is characterized as being fragmented and heterogeneous (Schmid-Holmes & Drickamer 2001). Fragmentation of habitat in an urban ecosystem may result in habitat use that differs from that in non-urban habitats because the fragmentation can cause natural habitats to exist as small, isolated patches. Mammals as diverse as bats (Gehrt & Chelsvig 2003) and coyotes (*Canis latrans*; Gehrt et al. 2009) have been found to alter habitat use in urban ecosystems. Studies of small mammals have shown urbanization to be an indirect factor in determining diversity (Ekernas & Mertes 2006) and habitat use (Dickman & Doncaster 1987). However, Sauvajot et al (1998) found direct responses by small mammals to the effects of urbanization.

Because urban habitats are fragmented, assessment of habitat

use by small mammals may need to be conducted at several smaller scales. Ladine & Ladine (1998) found differences in habitat use of white-footed mice (*Peromyscus leucopus*) when analyzed at small selected scales in a homogenous habitat. Urban ecosystems may have an abundance of diverse and disturbed habitats warranting study at such smaller scales.

Blarina carolinensis has been found in several types of habitat ranging from pastures, open woods (both pine stands and deciduous), and dense thickets (see Genoways & Choate 1998). McCarley (1959) and Schmidly (1983) noted that the species occurs throughout the pine-oak forests of eastern Texas. Mengak et al. (1989) found *B. carolinensis* to use a variety of disturbed sites, but Kirkland (1977) noted a decline in the closely related *B. brevicauda* in recently clearcut forests. There is a paucity of information concerning *B. carolinensis* in the western part of its range and urban settings. The objective of this study was to assess the microhabitat use of a population of *Blarina carolinensis* in an urban ecosystem.

Study Site

The study site was located on the campus of East Texas Baptist University (Marshall, TX; 32°33' N; 94°22' W) in a mixed pine-hardwood forested area. Residential areas were located 50 m on the south and west sides of the trapping grid. The north side of the trapping grid contained undisturbed woods for 200 m. The east side of the trapping grid was separated from an athletic field by an asphalt road. Several locations within the study site contained trash. Six weeks after the start of trapping, a 5m path on the west side of the site was cleared of all woody vegetation by the city of Marshall, Texas. This path ranged from 2-25 m from the trapping grid.

Dominant canopy trees found on the site were elm (*Ulmus* spp.), hickory (*Carya* sp.), loblolly pine (*Pinus taeda*), oak (*Quercus* spp.), and sweet gum (*Liquidambar styraciflua*). Poison ivy

(*Toxicodendron radicans*) and grape (*Vitis* sp.) were found in the canopy and poison ivy was extensive in the herbaceous layer. Understory vegetation was dominated by saplings of the canopy trees, flowering dogwood (*Cornus florida*), poison ivy, and green briar (*Smilax* spp.). Ground layer vegetation was sporadic and was primarily poison ivy and green briar.

Materials and Methods

A seven by seven trapping grid was established October 2002 using 8.9 cm by 8.9 cm by 22.9 cm folding Sherman traps (H. B. Sherman Traps, Inc.; Tallahassee, Florida) spaced 10 m apart. Trapping started on 8 October 2002 and continued through 21 December 2002. Traps were open nightly for three-week periods, checked daily at sunrise and baited with a mixture of peanut butter and oatmeal. Traps were closed and left on site for 10-day periods between each of the three-week trapping periods.

Data collected from captured *B. carolinensis* included mass and trap location. Live-captured short-tailed shrews were marked uniquely on the pelage with fingernail polish. White-footed mice (*Peromyscus leucopus;* two total captures) and pine vole (*Microtus pinetorum;* one capture) were also captured on the study site, marked and measured in the same manner as *B. carolinensis*. The southern flying squirrel (*Galucomys sabrinus)* was also captured at one trap station.

Due to severe violations of estimators for population density, population density was estimated using a bootstrap technique (Hilllborn & Mangel 1997) which was run using the poptools addin (Hood 2003) for Microsoft Excel. All data analyses except estimation of population density were conducted using R (R Development Core Team 2004).

Nineteen selected habitat variables (see Table 1) were measured within three circular areas (1 m^2, 5 m^2, and 10 m^2) of each trap site. The selection of scale was based on the movement patterns of *B.*

Table 1. Description of habitat variables measured at 49 trap sites for assessment of habitat use by *Blarina carolinensis* in an urban ecosystem in east Texas.

Habitat variable	Description
Canopy	Mean of four canopy cover measurements
Logs	Total number of horizontal woody stems on ground
Stems	Total number of vertical woody stems
Litter	Mean of seven leaf litter depths for each site
Understory cover	Percent green vegetation at 1 m height
Floor cover	Percent green vegetation at ground level
Plant species	Total number of different plant species
Stems < 10	Total number of vertical woody stems < 10 mm diameter
Stems 10 - 20	Total number of vertical woody stems 10-20 mm diameter
Stems 20 - 50	Total number of vertical woody stems 20-50 mm diameter
Stems > 50	Total number of vertical woody stems > 50 mm diameter
Logs <10	Total number of horizontal woody stems on ground < 10 mm
Logs 10 - 20	Total number of horizontal woody stems on ground 10-20 mm
Logs 20 - 50	Total number of horizontal woody stems on ground 20-50 mm
Logs > 50	Total number of horizontal woody stems on ground > 50 mm
Diameter logs	Mean diameter of horizontal woody stems on ground
Diameter stems	Mean diameter of vertical woody stems
Floor heterogeneity[1]	Variation of four measurement of floor cover
Log heterogeneity[1]	Variation of diameters of logs

[1] See text for a more detailed description of how the variation measurements were taken.

carolinensis reported by Gentry et al. (1971). Canopy cover was the average of four readings taken directly above the trap using a densiometer. Because understory and floor vegetation were not homogenous, a measure of heterogeneity was incorporated for these two variables. Understory cover and floor cover were measured by dividing the area around the trap into quarters, averaging the four readings and using the standard deviation of the mean as a measure of heterogeneity around the trap. This resulted in two variables each for understory cover and floor cover for analysis; mean understory and floor cover at the trap site and the variation of understory floor cover around the trap site. A measure for variation of log diameter was also included by using the standard deviation of the mean diameter of all logs around the site. Leaf litter depth was an average of seven measurements randomly taken around each trap.

