

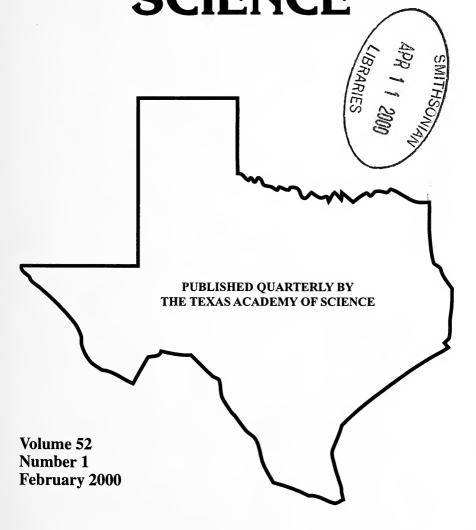






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#### FOSSIL CROCODYLIANS FROM THE EOCENE DEVIL'S GRAVEYARD AND CANOE FORMATIONS, BREWSTER COUNTY, TEXAS

#### Christopher A. Brochu

Department of Geological Sciences
University of Texas at Austin
Austin, Texas 78712
Present Address:
Department of Geology
Field Museum, 1400 S. Lake Shore Drive
Chicago, Illinois 60605

Abstract.—Fragmentary crocodylian remains from the Lower Eocene of west Texas represent at least three taxa; these are the extinct generalized taxon *Borealosuchus*, the terrestrial *Pristichampsus* and a derived alligatoroid closely resembling forms given the name *Allognathosuchus*. These remains are significant, as they preserve the youngest known occurrence of *Borealosuchus* and may be the southernmost known occurrence of *Allognathosuchus*.

Although most vertebrate paleontological work in the Eocene sequence of western Texas has focused on mammals (e.g., Wilson 1967; 1971; 1974; 1977; Wilson & Schiebout 1981; Gustafson 1986; Runkel 1988), nonmammalian tetrapods are known. These include crocodylians, turtles, squamates and lissamphibians (Wilson 1986; Runkel 1988). However, only one nonmammalian vertebrate has been described from the Eocene of this region, Pristichampsus, which may have been a terrestrial rather than semiaquatic predator (Busbey 1986). Material described herein derives from the Devil's Graveyard and Canoe Formations, both of which are exposed in Brewster County, Texas. The Devil's Gravevard Formation is a sequence of fluviolacustrine sandstones and mudstones interbedded with tuffs spanning the Uintan and Chadronian North American Land Mammal Ages (NALMAs; Stevens et al. 1984; Runkel 1988). The Canoe Formation is predominantly sandstone, and is correlative with the lower part of the Devil's Graveyard Formation (Runkel 1988). All localities from which crocodylians have been collected are regarded as early Uintan in age based on associated large mammals (Stevens et al. 1984; Wilson 1977; 1984; 1986; Wilson & Schiebout 1981) and K-Ar dates obtained from underlying and overlying volcanic deposits in the Devil's Graveyard Formation (Henry & McDowell 1986; Schucker & Nelson 1988).

Specimens described by Busbey (1986) and some of those described herein are derived from the Whistler's Squat Local Fauna, which refers to several localities in the Agua Fria region of Brewster County northwest of Big Bend National Park (Wilson 1986). Others are from assemblages and local faunas from elsewhere in Brewster County contemporaneous with Whistler's Squat Local Fauna (Runkel 1988).

The purpose of this report is to briefly describe the crocodylian taxa diagnosable from this unit based on material deposited in the Vertebrate Paleontology Laboratory, Texas Memorial Museum, Austin, Texas (TMM). One, *Pristichampsus*, has already been described from this area, representing a stratigraphic extension into the Uintan (Busbey 1986). A partial skeleton from the Canoe Formation indicates an extension into the Uintan for *Borealosuchus*, and a small alligatoroid of uncertain affinities (but closely resembling several blunt-snouted taxa from the North American Tertiary) can also be recognized.

#### SYSTEMATIC PALEONTOLOGY

EUUSUCHIA Huxley 1875 CROCODYLIA Gmelin 1789 Borealosuchus Brochu 1997

Referred material.—TMM 40146-6, associated vertebral centra, osteoderms, phalanges, teeth.

Occurrence.—Canoe Formation, Canoe A Local Fauna (Runkel 1988), White Amphitheater, Big Bend National Park, Brewster County, Texas.

Discussion.—The material catalogued under TMM 40146-6 was found in association and probably represents a single individual. The vertebral column is represented by procoelous mid-dorsal centra from which the neural arches have completely separated.

Among the osteoderms (Figure 1f) is the anterior ossification from a bipartite ventral element. Bipartite ventral osteoderms such as these are known in three separate crocodylian lineages: extant caimans, the European alligatoroid *Diplocynodon*, and *Borealosuchus*, a North American lineage including several taxa formerly classified as *Leidyosuchus* by Huxley (1859), Buscalioni et al. (1992) and Brochu (1997). By itself,

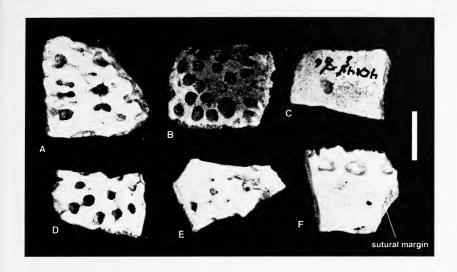


Figure 1. Osteoderm fragments (TMM 40146-6) from *Borealosuchus* sp. Item F is the anterior ossification from a bipartite ventral osteoderm; the sutural surface for its corresponding posterior element can be seen. Scale = 1 cm.

the isolated ventral element does not allow one to distinguish among these three lineages, but the remaining osteoderms, which include dorsal elements (e.g., Figure 1e), are unkeeled, arguing against affinities with both *Diplocynodon* and caimans. Given the absence of *Diplocynodon* in North America during the Tertiary, but the ubiquity of *Borealosuchus* in Lower Tertiary deposits of this continent, including the underlying Black Peaks Formation in Big Bend (Brochu 2000), identity with *Borealosuchus* appears most likely.

The anterior ventral element associated with TMM 40146-6 bears a single row of large pits immediately posterior to a broad, flat region that would have passed dorsal to the osteoderm immediately in front of it. In caimans and *Diplocynodon*, the anterior ossification usually bears small pits posterior to the imbrication zone, and these pits are not arranged in a single discrete row (e.g., Ludwig 1877: plate 14). However, a single row of large pits is characteristic of anterior ossifications in *Borealosuchus wilsoni* from the Lower Eocene of western North America. The nature of pits in *Diplocynodon* varies, and so caution must be observed when using this feature to diagnose ventral ossifications in crocodylians.

If these remains are from *Borealosuchus*, then they represent a stratigraphic extension of the taxon. The youngest previous occurrence of *Borealosuchus* is *B. wilsoni*, which ranges across the Wasatchian and Bridgerian NALMAs of the Bridger, Bighorn, and Green River Basins (Mook 1959; Bartels 1980; 1983; Brochu 1997). *Borealosuchus* has not been reported from units younger than the Bridgerian north of the Big Bend area.

#### PRISTICHAMPSINAE Kuhn 1968 Pristichampsus Gervais 1853

Referred specimens.—TMM 42952-113 (in part) teeth; TMM 42953-11, tooth. Many more dental, cranial, and mandibular specimens were listed by Busbey (1986).

Occurrence.—TMM 42952-113: Devil's Graveyard Formation, in an assemblage correlative with the Whistler's Squat Local Fauna (Runkel 1988), Dogie Mountain, Brewster County, Texas. TMM 42953-11: Canoe Formation, Canoe A Local Fauna (Runkel 1988), Crusher Big Yellow, Big Bend National Park, Brewster County, Texas. The specimens listed by Busbey (1986) were all from the Whistler's Squat Local Fauna.

Discussion.—The material described by Busbey (1986) includes cranial and mandibular remains as well as isolated teeth. This is important, as the flattened, serrated teeth characteristic of *Pristichampsus* and its presumed close relatives appeared independently in several other crocodyliform lineages (Benton & Clark 1988). The additional specimens listed here merely add to the number of formations and local faunas from which *Pristichampsus* has been found in the Uintan of Big Bend.

These remains are among the youngest reported for *Pristichampsus* in North America, which is otherwise best known from the Wasatchian and Bridgerian NALMAs of North America (Troxell 1925; Langston 1975; Golz & Lillegraven 1977; Bartels 1980; Gingerich 1989) and units correlative with the Bridgerian in Europe (Berg 1966; Kuhn 1938; Rauhe & Rossmann 1995; Efimov 1993; Rossmann 1998). Uintan pristichampsines have also been collected from California (Bramble & Hutchison 1971).

#### BREVIROSTRES von Zittel 1890 ALLIGATOROIDEA Gray 1844 GLOBIDONTA Brochu 1999

Referred material.—TMM 41576-7, fragments of left dentary and splenial; TMM 42952-113 (in part), isolated teeth.

Occurrance.—TMM 41576-7: Devil's Graveyard Formation, Whistler's Squat Local Fauna, Wax Camp, Brewster County, Texas; TMM 42952-113: Devil's Graveyard Formation, in an assemblage correlative with the Whistler's Squat Local Fauna (Runkel 1988), Dogie Mountain, Brewster County, Texas.

Discussion.—The rounded dorsal profile and stoutness of the symphysis (Figure 2a) suggests a placement deep within Alligatoroidea. The dentary bore a large fourth alveolus, but a small third. Alveoli posterior to the fourth are covered with matrix, but a natural mold of one of these alveoli, visible in cross section on the posterior surface of the specimen, indicates that they were very small. These are features consistent with members of Globidonta, which includes the crown-group Alligatoridae and a few of its closest extinct relatives such as Brachychampsa and Stangerochampsa; (refer to Brochu 1999). The relative sizes of the anterior alveoli are consistent with several extinct members of this assemblage, but are different from those of the horned alligatorid Ceratosuchus, in which the fourth alveolus is not much larger than the third (Bartels 1984).

The dentary symphysis extended back to approximately the same posterior extent as the sixth or seventh alveolus. The splenials met at the midline, and a distinct anterior foramen intermandibularis oralis is preserved (Figure 2a). Presence of a splenial symphysis rules out identity with the large alligatorid from the Paleocene Black Peaks Formation of Big Bend National Park (Brochu 1996), in which the splenials did not meet at the midline.

The second mandibular fragment represents a posterior segment of the left dentary (Figure 2b). The dorsal surface slopes anteriorly, and three alveoli are preserved. The splenial is not preserved, but the space for its attachment along the medial face of the dentary can be seen; the splenial nearly bordered the posteriormost of the preserved alveoli, and the dorsal splenial-dentary suture would have projected ventrally toward

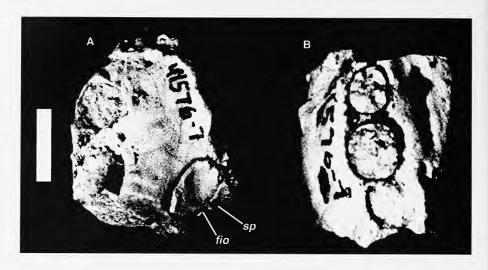


Figure 2. Fragments of left mandible from an alligatoroid (TMM 41576-7). (A) Symphyseal region of dentary and splenial. (B) Fragment of dentary probably comprising the tenth through twelfth alveoli. Abbreviations: fio - anterior foramen intermandibularis oralis; sp - splenial. Scale = 1 cm.

the symphysis. This fragment corresponds closely with the region of the dentary bearing the tenth through twelfth alveoli in *Allognathosuchus polyodon*, *A. wartheni*, *A. mooki* and *Wannaganosuchus brachymanus* (cf. Mook 1961; Case 1925; Simpson 1930; Erickson 1982); it is less similar with those of *Procaimanoidea* (cf. Gilmore 1946; Mook 1941; Wassersug & Hecht 1967) or basal members of *Alligator*, in which this region of the jaw is more elongate.

TMM 42952-113 comprises several isolated crocodylian teeth and osteoderms. Some of the teeth are flattened and serrated, and may be referred to *Pristichampsus* (see above), but two of them are dorsoventrally flattened and globular, closely resembling the enlarged teeth found in the back of the dentary and maxillary toothrows of several extinct alligatorids, such as *Allognathosuchus*, *Procaimanoidea*, *Hassiacosuchus*, *Ceratosuchus* and some extinct *Alligator*. Indeed, globular posterior teeth are also characteristic of the immediate sister taxa to Alligatoridae (Norell et al. 1994; Wu et al. 1996) and are very likely plesiomorphic at the level of the crown group (Brochu 1999).

These remains are most consistent with any of several taxa given the name Allognathosuchus. However, phylogenetic analysis of fossil alliga-

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torids (Brochu 1999) suggests the nonmonophyly of Allognathosuchus as currently used in the literature. As such, assigning this jaw to Allognathosuchus is premature; some taxa currently named Allognathosuchus (e.g., Allognathosuchus mooki) may be renamed in the future, and it is not currently known to which Allognathosuchus species the Texas alligatoroid is most closely related. Indeed, there are no characters in this specimen unambiguously placing it within the crown-group Alligatoridae. For these reasons, this specimen is not identified beyond the level of Globidonta. However, this material is biogeographically significant in representing the southernmost known occurrence of a blunt-toothed alligatoroid in the Tertiary of North America.

#### **ACKNOWLEDGMENTS**

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CAB at: cbrochu@fmppr.fmnh.org

#### AN ARCHAIC HUMAN BURIAL FROM YOAKUM COUNTY, TEXAS: THE CROSSROADS OF BIOARCHAEOLOGY AND FORENSIC ANTHROPOLOGY

#### Robert R. Paine and Patrick J. Lewis

Department of Sociology, Anthropology and Social Work Texas Tech University, Lubbock Texas 79409-1012 and Department of Biological Anthropology, Duke University Durham, North Carolina 27705-5000

Abstract.—A human burial from Yoakum County in northwest Texas was recently discovered and analyzed. Radiocarbon dating (ca.2940  $\pm$  40 B.P.) revealed this to be a late-Archaic period burial. The remains were identified as a female approximately 30 years old at the time of death. Skeletal evidence showed that she had suffered from mild non-life threatening health problems (i.e. slight osteoarthritic lesions of the back and hip). Dental examination indicated carious lesions and evidence of an early childhood nutritional deficiency. The only artifactual material recovered was red ochre. The burial is significant specifically because there is a lack of archaic period skeletal remains on the southern plains of west Texas. The lack of archaic burials limits the ability to draw specific conclusions concerning prehistoric Native American health for this time period and region.

Archaic human burials are seldom recovered on the Southern High Plains of Texas (Jurgens 1979; Owsley et al. 1989). In fact, less is known about the Archaic Period of the Southern High Plains than any other period (Holliday 1987:23). The lack of archaic burials for this region and the surrounding area is well illustrated by the work of Hartnady & Rose (1991). They examined the dental lesions of an archaic population from the Lower Pecos and for comparison purposes had to turn to the Indian Knoll archaic burial population (Hartnady & Rose 1991).

It is the intention of this study to provide paleopathological data recently obtained from the burial site found in Yoakum County, Texas; data that was recovered within the context of forensic anthropological services. A broader discussion of archaic burials from other southern plains localities is beyond the scope of this paper.

When a human burial with the potential of extreme antiquity was recently discovered on private property in Yoakum County, Texas, an extraordinary opportunity for study presented itself. At the time of discovery, Yoakum County had merely a single recorded archaeological site (41YK1), highlighting a lack of reported prehistoric material in the

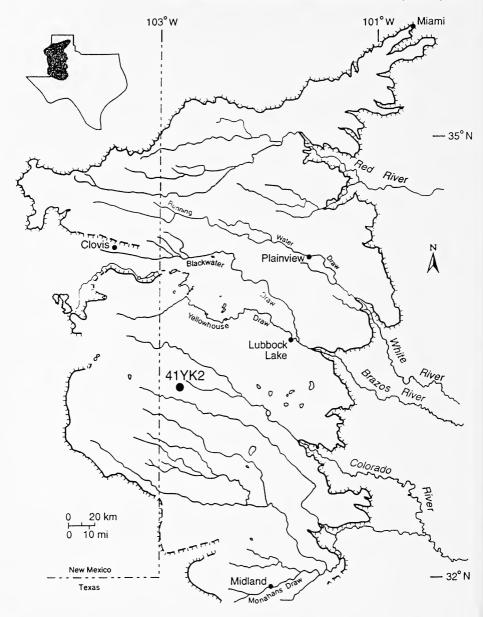


Figure 1. Southern High Plains map denoting the location of the Yoakum burial site (41YK2) (adapted from Holliday 1997).

region. The objectives of this study were to confirm the age of the remains through radiocarbon testing, compile a biological profile, and determine the state of health for the Yoakum County burial (41YK2).

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Forensic anthropological involvement with this burial began after a local artifact collector discovered the skeletal remains and reported the find to the Yoakum County law enforcement authorities. The Yoakum County criminal investigators were obligated to declare the site a crime scene until it could be shown to be otherwise. Subsequently, to determine the medicolegal significance of the remains the investigators contacted the Forensic Anthropology Lab at Texas Tech University, Lubbock, Texas, for assistance in an evaluation and recovery of the burial. Inspection of the site and examination of the dental morphology revealed that this burial was not of medicolegal importance. This was indicated by several criteria including: (1) the extreme occlusal wear of the teeth, typically seen in individuals who consume a course/gritty diet specific to prehistoric Americans; (2) the orientation the burial; (3) the extreme weathering of the bones indicative of prolonged exposure; (4) the presence of red ochre on the skeletal remains, and burned caliche and lithic debris in the immediate vicinity. Each of these criteria suggested that this was an archaeological burial rather than a recently deceased, modern individual.

The context of the burial, located in the sand dunes of west Texas (Fig. 1) suggested that it might be of archeological importance. Individual burials, some of extreme antiquity (Archaic & Paleoindian), have been reported in similar dune fields in western Texas (Brown 1979; Jurgens 1979; Holliday 1997), and as such, the burial from site 41YK2 warranted removal and analysis. Due to the threats of destruction that weathering and potential human disturbance imposed, immediate recovery of the burial was necessary. Local authorities aided in obtaining the landowner's permission for excavation and removal of the remains to the forensics lab at Texas Tech University. An agreement was made to ensure that the burial would be returned along with any artifactual material and reburied to a safe depth at its original location, and that a report of the findings would be provided to the Yoakum County Sheriff's department. The spatial distribution of the remains was recorded before the burial was removed and transported to Texas Tech University.

A site report and assessment of the remains were deemed appropriate due to the paucity of known archaic burials and the poor understanding of the Archaic Period on the Southern High Plains (Holliday 1987; Owsley et al. 1989). An assessment of the site, therefore, was performed and sent to the Texas Archeological Research Laboratory



Figure 2. A sketch of the Yoakum burial and the red ochre feature. The light gray shading shows the extent of the ochre stain, the dark gray shows ochre concentration. The skeletal elements are labeled: (1) atlas and axis; (2) scapula; (3) proximal humerus; (4) radius; (5) humeral shaft; (6) distal humerus; (7) right pelvis; (8) left pelvis; (9) sacrum; (10) proximal femur; (11) distal femur; (12) femoral shaft.

(TARL) for official archaeological site recording. Thus, this burial labeled 41YK2 has become the second recorded site in Yoakum County. The apparent lack of official sites in the county (n=2) is likely a result of a failure to recognize or record them rather than an actual absence of them. The apparent high frequency of artifact collecting by local avocational archaeologists in the region supports the notion of that there is considerable archaeological wealth for this area.

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#### SITE DESCRIPTION

The skeletal remains were found on the windward side of a large sand dune of the Lea-Yoakum dunefield. These anastomosing dunes are an eastward extension of the Mescalero Dunes that formed in the Pecos River Valley adjacent to the Southern High Plains escarpment (Holliday 1997). The burial setting indicated an Archaic Period age as the limited work within this dunefield suggests they are middle- to early-Holocene features (Johnson et al. 1986). The shifting nature of dune fields made stratigraphic correlation between the burial and nearby surface artifacts speculative. A test unit excavated near the burial exhibited uniform stratigraphy, and screening did not recover in situ artifacts. The ranch landmanager explained that the area surrounding the burial had yielded both Paleoindian and Archaic lithic artifacts. Burned caliche and lithic debris, primarily Edwards (outcrops found in central Texas) and Alibates chert (outcrops found in northern Texas Panhandle), were noted near the An ASM radiocarbon date performed by Beta skeletal material. Analytic lab on 10 grams of unexposed vertebral bone (sample #119926) returned a corrected age for the burial of ca. 2940 ± 40 years B.P. This places the burial in the late Archaic of the Southern High Plains, which extends from ca. 4500-2000 B.P. (Holliday 1987).

The exposed burial was discovered in a fetal position, laying on its right side, with the top of the cranium pointing east and the face looking north (Fig. 2). Due to severe weathering, most of the remains were extremely fragmented. The right side of the cranium, the right humerus, the proximal ends of the right forearm and femur, and several vertebrae were intact (Table 1). Most of the left side of the body, however, was missing or badly damaged.

A dark-red, grainy matrix appearing to be ochre was located at the mid-section of the burial (Fig. 2) and was the only artifactual material recovered. A preliminary analysis of the ochre preformed by Dr. B. L. Allen (Department of Plant and Soil Science, Texas Tech University) determined it to be hematite with a small amount of quartz. The red ochre probably originated from an outcrop of the Hickory Formation located near the Mason community of central Texas (Allen, pers. comm.), indicating either long range procurement of material or trade.

#### SKELETAL INVENTORY

Several cranial and post-cranial, partially intact bones from the right

Table 1. Cranial and postcranial elements available, excluding long bones.

CRANIAL BONES	<u>Left</u>	<u>Right</u>
Frontal		X
Parietal		X
Occipital		X
Temporal		X
TMJ		X
Maxilla	X	X
Palatine	X	X
Mandible		X
POSTCRANIAL BONES		
Clavicle	X	X
Scapula (body only)	X	X
Ribs	•	
1st	X	X
Os Coxae		
ilium	X	X
acetabulum	X	X
auricular surface	X	X
VERTEBRAE	CENTRA	NEURAL ARCHES
C1	X	X
C2	X	X
C7		X
T10		X
T11		X
T12		X
L2	X	X
L3	X	X
L4	X	X
L5	X	X
Sacrum	X	X
Vertebrae Grouped		
C3-C6	4	4
T1-T9		5

side of the body were suitable for analysis (Tables 1 and 2). Dental remains from the right side were also well represented allowing a detailed description of the teeth (Fig. 3). From the preserved elements, it was possible to construct a biological profile of the individual.

An age assessment was obtained by examining several criteria: the iliac articular surface wear pattern (Lovejoy et al. 1985; Buikstra & Ubelaker 1994); scoring the cranial anterior-lateral suture closure (Meindl & Lovejoy 1985; Buikstra & Ubelaker 1994); and estimating molar occlusal wear (Brothwell 1981; Hillson 1996). A general age estimation of 20-40 years was obtained using these criteria, with a narrower range of 25-35 years indicated once each criterion was cross-checked.

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Table 2. Long bone skeletal elements ava	ailable.
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Long Bones	Proximal Epiphysis	Proximal Third	Middle Third	Distal Third	Distal Epiphysis
Left Humerus				X	X
Right Humerus	X	X	X	X	X
Left Radius					X
Right Radius		X			
Right Ulna	X	X			
Left Femur	X	X			
Right Femur	X	X			
Left Tibia			X		

Since the skull is only partially represented by the right cranial bones (see Table 1), standard craniometric multivariate discriminant analyses (Giles & Elliot 1963; Giles 1970) used for sex determination are not possible. Therefore, sex determination was based on several quantitative and qualitative variables (Table 3). Qualitative indicators included the sciatic notch size, and supra-orbital ridge and margin (Buikstra & Ubelaker 1994; Bass 1995). The four quantitative measurements were taken: the mastoid length, femoral head diameter, humeral epicondylar breadth and glenoid fossa length. Each of these criteria taken as a group offers a convincing argument that this adult individual was a gracile person possessing feminine features. Hence, it is assumed that the remains are of an adult female.

#### HEALTH ASSESSMENT

Paleopathological assessment of the skeletal elements of this burial revealed that this female did not exhibit bony lesions that indicate long term or chronic health problems, which might be linked to her cause of death. Skeletal lesions found for this burial are indicative of health problems typical for archaic hunter and gatherers, who can be characterized as experiencing a very physically demanding way of life (Armelagos 1991; 1997).

A minor osteoarthritic problem of the lower back region is indicated in the lumbar and sacral bones by slight osteophytic lipping of the vertebral bodies (Kenndey 1989). Small periostitic lesions on the left femoral head and corresponding acetabulum also suggest a mild infection of the hip joint and the beginning of an osteoarthritic problem in this area as well (Mann & Murphy 1990).

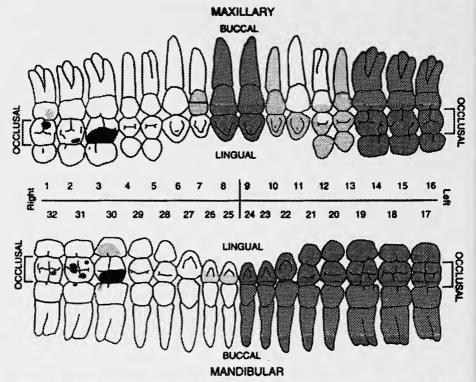


Figure 3. This diagram shows the dental remains recovered. Missing teeth are indicated with dark gray shading, black shading indicates caries, light gray shows weathering. The mandibular left central incisor exhibits an enamel hypoplasia line.

The teeth show moderate to extreme occlusal wear. The posterior teeth also exhibit large carious lesions (Fig. 3). These lesions are associated with dietary habits. In general, caries are the result of a bacterial build up from a sugar-based diet (Hillson 1996). The occlusal wear of the molars indicates a course diet that may have actually lessened the number and severity of the caries. High rates of dental caries have been seen for other Archaic populations in the southwest Texas region. Archaic populations (8000 to 1000 B.C.) of the Lower Pecos, for example, were found to have higher rates of caries than expected (Hartnady & Rose 1991). The high number of caries in these Archaic populations were suggested to be due to a high carbohydrate diet based on wild foods like sotal, prickly pear fruit and lechugilla (Hartnady & Rose 1991). These wild plants common the southwest Texas environment exhibit a sticky sugar-base diet that would account for the high frequency of posterior dental caries. These wild plants are most likely the major factor in the large number of carious lesions

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Table 3. Skeletal measurements taken from the right side of the body. (\* Indicates sexing criteria.)

Measurements	Length (cm)		
Cranial Length	17.2		
Cranial Height	12.9		
Orbital Height	3.4		
Orbital Width	3.4		
Mastoid Length*	2.5		
Palate Length	4.8		
Mandibular Ramus Height	6.2		
Symphysis Height	2.1		
Mandibular Ramus Breadth	3.3		
Lower 1st Molar Length	0.9		
Lower 1st Molar Width	1.2		
Femur Head Diameter*	3.8		
Humeral Length	26.6		
Humeral Midshaft Diameter	1.7		
Humeral Epicondylar Breadth*	5.1		
Ulnar Notch Height	1.8		
Sacral Wing Breadth	10.9		
Glenoid fossa Length*	2.6		

exhibited by the Yoakum burial posterior dentition.