Because a capture indicated that *B. carolinensis* was present around the trap, trap sites with at least one capture were classified as capture sites. Other sites were classified as no-capture sites. Sites were examined using principal components analysis to assess the possibility of any existing patterns at each scale. Stepwise analysis was conducted to determine if predictor variables existed for the presence of *B. carolinensis* based on capture occurrence. Each selected variable was then deleted from the stepwise analysis to determine any changes to the prediction. No deletion of a variable changed the prediction. Variables not initially selected were also added for analysis of changing the prediction. Only the variables initially selected from the stepwise analysis predicted the presence of *B. carolinensis*. Habitat variables selected in the stepwise analysis were compared between sites with captures and sites without captures using the nonparametric Kruskal-Wallis test.

Results

Thirty-two *B. carolinensis* were captured during 2,499 trap nights with 20 marked and released alive. There were 29 sites without captures and 20 sites with captures. Population density was estimated at 0.58 ± 0.56 shrews per ha.

Principal components analysis of habitat variables for the 1 m^2 scale indicated clustering of the sites according to status of captures at the site. Sites where shrews were not captured tended to be outliers or along the edge of the graphical extraction of the first three components. The first three components accounted for 83.6 % of the variation.

Principal components analysis of habitat variables for the 5 m^2 scale showed no discernable patterns between capture sites and no capture sites. The first three components indicated the existence of medium-diameter logs (20-50 mm) and low number of plant species at each site may discern sites with captures. Stepwise analysis showed neither variable to be important in discerning captures. The first three components accounted for 84.0% of the variation.

Principal components analysis of habitat variables for the 10 m^2 scale indicated a clustering of sites according to status of captures at the site. Sites where shrews were captured tended to be outliers to the model. Sites primarily grouped according to number of plant species and medium-diameter stems (20-50 mm). Both of these variables were shown to be important in the stepwise analysis in discerning captures. The first three components accounted for 81.1% of the variation.

Variables differed at each scale for predicting the presence of *B. carolinensis* (see Tables 2, 3, & 4). The predicting power (r^2) of the selected variables for each scale was 0.3918, 0.3249, 0.3177 for the 1m^2, 5 m^2, and 10 m^2 scales, respectively. Mean understory cover and variation of understory cover were shown to be important predictors of the presence of *B. carolinensis* at all three scales (Tables 2, 3, and 4). However, the predicting value differed among scales. At scales of 1 m^2 and 10 m^2, lower amounts of understory cover predicted captures (P = 0.0360 and 0.0997, respectively), while at the 5 m^2 scale, higher amounts of understory cover correlated to captures (P = 0.0794). Greater variation of understory cover was important in predictive value for captures at the 1 m^2 and 5 m^2 scales but not at the 10 m^2 scale (Table 2).

Discussion

Population density in this study (0.58 ± 0.56 shrews per hectare) was lower than previously reported densities (Calhoun 1941; Smith et al. 1971; Kaufman et al. 1977). The high error of this estimation can be attributed to mortality of shrews in live-traps. The difference between this study and previous studies were most likely due to the method of assessment. Previous studies used removal techniques. Shrews are not captured as readily as other small mammals assessed by using mark-recapture techniques (see Whittaker & Feldhammer 2000).

Miller & Getz (1977) found habitat use in the closely related *B. brevicauda* (northern short-tailed shrew) to be correlated to locations

Table 2. Mean ± one standard deviation, and probability of prediction (*P*) of habitat variables found to be significant using stepwise analysis to assess sites with captures and sites without captures of *Blarina carolinensis* in east Texas in a 1 m^2 circular area centered on trap sites. Predictive power for prediction of captures of *B. carolinensis* of the stepwise analysis was $r^2 = 0.3198$ ($P = 0.1253$). See Table 1 and text for descriptions of habitat variables.

Habitat variable	Capture	No capture	Predictive Value	*P*
No. of stems	4.35 ± 4.40	3.24 ± 3.54	-1.52	0.0003
Leaf litter depth	37.71 ± 12.67	36.65 ± 9.39	2.10	0.0136
Floor cover	9.54 ± 17.10	3.76 ± 5.58	2.17	0.0418
Understory cover	7.18 ± 8.59	14.87 ± 25.79	-2.82	0.0360
Stems < 10 mm	4.15 ± 4.37	2.79 ± 3.51	1.62	0.0074
Heterogeneity of understory cover	6.59 ± 7.27	4.85 ± 4.40	-1.87	0.0689
Logs > 50 mm	0.25 ± 0.55	0.07 ± 0.26	2.82	0.0074
Logs < 10 mm	0.45 ± 0.75	0.62 ± 1.11	1.87	0.0689
Mean diameter of logs	13.37 ± 18.51	15.63 ± 18.81	-2.45	0.0189

Table 3. Mean ± one standard deviation and probability of prediction (*P*) of habitat variables found to be significant using stepwise analysis to assess sites with captures and sites without captures of *Blarina carolinensis* in east Texas in a 5 m^2 circular area centered on trap sites. Predictive power for prediction of captures of *B. carolinensis* of the stepwise analysis was $r^2 = 0.3249$, ($P = 0.0084$). See Table 1 and text for descriptions of habitat variables.

Habitat variable	Capture	No capture	Predictive Value	*P*
Canopy cover	72.05 ± 28.19	75.92 ± 28.19	-1.72	0.0932
Number of logs	6.14 ± 3.87	4.05 ± 2.87	3.45	0.0013
Understory cover	9.83 ± 11.12	9.50 ± 16.95	-1.79	0.0794
Logs < 10 mm	2.00 ± 2.46	1.50 ± 2.01	-2.20	0.0333
Logs 10 - 20 mm	1.62 ± 1.57	1.25 ± 1.37	-1.33	0.1919
Heterogeneity of understory cover	10.62 ± 11.79	6.02 ± 6.12	3.33	0.0018

with greater than 50% herbaceous cover. Herbaceous cover (measured as floor cover) at the Marshall study site was never greater than 50% at any trap site. Also, leaf litter may provide habitat for prey of *B. carolinensis* (McCay 2001). Leaf litter depths were less than 75 mm at all sites. The habitat of this study site may be minimal for *B. carolinensis,* resulting in a lower density.

Table 4. Mean ± one standard deviation and probability of prediction (*P*) of habitat variables found to be significant using stepwise analysis to assess sites with captures and sites without captures of *Blarina carolinensis* in east Texas in a 10 m^2 circular area centered on trap sites. Predictive power for prediction of captures of *B. carolinensis* of the stepwise analysis was $r^2 = 0.3177$, ($P = 0.0203$). See Table 1 and text for descriptions of habitat variables.

Habitat variable	Capture	No capture	Predictive Value	*P*
Canopy cover	73.12 ± 28.89	74.13 ± 25.17	1.48	0.1474
Understory cover	14.75 ± 16.62	17.31 ± 14.96	1.68	0.0997
Number of plant species	5.18 ± 1.86	6.45 ± 2.11	-1.54	0.1304
Stems 20 - 50 mm	1.48 ± 1.45	2.63 ± 1.81	-2.20	0.0337
Stems > 50 mm	1.67 ± 1.64	2.23 ± 1.85	-1.65	0.1062
Heterogeneity of understory cover	13.83 ± 12.71	18.24 ± 9.92	1.86	0.0522
Logs 10 - 20 mm	3.67 ± 3.08	2.95 ± 1.85	-2.00	0.0709

Because *B. carolinensis* is carnivorous, individuals should be found in areas where their prey is found. However, because it is a prey item for other species (McCay 2001), habitat should provide refugia for the shrew. At the 1 m^2 scale, variables possibly associated with prey species (Ladine & Muñoz 2010) of *B. carolinensis* were those discerning captures. These variables (forest floor cover, slightly deeper leaf litter and logs > 50 mm) would potentially harbor more prey organisms. *Blarina carolinensis* should be expected to be actively foraging in these areas. Also, the number of logs of different sizes were important at the larger 1 m^2 and 5 m^2 scales, which may indicate exploratory foraging by the species. Principal components analysis at the 5 m^2 scale, may indicate the importance of exploratory foraging in selected vegetation. Principal components indicated logs with a diameter 20-50 mm may be important in discerning captures. Logs of this size could potentially harbor insect species that are potential prey items of *B. carolinensis*.

The importance of greater understory cover could be related to availability of refugia while foraging. Alternatively, habitat use of these areas could be an exploratory foraging behavior for these individuals. At the two smaller scales, greater variation in understory cover was associated with capture of the species. This may suggest

that individuals were moving into these areas in exploratory foraging. The 10 m^2 scale did not demonstrate the same association with understory cover as the two smaller scales. At this scale, variation may mean more open areas and greater exposure to predation in addition to the need for less exploratory behavior.

While the habitat use for *B. carolinensis* generally agrees with previous studies (McCay 2001), density estimates obtained during this study were lower than those previously reported for the species (McCay 2001) in natural undisturbed ecosystems. To the author's knowledge, this is the first study of *B. carolinensis* conducted in an urban ecosystem. However, factors involving the population demographics and microhabitat use of *B. caroinensis* have not been assessed in a directly modified urban ecosystem. Because of the nearness of this study site to direct influences by human disturbance (roadways, houses, lights on a soccer field within 50 m of the site), results may have been influenced by urbanization. More study is required to confirm whether the low density of *B. carolinensis* in this study was influenced by urbanization or natural vegetation factors. However, findings of previous studies of small mammals in urban ecosystems suggest weak influences by urbanization unless the habitat patch is directly modified (Dickman & Doncaster 1987, Sauvajot 1998).

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AVIFAUNA FROM CERRO EL POTOSÍ, GALEANA, NUEVO LEÓN, MÉXICO.

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Abstract.–Cerro El Potosí is located in the state of Nuevo Leon in northeastern Mexico and is one of the highest peaks of the Sierra Madre Oriental (3713 meters msl). Avian censuses were conducted on a monthly basis during 1995 in order to document the avifauna species known to inhabit this unusual habitat. Due to its high elevation, this peak exhibits six different vegetational types; these were grouped into two scrublands (*Quercus intricata* and *Pinus culminicula*), three pine forests (*Pinus cembroides, P. ayacahuite* and *P. hartwegii*) and one alpine grassland. Eighty species of diurnal birds in three different residency categories were recorded during this study; these were 54 permanent residents, 22 migrant and four unknown. Sixty-three of these species represent new distributional records for this mountainous region. The vegetational association of each species is also given. As a result of this study, the number of avian species known to occur in the state of Nuevo Leon is now increased to 366 species.