There is also an enamel hypoplasia, of moderate size, located on the left central mandibular incisor (Fig. 3). Developmental dental enamel defects are often recognized as critical evidence for the state of general health in prehistoric populations (Hillson 1996). The hypoplasia likely occurred when her adult teeth were formed and is the result of a mineral/dietary inadequacy. This dietary inadequacy most likely occurred during weaning (Goodman & Armelagos 1988; Hillson 1996; Katzenberg et al. 1996). Other less-likely causes for this enamel defect include a genetic anomaly or a parasitic infection (Hillson 1996).

Paleonutritional and paleoepidemological data for the Yoakum County burial showed a health pattern indicative of a hunter/gatherer lifestyle (Armelagos 1991; 1997). This human health pattern is referred to as the first epidemiological transitional (Armelagos 1997). This is a health pattern commonly seen in small mobile populations that rely on natural foods and resources available in their habitat (Armelagos 1991). Archaic hunter and gatherers were prone to (endemic) low numbers of ill individuals at a given time, and did not usually encounter high frequencies of bacterial and/or viral infections (epidemic events). The Yoakum burial health profile is consistent with the conventional interpretation for lifestyles of the Archaic peoples on the Southern High

Plains provided by research from other sites in the region (Holliday 1987; 1997). The skeletal lesions and corresponding health problems of the Yoakum County burial can be explained as a result of typical physical activities associated with collecting and consuming of local wild plant foods over a difficult landscape.

#### SUMMARY

The analysis of the Yoakum County burial found an apparently healthy female of 25-35 years of age who lived during the Archaic Period on the Southern High Plains of Texas. The cause of death for this individual is undetermined. She was intentionally buried with a significant amount of red ochre that had been brought in from central Texas. Recent exposure of the burial due to the erosion of the sand dune exposed it to severe weathering and decomposition. The weathering destroyed many of the skeletal elements of the burial and may have lead to the loss of most of the artifacts originally interred with the remains.

Intensified surface investigations of 41YK2 would likely expand the boundaries of the site, and provide additional artifactual evidence (i.e. lithics) that may improve the present knowledge of prehistoric life in the Yoakum County area. With the numerous reports of Paleoindian and Archaic lithics, and burned caliche and lithic debris apparent in the vicinity, it is possible that a habitation site would be discovered. The deliberate positioning of the burial further suggests the possibility of other burials in the region. As the dunes continue to shift over time, erosion may uncover additional skeletal remains. Yet for the moment, continued exploratory excavation of the Yoakum County dunes is not recommended. Specifically, because archaeological sites should be conserved and not excavated simply for the sake of doing so.

Data gathered from the Yoakum County burial offered a rare opportunity to study the life-ways Archaic Period culture on the Southern High Plains. Few lines of evidence about past cultures are as revealing as skeletal analysis. With the cooperation of the landowner and local law enforcement authorities, critical anthropological information was gathered and the remains have been returned to their original resting-place at a depth sufficient to prevent any disturbance for many years. Continued cooperation between landowners, local authorities, avocational archaeologists and professional bioarchaeologists should continue to yield positive results and increase the understanding of prehistoric cultures.

#### **ACKNOWLEDGMENTS**

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RRP at: robert.paine@ttu.edu

## A REVISION OF THE LAND SNAIL HELICINA ORBICULATA (GASTROPODA: PROSOBRANCHIA) FROM THE SOUTHERN UNITED STATES

#### Ned E. Strenth and Thomas G. Littleton\*

Department of Biology, Angelo State University San Angelo, Texas 76909 \*Deceased

Abstract.—Frozen tissue samples from living specimens of two subspecies of the land snail *Helicina orbiculata* (Say) from Texas and Florida were subjected to standard horizonal starch gel electrophoretic analyses in the laboratory. This included sympatric populations of both subspecies from two collection localities in Texas. A computer analysis of the resulting enzymatic variation in all specimens examined supports the existence of only a single species. *Helicina orbiculata tropica* Pfeiffer 1852 is synonymized with the nominate *Helicina orbiculata* (Say 1818).

Resumen.—Muestras de tejido helado de especímenes vivos de dos subespecies de caracol terrestre *Helicina orbiculata* (Say) de Tejas y Florida fueron examinadas usando electrofóresis en gel de almidón. Este estudio ha incluido poblaciones simpátracas de dos subespecies de dos localidades en Tejas. Un análisis de computadora de la variación enzimática resultante de todos los especímenes examinados apoya la existencia de una sola especie. *Helicina orbicula tropica* Pfeiffer 1852 es sinónimo de *Helicina orbiculata* (Say 1818).

Two subspecies of the land snail *Helicina orbiculata* are currently recognized as ranging from the southeastern United States west to central Texas and south to northeastern Mexico (Pilsbry 1948; Fullington & Pratt 1974; Hubricht 1985). Specimens of these two subspecies are identified and separated solely upon differences in shell morphology. *Helicina orbiculata orbiculata* exhibits a typical aperture lip which is common in many terrestrial gastropods. *Helicina orbiculata tropica* exhibits a "bevelled thickening beyond the reflected lip" (Pilsbry 1948:1084). While *Helicina orbiculata tropica* was earlier considered to be an "ill defined race" (Pilsbry 1897:46; Pilsbry & Johnson 1898:2), Pilsbry (1948) later maintained the existence of the two subspecies based upon geographical distributions. He stated (p. 1084) that while there was "no hard and fast line between them", the morphological differences were in fact "correlated with geographic range" and "therefore of subspecific significance".

Fullington & Pratt (1974) more recently noted the sympatric occurrence of both morphological types or subspecies from the same area of southeast Texas as well as from Tennessee. Hubricht (1985) noted

similar findings in specimens from Texas and Kentucky. Fullington & Pratt (1974) further questioned the validity of maintaining the existing subspecific classification and suggested that the differences in morphologies upon which the two subspecies were separated were possible ecologically induced modifications.

The problem, as it currently exists, is that the validity of these two subspecific taxonomic entities remains under question by researchers in this area of scientific study. This investigation was undertaken in an effort to resolve this taxonomic problem through the use of more recently developed biochemical techniques.

#### METHODS AND MATERIALS

Living specimens conforming to the descriptions of *Helicina* orbiculata orbiculata and *Helicina* orbiculata tropica were collected from four locations in north, central and south Texas as well as from the type locality of *H. orbiculata* near Jacksonville, Florida. Eight specimens from each collection site were biochemically analyzed in the laboratory except for Dallas County and Wilson County near San Antonio. The collections from these two counties were mixed and contained an equal number of specimens conforming to the description of each of the two subspecies. Eight specimens with the reflected lip and eight specimens without this kind of lip were analyzed from each of these two collection sites. A single specimen of *Helicina chrysocheila* Binney collected from near Ciudad Mante in the state of Tamaulipas, Mexico, was selected as an outgroup.

All specimens were initially held in the laboratory without feeding for at least seven days. Specimens were then frozen in cryotubes in liquid nitrogen and later stored in an ultracold freezer at a temperature of -80°C. The shells of these specimens were then fractured and tissue samples removed, sonicated in distilled water (chilled) and then subjected to a series of electrophoretic analyses using standard horizonal starch gel techniques (Selander et al. 1971; Murphy et al. 1990). Scorable data for 10 loci (Table 1) were obtained and analyzed (Figure 1) using the BIOSYS-1 computer program (Swofford & Selander 1981). To determine genetic similarity, Nei's (1978) unbiased genetic identity was calculated. This measure of genetic similarity was chosen due to the small number of individual specimens examined. An unweighted pair group method using arithmatic averages (UPGMA) cluster analysis was then performed using Nei's unbiased genetic identity. The aperture

Table 1. Isozymes with optimal buffer system.

Isozyme (E.C. No.)	Abbreviation	Buffer
Aspartate aminotransferase (2.6.1.1)	AAT-1	Tris-Citrate pH 8.0
,	AAT-2	Tris-Citrate pH 8.0
Hexokinase (2.7.1.1)	HK	Tris-Malate pH 7.4
Isocitrate dehydrogenase (1.1.1.42)	IDH-1	Tris-Malate pH 7.4
222	IDH-2	Tris-Malate pH 7.4
Malate dehydrogenase (1.1.1.37)	MDH-1	Morpholine Citrate
, , , , , , , , , , , , , , , , , , , ,		pH 6.1
	MDH-2	Morpholine Citrate
		pH 6.1
Mannose phosphate isomerase (5.3.1.8)	MPI	Tris-Citrate pH 8.0
NAD(P)H dehydrogenase (quinone) (1.6.99.2)	NDO	Tris-Citrate pH 8.0
6-Phosphogloconate dehydrogenase (1.1.1.44)	6-PGD	Tris-Citrate pH 8.0
, and a second s		1

lips from the shells of all specimens analyzed were removed and deposited in lots as voucher specimens with the holdings of the Strecker Museum (SM) at Baylor University in Waco, Texas.

Material electrophoretically examined.—Type-locality E of Jackson-ville, Duval County, Florida, 18 February 1993, eight specimens w/o thickened lip (SM 32382-89); Cedar Hill (Ellowi Girl Scout Camp), Dallas County, Texas, 26 June 1993, eight specimens w/thickened aperture lip (SM 32390-97), eight specimens w/o thickened lip (SM 32398-405); 13 km N of Stockdale, Wilson County, Texas, 12 May 1992, eight specimens w/thickened lip (SM 32406-13), eight specimens w/o thickened lip (SM 32414-21); 5 km S of Weslaco, Hidalgo County, Texas, 18 April 1992, eight specimens w/o thickened lip (SM 32422-29); Loma Alta, Val Verde County, Texas, 10 June 1993, eight specimens w/o thickened lip (SM 32430-37). Helicina chrysocheila; 20 km N of Ciudad Mante, Tamaulipas, Mexico, 21 May 1992, one specimen (SM 32438).

#### RESULTS AND CONCLUSIONS

The results (Figure 1, Table 2) of this study reveal the presence of a very high degree of genetic similarity in all specimens of *Helicina orbiculata* analyzed from both Texas and Florida. Two of the 10 presumptive loci of *H. orbiculata* were monomorphic within the specimens examined. The populations of both Dallas and Wilson counties were found to be genetically very similar for the 10 loci examined (Table 2). Less than 1% variation was observed either between or

Table 2. Allele frequencies in specimens from Texas, Florida and Mexico.

	Hidalgo County	Val Verde County	Wilson County w/o L	Wilson County w/Lip	Duval County Fla.	Dallas County w/o L	Dallas County w/Lip	Helicina chrysocheild (outgrp)
n	8	8	8	8	8	8	8	1
Locus								
IDH-1								
A	.000	.000	.000	.000	.000	.000	.000	1.000
В	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000
IDH-2	.000	.000	.063	.000	.000	.000	.000	1.000
A B	1.000	1.000	.938	1.000	1.000	1.000	1.000	.000
HK	1.000	1,000	.,,,,	1.000	1,000	1.000	1.000	,,,,,
A	.875	.625	1.000	1.000	1.000	.750	.750	1.000
В	.125	.125	.000	.000	.000	.250	.250	.000
C	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.000	.000	.000	.000	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.000
F	.000	.250	.000	.000	.000	.000	.000	.000
AAT-1								
A	.000	.000	.000	.000	.000	.000	.000	1.000
В	1.000	1.000	1.000	.875 .000	1.000	1.000	1.000	.000
C D	.000 .000	.000	.000	.000	.000 .000	.000	.000	.000
E	.000	.000	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.125	.000	.000	.000	.000
	.000	.000	.000	.123	.000	.000	.000	.000
AAT-2	1.000	1.000	1.000	1.000	.500	1.000	1.000	1.000
A B	.000	.000	.000	.000	.000	.000	.000	.000
Č	.000	.000	.000	.000	.000	.000	.000	.000
Ď	.000	.000	.000	.000	.000	.000	.000	.000
Ē	.000	.000	.000	.000	.000	.000	.000	.000
F	.000	.000	.000	.000	.500	.000	.000	.000
6-PGD								
Α	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
NDO								
Α	.500	.500	.500	.500	.500	.500	.500	.000
В	.500	.500	.500	.500	.500	.500	.500	1.000
MDH-1								
Α	.500	.750	.563	.438	.750	.500	.438	.000
В	.375	.125	.438	.563	.250	.500	.563	.000
C	.000	.000	.000	.000	.000	.000	.000	1.000
Ď	.000	.000	.000	.000	.000	.000	.000	.000
E F	.000 .125	.000 .125	.000 .000	.000 .000	.000 .000	.000	.000	.000
	.123	.125	.000	.000	.000	.000	.000	.000
MDH-2	.750	.875	1.000	1.000	.875	1.000	1.000	.000
A B	.000	.000	.000	.000	.000	.000	.000	1.000
Č	.000	.000	.000	.000	.000	.000	.000	.000
Ď	.000	.000	.000	.000	.000	.000	.000	.000
Ē	.000	.000	.000	.000	.000	.000	.000	.000
F	.250	.125	.000	.000	.125	.000	.000	.000
MPI								
Α	.000	.000	.000	.000	.000	.125	.000	.000
В	1.000	.750	1.000	.875	1.000	.875	.750	.000
C	.000	.000	.000	.000	.000	.000	.000	1.000
Ď	.000	.000	.000	.000	.000	.000	.000	.000
E F	.000	.000	.000	.000	.000	.000	.000	.000
Г	.000	.250	.000	.125	.000	.000	.250	.000

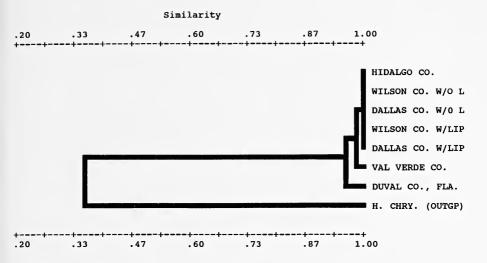


Figure 1. Phenogram of genetic similarity based upon electrophoretic analysis of specimens of *Helicina orbiculata* from Texas and Florida and *Helicina chrysocheila* from Tamaulipas, Mexico.

within these two populations which included an equal number of specimens conforming to the morphological descriptions of both subspecies. All Texas populations were found to be 98.5% similar or higher. The largest difference (1.5%) was observed in specimens from Val Verde County, which represents the westernmost boundary of the known distributional range of this species. The Florida specimens, which are located some 1400 km to the east of the Texas populations, were found to be similar at the 96.5% level.

The above results support the proposal that, while some degree of genetic variation exists between populations of *Helicina orbiculata* from Texas and Florida, there is no electrophoretic evidence supportive of maintaining the current subspecific classification within this species of land snail. The above demonstrated genetic similarity of specimens from Dallas and Wilson counties in conjunction with the reported sympatric occurrence of both morphological types from Texas, Tennessee and Kentucky (Fullington & Pratt 1974; Hubricht 1985) supports the existence of only a single species. *Helicina orbiculata tropica* Pfeiffer 1852 is hereby synonymized with the nominate *Helicina orbiculata* (Say 1818) with the following amended description.

#### Helicina orbiculata (Say 1818)

#### NEW SYNONYMY.

Olygyra orbiculata.—Say 1818:283.

Helicina orbiculata.—Gray 1824:70.—Binney 1851:352.—Pfeiffer 1852:375.—Binney 1865:108.—Singley 1893:311.—Pilsbry 1897:46; 1909:89; 1948:1082.—Strecker 1935:5.

Helicina tropica.—Pfeiffer 1852:374.—Singley 1893:311.

Helicina orbiculata tropica.—Pilsbry 1897:46; 1900:449; 1909:89; 1948:1084.—Pilsbry & Johnson 1898:2.—Pilsbry & Ferriss 1906:125.—Strecker 1908:65.—Baker 1926:39.—Fullington & Pratt 1974:8.—Hubricht 1985:3.

Oligyra orbiculata orbiculata.—Baker 1922:44.

Oligyra orbiculata tropica.—Baker 1922:44.

Helicina orbiculata orbiculata.—Baker 1926:40.—Fullington & Pratt 1974:8.

Type-locality.—East of Jacksonville, Duval County, Florida near the mouth of the river Saint Johns.

Amended description.—Shell aperture with or without expanded lip; many specimens exhibiting intermediate grades of thickening of bevelled lip development. The causative factor or factors responsible for the wide range of lip thickening in this species of land snail currently remains unresolved and in need of investigation.

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NES at: ned.strenth@angelo.edu

# EFFECTS OF THE AMERICAN SWALLOW BUG (OECIACUS VICARIUS) ON REPRODUCTIVE SUCCESS IN THE BARN SWALLOW (HIRUNDO RUSTICA)

## J. G. Kopachena, A. J. Buckley and G. A. Potts

Department of Biological and Earth Sciences Texas A&M University-Commerce Commerce, Texas 75428

Abstract.—The American swallow bug (Oeciacus vicarius) is a haematophagous ectoparasite that specializes on the cliff swallow (Hirundo pyrrhonota) as its host. During 1998, 41 of 166 barn swallow (Hirundo rustica) nests in northeast Texas were found infected with this parasite. This paper provides the first published account on the effect of O. vicarius on barn swallows. In general the effect parasitism was slight, being evident as reduced feather growth among broods of five young, but not among broods of four young. There was a slight, but not significant, tendency for broods of five to experience lower nesting success. These results suggest that O. vicarius was less detrimental to barn swallows than is often observed among cliff swallows. This could be a consequence of small colony sizes and wide nest spacing typical of barn swallows. These parameters may interfere with the ability of the parasites to establish populations in new colonies and to disperse within those colonies and may explain why O. vicarius is rarely associated with barn swallows.

Birds are hosts for several types of haematophagous ectoparasites. These parasites have been implicated as playing important roles in sexual selection (Moller 1991a), optimal clutch sizes (Moller 1991b), nest site selection (Brown & Brown 1986; Barclay 1988; Loye & Carroll 1998), and colony site selection (Brown & Brown 1986; Loye & Carroll 1991). However, the effects of ectoparasites on nestling growth and survival, and hence the importance of ectoparasitism in the evolution of avian reproductive strategies, seems to vary widely among and between host species. In some instances ectoparasites seem to have little or no effect. while in other instances ectoparasites appear to be detrimental to nestling growth and survival (e.g. Moss & Camin 1970; Brown & Brown 1986; Moller 1990; Burtt et al. 1991; Rogers et al. 1991; Johnson & Albrecht 1993; Richner et al. 1993). This observed variability in the effects of ectoparasites on their hosts emphasizes the need for additional research on the costs of parasitism and its influence on the reproductive biology of birds.

In North America, cliff swallow (*Hirundo pyrrhonota*) nests are frequently infected with the cimicid ectoparasite *Oeciacus vicarius* (American swallow bug). One alternate common name for *O. vicarius* is the "barn swallow bug". However, this is a misnomer, having arisen

from mistaking cliff swallows for barn swallows (*Hirundo rustica*) (Myers 1928). In fact, *O. vicarius* has been described as occurring almost exclusively on cliff swallows (Myers 1928; Usinger 1966; Loye 1985a) and the life history of this ectoparasite has evolved in close association with that of the cliff swallow (Smith & Eads 1978; Loye 1985a; Brown & Brown 1986).

The biology and life history of *O. vicarius* has been well studied (Myers 1928; Usinger 1966; Foster & Olkowski 1968; Loye 1985a). The nest cup is central to the host/parasite relationship and at any given stage of the host nesting cycle adults, nymphs and eggs of *O. vicarius* are likely to be present in the nest. Throughout their life cycle, the adult and nymphal stages of *O. vicarius* actively take blood meals from their hosts, especially at night. When inactive, the bugs hide in the nesting material or in cracks and crevices in and around the nest itself. They remain closely associated with the host nest where the adults and nymphs overwinter. There is a brief dispersal period in early spring when the bugs aggregate at the entrances of nests and attach themselves to adult swallows as they investigate the suitability of vacant nests as nesting sites. *Oeciacus vicarius* can be extremely long lived in the absence of the host and adults have been found alive in nests abandoned for three years (Loye 1985a).

The effect of *O. vicarius* on cliff swallow reproduction has been documented in Nebraska (Brown & Brown 1986; 1998), Oklahoma (Loye & Carroll 1991) and Texas (Chapman & George 1991). In Nebraska, Brown & Brown (1986) considered parasitism by *O. vicarius* to be a major cost of colonial nesting in cliff swallows. They found that parasitism by *O. vicarius* resulted in an average reduction in nestling body mass of 15% and a 50% decrease in nestling survivorship. In northeast Texas, Chapman & George (1991) showed that ectoparasites, primarily *O. vicarius*, resulted in a decrease in fledging success of 60%, a decrease in fledging mass of 9%, and a decrease in the length of the primary and rectrix feathers of 7% and 6% respectively. In Oklahoma, heavy infestations of *O. vicarius* led to the abandonment of nest and colony sites (Loye & Carroll 1991).

Data on the proportion of cliff swallow colonies infected with *O. vicarius* in different geographic areas have not been reported. Though broadly distributed within the range of the cliff swallow, *O. vicarius* is not found north of southern Canada or in the southeastern U.S. (Usinger 1966). Where it occurs, *O. vicarius* seems to be typical in colonies of

cliff swallows. It was described as the most "numerous and consistently present" ectoparasite of cliff swallows in Oklahoma where all of the study colonies were infected (Loye & Carroll 1991:224). Eight of 11 cliff swallow nests from a colony in Oklahoma were infected and harbored an average of 1779 O. vicarius per nest (Loye 1985b). Chapman & George describe O. vicarius as the most common ectoparasite in the nests of cliff swallows in north central Texas. In Nebraska, 28 percent of 706 nests were infected with O. vicarius (cf. Brown & Brown 1986). In Colorado, cliff swallow nests are reported to commonly contain "high population densities" of O. vicarius (cf. Smith & Eads 1978:26).

In contrast, *O. vicarius* is rarely observed in barn swallow nests (Usinger 1966; Smith & Eads 1978) and the effect of this parasite on barn swallow reproduction has not been reported. Myers (1928), who studied *O. vicarius* in California, never observed this species in the nests of barn swallows. He suggested that previous accounts of *O. vicarius* in the nests of barn swallows were based on cases in which cliff swallows had been mistakenly identified as barn swallows. There are only two subsequently published statements that associate *O. vicarius* with barn swallows. In his monograph, Usinger (1966) identifies the barn swallow as a host for *O. vicarius*, but no records are provided to substantiate this or indicate the extent to which barn swallows serve as a host species. Smith & Eads (1978:24) indicated that, in Colorado, *O. vicarius* has "on occasion [been found] in considerable numbers in barn swallow and bank swallow (*Riparia riparia*) nests".

During the current study at two colonies of barn swallows in northeast Texas, 41 nests were encountered that were infected with *O. vicarius*. This is of interest because, as described above, *O. vicarius* is not typically associated with barn swallows. Furthermore, the reproductive behavior and ecology of the barn swallow differs from that of the cliff swallow (Samuel 1971; Ramstack et al. 1998) in ways that could influence the host-parasite relationship. For example, cliff swallow colonies may contain up to 3000 densely spaced nests (Brown & Brown 1986), whereas barn swallows tend to nest in much smaller colonies with widely spaced nests (Snapp 1976). Differences in nest spacing and host population sizes could have a marked effect on parasite transmission between nests. Cliff swallows typically have only a single brood whereas barn swallows commonly have two broods per season (Samuel 1971). Barn swallows have larger broods than cliff swallows (Ramstack et al. 1998) and frequently re-use nests from previous broods (Barclay

1988). Larger clutches and double brooding should provide food for ectoparasites for a longer period of time during the nesting season, a factor that could influence ectoparasite population dynamics. Thus, the observations of *O. vicarius* in the nests of barn swallows provided a unique opportunity, first, to further document the occurrence of this ectoparasite in an alternate host species and, second, to evaluate the effect of this parasite on an alternate host. The purpose of this paper is to document *O. vicarius* in barn swallows, to describe how this species affected barn swallow reproductive success, and, where possible, to contrast these effects with published studies on the effect of *O. vicarius* on cliff swallows.

#### MATERIALS AND METHODS

Two barn swallow colonies were studied from 1 April to 3 August 1998. The north colony was in a concrete culvert 1.0 km south of the junction of Hwy 50 and Loop 178 in Commerce, Texas. This colony contained a maximum of 34 active nests on 11 May 1998. The south colony was located 1.3 km south of the north colony under two parallel two-lane bridges where Hwy 50 crosses the flood plain of the South Sulphur River. It contained a maximum of 44 active nests on 21 May 1998. Barn swallows at both colonies were double brooded. Thus, with second nests and replacement nests, 82 nests at the north colony and 84 nests at the south colony were studied.

For identification and to evaluate nest re-use, all nests were individually marked at the appearance of the first egg. Nests were monitored daily between 14:00 h and 17:00 h CDT from the time the first egg was laid until the last young left the nest. During these checks the number of eggs or young present in the nest were counted. These data were used to measure aspects of reproductive success and behavior. Hatching success was the number of eggs hatching. Hatch spread was the number of days between the hatching of the first egg and the hatching of the last egg. Fledging success was the number of young surviving to leave the nest. Fledging day was the number of days after the hatching of the first egg until the fledging of the last nestling.

Oeciacus vicarius was identified by Dr. Thomas Craig of the Texas Veterinary Diagnostic Laboratory at Texas A&M University – College Station from specimens collected at the south colony. Following the method described by Shields & Crook (1987) parasitism was quantified when the nestlings were weighed and measured (six and 12 days of age).

At these times the nest and nestlings were examined. Parasites were observed in or near the nesting material, in and among the feathers of the nestlings, and on their tarsi. Data were recorded on the number of *O. vicarius* observed. Estimating population sizes of *O. vicarius* in host nests is extremely difficult (Chapman & George 1991), however, because each nest and nestling was treated in exactly the same way, the number of bugs observed was used as a measure of the relative intensity of infection. It is important to note that, based on these criteria, some lightly infected nests were likely classified as uninfected. This is because *O. vicarius* typically rests within the nesting material and within cracks and crevices of the nest when not feeding (Myers 1928). Thus, in lightly infected nests, it is possible that none of the parasites present were feeding at the time the nestlings were examined.

Measurements of mass, tarsus length, the length of the right outer primary feather, the length of the right outer rectrix feather, and the length of the culmen were made when the nestlings were 6 and 12 days of age. Nestling mass was measured to the nearest 0.1 g using a 50 g spring scale. Tarsus length, primary length, rectrix length, and culmen length were all measured to the nearest 0.01 mm using digital callipers. The same observer made all measurements. Since parasitism can be influenced by nest density (Brown & Brown 1986) the nearest neighbor distance for each nest was measured to the nearest cm using the distance from the centers of the nest cups.

The statistical methods used are described in Sokal & Rohlf (1995) and were conducted using SAS (Version 6.12). Frequency data were evaluated using Chi-square contingency tables. All other data were tested for normality using the Shapiro-Wilk statistic (SAS Institute Inc. 1990). Two-sample comparisons with normally distributed data utilized t-tests and, where sample variances were unequal, the degrees of freedom were estimated using Satterthwaite's approximation (SAS Institute Inc. 1990). Two-sample comparisons with non-normal data were evaluated using Wilcoxon's Tests (Sokal & Rohlf 1995). Bivariate analyses of nestling measurements (normally distributed) were conducted using linear regression. Data on hatching success, hatch spread, fledging success, and fledging day were all non-normally distributed. For these variables, nests were assigned into three categories: no parasites observed (uninfected), one parasite observed (lightly infected), or more than one parasite observed (heavily infected). Comparisons were then made using Kruskal-Wallis Tests (Sokal & Rohlf 1995).