Resumen.–El Cerro El Potosí es el pico más alto de la Sierra Madre Oriental, con 3713 msns, en el estado de Nuevo León. Durante 1995 se realizo este trabajo en el área del Potosí con el objetivo de incrementar el conocimiento de la avifauna presente en este cerro. La altitud de este pico del Potosí genera diferentes tipos de vegetación, las cuales son agrupadas en 2 tipos de matorral: matorral de encino (*Quercus intricata*) y matorral de pino (*Pinus culminicula*) así como 3 tipos de bosque de pinos (*Pinus cembroides*; *Pinus ayacahuite* y *Pinus hartwegii*) y una pradera alpina. Durante el estudio fueron registradas ochenta especies las cuales 54 fueron categorizadas como residentes permanentes, 22 migratorias y 4 inciertas.

Cerro El Potosi (24°52'51.21"N & 100°12'50.13"W) is one of the highest peaks of the Sierra Madre Oriental with an elevation of 3713 meters (Google Earth 2011a). Due to its elevation, geology and geographical location, this mountainous region exhibits a unique set of environmental conditions in northern Mexico that are extremely important to ecological studies involving relict, endemic, threatened and endangered species of both plants and animals. Six different vegetational types were recognized by García (1989) and García-Arana (1996). In addition, anthropogenic activities have lead to the disappearance of some native vegetation as well as changes in some ecological succession.

This mountainous region of Nuevo Leon has been of particular historical importance to numerous ornithological investigations. This region was included in the surveys of Friedmann et al. (1950), Miller et al. (1957), Martin Del Campo (1959) and Phillips (1986). Martin Del Campo (1959) recorded 169 species from the state of Nuevo Leon state with five species reported from El Potosi.

Several species from the El Potosi region have received noteworthy attention. There are multiple sight records of the endangered Maroon-fronted Parrot (*Rhynchopsitta terrisi*) from El Potosi (Moore 1947; Friedmann *et al.* 1950; Martín del Campo 1959). Clark's Nutcracker (*Nucifraga columbiana*) was first reported from the area by Leopold (1947); a decade later Miller *et al.* (1957) reported a second sighting of this corvid. Phillips (1986) later reported a resident population of *N. columbiana* from El Potosi. Contreras-Balderas (1992a: 181) supported the earlier records of Phillips (1986) and indicated that the presence of this species on El Potosi represented a "stable isolated relict population". The Flammulated Owl (*Otus flammeolus*) was also reported from El Potosi by Contreras-Balderas (1992b). While this was the second record for the state of Nuevo Leon, it was the first record of this species from El Potosi.

This current study was undertaken in order to provide a more extensive listing of the avifauna of the Cerro El Potosi region of Nuevo Leon and determine the residency status of each species as well as the ecological relationships of these species within the six different vegetational types established by the studies of García (1989) and García-Arana (1996).

Study Area

Cerro El Potosi is one of the highest peaks of the Sierra Madre Oriental with an elevation of 3713 meters. It is located ≈15 km WNW (24°52'17.09"N & 100°13'56.92"W) of the municipality of Galeana in the state of Nuevo Leon (Google Earth 2011a). While the base of El Potosi is considered tropical, the weather encountered at higher elevations is considered cold with the mean

temperature of the warmest month less than 10°C (INEGI 1986). The area is affected by violent high winds during most of the year and snowfall may occur during the months of December through February with accumulations noted on the north and northeast slopes of the mountain.

This study recognizes the six different vegetational types established by the studies of García (1989) and García-Arana (1996). The following vegetation types are: 1) *Quercus intricata* scrubland which occurs between 2300 to 2600 meters in elevation. 2) *Pinus cembroides* forest (4-6 m high) which occurs between 2000 and 2500 meters and is found in association with *Pinus arizonica,* along with some *Agave sp., Arbutus xalapensis, Quercus mexicana and Rhus virens.* 3) *Pinus ayacahuite* forest (8 to 12 m high) between 2500 to 3500 meters and is associated with *Pinus gregii, Pinus pseudostrobus*, *Pseudotsuga menziesii* and *Abies vejari.* 4) *Pinus hartwegi* forest (4-20m high) between 2900 to 3000 meters and is associated with *Lupinus cacuminus, Euphorbia furcillata, Senecio coahuilensis, S. hintoniorum* and *S. carnerensis.* 5) *Pinus culminicola* scrubland between 3100 and 3650 meters and is associated with *S. loratifolius, Lupinus cacuminus* and *Arracacia schneideri.* 6) Alpine prairie (10-20 cm high) between 3600 to 3713 meters and is characterized primarily by the presence of *Potentilla leonina, Arenaria sp., Astragalus purpusii, Linum lewisii* and *Trisetum spicatum.*

Methods and Materials

Avian censuses of the study site were conducted on a monthly basis during all of 1995. Observations followed a narrow range 30 km long linear transect which started at Dieciocho de Marzo (elev. 2045 meters; 24°53'19.37"N & 100°10'54.97"W) (Google Earth 2011b) and continued to the peak of the mountain (3713 meters). Additionally, multiple transects were established through all six vegetational types and monitored monthly. Visual observations were made with binoculars; each specimen was recorded along with time of observation, vegetational association and elevation.

All censuses were conducted during daylight hours. As a consequence, no nocturnal species were included in this study.

Results

A total of 4147 individuals were observed during this study; these represented seven orders, 28 families, 62 genera and 79 species (Table 1). Four species were recorded on the study area for only one or two observational periods; as such, it is not possible to determine their residency status. Couch's Kingbird (*Tyrannus couchii*) was observed in March and September; the Rock Wren (*Salpinctes obsoletus),* was observed on the lower part of the mountain during September; two individuals of the Long-billed Thrasher (*Toxostoma longirostre*) were observed in March and the Swamp Sparrow (*Melospiza georgiana*) was observed only once in June.