Since most of the infected nests occurred at the south colony, evaluation of the effects of O. vicarius infection on reproductive success and nestling condition were restricted to nests at that colony in order to eliminate potential among colony effects. There were no differences in the interpretation of results from nestling measurements collected on days 6 and 12. Therefore, for the sake of brevity, only data measured on day 12 are presented here. Furthermore, the effects of parasites on reproductive success and nestling growth can vary markedly according to clutch size (Moller 1991b). Therefore, analyses of these parameters were limited to the two most common clutch sizes (four and five egg clutches) and analyzed separately for each clutch size. Lastly, for both brood sizes, mean nestling mass was strongly correlated with hatch date (Linear Regression; broads of four; f = 51.53; df = 1, 31; P = 0.0001; broods of five; f = 5.00; df = 1, 16; P = 0.040). To control for this effect, mass comparisons between infected and uninfected nests were conducted on residual mass calculated after regressing mean nestling mass against hatch date.

#### **RESULTS**

The two colonies, though of similar size, differed markedly in the proportion of nests in which O. vicarius was observed. At the south colony 34 of 84 nests (40.5%) were observed to be infected, while at the north colony only seven of 82 nests (8.5%) showed signs of O. vicarius infection (2 by 2 Contingency Table;  $X^2 = 22.76$ ; df = 1; P < 0.0001). Nest density, measured using nearest neighbor distance, also differed between colonies. At the north colony the average nearest neighbor distance was  $2.01 \pm 1.03$  m while at the south colony it was  $2.94 \pm 3.58$  m (T-test; t = 2.30; df = 100.9; P = 0.024). At the south colony there was no difference between the average nearest neighbor distance of infected nests ( $2.32 \pm 2.37$  m) and the average nearest neighbor distance of uninfected nests ( $3.32 \pm 4.21$  m) (T-test; t = 1.40; df = 79.9; P = 0.165).

Of the 34 infected nests at the south colony, 21 were only lightly infected. One *O. vicarius* was observed at each of these nests. At three nests, only two *O. vicarius* were observed. At the remaining 10 nests, three, four, five, eight, 11, 12, 14, 15, 18 and 23 bugs were observed. Larger clutches of eggs had heavier infections (Fig. 1) and broods of five were more heavily infected than broods of four (Wilcoxon's Test; S = 1351; P = 0.019). For both clutch sizes there was no relationship between the number of *O. vicarius* observed and hatching success, hatch

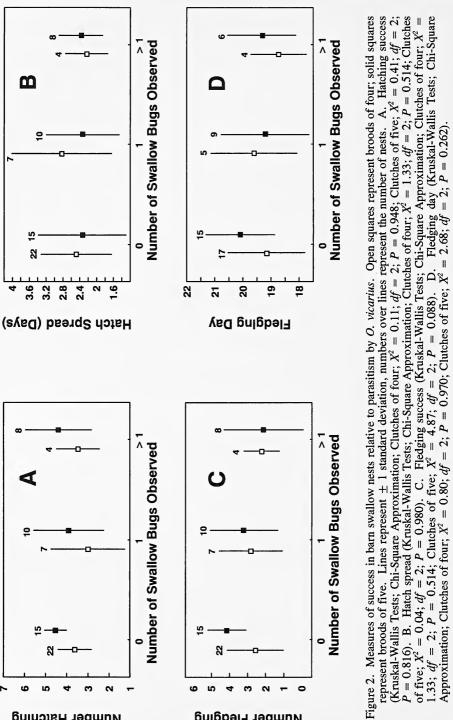


Figure 1. The relationship between clutch size and the mean number of O. vicarius observed. Lines represent  $\pm$  1 standard deviation, numbers over lines represent the number of nests. Larger clutches tended to be more heavily infected (Kruskal-Wallis Test; Chi-square Approximation;  $X^2 = 10.72$ ; df = 3; P = 0.013).

spread or fledging day (Fig. 2a, b, d). Among four-egg clutches there was also no relationship between the number of *O. vicarius* observed and nest success (Fig. 2c). Among five-egg clutches heavily infected nests tended to fledge fewer young, but this was not significant at the 5% level (Fig. 2c).

Residual day 12 mass was not found to be related to the level of infection for either brood size (Fig. 3). Among broods of four there were no significant trends relative to the number of *O. vicarius* observed and the lengths of the tarsus, primary, rectrix or culmen (Fig. 4a, b, c, d). Among broods of five the number of *O. vicarius* observed was unrelated to the length of the tarsus or the length of culmen (Fig. 4a, d). However, among broods of five, primary and rectrix feather lengths were shorter in more heavily infected nests (Fig 4b, c).

Number Hatching



Number Fledging

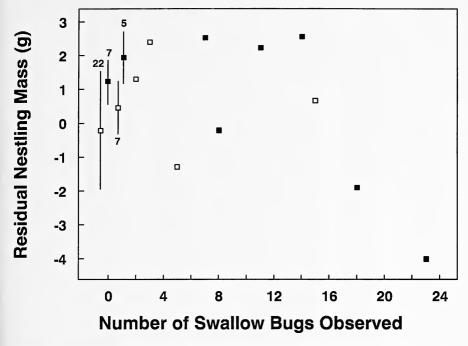
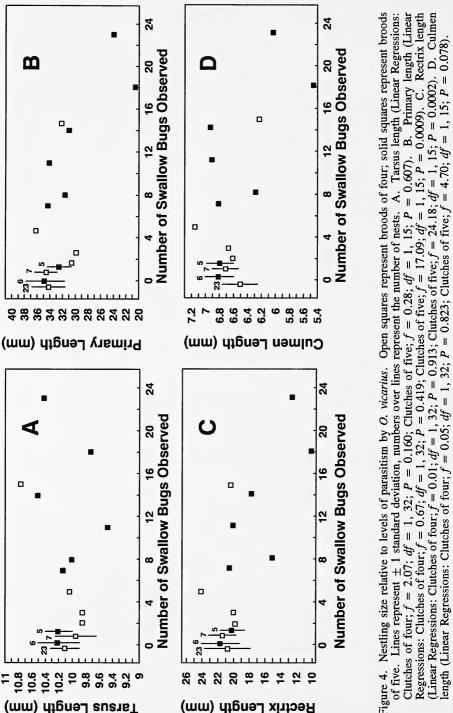


Figure 3. Relationship between residual nestling mass and parasitism by O. vicarius. Open squares represent broods of four; solid squares represent broods of five. Lines represent  $\pm$  1 standard deviation, numbers over lines represent the number of nests (Linear Regressions: Clutches of four; f = 0.38; df = 1, 31; P = 0.5062; Clutches of five; f = 19.48; df = 7, 9; P = 0.0005).

As a final analysis, the extent to which barn swallows avoided reusing infected nests was examined. Among both colonies 55 of 109 nests were re-used at least one time. The proportion of nests re-used was not lower at the more heavily infected south colony than it was at the less heavily infected north colony (Fig. 5) (2 by 2 Contingency Table;  $X^2 = 1.58$ ; df = 1; P = 0.100). At the south colony 61.9% of infected nests were re-used, while only 46.4% of uninfected nests were re-used. Thus, at the south colony, infected nests were actually more likely to be reused than uninfected nests (Fig. 5) (2 by 2 Contingency Table;  $X^2 = 4.21$ ; df = 1; P = 0.040).

#### DISCUSSION

Barn swallows are known to be hosts for a number of different ectoparasites (Moller 1994), but the American swallow bug (O. vicarius) has only rarely been found associated with this species and the effects of O. vicarius on the reproduction of barn swallows has not been documented.



Rectrix Length (mm)

Figure 4. Nestling size relative to levels of parasitism by O. vicarius. Open squares represent broods of four; solid squares represent broods of five. Lines represent ±

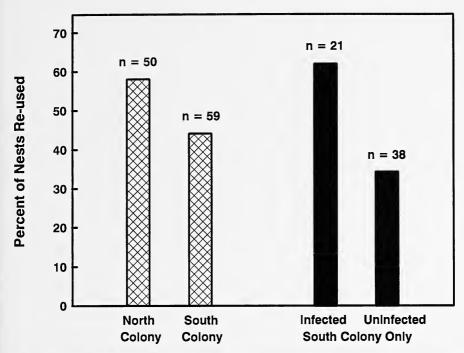


Figure 5. Percent of nests that were re-used. Hatched bars compare nest re-use between the north and south colonies. Solid bars compare nest re-use between infected and uninfected nests at the south colony.

A similar species, the European swallow bug (O. hirundinis), will occasionally infect nests of European barn swallows, but this parasite specializes on infecting the nests of sand martins (Riparia riparia) and house martins (Delichon urbica) (Rothschild & Clay 1957; Usinger 1966; Orszagh 1990; Christe et al. 1998). As is the case for O. vicarius, the effect of O. hirundinis on reproduction in the barn swallow has not been documented.

In the current study there was no evidence that parasitism was associated with differences in clutch size, hatch spread, or the time of fledging. There was little evidence that *O. vicarius* reduced nestling mass, tarsus length or culmen length. Nestlings did show signs of reduced feather growth, but this effect was limited to broods of five. There was also a slight, albeit insignificant, tendency for broods of five to suffer lower fledging success. These results were somewhat surprising since, in cliff swallows, *O. vicarius* has been associated with high levels of nestling mortality and dramatically reduced growth rates

(Brown & Brown 1986; Chapman & George 1991). Furthermore, on at least two occasions during the present study, nestlings in heavily infected nests were observed to die from apparent blood loss and general wasting. However, these heavily infected nests were exceptional, and barn swallow nestlings are prone to several other sources of mortality such as infanticide, starvation and nest falls (Shields & Crook 1987; Moller 1994). In the colonies studied here, nestlings also showed distress from heat, suggesting that this might also contribute to mortality and reduced mass. Frequent occurrences of nestling mass reduction and mortality associated with causes other than parasites would tend to obscure the effects of parasitism, especially if the parasitic effects were relatively slight. In addition, the methods used in this study may have caused some infected nests to be classified as uninfected. This type of error would diminish the perceived effect that parasitism had on the nestlings. Larger samples with more precise estimates of O. vicarius populations would help to define the effects of parasitism more precisely. However, it is also seems evident that, at the colonies studied here, O. vicarius did not have effects of the same magnitude as has been observed among cliff swallow colonies in Nebraska (Brown & Brown 1986) and Texas (Chapman & George 1991)

Though the data do not allow for direct comparison, the limited effects observed in this study seem to suggest that the levels of infection in the barn swallow nests were lower than those observed for cliff swallows where parasite populations in individual nests may number in the thousands. Several differences between cliff and barn swallow reproductive behavior and ecology might account for such a difference. Cliff swallow colonies may contain up to 3000 nests (Brown & Brown 1986), whereas barn swallows nest in much smaller colonies (Snapp 1976). Brown & Brown (1986) reported nest densities (based on total available substrate) among cliff swallows as ranging from about 0.5 nests/m to 15 nests/m. Measured in this way, the nest density of barn swallows in the current study was only 0.005 nests/m at the south colony. Brown & Brown (1986) found that O. vicarius only caused significant reductions in reproductive success in larger, denser, colonies where infection levels were much higher. Thus, observations during this study of only slight effects of O. vicarius infection among barn swallows could be a consequence of small colony size and low nest density, factors that tend to reduce the likelihood of nest colonization and spread by O. vicarius (cf. Brown & Brown 1986).

Unlike cliff swallows (Chapman & George 1991; Loye & Carroll 1991), parasitism by *O. vicarius* in barn swallows seemed to have no influence on nest site selection since infected nests were actually more likely to be re-used than uninfected nests. One possible explanation for this is that nests in preferred locations are more likely to be re-used (Shields 1984) and competition for such sites by numerous individuals might increase the likelihood their becoming infected. Thus, the pattern of nest infection observed in this study, might simply reflect heavy nest re-use among nests in favored locations and a lack of avoidance of infected nests by adults.

Given the relationship between density and level of infection among cliff swallows (Brown & Brown 1986) it is interesting that among the barn swallows there was no relationship between nest density within the south colony and *O. vicarius* infection. Furthermore, the south colony, which clearly had more infected nests had a much lower nest density than did the north colony. It is difficult to make conclusions on the basis of two colonies, but if *O. vicarius* infection is rare among barn swallows, then the distribution and relative abundance of *O. vicarius* within and among barn swallow colonies might be more a product of founder effects than of host density and host colony size. One obvious source of *O. vicarius* would be from nearby cliff swallow colonies. The nearest cliff swallow colony to the current study site is located on a bridge 20 miles to the northeast. This colony also supports a number of barn swallows. Given that barn swallows are known to switch between colony locations (Shields 1984), immigrants from this colony, or a similar colony, would be a plausible source of the parasites observed in this study.

Oeciacus vicarius is a haematophagous ectoparasite that specializes on the cliff swallow as its host. The high nest site fidelity, large colony sizes, and high nest densities exhibited by cliff swallows are important for the transmission and survival of O. vicarius (cf. Loye 1985a) and are, therefore, critical elements of the host/parasite relationship. Barn swallows also exhibit high nest site fidelity (Shields 1984; Barclay 1988) which should also make them good hosts for O. vicarius. In addition, barn swallows have larger broods than cliff swallows (Ramstack et al. 1998) and, unlike cliff swallows, will raise two broods per season (Samuel 1971; Ramstack et al. 1998), frequently re-using nests from the previous brood (Barclay 1988). These factors should make barn swallows even better hosts for O. vicarius by increasing the amount of food

available and by increasing the effective length of the nesting cycle. However, *O. vicarius* seems to have limited dispersal capability, hence the small colony sizes and low nesting densities typical of barn swallows may prevent this ectoparasite from fully exploiting the barn swallow as a host species. This, in turn, might explain why barn swallows are only rarely observed to be parasitized by *O. vicarius*.

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JGK at: Biojk@tamu-commerce.edu

## A VARIATION OF LINE INTERCEPT SAMPLING: COMPARING LONG TRANSECTS TO SHORT TRANSECTS

## Eric E. Jorgensen, Stephen Demarais and Tony Monasmith

708 S. Monte Vista, Ada, Oklahoma 74820
Department of Wildlife and Fisheries, Mississippi State University
Mississippi State, Mississippi 39762 and
Department of Range, Wildlife and Fisheries Management
Texas Tech University, Lubbock, Texas 79409

Abstract.—Line intercept transects are commonly used to measure vegetation although their length and number are variable among studies. Although line intercepts are used to compare other sampling techniques, few studies have investigated the effect of changing the number and length of transects on measurements. This study compared results from line intercept sampling with conventional 45 m transects to 4 m transects at 10 study sites in south-central New Mexico. In a plant community dominated by creosotebush (*Larrea tridentata*), tarbush (*Florensia cernua*), and bush mulhly (*Muhlenbergia porteri*) no differences were detected in measurements made with three 45 m transects compared to thirty 4 m transects or for six 45 m transects compared to one hundred 4 m transects (P > 0.05). However, sampling with the 4 m transects can be easily conducted by a single technician whereas the 45 m transects typically require a two person team. This study suggests that a substantial amount of sampling efficiency can be gained through use of short line transects with no loss in data quality regarding estimates of mean and variance.

Vegetation sampling is a critical component of research planning (e.g., Cook & Stubbendieck 1986). Notable among techniques is the line intercept, which is the method of choice for comparing other techniques (Cook & Stubbendieck 1986). Nonetheless, line intercept sampling is time intensive (Cook and Stubbendieck 1986, Higgins et al. 1996). This limitation is its greatest drawback.

Interestingly, although sampling techniques have been compared to one another (e.g., Cook & Stubbendieck 1986; Stohlgren et al. 1998) comparisons of results obtained from variations on the line intercept are few (Canfield 1941; Etchberger & Krausman 1997), despite the fact that number and length of transects is variable. Typically, short transects are more easily sampled and increase sampling efficiency. The time intensive nature of line intercept sampling may be substantially relaxed if short transects can be shown to produce results equivalent to long transects, especially if transects can be measured by a single investigator instead of a two person crew.

This study compared vegetation cover estimates determined with three to six 45 m line transects to estimates obtained from 30-100 to one

hundred 4 m transects in the Tularosa Basin of south-central New Mexico during the first two weeks of June, 1995. Vegetation measurements were made at 10 sites dominated by creosotebush (*Larrea tridentata*), tarbush (*Florensia cernua*) and bush muhly (*Muhlenbergia porteri*) within mixed desert scrub habitat.

#### MATERIALS AND METHODS

Each of 10 study sites was comprised of a 350 by 350 m plot (blocks). Within each plot, a 10 by 10 station sampling grid with 10 m sample spacing was centrally located forming a 100 by 100 m core area with 100 stations. Short (4 m) line intercept transects were measured at each sampling station within the core area. Transects formed an "x" shape centered at each sampling station with each oriented to a random azimuth and each arm of the "x" being 2 m long. Measurements with the short transects were made to the nearest 10 cm. For comparison, six 45 m long line intercept transects were sampled; three randomly located within the 100 by 100 m core area and three randomly located outside of the core area but within the 350 by 350 m research plot. Measurements with the long transects were made to the nearest 1 cm.

Measurements obtained with the short transects were compared to measurements obtained with long transects with paired t-tests (9 df). Two analyses are presented. The first compares cover estimates derived from the first 30 stations of short transect (120 m total) to the three long transects located within the core area (135 m total). The second compares cover estimates derived from all of the short transects (400 m total) to all of the long transects (270 m total). Analyses were conducted with Statistix (Analytical Software 1991).

## RESULTS AND DISCUSSION

Estimates of mean and error obtained with short line transects were indistinguishable from those obtained with long line transects for grass, creosotebush and tarbush cover using either thirty 4 m transects compared to three 45 m transects in, or one hundred 4 m transects compared to six 45 m line transects in mixed desert scrub habitat (Table 1). Also, it was noted that a single investigator using the 4 m transects measured the vegetation on 10 plots in 1.5 days ( $\approx 1.2$  person hours per plot or  $\approx 0.3$  person hours per 100 m of transect sampled). By comparison, in the same time a crew of two investigators measured the vegetation on four plots with 45 m transects ( $\approx 6.0$  person hours per plot or  $\approx 2.5$  person hours per 100 m of transect sampled). Therefore, the short transect technique is approximately five to eight times faster than the long transect technique in terms of person hours of effort.

Table 1. Cover estimates and errors obtained from long (45 m transects) and short (4 m transects) on 10 mixed desert scrub study plots in south-central New Mexico, June 1995. P represents probability of equality for estimates obtained by both estimate techniques (paired t-test, 9 df).

Cover variable	Technique (mean and error)				
	Long	SE	Short	SE	P
Test 1 135 m of Long transect 120 m of Short transect Grasses Larrea tridentata Flourensia cernua	34.7	3.7	31.7	1.9	0.418
	15.1	1.4	16.4	2.4	0.418
	5.7	1.8	5.3	2.0	0.684
Test 2 270 m of Long transect 400 m of Short transect Grasses Larrea tridentata Flourensia cernua	34.5	2.3	32.5	1.8	0.359
	17.6	1.7	17.8	1.7	0.852
	5.8	1.7	5.6	1.9	0.791

In contrast to the expectation that longer transects are needed in sparse vegetation (Canfield 1941), 4 m transects in this study sampled as well as 45 m transects for tarbush at less than 6% cover. Generally, transect length is chosen consistent with previous studies, as a matter of convenience, or in response to observed vegetation characteristics. Although any method can produce estimates of sampling mean and error, field techniques also need to be efficient. Technique selection depends upon the interaction of at least three factors: (1) logistical resources available, (2) statistical precision required and (3) ecological requirements (Krebs 1989; Higgins et al. 1996).

Line intercepts are conceptually equivalent to quadrat samples (Canfield 1941; Daubenmire 1968), they are quadrats with no width. In quadrat sampling, size and shape of quadrat are the two dimensions of concern (Krebs 1989). Generally, long thin quadrats are considered superior because they cross more vegetation patches (Daubenmire 1968; Krebs 1989). For instance, Bormann (1953) demonstrated that many small quadrats achieve greater precision for a given sample size than larger quadrats covering an equal area. Sampling theory holds that on average, estimate accuracy increases more rapidly by increasing plot number compared to increasing plot size (Daubenmire 1968). Thus, a large number of short line transects may produce equivalent results to a few long line transects with an improvement in sampling efficiency.

In part, the results observed in this study are attributable to the fact that 100 station sampling grids were already in place. Most or all of the sampling efficiency described herein would disappear if sampling grids

were set up for the sole purpose of measuring vegetation. However, concurrent studies frequently use grid positions (e.g., small mammal studies) and concurrent use of these positions for vegetation sampling would be an important efficiency.

This study was conducted in a typical habitat within the Chihuahuan Desert (Henrickson & Johnston 1986) and applied it to other research (Jorgensen 1996; Jorgensen & Demarais 1999; Jorgensen et al. 1995; 1998) with success. Although it is suspected that this technique will have wide applicability, this can only be determined by an examination of results from other biomes.

This study demonstrates that in cases where sampling grids are used (i.e., small mammal studies) substantial sampling efficiency can be gained with use of short line transects to measure vegetation. When logistic considerations limit both the number of personnel and time spent in the field, the efficiencies gained by use of short line transects make them attractive.

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EEJ at: Jorgensen.Eric@EPA.GOV

#### CENTROIDS IN UNITARY SPACES

#### Ali R. Amir-Moéz and Themistocles M. Rassias

Department of Mathematics and Statistics
Texas Tech University
Lubbock, Texas 79409-1042 and
National Technical University of Athens
Zografou Campus 15780
Athens, Greece

Abstract.—The centroid of the convex hull of a set of vectors in a unitary space is defined and its properties are studied. Notation of linear algebra is used (Amir-Moéz 1990).

Let  $\xi$  and  $\eta$  be two vectors (Fig. 1). Then the area of the parallegogram formed by these vectors is:

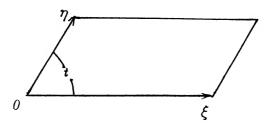


Figure 1.

$$A = \| \xi \| \| \eta \| \sin t$$

The square of A, that is,

$$A^2 = \|\xi\|^2 \|\eta\|^2 (1 - \cos^2 t)$$

implies

$$A^{2} = det \begin{pmatrix} (\xi, \xi) (\xi, \eta) \\ (\eta, \xi) (\eta, \eta) \end{pmatrix}$$

This determinant and its generalization are called Gram determinants.

Now let the origin be the centroid of a triangle and the vectors  $\propto$ ,  $\beta$ , and  $\gamma$  end on the vectices (Fig. 2). Then  $\propto + \beta + \gamma = 0$  and the three triangles formed by  $\{\propto,\beta\}$ ,  $\{\beta,\gamma\}$ , and  $\{\gamma,\infty\}$  have equal areas. This is equivalent to

$$det \begin{pmatrix} (\infty, \infty) & (\infty, \beta) \\ (\beta, \infty) & (\beta, \beta) \end{pmatrix} = det \quad \begin{pmatrix} (\beta, \beta) & (\beta, \gamma) \\ (\gamma, \beta) & (\gamma, \gamma) \end{pmatrix} = det \quad \begin{pmatrix} (\gamma, \gamma) & (\gamma, \infty) \\ (\infty, \gamma) & (\infty, \infty) \end{pmatrix}.$$

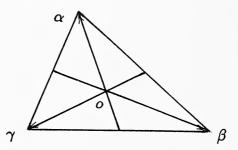


Figure 2.

There is also another property of the centroid, that is, for any point in the plane of the triangle, the sum of the squares of its distances from the vertices is minimum when the point is the centroid. These properties are generalized to a unitary (inner product) space.

Let  $\{\xi_1, \dots, \xi_n\}$  be a set of linearly independent vectors in a unitary space E. The centroid of the convex hull of  $\{0, \xi_1, \dots, \xi_n\}$ , is defined to be

$$\gamma = \frac{1}{n+1} \sum_{i=1}^{n} \xi_i \tag{1}$$

## **THEOREM**

Let  $\gamma$  be the same as (1). Then  $\frac{n+1}{n} \gamma = \mu$  ends in the centroid of the convex hull of the endpoints of  $\{\xi_1, \dots, \xi_n\}$ .

<u>Proof.</u> The hyperplane defined by  $\{\xi_1, \dots, \xi_n\}$  is

$$\xi = \sum_{i=1}^{n} p_{i} \xi_{i}, \sum_{i=1}^{n} p_{i} = 1$$
 (2)

From  $\mu = \frac{n+1}{n} \gamma$  one obtains that

$$\mu = \frac{1}{n} \sum_{i=1}^{n} \xi_i \tag{3}$$

It can easily be proved that  $\mu$  satisfies (3). Setting

$$\mu = \sum_{i=1}^{n} p_{i} \xi_{i}, \sum_{i=1}^{n} p_{i} = 1$$
 (4)

one obtains

$$\frac{1}{n} \sum_{i=1}^{n} \xi_{i} = \sum_{i=1}^{n} p_{i} \xi_{i}$$
 (5)

which implies

$$\sum_{i=1}^{n} \left( \frac{1}{n} - p_i \right) \ \xi_i = 0. \tag{6}$$

Since  $\{\xi_1, \dots, \xi_n\}$  is linearly independent (6) implies that

$$\frac{1}{n} - p_i = 0 \text{ for } i = 1, \dots, n,$$
 (7)

so

$$p_i = \frac{1}{n}, i = 1, \dots, n \text{ and } \sum_{i=1}^n p_i = n \left( \frac{1}{n} \right) = 1.$$
 (8)

## TRANSLATION TO THE CENTROID

Translating the centroid to the zero vector will simplify the study of it. Let  $\gamma$  be defined as (1).

Clearly  $\gamma = \frac{1}{n+1} \sum_{i=1}^{n} \xi_i$  implies that

$$\gamma + \sum_{i=1}^{n} (\gamma - \xi_i) = 0. \tag{9}$$

Now one can define

$$\eta_1 = \gamma, \eta_{i+1} = \gamma - \xi_i, i = 1, \dots, n.$$
(10)

So the centroid of  $\{\eta_i, \dots, \eta_{n+1}\}$  is at the zero vector.

# THEOREM (Least Square)

Let  $\{\eta_i\}$  be the same as (10) and  $\mu \in E$ . Then

$$\sum_{i=1}^{n=1} \| \eta_i - \mu \|^2$$
 (11)

is minimum if and only if  $\mu = \vec{0}$ .

Proof. It is clear that

$$\sum_{i=1}^{n=1} \eta_i = \vec{0} \tag{12}$$

One also observes that the inner product

$$(\mu, \sum_{i=1}^{n-1} \eta_i) = 0$$
 (13)

So (12) and (13) imply that

$$\sum_{i=1}^{n+1} \| \eta_i - \mu \|^2 = \sum_{e=1}^{n+1} \| \eta_i \|^2 + (n+1) \| \mu \|^2.$$
 (14)

Therefore (11) is minimum if and only if  $\mu = \vec{0}$ .

#### **THEOREM**

Let  $S = \{\eta_1, \dots, \eta_{n+1}\}$  be the same as in Section 2. Then the absolute value of the Gram determinant of  $\{\eta_{i1}, \dots, \eta_{in}\}$  is independent of  $i1, \dots, in$ , where  $\{i1, \dots, in\}$  is a subset of  $\{1, \dots, n+1\}$ 

Proof. Let

$$H_{in} = det \left( \frac{(\eta_{i1}, \eta_{i1}) \cdots (\eta_{i1}, \eta_{in})}{(\eta_{in}, \eta_{i1}) \cdots (\eta_{i1}, \eta_{in})} \right). \tag{15}$$

Let  $\eta$  be the member of S which is not in  $\{\eta_{i1}, \dots, \eta_{in}\}$ . Then it is clear that

$$\eta = -\sum_{k=1}^{n} \eta_{ik} . \tag{16}$$

Now adding the columns of (15) to the first, then adding the rows of the result to the first and considering (16), one obtains

$$H_{in} = det \left( \frac{(\eta, \eta) \cdots (\eta, \eta_{in})}{(\eta_{in}, \eta) \cdots (\eta_{in}, \eta^{in})} \right).$$
 (17)

which proves the theorem.

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ARA-M at: neaguirr@math.ttu.edu

# A NOTE ON EVENTUALLY COMPUTABLE FUNCTIONS

### T. G. McLaughlin

Department of Mathematics and Statistics Texas Tech University Lubbock, Texas 79409-1042

Abstract.—The importance of the class of computable (= recursive) functions is well known, both to mathematicians and to computer scientists. The class of numbertheoretic functions that are "combinatorial" (in the sense that all their Stirling coefficients are nonnegative) has been less widely studied, but nevertheless has played a key role in certain investigations involving computability. Computable functions may or may not be combinatorial, and vice-versa. Functions that have both properties are, naturally enough, termed computably combinatorial. A function f(x,y) is eventually computably combinatorial if there is a number n so that f(x+n,y+n) is computably combinatorial. This note will complete the catalog of relations between the notations "f(x,y) is combinatorial," "f(x,y) is eventually computably combinatorial," and "f(x,y) is computable," by showing that the first two of these notions, taken together, fail to imply the third. The argument proceeds by a classical diagonalization technique.

Let the nonnegative integers  $\{0,1,2,...\}$  be denoted by N. A function  $f: \mathbb{N}^{\kappa} \to \mathbb{N}$  is called *computable* if it can be coded as the input-output function of a Turing machine. Whether computable or not, f admits a unique representation in terms of its so-called *Stirling coefficients*  $c_{i1}..._{i\kappa}$ :

$$f(n_1, \dots, n_{\kappa}) = \sum_{i_1 \dots i_{\kappa}} \binom{n_1}{i_1} \dots \binom{n_{\kappa}}{i_{\kappa}}$$
. If all the  $c_{i_1 \dots i_{\kappa}}$  are  $\geq 0$ ,  $f$  is said to

be a combinatorial function (Myhill 1958; Nerode 1961; Dekker 1966). Combinatorial functions have a variety of pleasant features: they are closed under composition; they include many of the more important number theoretic functions such as x, x + y,  $x \cdot y$ ,  $(x + 1)^y$ , x!, projections, and the constant functions; and they are associated (Myhill 1958; Nerode 1961; Dekker 1966) with an interesting class of operators, called *combinatorial operators*, mapping k-tuples of subsets of N to subsets of N.

In addition to their relevance to certain questions in the foundations of Set Theory (Ellentuck 1969), a leading application of combinatorial functions and operators has been to the branches of computability theory known as theory of recursive equivalence types (Myhill 1958) and isol theory (Nerode 1961; Dekker 1966); a fairly extensive survey of the latter area has been given in (McLaughlin 1982). This application has

led to calling f computably combinatorial — c.c. for short — if f is both computable and combinatorial. One can further say that f is eventually c.c. if there exists a number n for which the function  $f(x_1 + n,...,x_k + n)$  is c.c. Eventually c.c. functions play a key role in isol theory, as docu-mented in chapter 12 of (McLaughlin 1982). The purpose of this note is to answer a question about these functions that was posed at the end of that reference. Specifically, on p. 366 of (McLaughlin 1982) it was asked: if  $f: \mathbb{N}^2 \to \mathbb{N}$  is both combinatorial and eventually c.c., must it be computable?

If k=1, then it is obvious that f(x) eventually c.c. implies f(x) computable; for this, f(x) need not be combinatorial (e.g., let g(x) be c.c. with g(0) < 10, and define f(0) = 10, f(n+1) = g(n)). More generally, suppose  $f: \mathbb{N}^k \to \mathbb{N}$  is "almost c.c.," in the sense that if h is any function obtained from f by replacing a proper subset of the variables of f by constants then h is eventually c.c. Then f is computable: this is easily shown by induction on k.

Things are quite different if  $k \ge 2$  and  $f: \mathbb{N}^k \to \mathbb{N}$  is merely assumed to be eventually c.c.: f may then be far from computable. For example, if one sets f(x+1,y+1)=x+y+2 for all x,y and defines f(0,y) and f(x,0) to be some arbitrary noncomputable functions of y,x respectively, then f is eventually c.c. but not computable. Similarly, if one chooses f(0,y) and f(x,0) to be computable but not combinatorial, with f(x+1,y+1)=x+y+2 as before, then, since constant functions are combinatorial and the combinatorial functions are closed under composition, f(x,y) will be eventually c.c. and computable, but not combinatorial.

Figure 1 displays the inclusion and noninclusion relations just discussed, where  $C_1$  is the class of c.c. functions,  $C_2$  the class of computable eventually c.c. functions,  $C_3$  the class of combinatorial eventually c.c. functions,  $C_4$  the class of eventually c.c. functions, and  $C^+$  denotes proper inclusion.

It seems natural to guess that Figure 1 is completed by the negative answer to the question, i.e.,  $C_3 \nsubseteq C_2$ . Such in fact is the case: it turns out that there is just enough "flex" in the relationship between the Stirling coefficients of g(x,y) and those of f(x+1,y+1) to enable one to construct f and g so that g(x,y) = f(x+1,y+1), g is c.c., f is combinatorial, and f is not computable. The construction is a case of "choosing f(0,y) and f(x,0)," as in the discussion above; but now the choice is not so trivial, since the combinatoriality of f(x,y) must be assured rather than discarded or ignored.

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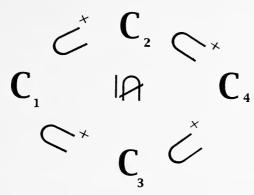


Figure 1. Previously documented inclusions and noninclusions between classes of c.c. and eventually c.c. functions.

Theorem. There exist functions  $f: \mathbb{N}^2 \to \mathbb{N}$  that are combinatorial and eventually c.c., but not computable.

*Proof.* Let doubly indexed sequences  $\langle a_{ij} \rangle$  of elements of N be understood to be arranged in the usual "Cantor ordering":  $a_{00}$ ,  $a_{01}$ ,  $a_{10}$ ,  $a_{02}$ ,  $a_{11}$ ,  $a_{20}$ , .... Clearly,  $h: \mathbb{N}^2 \to \mathbb{N}$  is computable if and only if its sequence  $\langle a_{ij} \rangle$  of Stirling coefficients is computable. One aims to define sequences  $\langle c_{ij} \rangle$  and  $\langle d_{ij} \rangle$  so that  $\langle c_{ij} \rangle$  is computable,  $d_{ij} \geq 0$  for all  $i, j, \langle d_{ij} \rangle$  is not computable,

$$g(x,y) = \sum c_{ij} {x \choose i} {y \choose j}, f(x,y) = \sum d_{ij} {x \choose i} {y \choose j}, \text{ and } g(x,y) = f(x+1, y+1).$$

As for  $\langle d_{ij} \rangle$  being noncomputable, it will clearly suffice to make sure that the subsequence  $d_{00}$ ,  $d_{01}$ ,  $d_{02}$ ,  $d_{03}$ , ... is noncomputable. All this can be arranged by taking advantage of the fact that in the equation

$$\sum c_{ij} {x \choose i} {y \choose j} = \sum d_{ij} {x+1 \choose i} {y+1 \choose j},$$

at least one "new"  $d_{ij}$  (i.e., a  $d_{ij}$  not occurring in any of the corresponding equations for  $\langle x', y' \rangle \langle \langle x, y \rangle \rangle$ ) will occur and is free to have its value assigned; while if x=0 then exactly two "new"  $d_{ik}$ 's will occur, namely,  $d_{0,y+1}$  and  $d_{1,y+1}$ . Let  $\zeta_0$ ,  $\zeta_1$ ,  $\zeta_2$ , ... be a listing of all computable sequences of 0's and 1's. One notes that if a sequence  $\langle a_{ij} \rangle$  of Stirling coefficients is computable and consists entirely of 0's and 1's, then there will be some  $\zeta_k$  such that  $\zeta_k (\frac{(i+j)(i+j+1)}{2} + i) = 0$  if  $a_{ij} = 0$  and  $\zeta_k (\frac{(i+j)(i+j+1)}{2} + i) = 1$  if  $a_{ij} = 1$ ; this simply reflects the identification of the pair  $\langle i,j \rangle$  with the number  $\frac{(i+j)(i+j+1)}{2} + i$  under the Cantor ordering.

To start the construction, one notes that  $g(0,0) = c_{00}$  and  $f(1,1) = d_{00} + d_{01} + d_{10} + d_{11}$ . Set  $c_{00} = 2$ . If  $\zeta_0(0) = 0$ , set  $d_{00} = 1$ ; otherwise, set  $d_{00} = 0$ . If  $\zeta_1(1) = \zeta_1 \frac{(0+1)(0+1+1)}{2} + 0 = 0$ , set  $d_{01} = 1$ ; otherwise, set  $d_{01} = 0$ . Then the value of  $d_{00} + d_{01}$  is 0, 1, or 2. Set  $d_{10} = 2 - (d_{00} + d_{01})$  and  $d_{11} = 0$ . Then g(0,0) = f(1,1), and one has prevented either  $\zeta_0$  or  $\zeta_1$  from witnessing computability for the sequence  $d_{00}$ ,  $d_{01}$ ,  $d_{02}$ , .... This completes Step 1.

Now suppose, carrying the construction forward, that one has effectively computed  $c_{00}, \ldots, c_{xy}$ , and have noneffectively assigned values to  $d_{00}, \ldots, d_{x+1+,y+1}$ , in such a way that g(x',y') = f(x'+1,y'+1) for  $\langle x',y' \rangle \leq \langle x,y \rangle$ ; and suppose that in doing this, one has effectively kept track of the maximum value  $m_{ij}$  that may have been assigned to  $d_{ij}$ ,  $\langle i,j \rangle \leq \langle x+1,y+1 \rangle$ . Let  $\langle x_0,y_0 \rangle$  be the next pair in the Cantor ordering following  $\langle x,y \rangle$ . There are two cases.

Case 1.  $\langle x_0, y_0 \rangle \neq \langle 0, x + y + 1 \rangle$ . Then  $\langle x_0, y_0 \rangle = \langle x + 1, y - 1 \rangle$ , and in  $\sum d_{ij} \binom{x+2}{i} \binom{y}{j}$  there will appear at least one "new" (i.e., not yet value-assigned)  $d_{ij}$ , namely,  $d_{x+2,y}$ . Using one's knowledge of the  $m_{ij}$ 's, one can now *effectively* compute a (nonnegative) value to assign to the "new" term  $c_{x+1,y-1}$  in the sequence  $c_{00}$ , ...,  $c_{x_0,y_0}$ , in such a way that if one distributes nonnegative values appropriately among the "new"  $d_{ij}$  appearing in  $\sum d_{ij} \binom{x+2}{i} \binom{y}{j}$  then one will have

$$\sum c_{ij} {x_0 \choose i} {y_0 \choose j} = \sum c_{ij} {x+1 \choose i} {y-1 \choose j} =$$

$$\sum d_{ij} {x+2 \choose i} {y \choose j} = \sum d_{ij} {x_0+1 \choose i} {y_0+1 \choose j};$$

i.e.,  $g(x_0, y_0) = f(x_0 + 1, y_0 + 1)$ . Obviously, this can be done in such a way that, once again, one has effective knowledge of the *maximum* value  $m_{ij}$  that may have been assigned to any  $d_{ij}$ .

Case 2.  $\langle x_0, y_0 \rangle = \langle 0, x+y+1 \rangle$ . Here one proceeds in essentially the same way as in Case 1; however, one can take advantage of the fact that there are now exactly two "new"  $d_{ij}: d_{0,x+y+2}$  and  $d_{1,x+y+2}$ . Let  $\zeta_w$  be the first term of  $\zeta_0$ ,  $\zeta_1$ ,  $\zeta_2$ , ... that has not yet been "spoiled," and set  $d_{0,x+y+2} = 1$  if  $\zeta_w$   $(\frac{(x+y+2)(x+y+3)}{2} + 0) = 0$ ,  $d_{0,x+y+2}$ 

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= 0 otherwise, thus spoiling  $\zeta_w$  as a candidate to witness computability of  $d_{00}$ ,  $d_{01}$ , ...,  $d_{0,x+y+2}$ , .... Using one's knowledge of the numbers  $m_{ij}$  from the previous step, one effectively assigns to  $c_{0,x+y+1}$  a nonnegative value sufficient to ensure that, once one has (noneffectively) specified a suitable corresponding nonnegative value for  $d_{1,x+y+2}$ , one will again have

$$\sum c_{ij} {x_0 \choose i} {y_0 \choose j} = \sum d_{ij} {x_0 + 1 \choose i} {y_0 + 1 \choose j};$$

i.e.,  $g(x_0, y_0) = f(x_0 + 1, y_0 + 1)$ .

That completes the construction. All  $c_{ij}$  are  $\geq 0$ , and the sequence  $\langle c_{ij} \rangle$  is computable (since one keeps effective track of the  $m_{ij}$ 's as the construction proceeds); hence g is c.c. All  $d_{ij}$  are  $\geq 0$ , so that f is combinatorial; and g(x,y)=f(x+1,y+1) for all x and y, so that f is eventually c.c. Finally, every  $\zeta_r$  is eventually "spoiled," via Step 1 and Case 2 of the general step; so f is not computable. The proof is complete.

*Remark.* To establish the same theorem in general, i.e., for  $f: \mathbb{N}^k \to \mathbb{N}$  for any  $k \ge 2$ , one needs, in essence, only to "complicate notation."

Postscript. The reader may wonder why the same straightforward procedure used to produce eventually c.c. functions that are computable but not combinatorial was not used for the theorem: let, say, f(x+1), y+1) = x+y+2 and define f(x,0) and f(0,y) "carefully," so as to make f an example of the theorem. The answer, at least as regards x + y, is that in order to ensure non-computability of f, one needs to define (say) the sequence  $d_{00}$ ,  $d_{10}$ ,  $d_{20}$ , ... in such a way that  $d_{i0} > 0$  holds for infinitely many i. But this negates the possibility of having f(x+1,1) =P(x+1,1), where P(x,y) is a polynomial with coefficients in N. (Starting at x+n,y+n instead of x+1,y+1 doesn't help, relative to the class of polynomials.) The author is unaware of any "textbook" c.c. function g(x,y) that would work in this way, certainly none that would obviously do so. Perhaps something like (x+y)!, or  $(x+1)^y$  with f(x+n,y+n)called upon to be c.c. for some n > 1, could be made to work; but if so, defining the  $d_{ii}$  would appear to be a bit of a serious exercise in its own right. Constructing f and g together, as done here, looks like the shortest path to a proof.

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TGM at: a3tgm@ttacs.ttu.edu

## **GENERAL NOTES**

# AN INEXPENSIVE METHOD TO AVOID TAIL DAMAGE TO KANGAROO RATS (*DIPODOMYS* SPP.) WHEN USING SHERMAN TRAPS

# J. Jeffrey Root\*, Nicole L. Marlenee, Eric E. Jorgensen and Stephen Demarais\*\*

Department of Range, Wildlife and Fisheries Management Texas Tech University, Lubbock, Texas 79409 Current addresses:

\*Department of Microbiology, Colorado State University Fort Collins, Colorado 80523 and \*\*Department of Wildlife and Fisheries, Mississippi State University Mississippi State, Mississippi 39762

The tails of long-tailed rodents occasionally are injured or severed when a sprung trap door comes into contact with them. This injury, which commonly occurs to long-tailed rodents (e.g., *Dipodomys* spp.) when caught in box live traps, is a methodological problem. Tail injury may cause trap-shy behavioral changes in capture probabilities (White et al. 1982) which could influence the results of mark-recapture studies. Additionally, a severed appendage may shorten the life span of some small mammals (Pavone & Boonstra 1985). Further, morphologically damaged specimens are less desirable for museum collections. Finally, concerns for humane treatment of animals warrant that the least harmful sampling technique be chosen (Schmidt 1990).

A simple solution to avoid tail injuries is to use larger traps when sampling for long-tailed rodents. However, this may not always be a feasible alternative. With this in mind, an inexpensive method which significantly reduces the frequency of tail injuries (without disfiguring traps) when using large Sherman folding, aluminum live traps (8 by 9 by 23 cm) was developed.

The device was made from a modified standard small binder clip. This study utilized binder clips from Charles Leonard, Inc. (#BC-02), but binder clips of the same size from any company could potentially be used. The binder clips were modified by bending one of the arms of the clip to a 70° angle inwards (towards the other arm when both arms are folded back) 1.3 cm from the end of the arm. The device is attached to

the trap by clamping it (similar to how one would clamp papers together with a standard binder clip) to the top left corner of the front trap door. The modified arm is positioned towards the outside of the door and folded back. The unmodified arm lies along the backside of the door, folded forward (towards the base of the door) and lies flush against the inside of the door. When the trap is opened, the modified arm rests on top of the interior plate (inside of the trap) which is located directly in front of the treadle. With proper modification and application of the binder clips, traps can be set as they would normally be without the device in place. When the trap is sprung, a one to one and one-half cm space remains open at the top of the trap door. The device can be attached through the back door when the trap is fully assembled or directly when the trap is apart.

Traps can still be folded with the tail-saver device in place. They cannot, however, be folded as flat as traps that do not have the device attached. Therefore, standard Sherman trap boxes can still be used, but placement of only seven traps into a standard 10-trap slot is recommended.

To test the device, 20 study plots were sampled in a saltbush (Atriplex canescens) community on Holloman Air Force Base in the Tularosa Basin of south-central New Mexico during July 1996. Ten study plots had the tail-saver device employed on all traps and 10 study plots did not have the tail-saver device employed. Within each study plot 50 traps spaced at 10 m intervals were placed within two, 250 m trap lines spaced 25 m apart. Traps were baited with quick oats and set for a single night. Traps were checked the next morning and tail status (injured with visible bleeding, severed or undamaged) was recorded for each kangaroo rat. Blood or tail pieces were observed for every animal that was classified as injured or severed, respectively. Additionally, captures of other species were recorded to determine if smaller rodent species were able to escape the traps when the tail-saver device was employed.

Thirty-five kangaroo rats (*Dipodomys merriami* and *Dipodomys ordii*) were captured in traps which had the tail-saver device. None of these subjects had severed or injured tails. Twenty-seven kangaroo rats were captured in traps which did not have the tail-saver device. Twelve of these individuals (44.4%) had either severed (nine) or injured (three) tails caused by trapping efforts from the previous evening. Because

injured or severed tails both indicate damage, these data were pooled for statistical analysis.

Data were converted to the percent of tails damaged and analyzed with Wilcoxon Signed Rank Test. Results showed that fewer tails were damaged on study plots which had traps with the tail-saver device (P=0.015, one-tailed test, 9 df). Hence, the tail-saver device significantly reduced the frequency of tail damage to kangaroo rats when applied to large Sherman live traps.

Chaetodipus penicillatus (n=14, n=6) and Perognathus flavus (n=1, n=1) were also captured in traps with the tail-saver device and in traps without the tail-saver device, respectively. These data provide limited support that small pocket mice cannot easily escape traps which have the tail-saver device attached. However, the space at the top of the trap left by the device is dependent on the angle that the arm of the binder clip is bent. Thus, workers should endeavor to bend their clips consistently.

The tail-saver device is an inexpensive means to help prevent damage to kangaroo rat tails. If bought in bulk, binder clips can be modified into tail-saver devices for a few cents each. This device could have applications for both mark recapture studies and museum collections. Workers should be cautious, however, if using traps that have doors that have been chewed when using this device. Once traps are serrated, small mice could wound themselves trying to escape.

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#### NEW COUNTY RECORD FOR THE MEXICAN LONG-TONGUED BAT (CHOERONYCTERIS MEXICANA) FROM TEXAS

#### Marie K. Fernandez, Steven A. Smith\* and Rodolfo Escamilla

U.S. Fish and Wildlife Service
Laguna Atascosa National Wildlife Refuge
Rio Hondo, Texas 78583 and
\*Department of Biology, Texas A&M University-Kingsville
Kingsville, Texas 78363

The Mexican long-tongued bat, *Choeronycteris mexicana*, (Family Phyllostomidae) is primarily found southward from northern Mexico to El Salvador and Honduras (Hall 1981). In the United States, it occurs as a summer resident in southeastern Arizona as well as the extreme southwestern portion of New Mexico (Schmidly 1991); the species also has been documented in southern California (Arroyo-Cabrales et al. 1987). These bats generally are found at elevations between 300 and 2,400 m (Arroyo-Cabrales et al. 1987) and usually prefer deep canyons where they use caves and mine tunnels for their daytime roosts (Davis & Schmidly 1994). Food habits for the species include fruit, pollen, nectar, and insects (Schmidly 1991).

In Texas, the Mexican long-tongued bat has been reported only from the Santa Ana National Wildlife Refuge in Hidalgo County (LaVal & Shifflet 1971; Schmidly 1991). Although some photographic documentation is available, all reports of *C. mexicana* in the state are from personal observations. Photographs of the first record of this species in Texas are deposited in the Texas Cooperative Wildlife Collections at Texas A&M University (Schmidly 1991). This report documents the first preserved specimen of *C. mexicana* in the state of Texas. This specimen also represents the first record of this species from Cameron County and the eastern-most record in the United States.

On 27 October 1998, a partially decomposed male Mexican long-tongued bat was found by a U.S. Fish and Wildlife Service employee, in an area adjoining the Bayside Drive at the Laguna Atascosa National Wildlife Refuge (UTM/UPS coordinates: 0665325 E, 2901951 N or 26° 13' 39.25" N latitude and 97° 20' 42.06" W longitude). This refuge is located approximately 40 km east of Harlingen in Cameron County and approximately 80 km northeast of the Santa Ana National Wildlife Refuge. Elevation at the collection site was approximately 5 m above sea level. The specimen was found on the ground about 1.5 m

off of Bayside Drive under a granjeno (Celtis pallida) in an area of thick thorn scrub with representative vegetation including granjeno, colima (Zanthoxylum fagara), honey mesquite (Prosopis glandulosa), Berlandier fiddlewood (Citharexylum berlandieri), Texas lantana (Lantana horrida), snake eyes (Phaulothamnus spinescens) and goat bush (Castela erecta). Unfortunately, the specimen had been partially scavenged by ants, but was collected, preserved in fluids, and deposited with the Texas Cooperative Wildlife Collections (TCWC 55310). Photographs of the specimen were deposited at the Laguna Atascosa National Wildlife Refuge.

#### **ACKNOWLEDGMENTS**

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SAS at: sa-smith@tamuk.edu

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ADDITIONAL OCCURRENCE OF THE FILARIOID NEMATODE, LITOMOSOIDES WESTI, IN GEOMYS SPP. IN TEXAS

#### Richard M. Pitts, Norman O. Dronen and John W. Bickham

Department of Wildlife and Fisheries Sciences Texas A&M University, College Station, Texas 77843

Filarioid nematodes of the genus *Litomosoides* have been reported to occur in species of several genera of mammals in the Nearctic and Neotropical regions (Burnham 1953; West 1962; Forrester & Kinsella 1973; Gardner & Schmidt 1986). *Litomosoides thomomydis* occurs in

disjunct populations within the range of the pocket gopher, *Thomomys talpoides*, in southern Colorado (Miller & Ward 1960). *Litomosoides westi* has been reported in both *Geomys bursarius* of the Great Plains region and *T. bottae* from the eastern foothills of the Rocky Mountains in Colorado (Gardner 1983; Gardner & Schmidt 1986). In Texas *L. westi* has only been reported from *G. personatus* from Duval and Zapata counties (Pitts et al. 1990).

Specimens of G. breviceps and G. bursarius examined during this study are deposited in the Sternberg Museum of Natural History (MHP) at Fort Hays State University. Specimens of L. westi from host gophers were stored in vials with glycerin and are deposited as voucher specimens (97-P7) in the Laboratory of Parasitology, Department of Wildlife and Fisheries Sciences at Texas A&M University.

While collecting Geomys breviceps and G. bursarius in Denton County, Texas nematodes identified as L. westi were collected from the pleural cavities of an adult male (MHP 33365) and an adult female (MHP 33366) G. breviceps, at the entrance of Isle Du Boris Unit, Lake Ray Roberts State Park. Additional L. westi were found in the pleural cavities of three adult G. bursarius, two males (MHP 33444, 33445) and one female (MHP 33457) 1.6 mi NW of Aubrey, Grubbs Road. The pocket gophers were captured using Victor gopher traps. The dead pocket gophers were immediately necropsied, to obtain tissue samples for karyotyping. The filarioid nematodes were collected from the pleural cavities using forceps. They were placed in vials with 70% ethanol. The vials were labeled with the host field catalogue number and shipped to Texas A&M University for identification. The filarioid nematodes were identical to those described by West (1962). This is the first record of L. westi from G. breviceps and the first reported occurrence of L. westi from G. bursarius in Texas. These records also fill in the range gap from southern Texas to the southern foothills of Colorado for L. westi.

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JWB at: j-bickham@tamu.edu

## FIRST RECORDS OF THE SUCKERMOUTH MINNOW PHENACOBIUS MIRABILIS FROM THE CANADIAN RIVER, TEXAS

#### Gene R. Wilde and Timothy H. Bonner

Department of Range, Wildlife and Fisheries Management Texas Tech University, Lubbock, Texas 79409

The suckermouth minnow (*Phenacobius mirabilis*) inhabits riffles in small permanent or semipermanent streams with moderate gradients (Miller & Robison 1973; Cross & Moss 1987). It is native to the Mississippi, Tennessee and Ohio river drainages, and also occurs naturally in rivers (Colorado, Trinity, Sabine and Pecos) that drain portions of the Gulf slope in New Mexico, Texas and Louisiana (Rohde 1980). *Phenacobius mirabilis* has been reported from the headwaters of the Canadian River in New Mexico (Rohde 1980; Sublette et al. 1990) and downstream in Oklahoma, near the present day site of Lake Eufaula (Rohde 1980). There is no published record of *P. mirabilis* from the Canadian River, or its tributaries, in Texas. Voucher specimens are deposited in the Texas Natural History Collection, University of Texas, Austin (TNHC).

The first specimens of *P. mirabilis* from the Canadian River drainage in Texas were collected by Larson et al. (1991) on 8 July 1990: three specimens of *P. mirabilis* were collected at the State Highway 70 crossing, Roberts County; and 17 specimens were collected at the US Highway 83 crossing, Hemphill County. More recent collections by the authors from the Canadian River confirm the presence of *P. mirabilis* 

at these sites. On 10 November 1995, one specimen of *P. mirabilis* (TNHC 26102) was collected at the State Highway 70 crossing and two specimens (TNHC 26101) were collected at the US Highway 83 crossing. On 26 February 1998, four additional specimens of *P. mirabilis* (TNHC 26103) were collected at the US Highway 83 crossing.