Discussion and Conclusions

This study documents the presence of 79 species of diurnal birds from Cerro El Potosi; 63 of these represent new species records for this mountainous region. The residency statuses of the species observed are: 41 permanent residents, 10 summer residents, three occasionals and two species of unknown status. Seasonal patterns of observation indicated that there were 53 species in summer, 47 in spring, 37 in fall and 33 in winter. The vegetational association is given for all but four species (Table 1). Noteworthy among this listing is the fact that two species observed during this study are of special concern. The Golden Eagle (*Aquila chrysaetos*) is listed as Threatened (SEMARNAT-2001) and the Maroon-fronted Parrot (*Rhynchopsitta terrisi*) is listed as Endangered (IUNC 2012). The documentation of the Swamp Sparrow (*Melospiza georgiana)* during this study increases the avifauna listing for the state of Nuevo Leon to 366 species.

Table 1. Avifauna present from Cerro El Potosí during 1995. Residency of each species (1=breeding, 2=migrants, 3=unknown). Vegetational association of each species (1=*Quercus intricata* scrubland, 2=*Pinus cembroides* forest, 3=*Pinus ayacahuite* forest, 4=*Pinus hartwegi* forest, 5=*Pinus culminicola* scrubland, 6=Alpine grassland and 7=all types of vegetation. Taxonomic assignments follow those of the AOU (1998).

	Residency	Vegetational association
ORDER FALCONIFORMES		
FAMILY CATHARTIDAE		
Cathartes aura (Zopilote aura; Turkey Vulture)	1	7
FAMILY ACCIPITRIDAE		
Accipiter cooperi; (Gavilán de Cooper - Cooper´s Hawk)	2	3
Parabuteo unicinctus; (Agulilla rojinegra - Harris´s Hawk)	1	5
Buteo jamaicensi;. (Agulilla cola roja - Red-Tailed Hawk)	1	7
Aquila chrysaetos; (Aguila real - Golden Eagle)	1	2,3,4,5 & 6
FAMILY FALCONIDAE		
Falco sparverius; (Cernicalo Americano - American Kestrel)	1	2,3,4,5 & 6
ORDER COLUMBIFORMES		
FAMILY COLUMBIDAE		
Columba fasciata; (Paloma de collar - Band-tailed Pigeon)	1	2,3,4 & 5
Zenaida macroura; (Paloma huilota - Mourning Dove)	1	1 & 2
Columbina inca; (Tortola colalarga - Inca Dove)	1	1
ORDER PSITTACIFORMES		
FAMILY PSITTACIDAE		
Rhynchopsitta terrisi; (Cotorra serrana oriental - Marron-fronted Parrot)	1	1,2,3 & 4
ORDER APODIFORMES		
FAMILY APODIDAE		
Aeronautes saxatalis; (Vencejo pecho blanco - White-throated Swift)	1	2, 3 & 4
FAMILY TROCHILIDAE		
Lampornis clemenciae; (Colibrí garganta azul - Blue-throated Hummingbird)	2	3
Archilocus colubris; (Colibrí garganta rubi - Ruby-throated Hummingbird)	2	3
Archilocus alexandri; (Colibrí barba negra - Black-throated Hummingbird)	1	2,3 & 4
Selasphorus platycercus; (Zumbador cola ancha - Broad-tailed Hummingbird)	2	1, 2, 3 & 4
ORDER PICIFORMES		
FAMILY PICIDAE		
Melanerpes formicivorus. (Carpintero bellotero-Acorn Woodpecker)	1	2, 3,4 & 5
Melanerpes aurifrons; (Carpintero cheje - Golden-fronted Woodpecker)	1	1, 2, 3 & 4
Picoides scalaris; (Carpinterillo mexicano - Ladder-backed Woodpecker)	1	7
Picoides villosus; (Carpintero velloso mayor - Hairy Woodpecker)	1	3 & 5

Table 1. Cont.

	Residency	Vegetational association
Colaptes auratus; (Carpintero de pechera - Northern Flicker)	1	7
ORDER PASSERIFORMES		
FAMILY TYRANNIDAE		
Contopus borealis; (Pibi boreal - Olive-sided- Flycatcher)	2	3
Contopus virens; (Pibi oriental - Eastern Wood-Pewee)	2	3
Empidonax sp. (Mosquero)	2	1, 2,3,4 & 5
Myiarchus tyrannulus; (Papamoscas tirano - Brown-crested Flycatcher)	2	1
Tyrannus couchii; (Tirano silbador - Couch´s Kingbird)	3	1
FAMILY HIRUNDINIDAE		
Hirundo rustica; (Golondrina tijereta - Barn Swallow)	1	1 & 2
FAMILY CORVIDAE		
Cyanocitta stelleri; (Chara crestada - Steller´s Jay)	1	2,3,4,5 & 6
Aphelocoma ultramarina; (Chara pecho gris - Mexican Jay)	1	1,2,3,4, & 5
Nucifraga columbiana; (Cascanueces Americano - Clark´s Nutcracker)	1	3,5 & 6
Corvus corax; (Cuervo comun - Common Raven)	1	7
FAMILY PARIDAE		
Poecile sclateri; (Carbonero mexicano - Mexican Chickadee)	1	3,4,5 & 6
FAMILY SITTIDAE		
Sitta carolinensis; (Sita pecho blanco - White-breasted Nuthatch)	1	3
Sitta pygmaea; (Sita enana - Pygmy Nuthatch)	1	2,3,4,5 & 6
FAMILY CERTHIDAE		
Certhia Americana; (Trepador Americano - Brown Creeper)	2	2,3 & 4
FAMILY TROGLODYTIDAE		
Salpinctes obsoletus; (Chivirin salta roca - Rock Wren)	3	2
Thryomanes bewickii; (Chivirin de cola oscura - Bewick´s Wren)	1	1 & 2
Troglodytes aedon; (Chivirin saltapared - House Wren)	1	1,2,3,4 & 5
FAMILY REGULIDAE		
Regulus calendula; (Reyezuelo de rojo - Ruby-crowned Kinglet)	2	1 & 2
FAMILY TURDIDAE		
Sialia sialis; (Azulejo garganta canela - Eastern Bluebird)	1	1
Sialia mexicana; (Azulejo garganta azul - Western Bluebird)	1	1,2,3 & 4
Turdus migratorius; (Mirlo primavera - American Robin)	1	3,4,5 & 6
FAMILY MIMIDAE		
Oreoscoptes montanus; (Cuitlacoche de chias - Sage Thrasher)	2	1
Toxostoma longirostre; (Cuitlacoche pico largo - Long-billed Trasher)	3	1
Toxostoma curvirostre; (Cuitlachoce pico curvo – Curved-billed Trasher)	1	1 & 2