On 11 May 1999, three specimens of *P. mirabilis* were collected from two additional sites in the Canadian River in Texas. One specimen, not preserved, was collected at the mouth of Dixon Creek, a small intermittent tributary of the Canadian River located about 22 km downstream from the State Highway 207 crossing, Hutchinson County. Two specimens (TNHC 26104) were collected at the State Highway 207 crossing, Hutchinson County. This location is near the head of permanent waters in the Canadian River downstream from Lake Meredith. Collectively, the records reported herein document the presence and persistence of *P. mirabilis* across most of the approximately 150 km reach of the Canadian River in Texas, downstream from Lake Meredith.

Two additional specimens of *P. mirabilis* (TNHC 26105) were collected on 11 May 1999 from White Deer Creek, a perennial tributary of the Canadian River in Hutchinson County. White Deer Creek was briefly sampled in July 1954 by Lewis & Dalquest (1955). Although Lewis and Dalquest failed to detect the presence of *P. mirabilis*, their limited collections, presumably made at or near the lower end of the creek, might easily have missed the species. *Phenacobius mirabilis* should be considered a natural member of the White Deer Creek fish assemblage.

The Canadian River was impounded in 1965 to form Lake Meredith. All records of *P. mirabilis* reported herein are from sites located downstream from the lake, an area from which it historically was absent based on the extensive sampling by Lewis & Dalquest (1955). Presence of *P. mirabilis* in this reach of the Canadian River is consistent with changes in the fish assemblage that have occurred since the river was impounded. Historically, the Canadian River was a large, braided stream, with dramatic fluctuations in flow, salinity and turbidity. The historic fish assemblage was dominated by species that commonly inhabit the main channels of larger prairie streams, including the Arkansas River shiner (*Notropis girardi*), plains minnow (*Hybognathus placitus*), flathead chub (*Platygobio gracilis*) and speckled chub (*Macrhybopsis aestivalis*) (Cross et al. 1955; Lewis & Dalquest 1955). Downstream

from Lake Meredith, the Canadian River now has a well-defined channel, lined by woody vegetation (salt cedar and cottonwood), with greatly reduced flows (flows at the US Geological Survey gauge at US Highway 83 average only 15% of historic, unregulated flows). The fish assemblage in this reach of the Canadian River is now dominated by species, including red shiner (*Cyprinella lutrensis*), sand shiner (*N. stramineus*), plains killifish (*Fundulus zebrinus*) and mosquitofish (*Gambusia affinis*), that historically occurred infrequently in the mainstem river, but which were common in tributary streams (Lewis & Dalquest 1955). Similar changes in the composition of prairie stream fish assemblages in response to decreased stream flows, resulting from impoundment, water diversion and groundwater withdrawal, were described by Cross & Moss (1987) and Pflieger & Grace (1987).

Two possible explanations are offered for the recent occurrence of *P. mirabilis* in the Canadian River, Texas. First, the species may be expanding its distribution upstream in the Canadian River, from Oklahoma, as a result of habitat changes associated with impoundment of the river to form Lake Meredith. Although *P. mirabilis* has been infrequently collected from the Canadian River in Oklahoma, it is widely distributed in tributaries to the river (Miller & Robison 1973), which may have served as sources for fish that colonized the river. The presence of *P. mirabilis* in White Deer Creek may represent a secondary range expansion, from the Canadian River into suitable tributary streams. Alternatively, *P. mirabilis* may be native to White Deer Creek, and other suitable tributaries in Texas, and it is from these populations that those in Texas portions of the Canadian River are derived. The latter explanation is more parsimonious and appears more probable.

#### **ACKNOWLEDGMENTS**

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GRW at: gwilde@ttu.edu

#### **BOOK REVIEW**

Texas Wildlife Resources and Land Uses edited by Raymond C. Telfair II. University of Texas Press, 1999, First Edition, 404 + xi pages, 26 numbered chapters in 5 Parts plus Introduction and Prognosis, 4 appendices, references. ISBN 0-292-78159-8 PBK.

On first glance one might be prepared to find this rework of a 1982 symposium of the Texas Chapter of The Wildlife Society as only historically relevant. However, that initial impression would be quite wrong; this book has something important to ponder for everyone in the conservation field. Many of its chapter authors are leading figures in modern Texas wildlife conservation.

The book is introduced with an excellent summary of the ecological regions of Texas by Editor Telfair. Not only does Telfair include 57 reference sources to his Introduction, but every chapter includes a Literature Cited. As with most published symposia, readers are likely to pick and choose chapter offerings rather than read the book through. Appropriately then, chapters are grouped into parts: (I) Perspectives on Texas Wildlife Resources, (II) Future Expectations In Land Use, (III) The Public and Future Demands For Wildlife, (IV) Wildlife Management and Research, and (V) Wildlife Management on Public Lands. The book ends with a depressing but important prognosis for Texas wildlife.

Despite the updating of original 1982 papers, some information remains more dated than one would like. Data presented on game populations (chapter 13) and the analysis of wildlife research in Texas (ch. 17), for examples, are each 10 years old in these otherwise well-written chapters.

As a natural historian concerned with all of Texas' biota, this reviewer was prepared to find many of his vertebrates of interest labeled "nongame" or "furbearer". After all, wildlife management has its roots in a successful history of managing habitat and populations of largely wild vertebrates that we take for sport or food (game). Hunters and anglers continue to buy their interests from government agencies by putting over \$2.7 billion annually into the state economy (ch. 9), and so hawks, lizards, armadillos and killifishes are codified as "nongame".

But what is really fascinating in this book is a give and take on the new directions and issues facing management of Texas' natural living resources. The growing conflict between the interests of largely nature-naive urban masses (80% of Texans, ch. 9) and the politically powerful private property rights rural landowners (ch. 11) is given datarich coverage (all or parts of at least 9 chapters) and is a must read.

The rancher landowners plead for an understanding of the "true rancher - hunter - conservationist" with a poignant (and mostly sympathetic) longing for seclusion that usually allows only the seasonal visitation of "their" relatively few fee-lease hunters. Endangered species and wetlands concerns are only an excuse for big government seizure of private property "in the name of conservation."

In contrast, the professional wildlifers point out (7 chapters) the need for more nonconsumptive wildlife opportunities on Texas rural land. In a survey (ch. 12), up to 67% of respondents called for more access to rural Texas (97% privately owned) via establishment of public recreation areas. The call is a serious one considering that today only about 12% of Texas' citizens hunt but 25-55% enjoy hiking, camping and observing native plants, birds and other wildlife. The ranchers' disdain for this want of the urban nature enthusiasts is described (ch. 11) by once native range now "stocked by minivans and people in sneakers." This reviewer admires their courage, but questions their tactic in verbally razzing the thousand pound (and growing daily) gorilla of urban Texas. Certainly, a more sympathetic landowner concern is for the "increased incidence of litigation associated with ownership."

How is Texas to resolve the conflict between a call for a Texas land ethic (ch. 1) that acknowledges the end of frontier days and that would empower all Texans (including sneaker-clad minivan drivers) in management issues with the independence and private property rights advocacy of traditional rural Texans? Clearly, the modern wildlife manager faces a challenge to satisfy wildlife resource and land use needs of an urban 21st century Texas. This book is a good beginning to fact-based thought on the issues.

Terry C. Maxwell Department of Biology Angelo State University Terry.Maxwell@angelo.edu

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- Smith, J. D., & G. L. Davis. 1985. Bats of the Yucatan Peninsula. Occas. Pap. Mus., Texas Tech Univ., 97:1-36.

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Jones, T. L. 1975. An introduction to the study of plants. John Wiley & Sons, New York, 386 pp.

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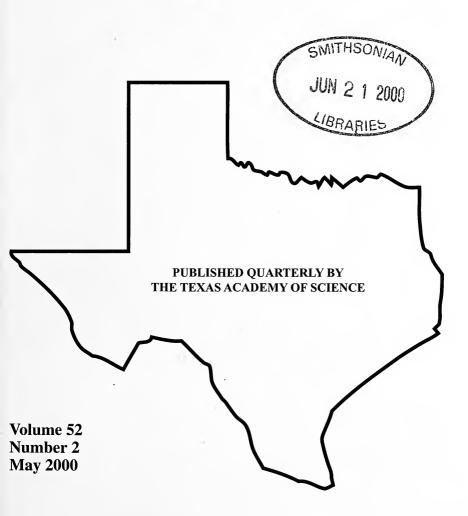
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#### FOOD HABITS OF THREE SPECIES OF STRIPED WHIPSNAKES, MASTICOPHIS (SERPENTES: COLUBRIDAE)

#### Jeffrey D. Camper\* and James R. Dixon

Department of Wildlife & Fisheries Sciences, Texas A&M University
College Station, Texas 77843-2258
\*Present Address:
Department of Biology, Francis Marion University
Florence, South Carolina 29501-0547

Abstract.—The food habits of the striped whipsnake (Masticophis taeniatus), the Sonoran whipsnake (Masticophis bilineatus) and Schott's whipsnake (Masticophis schotti) were studied by examining the stomach contents of museum specimens. All three species ate lizards most frequently. Lizards of the genera Cnemidophorus and Sceloporus were the most abundant in the sample. Only larger whipsnakes ate mammals. Snakes, frogs and insects were found in low frequencies in the diet of striped whipsnakes. Geographic variation in the diet of M. taeniatus based upon a comparison of the two subspecies showed that the more northerly M. taeniatus taeniatus fed more frequently upon phrynosomatid lizards and less frequently upon Cnemidophorus and Crotaphytus than the southern populations assigned to M. taeniatus girardi. A positive relationship between prey mass and snake mass was found for the striped whipsnake.

Although the diet of *Masticophis taeniatus* has been well documented (Parker & Brown 1980; Brown & Parker 1982; Camper 1996c), little information exists concerning the diets of *Masticophis bilineatus* (cf. Camper 1996a) and *Masticophis schotti* (cf. Camper 1996b). Most reports concerning the food habits of *M. taeniatus* are based upon data from the northern subspecies, *M. taeniatus taeniatus*, which occurs in the Great Basin Desert of the western U.S.A.; however, there has been little reported on the diet of the southern form, *M. taeniatus girardi*, that inhabits the Chihuahuan Desert and Edwards Plateau of Texas and México (Camper 1996c). The objectives of this paper are to (1) report the diets of *M. bilineatus*, *M. schotti* and *M. taeniatus*, (2) examine geographic variation in the diet of *M. taeniatus* and (3) evaluate size relationships among *M. taeniatus* and its prey, based upon stomach contents removed from museum specimens.

Masticophis taeniatus, M. schotti and M. bilineatus are parapatrically distributed (Camper & Dixon 1994). Masticophis taeniatus is found in the Chihuahuan and Great Basin Deserts, the Edwards Plateau of central Texas, and western portions of the Mexican Plateau, whereas M. schotti is found south of the Edwards Plateau and along eastern portions of the Mexican Plateau east to the Gulf of Mexico. Masticophis bilineatus

occurs in the Sonoran Desert of southern Arizona, along the west coast of México south to the state of Colima, and east to the Continental Divide. Narrow zones of sympatry exist between *M. taeniatus* and *M. schotti* along the Balcones Escarpment in central Texas, between *M. bilineatus* and *M. taeniatus* in central Arizona and possibly all three in central México.

#### **METHODS**

A total of 1317 *M. taeniatus*, 316 *M. schotti* and 335 *M. bilineatus* museum specimens, that were collected from throughout the geographic ranges of each species, were examined for the presence of stomach contents (locality data from specimens examined is available from the author). Stomach contents were identified to species whenever possible. Age, mass and sex of each snake and each prey item were recorded. Juvenile and adult snakes were distinguished by ontogenetic color pattern differences (Camper & Dixon 1994). A *P* value of 0.05 or less was considered significant in all statistical tests.

#### RESULTS

Few whipsnakes examined contained prey. Only 9% of *M. taeniatus* and 5% of both *M. schotti* and *M. bilineatus* contained stomach contents. Thirty-five prey species for *M. taeniatus* and eight for both *M. bilineatus* and *M. schotti* were found. Unidentified lizards and snakes were not included (Table 1). Lizards were the most frequent category of prey and made up 88.9% of the diet of *M. taeniatus*, 91% of the *M. bilineatus* sample, and 78.3% of the *M. schotti* sample. Mammals and snakes made up only a small part of the diet by frequency. Intact insects were ingested by three adult *M. taeniatus* > 775 mm SVL. They were ingested intentionally because they were fairly large (two lepidopteran larvae and one cicada) and were the only stomach contents present in those specimens.

Most whipsnakes with stomach contents contained single prey items. However, 19 adult specimens, > 672 mm SVL, contained multiple prey. One *M. bilineatus* and one *M. schotti* each contained three different species of lizards. Eleven adult *M. taeniatus* contained two prey. Five contained conspecific lizards, one contained two mammals, and three *M. taeniatus* contained different prey species. Two *M. bilineatus* contained two lizards each. Four *M. schotti* had each eaten two prey. Two contained both lizards and mammals, whereas two had eaten lizards only, with one *M. schotti* harboring conspecific lizards.

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Table 1. Frequency of occurrence of prey found in 118 Masticophis taeniatus, 18 Masticophis bilineatus, and 17 Masticophis schotti from throughout the species' geographic ranges. N is the number of prey items in the sample. Numbers in parentheses after prey taxa indicate the number of species from that taxon identified in the sample.

Prey category		Snake species	
	Masticophis taeniatus n (%)	Masticophis bilineatus n (%)	Masticophis schotti n (%)
Mammalia	7 (5.5)	2 (9)	5 (21.7)
Reptilia: Sqaumata Anguidae (1) Crotaphytidae (1)	2 (1.6) 7 (5.5)	0	0
Phrynosomatidae Callisaurus Cophosaurus (1) Holbrookia Phrynosoma (2) Sceloporus (9) Urosaurus (1) Uta (1)	1 (0.8) 5 (4) 1 (0.8) 2 (1.6) 49 (37.6) 2 (1.6) 11 (8.4)	0 1 (4.6) 0 0 3 (13.7) 2 (9) 1 (4.6)	0 0 0 0 8 (34.7) 0
Polychridae (1)	1 (0.8)	0	1 (4.4)
Scincidae Eumeces Scincella	0 0	0 0	1 (4.4) 1 (4.4)
Teiidae <i>Cnemidophorus</i> (12)	32 (24.5)	12 (54.5)	6 (26)
Unidentified lizards	1 (0.8)	1 (4.6)	1 (4.4)
Colubridae Bogertophis Sonora (1) Thamnophis	1 (0.8) 2 (1.6) 1 (0.8)	0 0 0	0 0 0
Unidentified snakes	1 (0.8)	0	0
Amphibia: Pelobatidae Scaphiopus	1 (0.8)	0	0
Insects	3 (2.4)	0	0
Total Prey	130	22	23

Direction of ingestion was inferred by examining the position of the prey in the stomachs of the snakes. Significantly more striped whipsnakes (88.5%) ingested their prey head-first ( $\chi^2 = 64.7$ , df = 1, P < 0.05) indicating that they may manipulate prey prior to ingesting it. Tail-first ingestion of prey by *M. taeniatus* were limited to smaller prey, less than 7% of the mass of the snake. *Masticophis bilineatus* consumed 86.7% of its prey head-first and *M. schotti* ate 85.7% of its prey head-first.

Table 2. Comparison of the frequency of mammals, lizards and snakes found in 65 Masticophis taeniatus taeniatus and 55 Masticophis taeniatus girardi museum specimens. N is number of prey items.

Prey category	Snake subspecies				
	Masticophis teaniatus taeniatus n (%)	Masticophis teaniatus girardi n (%)			
Mammalia	4 (6)	3 (5)			
Reptilia: Squamata Crotaphytidae Phrynosomatidae Teiidae Colubridae	1 (1.5) 45 (69) 14 (22) 1 (1.5)	6 (11) 24 (44) 18 (33) 4 (7)			
Total prey $\chi^2 = 11.6$ , df = 4, P < 0.05	65	55			

Juvenile whipsnakes ate the same prey taxa as the adults. Juvenile M. taeniatus (N=19) ate Sceloporus, Cnemidophorus, Uta, Anolis and a neonatal Thamnophis elegans. Adults and juveniles consumed similar proportions of Cnemidophorus, phrynosomatids, and snakes ( $\chi^2 = 0.15$ , df = 2, P > 0.05). One juvenile male M. taeniatus, 286 mm SVL, consumed two juvenile Sceloporus graciosus. The T. elegans was 247 mm total length and was eaten by a 279 mm SVL M. taeniatus. The relative prey mass (RPM = prey mass/snake mass) was 0.45 for this specimen. The whipsnake may have been collected while ingesting the garter snake, or may have died during the ingestion attempt, because the posterior quarter of the Thamnophis protruded from the mouth of the whipsnake. One juvenile M. bilineatus contained a Urosaurus ornatus, and three juvenile M. schotti ate juvenile Anolis carolinensis and Cnemidophorus gularis, and an adult Sceloporus grammicus.

Geographic variation in the diet of *M. taeniatus* was examined by comparing the frequency of prey in the diets of both subspecies whose geographic ranges correspond to the Great Basin and Chihuahuan Deserts (Table 2). The desert striped whipsnake, *M. taeniatus taeniatus*, consumed phrynosomatid lizards more frequently and *Cnemidophorus* and *Crotaphytus* less frequently than the Central Texas whipsnake, *M. taeniatus girardi*. *Sceloporus graciosus* and *Uta stansburiana* occurred at frequencies of 27.5% and 14.5%, respectively, in the sample of *M. taeniatus taeniatus*. However, the most frequent taxon in the sample of *M. taeniatus girardi* was *Crotaphytus collaris*, which occurred at a frequency of only 10.2%. Data from stomach contents of museum

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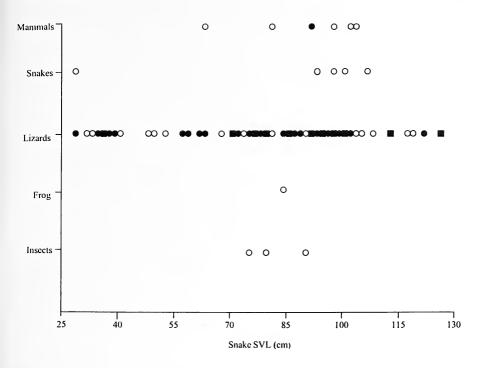


Figure 1. Relationship between prey category and snake body size (SVL) in *Masticophis taeniatus* (N = 118). Circles indicate one prey item, dots two prey and black squares three prey.

specimens may differ from data gathered in field studies from single localities. The relative frequencies of mammals, phrynosomatid lizards, teiid lizards and snakes in the diet of a population of M. taeniatus in northern Utah (Parker & Brown 1980) was compared with this sample. The Utah population ate mammals at a higher frequency and lizards less frequently than the sample from museum specimens ( $\chi^2 = 13.6$ , df = 3, P < 0.05).

Striped whipsnakes of all sizes ate lizards, whereas only large specimens, > 630 mm SVL, consumed mammals (Fig. 1). With the exception of one juvenile, only specimens > 950 mm SVL ate snakes. Even though large striped whipsnakes retain small prey in their diet, there was still a significant relationship between snake mass and prey mass (Fig. 2). Relative prey mass for adult *M. taeniatus* ranged from 0.011-0.313,  $\bar{x} = 0.065 \pm 0.059$ .

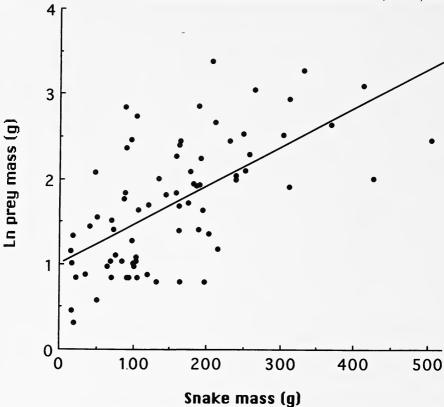


Figure 2. Ln-transformed prey mass as a function of snake mass in *Masticophis taeniatus* (adjusted  $r^2 = .39$ , F = 48.09, df = 1, 74, N = 76, P < 0.05).

#### **DISCUSSION**

Despite some discrepencies with the literature, this sample agrees fairly well with published reports of the diet of *M. taeniatus*. Lizards have been reported as occurring in the diet of *M. taeniatus* by Parker & Brown (1980), Brown & Parker (1982) and several anecdotal reports reviewed by Camper (1996c). Mammals have been reported in the diet of *M. taeniatus* by Parker & Brown (1980) and Reynolds & Scott (1982) and insects were reported by Fautin (1946). Predation upon birds and bats was reported for *M. taeniatus* by La Rivers (1944) and Herreid (1961), respectively. However, neither birds nor bats were found in this study. There is little agreement between published accounts of the diet of *M. bilineatus* and this sample. Birds and snakes have been reported as the prey of *M. bilineatus* (Stejneger 1902; Van Denburgh 1922; Ortenburger 1928); however, no birds or snakes were found in this sample. It is unclear if the large frequency of mammals eaten by *M*.

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schotti is real or an artifact of a small sample size. The only literature report for this species lists rodents as well as birds, bird eggs, snakes and frogs fed to captive specimens (Gloyd & Conant 1934), however, only lizards and mammals were found in this sample.

Differences in the diet between the two subspecies of *M. taeniatus* probably reflect prey availability in the different geographic regions they inhabit. The lizard faunas of the Great Basin and Chihuahuan Deserts differ at the species level (Stebbins 1985; Conant & Collins 1991). The majority of the diet, by frequency, of both subspecies of *M. taeniatus* consisted of phrynosomatid lizards and *Cnemidophorus*. However, there was little overlap of lizard species in the diets of the two *M. taeniatus* subspecies. Eleven species of *Cnemidophorus* and 13 species of phrynosomatid lizards were found in this sample, with zero and three, respectively, being eaten by both subspecies of *M. taeniatus* (cf. Camper 1990). Geographic variation in diet resulting from prey availability has been reported in other snake species (Kephart 1982; Kephart & Arnold 1982; Greene 1984). Variation in diet between subspecies was found in the northern water snake (*Nerodia sipedon*) by King (1993) and the gopher snake (*Pituophis catenifer*) by Rodríguez-Robles (1998).

The relationships between snake size and prey size found in this study are similar to what has been found in other colubrid snakes. A positive relationship between snake mass and prey mass is probably common in snakes (Seib 1984; Jayne et al. 1988; King 1993; Rodríguez-Robles et al. 1999a; 1999b). Data from this study and from Parker & Brown (1980) show that only larger striped whipsnakes eat mammals. This pattern has been seen in other saurophagous racer-like snakes (Franz & Gicca 1982; Seib 1984; Rugiero & Luiselli 1995).

Mean RPM for *M. taeniatus* was lower than in many other colubrid snakes (Shine et al. 1996; Rodríguez-Robles et al. 1999a; 1999b). The low mean RPM for *M. taeniatus* occurs because the species does not drop small prey from its diet, a pattern that is relatively uncommon in snakes (Arnold 1993). Retention of small prey in the diet appears to occur more frequently in snakes that specialize on only one or two prey types, such as crayfish (*Regina*; Godley et al. 1984), fish (*Enhydrina*; Voris & Moffett 1981), lizards (*Uromacer*; Henderson et al. 1987) or frogs (*Notechis*; Shine 1977). Specializing on relatively small prey such as lizards and retaining small prey in the diet may explain the low mean RPM seen in this sample. The low mean RPM found in this study is

comparable to that reported for other attenuate colubrid snakes that feed mainly on lizards and frogs including neotropical racers *Drymobius chloroticus* (RPM:  $\bar{x}=0.041$ ) and *D. margaritiferus* (0.053; Seib 1984), a Hispaniolan vine snake (*Uromacer frenatus*; 0.125; Henderson et al. 1987), and a European racer (*Coluber viridiflavus*; 0.076; Rugiero & Luiselli 1995). It is hypothesized that other snakes that specialize on relatively small prey will retain small prey in their diets and exhibit low RPM values.

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JDC at: jcamper@fmarion.edu

#### DIET COMPOSITION OF THE COLLARED LIZARD (CROTAPHYTUS COLLARIS) IN WEST-CENTRAL TEXAS

Jerry F. Husak\* and J. Kelly McCoy

Department of Biology, Angelo State University
San Angelo, Texas 76909
\*Present Address:
Department of Zoology, Oklahoma State University
Stillwater, Oklahoma 74078

Abstract.—Crotaphytus collaris is a species of lizard widely distributed in the southwestern United States, but there are no quantitative studies of its diet from Texas, where the species is widespread and common. Studies of this species from other locations have shown sexual differences in diet, suggesting differential niche utilization between the sexes that may act as a selective force for the sexual dimorphism that is evident in this species. This study was conducted to provide a descriptive and quantitative account of the diet of C. collaris in west-central Texas in general, and to look for sexual differences in diet. Two-sample t-tests revealed no significant difference between sexes in total volume of prey, total weight of prey, number of prey per stomach, and number of kinds of prey per stomach. Levins' niche breadth values were calculated for each sex and found to be similar and low. Discriminant analysis revealed no significant differences in composition of diet. There is a lack of evidence supporting the hypothesis that sexual differences in diet are acting as a selective force driving the evolution of sexual dimorphism in this population.

Crotaphytus collaris has a broad distribution in the southwestern United States, ranging from eastern Utah and Colorado to southwestern Illinois, and south into Mexico. Its optimal habitat is rocky, limestone outcroppings and rocky areas (Smith 1946; Conant & Collins 1991). It has been the subject of numerous ecological studies, including its diet (see Best & Pfaffenberger 1987 for a complete listing). Diet has been studied quantitatively and anecdotally for this species in New Mexico (Best & Pfaffenberger 1987), Kansas (Fitch 1956), Oklahoma (Blair & Blair 1941), Arkansas and Missouri (McAllister 1985), and Utah (Knowlton 1938), but not in Texas, a state where the species is widespread and relatively common. Other quantitative studies have focussed on this species at the periphery of its range (e.g., McAllister 1985,) or in areas of habitat other than limestone outcroppings (e.g., Best & Pfaffenberger 1987). The general feeding ecology of this species has been thoroughly described, but this study focuses on sites that have optimal habitat for the species, so that a better understanding of the geographic variation in diet can be determined by comparison to other studies.

Crotaphytus collaris is significantly and variably sexually dimorphic in head width and length, with males having larger heads than females (McCoy et al. 1994; McCoy et al. 1997). One hypothesis for the evolution of sexual dimorphism proposes that the sexes differ in morphology so that they can utilize different resources and minimize niche overlap, reducing intraspecific competition (Selander 1966). Males and females could consume different types and/or sizes of prey items as part of the resource partitioning. The larger heads of males may be explained by this hypothesis if males were found to eat larger and/or harder prey items (e.g., beetles as opposed to grasshoppers) to account for the increased musculature. Best & Pfaffenberger (1987) did not find a difference in size of prey consumed by males and females, but they did find sexual differences in diet composition. They postulated that this difference may reduce intraspecific competition. A goal of this study was to determine if there is a difference in diet between sexes, and whether or not the data would lend support to the hypothesis that differential niche use is a selective force driving the evolution of sexual dimorphism in this population.

#### METHODS AND MATERIALS

Collared lizards (N=48) were captured by noosing in June – July 1997 and 1998 at six locations in west-central Texas: a private ranch in Irion County, Texas consisting of rocky limestone outcroppings on a redberry juniper (Juniperus pinchottii) and mesquite (Prosopis glandulosa) dominated hillside; a private ranch in Concho County, Texas consisting of a dry, limestone creekbed surrounded by mesquite brushland; and four sites in Tom Green, Runnels and Coke counties, consisting of rip-rap boulder dams surrounded by mesquite brushland.

Upon capture, lizards were returned to the lab where they were measured (SVL, total length, head width, and head length, all to the nearest 0.1 mm), euthanized and necropsied. The gastrointestinal tract was removed and the stomach was separated and preserved with its contents in 10% formalin. Stomach contents were later removed and prey items were counted and identified to the lowest possible taxon following Borror et al. (1989). Total weight of prey items was determined to the nearest 0.01 g and total volume of prey items was determined to the nearest 0.01 mL by volumetric displacement of water in a small, calibrated syringe. Volume and weight for individual prey items were not determined to avoid any discontinuity that might result by not including prey items in advanced stages of digestion.

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Table 1. Stomach contents of 48 collared lizards (*Crotaphytus collaris*) from west-central Texas presented by sex. Sample sizes are given in parentheses and occurrence data are given as total items of that category in the stomachs.

Category	Male (26)	Female (22)	Total (48)
Arachnida	2	0	2
Insecta			
Orthoptera	35	23	58
Hemiptera	2	1	3
Homoptera	5	9	14
Coleoptera	9	17	26
Hymenoptera	9	26	35
Odonata d	3	0	3
Plant tissue	4	3	7
Total	69	79	148

Two-sample t-tests were performed to compare total volume, total weight, number of prey items per stomach and number of kinds of prey items per stomach between sexes. An artificial average volume and average weight for each prey item was calculated and tested between sexes with a two-sample t-test. Levins' (1968) value for niche breadth was calculated for each sex individually and for both sexes combined. Discriminant analysis was used to statistically compare composition of diet between sexes. Procedures for using discriminant analysis in dietary analysis follow Best & Gennaro (1984). There were no significant differences (two-sample t-tests) found in total volume, total weight, number of prey items per stomach, or number of kinds of prey items per stomach between the rip-rap dam and natural habitat, nor were there significant differences in the four categories between sexes in the two different habitat types (ANOVA) at the 0.05 significance level. Therefore, data were pooled from all of the sites for all comparisons between sexes.

#### RESULTS

A summary of the diet of *C. collaris* in west-central Texas is presented in Table 1. The mean number of prey items per stomach was 3.06. The mean number of kinds of prey items per stomach was 2.29. The mean total volume of prey items per stomach was 0.99 mL. The mean total weight of prey items per stomach was 1.11 g. The prey items with the highest frequency of occurrence (number of specimens containing a prey item / total number of specimens x 100) were: Orthoptera, 87.5%; Coleoptera, 31.3%; and Hymenoptera, 25.0%.

Within the orthopterans, the family Acrididae had a frequency of occurrence of 85.4%. Coleopterans were evenly spread among the six families identified, but were dominated by Curculionidae with a frequency of occurrence of 10.4%. Hymenopterans were represented by four families, and were dominated by Formicidae with a frequency of occurrence of 18.8%. Orders that represented the highest percentages of the prey items (number of prey items in a category / total number of prey items x 100) were: Orthoptera, 39.2%; Hymenoptera, 23.6%; Coleoptera, 17.6%. Homopterans were less common, accounting for only 9.5% of the prey items and being present in only 20.8% of the lizards. Hemiptera and Odonata were even less common, both accounting for only 2.0% of the prey items and being present in only 6.3% and 4.2% of the lizards respectively. Plant material of various sorts accounted for 4.7% of the prey items and was found in 12.5% of the lizards. This is believed to be due to incidental ingestion as suggested by McAllister (1985). Small pebbles (2-3 mm) were common, being found in 29.2% of the lizards. These are probably used as digestive aids (Johnson 1966). All of the pebbles were of approximately uniform size and shape, suggesting that they are used for breaking up arthropod exoskeletons.

Two-sample *t*-tests revealed no significant difference (p > 0.05) in total volume, total weight, number of prey items per stomach, number of kinds of prey items per stomach, average volume, or average weight between sexes. Despite the lack of significant difference between sexes, there were very evident trends in dietary composition that were not accounted for by the statistical analyses (Figure 1). Females appeared to consume more, smaller prey items than males, but the number of kinds of prey items per stomach was similar. Females also appeared to eat more hymenopterans (32.9%) and coleopterans (21.5%) and less orthopterans (29.1%) than males (13.0%, 13.0% and 50.7% respectively).

Levins' measures of niche breadth for the males (B=3.30) and females (B=3.94) were similar. For the sexes pooled (B=3.96), it was more similar to that of females. Discriminant analysis revealed no significant difference in diet between sexes. For both sexes, a total of 75% were classified as the correct sex based on diet, with 85% of the males being classified and 64% of the females being classified correctly.

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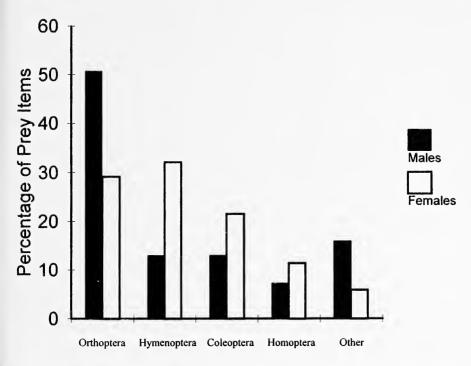


Figure 1. Percentages of five prey categories found in the stomachs of 48 collared lizards (*Crotaphytus collaris*) from west-central Texas presented by sex.

The jackknifed matrix, however, only classified 38% total correctly, with 46% of the males and 27% of the females correctly classified. The jackknifing procedure, which drops each observation one at a time and repeatedly re-classifies groups, is not as influenced by outliers during the classification process when groups (e.g., sexes) are being predicted based on certain variables (e.g., diet components). This procedure represents a better way of predicting and classifying groups based on true separations of the variables being used to do the classifications.

#### DISCUSSION

The diet composition for this population of *C. collaris* differed from other studies in New Mexico, Kansas and Arkansas and Missouri as should be expected in populations that are geographically separated. Orthopterans made up a much greater portion of the diet in this study, followed by hymenopterans and coleopterans. It is, however, consistent with findings in northeastern Oklahoma (Blair & Blair 1941) and Utah

(Knowlton 1938) where orthopterans were by far the most abundant food items taken.

The Levins' niche values are similar, suggesting that there is little variation in diet niche breadth between the sexes. They are also relatively low values (see Pianka 1987), suggesting that C. collaris in westcentral Texas does not have as diverse a diet as might be expected for a lizard that is usually considered a strict opportunist (e.g., B=7.34 for Uta stansburiana, B=5.83 for Cnemidophorus tigris; Pianka 1987). This is evident by the extreme dominance of certain prey groups such as orthopterans and hymenopterans. Other studies have suggested that this species is an extreme generalist, such that availability was more important than preference (McAllister 1985). More specifically, it is worth noting that no dipterans, lepidopterans, blattids, scorpions or vertebrates were found, all of which are common in the area. It is not surprising how important orthopterans were found to be in the diet, but it is surprising how relatively unimportant abundant groups such as coleopterans and arachnids were found to be to the lizards' diet overall when compared to other studies. Blair & Blair (1941) found similarly high values for orthopterans in northeastern Oklahoma, but suggested that it was due to availability. Knowlton (1938) found similar dominance by orthopterans, but they constituted a smaller percentage of the diet and had a lower frequency of occurrence than found in this study. Hymenopterans were found to be more common and coleopterans were found to be about the same as found in this study. Best & Pfaffenberger (1987) hinted that preference may play a role in the diet of C. collaris based on the dominance of certain prey taxa. There was a dominance of certain taxa to an even higher degree in this study. It is unclear whether this population may be discriminating to any degree as is predicted by optimal foraging theory. They apparently have an abundant supply of diverse, palatable prey items, but they are consuming one prey item much more than any other taxa. Prey abundances were not determined in this study, but grasshoppers often appeared to be the most abundant prey item at the study sites. Quantitative studies of prey densities and diet are needed to confirm this.

Discriminant analysis produced a high degree of correct classification, but the jackknifed matrix percentages were all very low. This gives a better understanding of the true separation, or lack thereof, because outliers do not play a large role in the jackknifed classification. This shows that there is no significant separation in prey composition between

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the sexes. Best & Pfaffenberger (1987) found a significant difference in diet composition between sexes in New Mexico and suggested that it might provide evidence that differential niche utilization by the sexes was a force driving sexual dimorphism in head size. The lack of significant difference between the sexes in this study does not support the theory that differential niche utilization by the sexes is a strong selective force driving sexual dimorphism in this population, at least in the sense of differential foraging niches based on diet. Although there were trends evident between the sexes, none are significant and many are due to a few outlying individuals. The trends were also contradictory to this hypothesis. Females were found to eat more, smaller prey, but they ate more hard prey items (more coleopterans and hymenopterans and less orthopterans) than males. Sexual dimorphism has been shown to vary geographically for this species (McCoy et al. 1994; McCoy et al. 1997), so it may be that other selective forces (e.g., different parasite loads, different predation rates, different densities of potential mates) are acting at higher degrees on this population, when compared to the populations examined in other studies. Differential niche utilization may still be a factor, but in this case, they may be partitioning resources other than prey.

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JFH at: husak@okstate.edu

## REPRODUCTION IN THE MOJAVE RATTLESNAKE, CROTALUS SCUTULATUS (SERPENTES: VIPERIDAE)

#### Stephen R. Goldberg and Philip C. Rosen

Department of Biology, Whittier College Whittier, California 90608 and Department of Ecology & Evolutionary Biology University of Arizona, Tucson, Arizona 85721

Abstract.—Reproductive tissue was examined from 145 museum specimens of *Crotalus scutulatus* from Arizona, New Mexico, Texas and México. Males follow a seasonal testicular cycle with sperm produced June-September; regressed testes were present March-May and October. Recrudescence occurred March-July. Timing of this cycle is similar to that of several other North American rattlesnakes. Sperm was present in the vasa deferentia March-October suggesting *C. scutulatus* has potential for breeding throughout this period. Females appear to have a biennial reproductive cycle with yolk deposition completed over two activity seasons. Mean litter size for 35 *C. scutulatus* was  $8.2 \pm 2.36$  SD, range 5-13 young. Yearly percentages of gravid females appear to be related to food abundance.

The Mojave rattlesnake, *Crotalus scutulatus* ranges from southern Nevada to Puebla, México and the western edge of the Mojave Desert, California to extreme western Texas from near sea level to around 2530 m elevation (Stebbins 1985). The *C. scutulatus* studied here frequent desert scrub, desert grassland and grassland in non-rocky habitats (Lowe 1964; Reynolds & Scott 1982; Tennant 1984; Degenhardt et al. 1996). There are only anecdotal reports on reproduction in this species (Gloyd 1937; Gates 1957; Minton 1958; McCoy 1961; Tennant 1984; Lowe et al. 1986; Price 1998). Jacob et al. (1987) provided information on the testicular cycle. Klauber (1972) reported on brood (= litter) sizes. Price (1982) summarized information on the biology of this species. The purposes of this study are to provide information on the seasonal ovarian and testicular cycles of *C. scutulatus* from a histological examination of museum specimens. Information on litter sizes and the relation of food abundance to production of litters is also presented.

#### MATERIALS AND METHODS

A sample of 145 specimens of C. scutulatus (48 females, mean Snout-Vent Length, SVL = 692 mm  $\pm$  98.3 SD, range = 508-900 mm; 97 males, SVL = 682 mm  $\pm$  159.8 SD, range = 411-1098 mm) from Arizona, New Mexico, Texas and México was examined from the herpetology collections of Arizona State University, Tempe (ASU), The

Natural History Museum of Los Angeles County (LACM) and The University of Arizona, Tucson (UAZ). Neonate sizes (SVL) for three litters from Pima County, Arizona are also presented. Snakes were collected 1946-1994. Counts were made of enlarged ovarian follicles (> 12 mm length), embryos or full-term young. None of the females contained oviductal eggs. The left testis and vas deferens were removed from males; the left ovary was removed from females for histological examination. Tissues were embedded in paraffin and sectioned at  $5 \mu m$ . Slides with tissue sections were stained with Harris' hematoxylin followed by eosin counterstain. Testes slides were examined to determine the stage of the male cycle; ovary slides were examined for the presence of yolk deposition (secondary yolk deposition sensu Aldridge 1979a). Because some of the specimens were road kills, not all tissues were available for histological examination due to damage or autolysis. Number of specimens examined by reproductive tissue were: testis = 97, vas deferens = 65, kidney = 83, ovary = 48. In addition, information is provided on yearly percentages of gravid C. scutulatus from a sample of 32 adult females captured 1987-1996 in desert scrub in southern Pima County, Arizona, primarily in the Valley of the Ajo (32°05'N, 112°50'W, elevation 500 m). Reproductive condition was ascertained by palpating May-August females, the period during which gravidity is determinable.

Material examined.—The following specimens of Crotalus scutulatus were examined: ARIZONA: COCHISE COUNTY, (ASU 2471, 3792, 21260, 21489, LACM 2978, 116024, 123776, UAZ 27760, 27766, 32584, 35083, 40083, 43571, 46836-46838, 48220, 50015); GILA COUNTY, (ASU 3107, LACM 2977, UAZ 43035); GRAHAM COUNTY, (ASU 7036, 7039, 15725, 22510, 22511, 22519, 22520, UAZ 42695, 42696, 46547); GREENLEE COUNTY, (UAZ 42350); LA PAZ COUNTY, (LACM 70540, 116041, UAZ 36202, 36260, 49343); MARICOPA COUNTY, (ASU 1418, 2918, 22411, 22478, LACM 105059, 105060, 125259, UAZ 27772, 42981, 43584, 46425, 46942, MOHAVE COUNTY, (LACM 122371, UAZ 40129, 42298), PIMA COUNTY, (LACM 64282, 64284, 105063, 105066, 105067, 105071, 116027, 116029, 116032, 116035, 116038, 134045, UAZ 10109, 27652, 27665, 27666, 27668, 27669, 27671, 27672, 27675, 27677, 27680, 27686, 27716, 27717, 27769, 27713, 27741, 27745, 27746, 27749, 27753, 27757-27759, 27770, 27774, 27784, 41097, 41719, 41722, 42892, 42980, 44070, 47360, 48176, 50040, 50156, 50651, 51015, 51056); PINAL COUNTY, (LACM 68843, UAZ 27687, 41091,

Table 1. Monthly distribution of conditions in seasonal testicular cycle of *Crotalus scutulatus*. Values shown are the numbers of males exhibiting each of the three conditions.

Month	n	Regressed	Recrudescence	Spermiogenesis	
March 3		1	2	0	
April	11	3	8	0	
May	9	3	6	0	
June	12	0	8	4	
July	16	0	3	13	
August	26	0	0	26	
September	17	0	0	17	
October	3	3	0	0	

42978, 42982); SANTA CRUZ COUNTY, (LACM 105073, UAZ 27678, 27684); YAVAPAI COUNTY, (ASU, 4114, LACM 20051, 134046); YUMA COUNTY, (LACM 116039, UAZ 27775, 33818, 36566, 44069).

NEW MEXICO: HIDALGO COUNTY, (UAZ 27793).

TEXAS: CULBERSON COUNTY, (LACM 116043); HUDSPETH COUNTY, (UAZ 27797); PRESIDIO COUNTY, (LACM 109531).

**MÉXICO**: **CHIHUAHUA**, (UAZ 34319, 34424, 34797-34799, 34801, 34803, 35003, 35007, 35196, 37841, 39850, 42435, 42906, **SONORA**, (LACM 9212, 52567, 105099, 105102, UAZ 27795, 32779, 33819).

#### RESULTS AND DISCUSSION

Testicular histology was similar to that reported by Goldberg & Parker (1975) for two colubrid snakes, Masticophis taeniatus and Pituophis catenifer (= P. melanoleucus) and the viperid snake, Agkistrodon piscivorus by Johnson et al. (1982). In the regressed testes, seminiferous tubules contained spermatogonia and Sertoli cells. recrudescence, there was renewal of spermatogenic cells characterized by spermatogonial divisions; primary and secondary spermatocytes were typically present. Some spermatids were occasionally seen. In spermiogenesis, metamorphosing spermatids and mature sperm were present. Males undergoing spermiogenesis were found June-September; regressed testes were found March-May and October. Testes in recrudescence were found March-July (Table 1). The smallest spermiogenic male measured 411 mm SVL, and other males close to this size that were undergoing spermiogenesis measured 440, 441, 460, 465 and 478 mm SVL. Jacob et al. (1987) reported spermiogenesis occurred in male C. scutulatus collected July-August from Chihuahua, México. The testicu-

Table 2. Monthly distribution of conditions in seasonal ovarian cycle of *Crotalus scutulatus* from examination of museum specimens. Values shown are the numbers of females exhibiting each of the four conditions.

Month	n	Inactive	Early yolk deposition	Enlarged follicles > 12 mm length	Young
March	2	0	0	2 a	0
April	7	0	0	7	0
May	3	3	0	0	0
June	5	3	1	0	1.
July	9	7	0	1 <sup>a</sup>	1 b
August	18	16	2	0	0
September	3	1	1	0	1 c
November	1	0	1	0	0

<sup>&</sup>lt;sup>a</sup> Each sample contains one female with squashed enlarged follicles.

lar cycle of C. scutulatus fits the "postnuptial" (aestival) breeding pattern with maximum spermatogenic activity occurring in late summer and fall (Saint Girons 1982; Seigel & Ford 1987). Testicular cycles of other North American rattlesnakes (Crotalus viridis, Crotalus tigris, Crotalus molossus; Aldridge 1979b, Goldberg 1999a; 1999b) also appear to fit this pattern. Vasa deferentia of the following C. scutulatus males contained sperm: 3/3 (100%) March; 6/6 (100%) April; 4/4 (100%) May; 2/4 (50%) June; 6/7 (86%) July; 24/24 (100%) August; 14/14 (100%) September: 3/3 (100%) October. The presence of sperm in the vasa deferentia throughout the activity season indicates C. scutulatus has the potential of breeding throughout this period and lends support to Price's (1998) report that mating might occur at any time during the summer activity season. However, Lowe et al. (1986) reported that breeding occurs mainly in the spring (late February through May). The kidney sexual segments of C. scutulatus were enlarged and contained secretory granules in the following males: 3/3 (100%) March; 10/10 (100%) April; 7/7 (100%) May; 8/9 (89%) June; 9/11 (82%) July; 24/24 (100%) August; 16/16 (100%) September; 3/3 (100%) October. Because breeding coincides with hypertrophy of the kidney sexual segment (Saint Girons 1982), these results also indicate that C. scutulatus has the potential for a prolonged breeding season. observations of mating are needed to ascertain the natural duration of breeding in C. scutulatus.

Females with enlarged follicles or developing embryos were found March, April, June, July (Table 2). Minton (1958) reported a gravid C.

b Collected 11 July, gave birth 18 July in captivity.

<sup>&</sup>lt;sup>c</sup> Collected 14 June, gave birth 24 September in captivity.

Table 3. Litter sizes for 14 *Crotalus scutulatus* (estimated from counts of yolked follicles > 12 mm length, developing embryos, palpations or young born in captivity).

Date	SVL Litter (mm) size		Locality	Source		
14 March	802	7	Maricopa Co., AZ	ASU 1418		
4 April	833	8	Pima Co., AZ	UAZ 27666		
16 April	813	13	Graham Co., AZ	UAZ 42695		
18 April	793	10	Pima Co., AZ	UAZ 42980		
24 April	693	5	Maricopa Co., AZ	UAZ 42981		
24 April	742	6	La Paz Co., ÁZ	UAZ 49343		
28 April	743	12	Pinal Co., AZ	UAZ 42982		
30 April	740	10	Pima Co., AZ	UAZ 27669		
7 May	699	6 <sup>a</sup>	Pima Co., AZ	this paper		
5 June	707	7 a	Pima Co., AZ	this paper		
20 June	611	8 b	Presidio Co., TX	LACM 109531		
18 July	647	8 <sup>c</sup>	Chihuahua, MX	UAZ 34319		
26 August	751	5 d	Pima Co., AZ	this paper		
24 September	696	12 e	Chihuahua, MX	UAZ 35196		

a Determined by palpating live females.

scutulatus female collected in July. All seven C. scutulatus females collected in April had enlarged follicles (> 12 mm length) and probably would have ovulated later in the year. However, as they were from five different years, these observations cannot be taken as an indication that all April S. scutulatus females are gravid in any one year. Similarly, the absence of females with enlarged follicles or oviductal eggs in May (Table 2) probably reflects a small monthly sample size (n = 3), rather than suggesting that gravid females do not occur during May. smallest reproductively active C. scutulatus female (LACM 109531) contained eight developing embryos and measured 611 mm SVL (Table 3). Four other reproductively active females in the 600-699 mm SVL class are listed in Table 3. One smaller female (UAZ 34797) from June that measured 543 mm SVL was undergoing early yolk deposition (i.e. secondary vitellogenesis sensu Aldridge 1979a). However, it is not possible to know if the follicles in this snake would have completed their development.

Mean litter size from 14 C. scutulatus females (Table 3) was  $8.4 \pm 2.65$  SD, range 5-13. This value may be slightly higher than what actually occurs since litter sizes for eight of the 14 females (Table 3)

b Contained developing embryos.

<sup>&</sup>lt;sup>c</sup> Collected 11 July, gave birth in captivity.

d Collected 22 July, gave birth in captivity.

e Collected 14 June, gave birth in captivity.

-				•						
Year	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996
Sample Size	4	6	4	4	1	5	4	1	1	2
Gravid	3	3	1	2	1	4	3	0	1	0
not gravid	1	3	3	2	0	1	1	1	0	2
% gravid	75	50	25	50	100	80	75	0	100	0

Table 4. Yearly percentages of gravid versus non-gravid adult female *Crotalus scutulatus* captured May-August from the Valley of the Ajo, Pima County, Arizona 1987-1996.

came from counts of follicles > 12 mm length. There is a chance that not all enlarged follicles would have completed development. Mean brood (= litter) size value from 21 females from Klauber (1972) was  $8.1 \pm 2.21$  SD, range 5-13. There was no significant difference between these two data sets (t = 0.317, P > 0.7). Combining these data gave a mean litter size of  $8.2 \pm 2.36$  SD, range 5-13 for 35 C. scutulatus. This value is within the eight to 10 per brood (= litter) reported for "our commonest" rattlesnakes of the United States by Klauber (1972).

Three females gave birth to litters in captivity (Table 3). One litter (from UAZ 35196) was born 24 September and consisted of 12 young with a mean SVL of 220 mm  $\pm$  3.4 SD, range 213-224 mm. The second litter (from UAZ 34319) was born 18 July and consisted of eight young with a mean SVL of 216 mm  $\pm$  3.8 SD, range 211-221 mm. The third litter was born 26 August and consisted of five young with a mean SVL of 270 mm  $\pm$  5.1 SD, range 264-278 mm. Young C. scutulatus are born July through September with a peak in August (Ernst 1992).

Twenty-eight percent (14/50) of females from March-August contained enlarged follicles > 12 mm length, or developing young and would have likely produced young. This sample includes 11 litters from 47 museum specimens collected March-September, two litters from palpations, and one litter from a female that gave birth in captivity. This is within the range of 7-70% for annual percentages of females reproducing reported in a survey of 85 snake species (Seigel & Ford 1987).

Yearly percentages (1987-1996) of gravid *C. scutulatus* from Valley of the Ajo, Pima County, Arizona are given in Table 4. While sample sizes are too small to ascertain what percent of the female population bears young each year, it is clear that in most years, not all females

were gravid. Furthermore, trapping surveys revealed a low point in rodent density during 1989, 1990, high densities during 1991-1995 and a marked decline by 1996 in the Valley of the Ajo (unpublished data). During the years of high rodent density (1991-1995), 75% (9/12) of female C. scutulatus were gravid compared to the years of low rodent density (1989-1990, 1996), when 30% (3/10) were gravid (Fisher's exact test, P = 0.05). Although this sample size is clearly limited, these data suggest there may be a relationship between food availability and production of young in C. scutulatus.

Crotalus scutulatus females from June, August-September, November (Table 2) were undergoing early yolk deposition (vitellogenic granules = secondary yolk deposition sensu Aldridge 1979a) at which time it would not have been possible to complete the process and ovulate during the current activity season. This suggests that C. scutulatus females have a biennial reproductive cycle in which yolk deposition begins in summer followed by ovulation the next year. This cycle was reported for C. viridis in Wyoming by Rahn (1942), C. atrox in Texas by Tinkle (1962) and was suggested to occur in C. tigris and C. molossus by Goldberg (1999a; 1999b). The frequency of reproduction in North American rattlesnakes is apparently variable, with some populations producing litters each year (Fitch 1985; Fitch & Pisani 1993) and the possibility of biennial, triennial or quadraennial cycles occurring within the same species (Ernst 1992). To definitively answer this question will require a long-term field study in which individual females are re-captured for several years and their reproductive condition noted.

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SRG at: sgoldberg@whittier.edu

#### SYNTHESIS OF A NEW HETEROCYCLIC AMIDE-IMINE

### Ben A. Shoulders, C. W. Schimelpfenig and Gretchen M. Schimelpfenig\*

Department of Chemistry, University of Texas at Austin Austin, Texas 78712 and \*Department of Chemistry, University of North Texas Denton, Texas 76203

Abstract.—Methyl glutarate and ethylenediamine react at 162°C to form 1,7-diaza-2-oxobicyclo[4.3.0]non-6-ene. This unexpected product may be the result of a transannular amide-amide reaction with loss of water from the first-formed nine-membered ring ethylene glutaramide. This bicyclic amide-imine has been reduced with lithium aluminum hydride to 1,7-diazabicyclo[4.3.0]nonane.

The difficulty of making nine-membered ring compounds from non-cyclic precursors has been demonstrated. Examples can be found in discussions of the unusual properties of medium-sized rings (Prelog 1950; Huisgen 1957; Sicher 1962; Eliel et al. 1965; Dale 1971). The difficulty is caused by overlap of van der Waals radii and repulsion before the two ends of a chain may become joined and, in the ring, by repulsions between transannular hydrogens and by unfavorable dihedral angles. The steric reasoning was first proposed over 60 years ago (Stoll & Stoll-Comte 1930). However, a discussion of torsional constraints (planar groups such as amide and benzo) predicted that ring formation should be easier if these groups were present (Alder & White 1988).

An interesting example of a medium ring with torsional constraint groups was presented by the observation that the nine-membered ring, cyclic ethylene glutaramide, could be made by reaction of ethylene-diamine and a diester of glutaric acid in a heated solution of an oxygen-containing solvent (Lippert & Reid 1939). Experimental details for this particular synthesis were not presented in the patent examples, but related examples, reactions of ethylenediamine and other diesters, involved refluxing methanol solvent (b.p. 65°C) and times of 40 to 50 hours. Over three decades later cyclic ethylene glutaramide was again prepared (Grinberg et al. 1975). These authors tried to dehydrate cyclic ethylene glutaramide by azeotropic distillation, but they were unsuccessful.

This study of the reaction between methyl glutarate and ethylene-diamine involved refluxing diglyme solvent (b.p. 162°C) for 96 hours.

The major product was 1,7-diaza-2-oxobicyclo[4.3.0]non-6-ene (Compound 1), a new compound. Reaction at 162°C appears to provide an example of a transannular reaction between two amide groups of cyclic ethylene glutaramide.