Table 1. Cont.

	Residency	Vegetational association
FAMILY BOMBYCILLIDAE		
Bombycilla cedrorum; (Ampelis chinito - Cedar Waxwing)	2	2
FAMILY PTILOGONATYDAE		
Ptilogonys cinereus; (Caolineri gris – Gray Silky-flycatcher)	1	1 & 2
Phainopepla nitens; (Cardenal negro - Phainopepla)	1	1 & 2
FAMILY VIREONIDAE		
Vireo griseus; (Vireo ojos blancos – White-eyed Vireo)	1	2
FAMILY PARULIDAE		
Vermivora celata; (Chipe corona anaranjada - Orange-crowned Warbler)	2	1
Dendroica coronata; (Chipe coronado-Yellow-rumped Warbler)	2	7
Dendroica towsendi; (Chipe negro Amarillo - Towsend´s Warbler)	2	1
Mniotilta varia; (Chipe trepador – Black-and-white Warbler)	2	1
Wilsonia pulsilla; (Chipe corona negra - Wilson´s Warbler)	2	1
Myioborus pictus; (Chipe ala blanca - Painted Redstart)	1	1
FAMILY PEUCEDRAMIDAE		
Peucedramus taeniatus; (Ocotero enmascarado - Olive Warbler)	1	3,4,5 & 6
FAMILY THRAUPIDAE		
Piranga flava; (Tangará encinera - Hepatic Tanager)	1	2
Piranga rubra; (Tangara roja - Summer Tanager)	1	2
FAMILY CARDINALIDAE		
Cardinalis sinuatus; (Cardenal pardo - Pyrrhuloxia)	1	1
Pheucticus melanocephalus; (Picogordo tigrillo - Black-headed Grosbeak)	1	2
Guiraca caerulea; (Picogordo azul - Blue Grosbeak)	1	1
FAMILY EMBERIZIDAE		
Atlapetes pileatus; (Atlapetes gorr rufa - Rufous-capped Brush-Finch)	1	2
Pipilo erythropthalmus; (Toquí oriental - Eastern Towhee)	1	7
Pipilo fuscus; (Toqui pardo - Canyon Towhee)	1	1 & 2
Spizella passerina; (Gorrión ceja blanca - Chipping Sparrow)	1	1 & 2
Spizella pallida; (Gorrión pálido - Clay-colored Sparrow)	2	1
Spizella pusilla; (Gorrión pusila - Field Sparrow)	1	1
Spizella atrogularis; (Gorrión barba negra - Black-chinned Sparrow)	1	1
Melospiza melodia; (Gorrión cantor - Song Sparrow)	2	5 & 6
Melospiza georgiana; (Gorrión pantanero - Swamp Sparrow)	3	1
Junco hyemalis; (Junco ojo oscuro-Dark-eyed Junco)	2	1
Junco phaeonotus; (Junco ojo de lumbre - Yellow-eyed Junco)	1	7
FAMILY ICTERIDAE		
Sturnella magna; (Pradero tortilla con chile - Eastern Meadowlark)	1	1
Sturnella neglecta; (Pradero occidental - Western Meadowlark)	1	1
Quiscalus mexicanus; (Zanate mexicano - Great-tailed Grackle)	1	1 & 2
Molothrus aeneus; (Tordo ojo rojo - Bronzed Cowbird)	1	1
Icterus parisorum; (Bolsero tunero - Scott´s Oriole)	1	2
FAMILY FRINGILLIDAE		
Carpodacus mexicanus; (Pinzon mexicano - House Finch)	1	1
Carduelis pinus; (Jilguero pinero - Pine Siskin)	2	2, 3, 4, 5 & 6
Carduelis psaltria; (Jliguero dominico - Lesser Goldfinch)	1	1
Carduelis tristis; (Jilguero americano - American Goldfinch)	2	3

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GENERAL NOTES

ADDITIONAL RECORDS OF MAMMALS FROM THE SOUTHERN ROLLING PLAINS

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Mammal survey studies of Texas continue to reveal additional aspects as well as changes in distribution of the state's mammal fauna even after decades of sampling (Bailey 1905; Hall 1981; Schmidly 2004). This study presents new records documenting the presence of small mammal species in the Southern Rolling Plains. Voucher specimens, including: study skins, skeletal material, and tissue samples are deposited in the Abilene Christian University Natural History Collection (ACUNHC) and the Mammal Collection at McMurry University (MCMM). All but one of the bat records presented here are from wind turbine sites located in Callahan, Nolan, and Shackelford counties. For the description of the habitat in the counties reported here, see Hanson et al. (1998).

Scalopus aquaticus.–The eastern mole is common in the eastern two-thirds of Texas (Schmidly 2004). One specimen (ACUNHC 870) was collected in Callahan County, 10 km south of Putnam. The specimen is on the western edge of its range (Schmidly 2004). An additional record (ACUNHC 1540) is from Jones County, 9.4 km northeast of Hawley. A third specimen (MCMM 98) was collected 24 km south of Abilene in Taylor County. These specimens were found in sandy soils. Few records are known from the Southern Rolling Plains (Schmidly 2004). This species was previously recorded in Comanche, Erath, and Eastland counties to the east and Garza County to the west of this current study (Goetze 2000; 2009; Schmidly 2004).

Pipistrellus subflavus.–Until recently all records of this species were known from the eastern forests of Texas. *Pipistrellus subflavus*

is now known from many locations in western Texas (Schmidly 2004). New records for the species include one specimen (ACUNHC 1281) collected on the campus of Abilene Christian University (in Abilene) in Taylor County. An additional specimen (ACUNHC 1513) was found 9.3 km north of Clyde in Callahan County. A third specimen (ACUNHC 1533) was found 19.6 km northeast of Abilene in Shackelford County. The most proximal records for *P. subflavus* are from Lubbock and Tom Green counties (Schmidly 2004).

Lasiurus cinereus.–This species is known as a statewide migratory species through Texas (Cryan 2003). Specimens ACUNHC 1356 and 1357 were found 20 km east and 10 km south of Sweetwater in Nolan County. An additional specimen (ACUNHC 1529) was found 9.3 km north of Clyde in Callahan County. A third specimen (ACUNHC 1538) was found 19.6 km northeast of Abilene in Shackelford County. The most proximal record is in Coke County (Schmidly 2004).

Lasiurus borealis.–A specimen (ACUNHC 1512) was found 9.3 km northeast of Clyde in Callahan Co, and ACUNHC 1534 was found 19.63 km northeast of Abilene in Shackelford County. The most proximate records for this species are in Brown and Taylor counties (Campbell et al. 2002; Schmidly 2004).

Tadarida brasiliensis.–A specimen (ACUNHC 1542) was found 19.63 km northeast of Abilene in Shackelford County. *Tadraida brasiliensis* has been recorded in Callahan, Jones, Stephens, and Taylor counties west, east, south and southwest of Shackelford (Schmidly 2004).

Taxidea taxus.–A specimen (ACUNHC 1402) was taken 40 km north of Albany at the intersection of County Road 2548 and US 283 in Throckmorton County. The habitats of badgers are variable, but they are common in prairie and desert ecosystems (Schmidly 2004). This specimen was found 1 km north of a patch of cross timbers (post oak forest) in grassland habitat. The most proximal records of *Taxidea taxus* are in Brown, Taylor and Young counties (Goetze 2004; Hanson et al.1998; Schmidly 2004).

Peromyscus maniculatus.–One specimen (ACUNHC 957) was collected in the town of Hawley, Jones County. Fisher and Taylor counties adjacent to Jones County hold records for this species (Schmidly 2004).

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NOTES ON REPRODUCTION OF THE LITTORAL SKINK *EMOIA ATROCOSTATA* (SQUAMATA: SCINCIDAE) FROM OCEANIA

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The littoral skink, *Emoia atrocostata* (Lesson 1830) is widely distributed in Oceania and occurs in southern parts of Taiwan (Ota 1998) the Philippines, parts of the Malay Peninsula, Caroline

Islands, Indonesia, New Guinea, Cape York in northern Australia, Solomon Islands and Mariana Islands (Brown 1991). Knowledge of reproductive characteristics is important for assessing the feasibility of potential management programs. Information on reproduction of *E. atrocostata* from the Philippines indicates continuous breeding, with clutches of 1-3 eggs produced (Alcala & Brown 1967; Auffenberg & Auffenberg 1989). Cogger et al. (1983) reported that clutches of 1-2 eggs were produced on Christmas Island, Australia. Greer (1968), Brown (1991) and McCoy (2006) reported clutches of two eggs and Das (2011) reported clutches of 1-3 eggs. The purpose of this note is to provide additional information on the reproductive cycle of *E. atrocostata* based on a histological examination of museum specimens.

A sample of 45 *E. atrocostata* consisting of 27 adult males, 15 adult females and three juveniles, deposited in the Bernice P. Bishop Museum (BPBM) Honolulu, Hawaii, USA was examined. The *E. atrocostata* specimens were collected in the period 1962 to 2008 from the Caroline Islands, Irian Jaya, Malaysia, Northern Mariana Islands, Papua New Guinea and Solomon Islands.

Material examined.– Malaysia Pulau Jarak, (7895, 7896, 7898); Northern Mariana Islands: Guguan (26863), Saipan (26853), Tinian (31708); Caroline Islands: Yap (34541-34544); Irian Jaya (3722); Papua New Guinea: Milne Bay Province (16706-16710, 19967, 19969-19974, 19976-19979), Madang Province (11758, 13568), Morobe Province (13568), New Ireland Province (12015), West Sepik Province (13496-13501, 13503); Solomon Islands: Western Province, Choiseul Island (12774), Ranongga Island (12827-12830), Vangunu Island (12922, 12923), Uipi Island (12982).