Transannular reactions between one amide group and one other group have already been observed (Witkop et al. 1951; Cohen & Witkop 1955; Shemyakin et al. 1965). Moreover, transannular amide-amide reactions have been observed to give monocyclic products (Glover & Rapaport 1964; Glover et al. 1965). Also noteworthy are reactions in which medium-sized ring dipeptides gave bicyclic products by azeotropic distillation of water (Shemyakin et al. 1965).

When Compound 1 was reduced with lithium aluminum hydride, the product was 1,7-diazabicyclo[4.3.0]nonane (Compound 2).

The structure of Compound 1 was proved by nuclear magnetic resonance (NMR) and by high resolution mass spectrometry (HRMS). The <sup>13</sup>C chemical shifts are consistent with chemical shifts in similar bicyclic acylamidines (Huang & Wamhoff 1984). Compound 2 has been reported (Alder et al. 1982), but physical properties and analytical information about Compound 2 were not mentioned in the article; therefore, the data are included below.

#### SYNTHESIS AND ANALYSIS OF EACH COMPOUND

Preparation of compound 1.—1,7-Diaza-2-oxobicyclo [4.3.0] non-6-ene (Figure 1). To a flask previously flushed with argon, which was equipped with a magnetic stirrer and a reflux condenser, were added 300 mL of diglyme, 16.0 g (0.1 mol.) of methyl glutarate, and 6.0 g (0.1 mol.) of ethylenediamine. The reaction mixture was stirred and refluxed for 96 hours. During the first 48 hours the solution was transparent, but a precipitate began forming after 48 hours. At room temperature the solid was removed by gravity filtration. It was dried in a vacuum desiccator over phosphoric anhydride. The pale yellow powder weighed 1.72 g and melted with decomposition at 260-270°C - reported for cyclic ethylene glutaramide, 287-289°C (Grinberg et al. 1975). Diglyme was removed from the filtrate by distillation. The residue was distilled. Compound 1 distilled at 90-92°C (0.2 torr). The colorless distillate was a super-cooled liquid which slowly solidified; the solid melted at 45°C. This colorless distillate weighed 7.7 g (56% yield). The structure was determined by HRMS (molecular ion 139.087349,

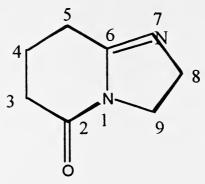


Figure 1. Compound 1.

 $C_7H_{11}N_2O^+$ ) and two dimensional NMR (COSY, NOSY, HMQC, HMBC): C2,  $\delta$  157.6; C3,  $\delta$  24.5; C4,  $\delta$  18.1; C5,  $\delta$  31.4; C6,  $\delta$  167.8; C8,  $\delta$  40.2; C9,  $\delta$  52.3; H3,  $\delta$  2.08; H4,  $\delta$  1.10; H5,  $\delta$  1.95; H8,  $\delta$  3.35; H9,  $\delta$  3.46. All NMR spectra were taken in  $C_6D_6$ . The infrared spectrum of Compound 1 exhibited strong absorption bands at 1692 cm<sup>-1</sup> and 1652 cm<sup>-1</sup>, but not any absorption at a higher frequency than 3000 cm<sup>-1</sup>.

The perchlorate, colorless plates from ethanol, melted at 120-121°C.

Anal.: Calc. for  $C_7H_{10}N_2O \cdot HC1O_4$ : C, 36.03; H, 4.59; N, 11.59 Found: C, 35.79; H, 4.54; N, 11.54

The infrared spectrum of the perchlorate exhibited strong absorption bands at 3200, 1710, and 1645 cm<sup>-1</sup>.

The picrate, yellow needles from ethanol, melted at 203-204°C Anal.: Calc. for  $C_7H_{10}N_2O \cdot C_6H_3N_3O_7$ : C, 42.51; H, 3.57; N, 19.07 Found: C, 42.73; H, 3.44; N, 18.88

Preparation of compound 2.—1,7-Diazabicyclo[4.3.0]nonane (Figure 2). To a flask previously flushed with argon, which was equipped with a magnetic stirrer and a reflux condenser, were added 125 mL of ethyl ether, 3.8 g of lithium aluminum hydride powder, and 2.0 g of Compound 1. The mixture was stirred at room temperature for 14 hours and then was heated to reflux for two hours. The mixture was chilled with an ice-water bath and 10 mL of water was added dropwise. The solid was removed by filtration and the filtrate was concentrated to a yellow/orange residue. The residue was distilled. There was obtained 0.70 g of colorless Compound 2, which distilled at 26°C (0.2 torr),

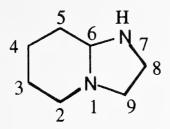


Figure 2. Compound 2.

38% yield. The structure was studied by HRMS (molecular ion 127.123819,  $C_7H_{15}N_2^+$ ) and two dimensional NMR (COSY, NOSY, HMQC): C2,  $\delta$  50.3; C3,  $\delta$  25.1; C4,  $\delta$  23.4; C5,  $\delta$  29.9; C6,  $\delta$  78.6; C8,  $\delta$  42.8; C9,  $\delta$  52.2; H2,  $\delta$  2.97 and  $\delta$  1.94; H3,  $\delta$  1.52 and  $\delta$  1.41; H4,  $\delta$  1.72 and  $\delta$  1.20; H5,  $\delta$  1.77 and  $\delta$  1.14; H6,  $\delta$  2.38; H8,  $\delta$  2.88 and  $\delta$  2.68; H9,  $\delta$  2.97 and  $\delta$  2.00. All NMR spectra were taken in DMSO-d<sub>6</sub>.

The infrared spectrum of Compound 2 exhibited no absorption in the region between 1500 cm<sup>-1</sup> and 2700 cm<sup>-1</sup>. A medium band at 2795 cm<sup>-1</sup> and a weak band at 3320 cm<sup>-1</sup> were in Compound 2 spectrum, but not in the Compound 1 spectrum.

The perchlorate, colorless plates from ethanol, melted at 135-137°C (dec.).

Anal.: Calc. for C<sub>7</sub>H<sub>14</sub>N<sub>2</sub>·2HClO<sub>4</sub>: C, 26.00; H, 4.93

Found: C, 25.72; H, 4.90

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BAS at: bens@utxms.cc.utexas.edu

# CONSTRUCTION OF A TISSUE-SPECIFIC PUNCH PROMOTER::LACZ EXPRESSION VECTOR THAT ENCODES GTP CYCLOHYDROLASE IN THE FRUIT FLY DROSOPHILA MELANOGASTER

#### William J. Mackay and Lisa A. Phelps

Edinboro University of Pennsylvania Department of Biology & Health Services Edinboro, Pennsylvania 16444

Abstract.—The Punch locus of Drosophila encodes GTP cyclohydrolase I, a multimeric enzyme that catalyzes the first step in pterin biosynthesis. One pterin, tetrahydrobiopterin (BH4), is a necessary cofactor for tyrosine hydroxylase (TH) which is involved in neurotransmitter biosynthesis. One Punch clone, pO.380, overlaps the 5' end of a 1.75 kb transcript. Punch mutations which alter the levels of this transcript also affect TH activity. A restriction map of pO.380 was generated; two restriction sites, EcoRI and NheI, were found to flank the promoter sequence. A 2.3 kb EcoRI - NheI DNA fragment was gel purified and ligated into the polycloning site of the expression vector, pJY505, which is upstream and in frame of the lacZ gene that encodes ß galactosidase. This Punch pO.380 promoter::lacZ vector can be injected into Drosophila embryos, and tissue specific expression of this construct can be detected by staining tissues with the ß galactosidase chromogenic substrate, X Gal.

Catecholamines, including dopamine, noradrenaline and adrenaline, are key signaling agents in the mammalian brain, sympathetic ganglia and in the adrenal medulla. A variety of disorders are associated with changes in the biosynthetic pathways of these agents. For example, both Parkinson's disease and schizophrenia are associated with low and high levels, respectively, of dopamine in the brain. Tyrosine hydroxylase (EC 1.14.16.2, TH) catalyzes the hydroxylation of tyrosine to 3,4 dihydroxyphenylalanine (L DOPA) with the oxidation of tetrahydrobiopterin (BH4), and is the rate limiting reaction in the synthesis of dopamine, a precursor molecule for epinephrine and norepinephrine (Nagatsu et al. 1964; Zigmond et al. 1989; Stathakis et al. 1999). BH4 is absolutely required as a cofactor for the activity of TH. BH4 is synthesized from guanosine triphosphate (GTP), and the rate limiting reaction in this biosynthetic pathway is catalyzed by GTP cyclohydrolase I (EC 3.5.4.16, GTPCH) (reviewed by Brown 1985). Failure to synthesize adequate amounts of GTPCH leads to a neurological syndrome in humans associated with deficits in catecholamines, as well as serotonin and other BH4 dependent products (reviewed by Scriver & Chow 1980; Scriver et al. 1994).

The fruit fly, *Drosophila melanogaster*, is an ideal organism to study developmentally regulated pathways and has been the object of molecular, genetic and biochemical experimentation for more than 85 years. The biosynthetic pathway that produces BH4 in Drosophila has been subjected to extensive genetic, biochemical and molecular analyses with an emphasis on the first and rate limiting enzyme, GTPCH (Mackay & O'Donnell 1983; Mackay et al. 1985; McLean et al. 1990; 1993). This multimeric enzyme, which functions in the synthesis of pteridine eye pigments as well as BH4 cofactor, exists as multiple isoforms that are expressed in a developmentally and functionally specific manner (O'Donnell et al. 1993). The Punch (Pu) locus encodes four mRNA transcripts which translate into multiple isoforms of GTPCH, and a 1.75 kb transcript was shown to be associated with TH activity (O'Donnell pers. comm.). The following were completed during this study: (a) construction of a comprehensive restriction map of a 3.8 kb Pu clone, pO.380, which overlaps the 5' end of the 1.75 kb transcript; (b) identified two unique sites within pO.380, *Eco*RI and *Nhe*I, that flank the 5' promoter for the 1.75 kb transcript; and (c) ligated the 2.3 kb *Pu* EcoRI - NheI promoter fragment into a lacZ expression vector, pJY505, in order to monitor tissue specific promoter expression.

#### MATERIALS AND METHODS

Isolation of plasmid vector.—Plasmids pO.380, a vector which contains 3.8 kb of Pu DNA subcloned into the EcoRI restriction site within the polylinker region of pBluescribe (Stratagene) (McLean et al. 1990), and the lacZ expression vector, pJY505 (Neckameyer pers. comm.), were isolated using a modified Triton Lysis miniprep procedure (Ausubel et al. 1989). DNA was visualized using agarose gel electrophoresis (0.08% agarose gel, 100 volts, 2 hours). One microgram of HindIII (New England Biolabs) digested  $\lambda$ DNA was included on each gel as a molecular size standard.

Restriction mapping of pO.380.—pO.380 was digested with a variety (approximately 30) of restriction endonucleases (New England Biolabs; Promega) according to the manufacturer's instructions. DNA fragments were separated and analyzed by agarose gel electrophoresis.

Fusion of the Pu EcoRI - Nhel promoter fragment into pJY505.— pO.380 (approximately 5  $\mu$ g) was digested with EcoRI (New England Biolabs) (2 hr; 37°C). The resulting digested solution was extracted

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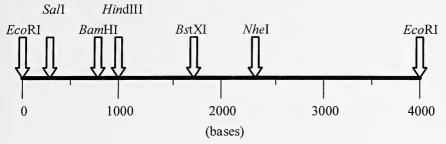


Figure 1. Restriction map of pO.380.

with phenol; chloroform to remove the endonuclease, and the DNA was concentrated by ethanol precipitation. The EcoRI treated DNA was then digested overnight (37°C) with NheI. The resulting double digested solution was then extracted with phenol:chloroform to remove the second endonuclease, and the DNA was concentrated by ethanol precipi-The resulting 2.3 kb restriction fragment was separated by agarose gel electrophoresis. The fragment was excised from the agarose gel using a scalpel and gel purified using a GlassMAX® DNA Isolation Spin Cartridge System according to the manufacturer's instruction (GIBCO BRL). pJY505 (approximately 5  $\mu$ g) was double digested with EcoRI and XbaI (New England Biolabs) (2 hr; 37°C). The resulting double digested solution was extracted with phenol:chloroform to remove the restriction endonucleases, and the DNA was concentrated by ethanol precipitation. The double digested pJY505 was treated with calf intestinal phosphatase (CIP) (Promega) (final CIP concentration 0.05% total volume; 2 hr at 37°C), an enzyme that catalyzes the hydrolysis of 5' phosphate residues and prevents recircularization of vector DNA due to self ligation of linear vector molecules (Ausubel et al. 1995). The treated vector was extracted with phenol:chloroform to remove the CIP, and the DNA was concentrated by ethanol precipitation. purified insert DNA was ligated overnight into the EcoRI - XbaI restriction sites within the polylinker region of the pJY505 (16°C; ratio of insert DNA to vector DNA was approximately 10:1). The circular, ligated vector plus insert was transformed into CaCl<sub>2</sub> treated competent ampicillin sensitive JM101 cells and transformants were isolated by their growth in LB media containing ampicillin (Ausubel et al. 1995). Plasmid DNA from ampicillin resistant cells was purified, double digested with EcoRI and KpnI, and separated and analyzed by agarose gel electrophoresis that confirmed the presence of the 2.3 DNA fragment in the vector.

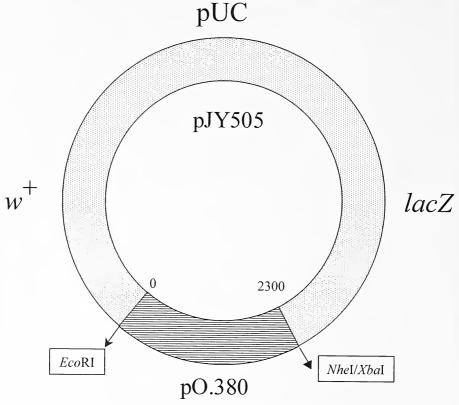


Figure 2. Insertion of the *EcoRI - NheI* pO.380 restriction fragment into the expression vector pJY505 (see Materials and Methods for details of construction).

#### RESULTS AND DISCUSSION

Previous reports have shown that a 3.8 kb Pu clone, pO.380, contains DNA sequences which overlap the 5' end of a 1.75 kb Pu transcript (McLean et al. 1990; 1993). Subsequent genetic and molecular analyses have demonstrated that Pu mutations which map within pO.380 and affect the levels of the 1.75 kb transcript also affect TH activity (O'Donnell pers. comm.). Figure 1 represents the construction of a comprehensive restriction map of this clone. pO.380 was digested with approximately 30 different restriction endonucleases (New England Biolabs; Promega) according to the manufacturer's instruction. The restriction mapping analysis of pO.380 identified 10 unique restriction sites within the 3.8 kb DNA fragment (five restriction sites are illustrated in Figure 1). DNA sequencing and transcript analyses positioned an NheI site between the start sites for transcription and

translation (O'Donnell pers. comm.). Thus, two restriction sites, EcoRI and NheI, which are separated by 2.3 kb, should contain most or all of the necessary eukaryotic promoter sequences for the 1.75 kb Pu transcript. The 2.3 kb EcoRI - NheI Pu DNA fragment was separated by agarose gel electrophoresis and gel purified (see Materials and Methods). This fragment was ligated into the EcoRI - XbaI restriction sites within the polylinker region of the lacZ expression vector pJY505 (Figure 2). pJY505 is a 12 kb plasmid which contains a white gene as a selectable marker in order to identify transgenic Drosophila that receive a stable recombinant promoter::lacZ construct via P-element mediated transformation as well as multiple cloning sites upstream of a truncated hs43 promoter and a ß galactosidase gene. The *Punch* pO.380 promoter::lacZ vector will be injected into Drosophila white embryos (Spradling 1986), and transgenic offspring will be identified as flies with pigmented eyes (i.e.,  $w^+$  phenotype). Tissue-specific localization of the reporter gene activities will be detected by staining dissected tissues of transgenic flies with the ß galactosidase chromogenic substrate, X Gal (Gönczy et al. 1992). These experiments foreshadow the long range goals of an effort to understand the structure, function and regulation of a specific pteridine biosynthetic gene during the development of a multicellular eukaryotic organism.

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WJM at: bamackay@erie.net

## HARRINGTON'S EXTINCT MOUNTAIN GOAT (OREAMNOS HARRINGTONI STOCK 1936) FROM MUSKOX CAVE, NEW MEXICO

Christopher N. Jass\*, Jim I. Mead and Lloyd E. Logan

Laboratory of Quaternary Paleontology, Quaternary Studies Program, Box 5644
Northern Arizona University, Flagstaff, Arizona 86011-5644
Department of Geology, Northern Arizona University
Flagstaff, Arizona 86011 and
Chase Studio, 205 Wolf Creek Road, Cedarcreek, Missouri 65627
\*Present address:
Department of Geological Sciences
University of Texas, Austin, Texas 78712

Abstract.—Oreamnos harringtoni (Harrington's extinct mountain goat) (Artiodactyla: Bovidae) remains are relatively uncommon in the fossil record. Pleistocene-age skeletal remains from Muskox Cave, Guadalupe Mountains, New Mexico, are referred to O. harringtoni. Qualitative and quantitative characters of recovered skeletal elements fit previous descriptions for Harrington's extinct mountain goat. This specimen is the first from the region and extends the known range of the species eastward.

The Guadalupe Mountains of New Mexico/Texas have numerous caves containing a wealth paleontological remains of Pleistocene and Holocene age (Harris 1993). Several caves along with various portions of the fossil faunas have been described (Ayer 1936; Gelbach & Holman 1974; Harris 1970; Howard 1932; Logan 1983; Logan & Black 1979; Lundelius 1979; Rickart 1977; Schultz & Howard 1935). Of particular importance here are the largely undescribed remains from Muskox Cave.

Muskox Cave is situated at approximately 1,600 m (5,250 ft) elevation on the southwestern slope of a side canyon in the arid, eastern face of the northern Guadalupe Mountains, Carlsbad Caverns National Park, Eddy County, New Mexico. The present entrance is small, with the cave floor situated approximately 35 m below the surface (Logan 1981). During the Pleistocene, various extinct and extant animals were naturally trapped by a now-choked entrance (Logan 1981). Radiocarbon dates between  $25,500\pm1100$  and  $18,140\pm200$  YBP indicate that the age of the Muskox Cave fauna is within the late Wisconsinan Glaciation of the late Rancholabrean Land Mammal Age (Harris 1993; Logan 1981).

In a preliminary review of Pleistocene-age fossils from Muskox Cave, Logan (1981) indicates recovery of an undescribed mountain goat-like bovid. Mountain goats are a form of caprine bovid (Artiodactyla: Bovidae: Caprinae) and typically are placed within the problematic, and

likely polyphyletic, tribe Rupicaprini (Gentry 1992). Based on several skeletal elements, it was thought at the time that the undescribed bovid might represent a new taxon. Both cranial and postcranial elements were recovered and are currently curated at the United States National Museum (USNM 244235). It is believed that all of these elements represent a single individual. As will be shown, these bovid remains from Muskox Cave can be referred to *Oreamnos harringtoni*. A detailed analysis of the osteology of *O. harringtoni* from the Grand Canyon of Arizona, Nevada and Utah (Mead & Lawler 1994) provides data for quantitative comparison.

Oreamnos harringtoni is not just a smaller mountain goat (approximately 66% the size) as compared to the living species (O. americanus), as alluded by Stock (1936). Although often smaller in some aspects, O. harringtoni is proportionally different. Based on data presented in Mead & Lawler (1994), O. harringtoni has the following appearance in comparison to the living O. americanus: (1) the tooth row, therefore the greater part of the face, is as long or slightly longer; (2) the palate is often narrower; (3) although there is overlap in measurements, the M3/ is typically larger and more robust; (4) the area between the orbits is narrower, making a narrower-appearing face; (5) the mandible is more often than not wider, meaning a more massive or robust jaw bone; (6) horncores are rarely as large as those of the smaller adults (females of the living species); and (7) metacarpals and metatarsals are considerably smaller (by about 25%) at the proximal end and in total length, indicating the animal had a shorter stature but still a robust foot. In summary, O. harringtoni was considerably shorter; its masticatory region was robust but situated on a narrower face, which was accentuated by the presence of thinner and smaller horns.

## MUSKOX CAVE *OREAMNOS* (Figures 1 & 2)

Mountain goat remains from Muskox Cave described here include a skull, both dentaries (= mandibles) and a left metatarsal. These elements allow for morphometric and qualitative comparisons with published data about the living form of mountain goat (O. americanus) and the extinct O. harringtoni. The humerus, femur, tibia and calcaneum, also part of the same Muskox Cave specimen, will be described as part of a separate project when those elements from Oreannos are better diagnosed. Mead & Lawler (1994) described qualitative and morphometric characters of the skull, mandible, horncores, keratinous hornsheaths and metapodials of O. harringtoni and O. americanus.

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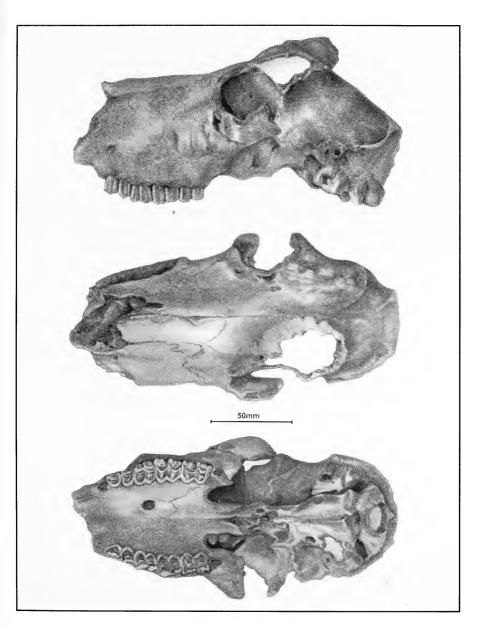


Figure 1. Skull of Oreamnos harringtoni from Muskox Cave. Scale in mm.

Skull.—The majority of the skull is intact although certain portions, important in this analysis, are fragmented or missing (Fig. 1). The left horncore is missing; the right is broken and poorly preserved. Due to the state of preservation, only two horncore measurements were possi-

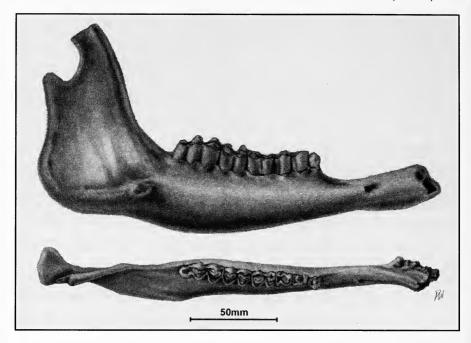


Figure 2. Mandible of Oreamnos harringtoni from Muskox Cave. Scale in mm.

ble. Dorso-lateral portions of the orbits are not well-preserved. The premaxilla is absent, as are the anterior portions of the maxilla, anterior to the P3/. No hornsheaths were recovered.

Measurements (Table 1) follow Mead & Lawler (1994). measurements of the skull lie outside the previously known range of Oreamnos harringtoni stated in Mead & Lawler (1994). In particular, the alveolar length of P2/-M3/ is smaller than is found in O. harringtoni from the Grand Canyon (Table 1). When measurements of the facial region are plotted against data from Mead & Lawler (1994), the skull appears to be closer to O. americanus, possessing a wider palate with a smaller tooth row than what is known to be O. harringtoni (Fig. 3a and 3b). Mead & Lawler (1994) recognized that there is not significant difference between O. harringtoni and O. americanus in measurements of the facial region. However, the data presented here indicates a greater range of variation in the skull of O. harringtoni than was previously known. Such differences may indicate geographic variation in O. harringtoni as the majority of data provided by Mead & Lawler (1994) for the skull represents specimens from the Grand Canyon, Arizona. However, due to the state of preservation, the skull

Table 1. Measurements (mm) of the skull, horncore, mandible and metatarsal of *Oreamnos harringtoni* from Muskox Cave, Guadalupe Mountains, New Mexico in comparison to those of *O. harringtoni* and *O. americanus* reported in Mead & Lawler (1994: observed range). For consistency, measurements of the upper dentition, lower dentition and dentary width of Muskox Cave *Oreamnos* are from the right side.

Measurement	Muskox Cave Oreamnos	O. harringtoni	O. americanus
	SKULL		
Alveolar length: P2/ - M3/ *	73.1	78.5-85.6	67.5-81.2
Palatal width (middle of P2/) *	30.0	22.2-32.9	28.2-49.0
Palatal width (middle of M3/)	49.9	38.3-44.7	37.2-53.5
Alveolar length: M1/ - M3/	48.4	50.7-62.9	47.0-55.2
Alveolar length: P2/ - P4/ *	23.5	22.5-26.9	24.0-25.3
Least width between orbits *	60.0	60.0-69.5	71.0-97.0
	HORN CORE		
Nasal-nuchal length at burr base *	30.1	19.0-36.0	31.3-48.3
Medial-lateral width at burr base	27.3	15.9-34.8	26.8-41.4
	MANDIBLE		
Length of tooth row	78.1	69.4-90.7	66.6-87.0
Length of m/3	22.1	26.5-32.6	21.8-29.2
Width of m/3 Greatest buccal-lingual width	10.4	9.7-11.6	7.4-10.1
below tooth row	19.0	15.1-21.5	12.2-16.2
	METATARSAL		
Greatest proximal-distal length	108.3	79.8-116.3	114.2-137.4
Medio-lateral width (proximal)	26.2	23.4-26.3	28.3-34.7
Antero-posterior depth (proximal)	20.4	19.3-22.4	24.0-31.7
Medio-lateral width (distal)	30.5	20.0-31.1	37.3-43.7

<sup>\*</sup>Measurements are approximate due to somewhat poor preservation of specimen.

discussed here may not be the best candidate for making such an argument. As mentioned, the P2/ portion of the tooth row is missing. As a result, estimates of alveolar length and width of palate across P2/ may be imprecise. Furthermore, the preservation state of the orbits likely contributed to a somewhat low measurement for the width between orbits, giving the Muskox Cave specimen the appearance of a narrower face than is typical for *O. harringtoni*.

In all qualitative characters, the skull is more similar to *O. harringtoni* than *O. americanus*. In particular, the sphenoid and the vomer form a "V", as is found in *O. harringtoni* (versus *O. americanus* 

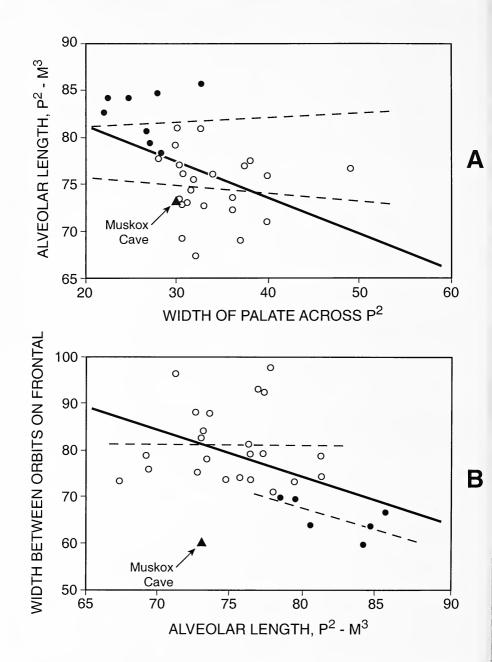


Figure 3. Diagram of the measurements of the facial region of *Oreamnos harringtoni* vs. O. americanus. (A) Plot of palatal width across P2/ vs. alveolar length of maxillary tooth row. (B) Plot of alveolar length of maxillary tooth row vs. width of orbitals on frontals. Measurements in mm. Lines represent regressions (not including Muskox Cave data) with the heavy line equal to the overall regression for the genus and the dashed lines equal to the regression for each species. Graphs modified from Mead & Lawler (1994). Solid dots = O. harringtoni. Circles = O. americanus.