The left gonad was removed from each skink and embedded in paraffin. Histological sections were cut at 5 μm and stained by hematoxylin followed by eosin counterstain (Presnell & Schreibman 1997). Enlarged follicles > 4 mm length and oviductal eggs were counted. Histology slides were deposited in BPBM. The

snout-vent length (SVL) of each specimen was measured from the tip of the snout to the posterior margin of the vent. An unpaired *t*-test was used to compare *E. atrocostata* male and female mean body sizes (SVL) utilizing Instat (vers. 3.0b, Graphpad Software, San Diego, CA).

There was no significant size difference between the males (mean SVL = 72.7 mm ± 8.4 SD, range = 53-88 mm), and females (mean SVL = 67.9 mm ± 6.6 SD, range = 57-88 mm) (unpaired *t*-test, $df = 40$, $t = 1.91$, $P = 0.063$). Juveniles averaged (SVL = 49.7 mm ± 1.5 *SD*, range = 48-51 mm). The smallest reproductively active male (spermiogenesis in progress) measured 53 mm SVL (BPBM 13500) and was collected in September. Two stages were noted in the testicular cycle: (1) spermiogenesis (= sperm formation), in which the seminiferous tubules were lined by clusters of sperm or clusters of metamorphosing spermatids; and (2) regressed, in which the seminiferous tubules were reduced in size and contain mainly spermatogonia and interspersed Sertoli cells. All males > 53 mm were undergoing spermiogenesis. These were by month: January: Papua New Guinea ($n = 3$); February: Solomon Islands ($n = 4$); March: Solomon Islands ($n = 1$); April: Papua New Guinea ($n = 7$), June: Northern Marianas 1, Malaysia 2 ($n = 3$); July: Caroline Islands 1, Northern Marianas 1 ($n = 2$); August: Caroline Islands, 1, Irian Jaya 1, Papua New Guinea, 1, ($n = 3$); September: Papua New Guinea ($n = 3$). One male from July (BPBM 7898) measured 48 mm SVL and contained a testis with regressed seminiferous tubules, so it was considered to be a juvenile.

Four stages were present in the ovarian cycle (Table 1): (1) quiescent, no yolk deposition present; (2) early yolk deposition, with basophilic yolk granules in the ooplasm; (3) enlarged ovarian follicles > 4 mm diameter; (4) oviductal eggs present in the oviducts. The smallest reproductively active females (2 follicles > 4 mm) each measured 64 mm SVL. They were (BPBM 13499), which was collected in September and (BPBM 31708), which was

Table 1. Monthly stages in the ovarian cycle of 15 *Emoia atrocostata*. *One female from September with enlarged follicles > 4 mm was undergoing concurrent yolk deposition in a smaller follicle for a subsequent clutch. *Emoia atrocostata* were from: C = Caroline Islands, NM = Northern Marianas, PNG = Papua New Guinea, S = Solomon Islands.

Month	*N*	Quiescent	Early yolk deposition	Enlarged follicles > 4 mm	Oviductal Eggs
January	3	1 (PNG)	1 (PNG)	1 (PNG)	0
February	1	1 (S)	0	0	0
March	2	0	0	1 (S)	1 (S)
April	3	1 (S)	0	1 (S)	1 (S)
May	1	0	0	0	1 (PNG)
July	2	0	0	1(NM)	1(C)
September	3	2 (PNG)	0	1*(PNG)	0

collected in July. One female from West Sepik Province, Papua New Guinea from September (BPBM 13499) contained enlarged follicles > 4 mm and was undergoing concurrent yolk deposition in a smaller follicle for a subsequent clutch, indicating *E. atrocostata* can produce multiple clutches in the same year. Nine females contained an invariant clutch size of 2.0 eggs. Two smaller females (BPBM 34543, SVL = 51 mm; BPBM 19967, SVL = 50 mm) contained quiescent ovaries and were considered as juveniles.

An extended reproductive cycle was reported for *E. atrocostata* by Auffenberg & Auffenberg (1989) in the Philippines, where the prolonged reproduction of *E. atrocostata* may be feasible because of the continuously warm, relatively aseasonal habitat occupied by this littoral species. Auffenberg & Auffenberg (1989) concluded sperm production in *E. atrocostata* in the Philippines was spread evenly through most of the year but did not record a minimum size of maturity in males.

Auffenberg & Auffenberg (1989) reported that the smallest mature female *E. atrocostata* from the Philippines measured 71 mm SVL. During this study, one female from West Sepik Province, Papua New Guinea (BPBM 13499) and one female from Tinian Island, Northern Marianas (BPBM 31708) were found that were

mature at 64 mm SVL, indicating females mature at a larger minimum size in the Philippines. An extended period of egg production was reported in the Philippines, as young were produced continuously during the year (Auffenberg & Auffenberg 1989). *Emoia atrocostata* produced small clutches of 1-3, usually two eggs, in the Philippines and no evidence of multiple clutches was reported, although it was believed to be possible (Auffenberg & Auffenberg 1989). Herein is provided histological evidence that multiple clutches likely are produced by *E. atrocostata.*

Information on reproduction in other species of *Emoia* skinks from Oceania reveals prolonged reproductive cycles and small clutch sizes of predominately two eggs (Baker 1947; Schwaner 1980; McCoy 2006; Goldberg & Kraus 2008) and the production of multiple clutches also appears frequently (Goldberg & Kraus 2008). An exception is *Emoia sanfordi*, endemic to the Vanuatu Archipelago, which produces clutches of 2-7 eggs (Hamilton et al. 2008).

While reproductive activity was exhibited (by either males, females or both) of *E. atrocostata* in all months sampled, (January to September), larger monthly samples from different parts of its range will be needed to ascertain geographic variations in its reproductive cycle.

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