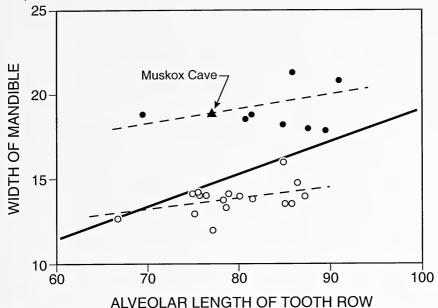


Figure 4. Plot of alveolar length of mandibular tooth row vs. width of mandible for *Oreamnos*. Lines represent regressions (not including Muskox Cave data) with the heavy line equal to the overall regression for the genus and the dashed lines equal to the regression for each species. Data modified from Mead & Lawler (1994). Solid dots = O. harringtoni. Circles = O. americanus. Measurements in mm.

where the same bones tend to form a "U"; Mead 1983). Additionally, the portion of preserved horncore appears to be directed more posteriorly, as in *O. harringtoni*, than dorsally, as in *O. americanus*.

Mandible.—Measurements (Table 1) of the dentary correspond well with data on Oreamnos harringtoni presented in and Mead & Lawler (1994). Both left and right dentaries are in good condition and contain complete cheek tooth rows (Fig. 2). When the alveolar length of the tooth row is plotted against the thickness of the mandible below the tooth row, the Muskox Cave specimen and O. harringtoni show significant difference from that of O. americanus (Figure 4 this study; Mead & Lawler 1994). The Muskox Cave specimen lies well within the line of regression for known O. harringtoni. The mandible of O. harringtoni is considerably larger than O. americanus, which may reflect a dietary adaptation (Mead 1983).

Metatarsal.—Measurements of the metatarsals of O. harringtoni and O. americanus show significant differences when the proximal width is plotted against the total length (Table 1 this study; Mead & Lawler

#### **METATARSAL**

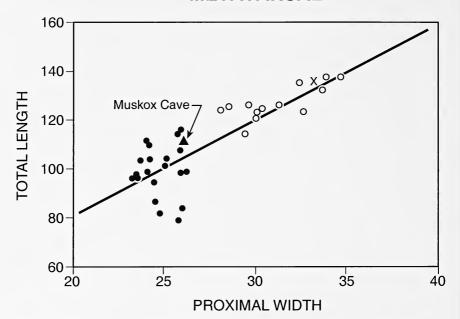


Figure 5. Plot of the proximal width of the metatarsal vs. total length for *Oreamnos*. Regression line does not include data from Muskox Cave. Data modified from Mead & Lawler (1994). Solid dots = O. harringtoni. Circles = O. americanus. X = Rancholabrean-age O. americanus. Measurements in mm.

1994). Such measurements on the Muskox Cave specimen indicate that it is well within the plot for other *O. harringtoni* (Fig. 5).

#### DISCUSSION

Stock (1936) first described *Oreamnos harringtoni* from Smith Creek Cave, Nevada. Compared to other Pleistocene mammals, mountain goat remains are fairly uncommon, possibly due to a lack of preservation in their preferred habitat. Today, mountain goats live in mountainous habitats which are typically unconducive to fossil preservation (Harington 1971). *Oreamnos harringtoni* remains which date to the Rancholabrean Land Mammal Age come from the Grand Canyon (Mead 1983; Mead & Lawler 1994), elsewhere on the Colorado Plateau (Mead et al. 1987), the Great Basin (Stock 1936; Mead & Lawler 1994), U-Bar Cave in extreme southwest New Mexico (Mead & Lawler 1994: Table 1 footnote), and San Josecito Cave, Mexico (Wilson 1942). One other deposit, Porcupine Cave, Colorado, contains remains referred to *O*.

harringtoni which date to the Irvingtonian Land Mammal Age (Mead & Taylor 1998). The skeletal elements from Muskox Cave described herein extend the known geographic range of Rancholabrean O. harringtoni, but lie well within the expected distribution hypothesized by Mead & Lawler (1994). These remains are the first record of O. harringtoni from the Guadalupe Mountains.

Today, portions of western North America previously inhabited by Oreamnos harringtoni are characterized by widely divergent environmental settings. Unlike the Pleistocene record of O. harringtoni from the Grand Canyon, which contains direct evidence of the contemporaneous environment in the form of dung remains (Mead et al. 1986), the paleoenvironmental surroundings of extinct mountain goat from the Guadalupe Mountains can only be inferred. During the Wisconsinan Glaciation (≈80,000 - 11,000 YBP), plant and faunal communities in the Guadalupe Mountains were quite different than modern. Today, Muskox Cave is surrounded by a complex Chihuahuan desertscrub community with chaparral and grassland species (Logan 1981; Van Devender 1990). Regional packrat (Rodentia, Neotoma) midden studies and other faunal evidence provide a basis for the interpretation of paleoenvironmental conditions at Muskox Cave.

Van Devender (1990) reviewed a total of 220 packrat middens with 259 associated radiocarbon dates for the entire present Chihuahuan Desert region. This region shows an overall latitudinal trend, with increasing similarities between Wisconsinan Glacial floras/faunas and modern floras/faunas towards the south (Van Devender 1990; Van Devender & Bradley 1990). In contrast, based on the packrat midden record, it appears that Late Pleistocene plant community composition for the more northerly Guadalupe Mountains would have been substantially different than modern.

In the southern Guadalupe Mountains, during the Wisconsinan Glaciation, mixed-conifer forest transitioned into various types of pinyon/juniper/oak woodlands near 1500 m (Van Devender 1990). Today, the mixed-conifer component in the southern Guadalupe Mountains is situated at 2400 m (Van Devender 1990) and the northern Chihuahuan Desert now occupies the elevational gradient in which the pinyon/juniper/oak woodlands previously occurred (Van Devender & Bradley 1990). Differences in the fossil and modern botanical record may be a result of cooler summers and greater winter rainfall in the late

Wisconsinan Glacial (Van Devender 1990). A similar climatic pattern for southeastern New Mexico has been interpreted based on the Full Glacial portion of the mammalian fauna from Dry Cave (Harris 1989).

Logan (1981) indicates that the area surrounding Muskox Cave during the Pleistocene likely contained spruce-fir forest with intermittent grassy meadows. Harris (1985) suggests coniferous forest with a boreal aspect and grassy areas with woodland elements for the Muskox Cave area. Either interpretation appears consistent with the packrat midden record, the faunal record, as well as data from Mead et al. (1986) on the diet of *O. harringtoni* from the Grand Canyon. Faunal communities of the late Wisconsinan were varied and ever-changing, as was the vegetation. *Oreannos harringtoni* from Muskox Cave was associated with these dynamic communities.

#### **CONCLUSIONS**

Oreamnos harringtoni and O. americanus have similar morphometric features of the facial region while other skeletal elements show significant differences between the two species. In some cranial respects, the Muskox Cave specimen seems to contain a mixture of characters intermediate in size between O. harringtoni and O. americanus. As a result, facial features were characterized by a narrow face with a wide mouth and robust dentary tooth row. The small horns, in addition to the qualitative characters of the skull, are indicative of O. harringtoni (Mead 1983). Graphs of morphometric characters of the mandible and metatarsal correspond well with previous data on O. harringtoni. The mountain goat from Muskox Cave would have been of short stature, as with all other specimens of O. harringtoni. Overall qualitative and morphometric characters of mountain goat fossils from Muskox Cave indicate that the remains are those of O. harringtoni.

Mead & Taylor (1998) provide the known distribution of *Oreannos harringtoni*, which now extends to southeastern New Mexico. The only reported *O. harringtoni* remains in New Mexico occur in U-Bar Cave (Mead & Lawler 1994: Table 1 foot note). These remains come from approximately the same latitude as those from Muskox Cave. The record presented here places *O. harringtoni* in the Guadalupe Mountains, the southern extension of the North American Rocky Mountains. It was assumed that *O. harringtoni* existed in the Rocky Mountains (Mead & Lawler 1994), which was probably the avenue for southward

migration to the mountainous region around San Josecito Cave, Nuevo Leon, Mexico, the apparent southernmost range extension.

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JIM at: james.mead@nau.edu

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#### MICROHABITAT FEATURES ASSOCIATED WITH THE SONG PERCHES OF PAINTED AND INDIGO BUNTINGS (PASSERIFORMES: CARDINALIDAE) IN NORTHEAST TEXAS

#### Jeffrey G. Kopachena and Christopher J. Crist

Department of Biological and Earth Sciences Texas A&M University-Commerce Commerce, Texas 75429

Abstract.—Microhabitat features surrounding the song perches of 26 Indigo Buntings (Passerina cyanea) and 24 Painted Buntings (Passerina ciris) in northeast Texas were compared to determine whether these species were segregated according to habitat. Analyses of the physical structure of vegetation in randomly selected field sites and randomly selected wooded sites surrounding the song perches failed to identify any parameters that differed between Indigo and Painted Buntings. However, the species of trees used as song perches and the species of trees identified in areas surrounding the song perches of the two species did differ. The data suggest that the two species occur in similar microhabitats, but may be ecologically segregated at a different scale, or through floristic associations rather than through the physical structure of the habitat.

Indigo Buntings (*Passerina cyanea*) are common passerines that breed through much of North America east of the Great Plains. In central and west Texas, the Indigo Bunting is largely replaced by the congeneric Painted Bunting (*Passerina ciris*). There is a broad zone of overlap between the two species that extends across much of Oklahoma, east Texas and Louisiana (Sauer et al. 1997).

Painted Buntings are neotropical migrants and, like many neotropical migrant birds, they have recently experienced population declines (Sauer et al. 1997). There are many possible reasons for these declines, but factors associated with reduced breeding performance have been cited as critically important (Terborgh 1992). For Painted Buntings, it has been suggested that, at least in some parts of their distribution, population declines may, in part, be due to competition with Indigo Buntings (Cely 1997). This seems like a plausible hypothesis because much of the available information on these two species suggests that they are ecologically equivalent (Sprunt 1968; Taber & Johnston 1968). In the popular literature (e.g. Peterson 1947; Robbins et al. 1983) both Indigo and Painted Buntings are described as occupying the same breeding habitats; these being brushy areas, river and streamside thickets and forest edges.

On the other hand, Parmelee (1959), who described the breeding behavior of Painted Buntings in southern Oklahoma, seldom observed Painted and Indigo Buntings in the same areas. This suggests that, at least in western sympatric populations, the two species might occupy different habitats and, hence, should not compete directly for resources. Unfortunately, there is no quantitative data available to test this idea. In fact, while Indigo Buntings have been well studied with respect to their association with habitat features (Posey 1974; Conner et al. 1983; Yahner 1986; Best et al. 1995), ecological gradients (Johnston & Odum 1956; Shugart & James 1973), and habitat alteration (Stauffer & Best 1980; Strelke & Dickson 1980; Triquet et al. 1990; Yahner 1993), there is no quantitative data on the habitats occupied by Painted Buntings.

The objective of the current study was to provide a quantitative description of microhabitat features associated with the song perches of territorial male Indigo and Painted Buntings in northeast Texas. In so doing, this study provides the first quantitative description of microhabitat features associated with the breeding territories of Painted Buntings. The data are contrasted between the two species to test the hypothesis that, in northeast Texas, Indigo and Painted Buntings are segregated according to habitat.

#### MATERIALS AND METHODS

Study area.—The study was conducted on the agricultural property of Texas A&M University-Commerce near the city of Commerce in Hunt County, Texas. The study area consisted of 5.5 km² of land that varied considerably in elevation and habitat types, but which reflected variation typical of this region. About 60% of the area was composed of managed and unmanaged pasture which contained a variety of wooded streamsides, bottomland woods, upland woods and fencerows. These woodland inclusions varied considerably and represented a wide variety of secondary and mature growths. Cropland, active and idle, made up another 15% of the area. These fields contained no woody vegetation, but were bordered by fencerows. About 10% of the area was managed and unmanaged hay fields. These areas were bounded by fencerows and, in one case, contained a streamside thicket and isolated stands of trees. The remainder of the study area was composed of bottomland woods and idle land in various stages of succession. Dominant trees on the study area were black oak (Quercus velutina), hickory (Carya sp.),

green ash (Fraxinus pennsylvanica), sugarberry (Celtis laevigata), pecan (Carya illinoinensis), cedar elm (Ulmus crassifolia), bois d'arc (Maclura pomifera), honey locust (Gleditsia triacanthos), post oak (Quercus stellata), red cedar (Juniperus virginiana), slippery elm (Ulmus rubra) and persimmon (Diospyros virginiana).

Data collection and analyses. - Song perches of male Indigo and Painted Buntings were located from 5 May 1996 through 25 June 1996. Singing males were identified and the species of tree in which the song perch was located was recorded. To discriminate individuals and to verify that adjacent perches represented different individuals a spotmapping technique was used (Robbins 1970). Thus, when a song perch was identified, the location of the perch was plotted onto an aerial photograph of the study area along with the locations of immediate neighbors. These simultaneous registrations (Robbins 1970) ensured that adjacent song perches did not belong to the same individuals. At least three visits were made to each song perch to verify the locations of the owner and his neighbors. In this way, each individual could be represented on the photographs as a cluster of points. However, spot mapping can cause some individuals to be overlooked while others are duplicated (see review in Verner 1985). Thus, additional measures were taken to eliminate the possible duplication of individuals in the sample. Notes were made of the song types of each male and his neighbors (distinctive syllables, phrases and accents). Painted Buntings do not share song types with neighbors (Forsythe 1974) and some individuals could be recognized this way. Also, as has been observed in South Carolina and Georgia (Forsythe 1974), many Indigo Buntings sang recognizably distinctive songs that differed from those of their neighbors. Notes on song characteristics were consulted to corroborate the identity of the resident male on subsequent checks to ensure that adjacent song perches did not belong to the same individuals. Thus, using a combination of simultaneous registrations, recognition of individuals when possible and corroborating multiple observations of singing males with the locations of specific song perches, all the song perches used were determined to be those of different individuals. Song perches of 26 Indigo Buntings and 24 Painted Buntings were used for analyses.

Ten sample points distributed in random directions within 30 m of each song perch were located by using a random number generator to

Table 1. Microhabitat variables measured in 1 m² quadrats at randomly selected field sites located within 30 m of the song perches of Indigo and Painted Buntings.

Measure	Indigo Bunting Mean ± D	Painted Bunting Mean ± SD	P a
Percent cover - grasses	28.8 ± 16.1	36.0 ± 11.0	0.074
Height of grasses (cm)	$75.1 \pm 32.5$	$67.2 \pm 21.4$	0.634
Percent cover - forbs	$35.2 \pm 16.9$	$32.4 \pm 15.4$	0.580
Height of forbs (cm)	$76.6 \pm 34.5$	$66.0 \pm 26.3$	0.420
Percent cover - bare ground	$36.6 \pm 17.8$	$31.1 \pm 10.5$	0.449
Number of woody plants	$0.49 \pm 0.45$	$0.64 \pm 0.63$	0.531
Number of thorny plants	$0.49\pm0.58$	$0.50 \pm 0.44$	0.561

Probabilities based on Wilcoxon's Rank-Sum Tests. n = 26 for Indigo Buntings, n = 24 for Painted Buntings.

assign distances and compass directions. Data on vegetation characteristics were then collected at these sample points. Because the song perches were invariably adjacent to habitat edges, the sample points either fell within fields or wooded areas. Fields were those locations where the dominant vegetation was grasses and forbs and there was no overhead cover greater than 2 m in height. Wooded sites were locations where the dominant vegetation was woody and there was overhead cover greater than 2 m in height. The type of data collected varied according to whether the sample point represented a field site or a wooded site. At field sites, data were collected within a 1 m² quadrat. Data were collected on the percent cover and maximum height of grasses and forbs, the percent of bare ground, and the number of woody and thorny plants present. For each song perch the data from all of the field sites were averaged to provide a single mean value for each variable at each song perch.

When a sampling site was wooded, data were collected on vegetation features within a circular area 4 m in diameter and centered at the sample point. In this case, data were collected on the identity of the nearest tree, the height and distance to the nearest shrub and tree, and the number of vines and thorny plants present within the sample area. For this study, trees were defined as any woody species with a diameter at breast height (dbh) of 5 cm or more, shrubs were woody plants with a dbh of less than 5 cm. Vegetation height was measured using a tape measure or, in the case of taller plants, by using a clinometer. For each

Table 2. Microhabitat variables measured at randomly selected wooded sample points located within 30 m of the song perches of Indigo and Painted Buntings.

Measure	Indigo Bunting	Painted Bunting	P a
	Mean ± SD	Mean ± SD	
Distance to nearest shrub (cm)	53.9 ± 29.9	53.4 ± 22.1	0.977
Height of nearest shrub (cm)	$158.8 \pm 89.5$	$141.7 \pm 69.5$	0.717
Distance to nearest tree (cm)	$131.0 \pm 42.6$	$115.0 \pm 55.5$	0.438
Height of nearest tree (cm)	$581.8 \pm 312.7$	$675.5 \pm 287.9$	0.387
Number of vines within 2 m	$1.1 \pm 0.64$	$1.2 \pm 0.57$	0.847
Number of thorny plants within 2 m	$1.1 \pm 0.73$	$1.2 \pm 0.70$	0.138

Probabilities based on Wilcoxon's Rank-Sum Tests.

song perch the values collected at all the wooded sites were averaged to provide a single value for each variable for each song perch.

Variables were tested for normality using Wilk's Statistic (SAS Institute Inc. 1990). Because most of the variables were not normally distributed, Wilcoxon's Rank Sum Tests were used for making comparisons between species (Pratt & Gibbons 1981, Sokal & Rohlf 1995). In cases where frequency tables were evaluated, Fisher's Exact Multiway Tables were used to determine significance (SAS Institute Inc. 1990).

#### **RESULTS**

Analyses of the data collected at random field sites are presented in Table 1. There were no significant differences between Indigo and Painted Buntings with regard to the coverage or height of grass, the height or coverage of forbs, the amount of bare ground exposed, or the number of woody and thorny plants present. Thus, the vegetation characteristics of the open areas surrounding Indigo and Painted Bunting song perches were essentially the same.

Analyses of data collected at the random wooded sites are presented in Table 2. Shrub density, measured as the distance to the nearest shrub from the sample point, and tree density, measured as the distance to the nearest tree from the sample point, were not found to differ for wooded areas associated with Indigo or Painted Buntings (Table 2). There was also no difference between bunting species with regard to overstory

n = 26 for Indigo Buntings, n = 24 for Painted Buntings.

Table 3. Species of Trees used as song perches by Indigo and Painted Buntings and species of trees found in randomly selected wooded sites surrounding the song perches of Painted and Indigo Buntings. Numbers represent the number of observations.

Tree Species	Song	Perches	Wood	ed Sites
	Indigo Bunting	Painted Bunting	Indigo Bunting	Painted Bunting
Honey Locust				
(Ğleditsia triacanthos)	7	2	16	7
Green Ash				
(Fraxinus pennsylvanica)	7	0	13	6
Bois d'Arc	2	•	2	•
(Maclura pomifera)	3	2	3	0
Pecan	2	2	2	
(Carya illinoensis)	2	2	2	6
Sugarberry (Celtis laevigata)	2	8	5	13
American Elm	2	0	3	13
(Ulmus americana)	1	0	1	0
Black Willow	•	· ·	•	ŭ
(Salix niger)	1	0	1	0
Post Oak				
(Quercus stellata)	1	2	0	0
Dead Tree	2	1	0	0
Hercules Club				
(Zanthoxylum clava-herculis)	0	2	1	2
Cedar Elm				_! !
(Ulmus crassifolia)	0	1	1	7
Slippery Elm				_
(Ulmus rubra)	0	1	6	0
Bumelia	0	1	0	
(Bumelia lanuginosa)	0	1	0	1 .
Hawthorn (Crataegus sp.)	0	1	0	2
Red Cedar	U	1	U	2
(Juniperus virginiana)	0	1	0	0
Water Oak	U	1	· ·	O
(Quercus nigra)	0	0	0	3
Winged Elm	· ·		· ·	,
(Ulmus alata)	0	0	0	2
Persimmon				
(Diospyros virginiana)	0	0	0	1
Elderberry				
(Sambucus canadensis)	0	0	1	0
Redbud		Α ``		
(Cercis canadensis)	0	0	1	0
Possomhaw	•	•	_	
(Ilex decidua)	0	0	1	0
Black Oak	0	0		0
(Quercus velutina)	0	0	1	0
Burr Oak (Quercus macrocarpa)	0	0	1	0
Hickory	U	U	1	U
(Carya sp.)	0	0	1	0
Buckthorn	U	J	1	Ū
(Rhamnus caroliniana)	0	0	1	0
Chinaberry	A	-	•	
(Melia azedarach)	0	0	1	0

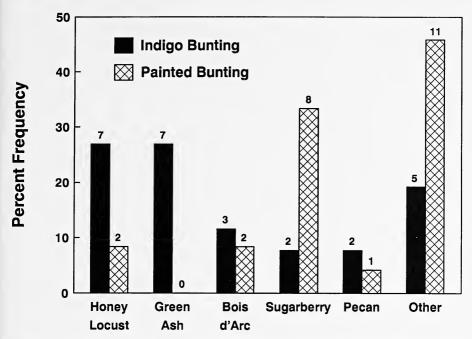


Figure 1. The five most common species of trees used by Indigo and Painted Buntings as song perches. Data are expressed as percent frequency; numbers over bars represent actual frequency. For scientific names of species see Table 3.

height (measured as tree height) or understory height (measured as shrub height) (Table 2). Similarly, Indigo Buntings and Painted Buntings did not differ with respect to the number of vines or thorny plants present in the sample areas surrounding their song perches (Table 2). Thus, the vegetation characteristics of the wooded areas surrounding Indigo and Painted Bunting song perches also appeared to share the same physical structure.

Despite the lack of differences associated with the physical structure of the fields and woods surrounding the song perches of the two species, there were differences in the types of trees associated with Indigo and Painted Buntings. Indigo Buntings were observed singing from nine different species of trees (Table 3), but most commonly were observed in honey locust, green ash and bois d'arc (Fig. 1). These three species accounted for 65.4% of all the song perches observed for this species. Painted Buntings sang from 12 different species of trees (Table 3), but were most commonly observed singing from sugarberry trees (Fig. 1).

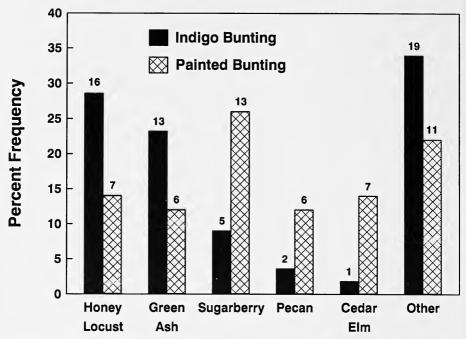


Figure 2. The five most common species of trees observed in randomly selected wooded sites surrounding the song perches of Indigo and Painted Buntings. Data are expressed as percent frequency; numbers over bars represent actual frequency. For scientific names of species see Table 3.

These trees accounted for 33.3 % of all the song perches observed for this species. Use of the five most common song perch tree species was found to differ significantly between Indigo and Painted Buntings (Fisher's Exact Multiway Table, P = 0.0029).

Trees found in the general vicinity of the song perches of Indigo and Painted Buntings were identified from the randomly selected wooded sites. As with the song perches, there were differences in the species of trees associated with each species of bunting (Fig. 2) and these differences were parallel to those observed for song perches. Thus, Indigo Buntings were most frequently associated with honey locust and green ash, whereas Painted Buntings were most often associated with sugarberry, and to a lesser extent, with pecan and cedar elm. These differences were statistically significant (Fisher's Exact Multiway Table, P = 0.0027). Eighteen tree species were found in areas around the song perches of Indigo Buntings, whereas only 11 species of trees were found in areas around the song perches of Painted Buntings (Table 3).

#### DISCUSSION

Both Indigo and Painted Buntings are birds characteristic of areas where there is a mixture of wooded and field habitats. In Oklahoma, Parmelee (1959) described Painted Buntings as occurring in areas where there was a mixture of wooded stands interspersed among fields and in areas where there were wooded ravines in otherwise open habitats. Painted Buntings were found in similar areas during the current study. Indigo Buntings have been described in a wide variety of habitats. For example, in Iowa, they were documented in 12 different types of habitat, ranging from tilled row crops and herbaceous fence rows to upland and bottomland forests (Best et al. 1995). On an east Kansas flood plain, Indigo Buntings were found in cropland, oldfields, and mature hardwood forests (Zimmerman & Tatschl 1975). In northwestern Arkansas, Indigo Buntings occurred in xeric forests, woodland edges, oldfields, shrubby fields and mesic forests (Shugart & James 1973). In Illinois, Indigo Buntings were found in early successional shrub habitats, late successional shrub habitats, bottomland forests and a mature upland forest (Karr 1968).

The preceding description suggests that Indigo Buntings are highly adaptable and capable of occupying a wide range of physical habitat. Indigo Buntings, therefore, have the potential to be competitors with Painted Buntings for habitat. The observation that the two species did not differ relative to microhabitat features would tend to support this hypothesis. However, the current study also demonstrated that Painted Buntings and Indigo Buntings were associated with different species of trees, suggesting that some form of habitat segregation, possibly based on floristic associations, may be occurring. As such, Indigo Buntings and Painted Buntings may not be ecologically equivalent. This finding is consistent with the observation by Rotenberry (1985) that, within a given general habitat type, plant species composition may be more important in explaining the local distribution and abundance of bird species than is the physical structure of the vegetation. However, much more detailed study would be needed to determine whether the differences in tree species associated with Painted and Indigo Buntings found in this study truly represent habitat segregation based on floristic associations or whether they represent more subtle differences in microhabitat correlated with the occurrence of the different tree species.

It is also possible that the tendency for Indigo Buntings in northeast Texas to be associated with honey locust and green ash and the tendency for Painted Buntings in the same area to be associated with sugarberry may be correlated to differences in habitat structure on a scale other than at the microhabitat level. For example, at the mesohabitat level, it is reported elsewhere (Crist 1998) that in the same study area, Indigo Buntings occurred more frequently in disturbed bottomlands whereas Painted Buntings occurred more often in more heterogenous, less disturbed, sites. However, given that Indigo Buntings are observed to occupy so many different physical habitat types in other areas, including uplands, bottomlands, disturbed, and undisturbed sites (Taber & Johnston 1968), it seems unlikely that they should be strongly constrained by physical aspects of vegetation structure in northeast Texas. Clearly, more detailed study of the physical and floristic environments associated with Indigo and Painted Buntings is necessary to fully explain the results of the current study.

In summary, this study found no evidence of microhabitat differences relative to vegetation structure surrounding the song perches of Indigo and Painted Buntings in northeast Texas. However, the two species were found to be associated with different species of trees, suggesting that some form of habitat segregation is occurring. For this reason, it seems unlikely that Indigo and Painted Buntings are strong competitors. Therefore, at least in northeast Texas, it is unlikely that recent population declines of Painted Buntings can be attributed to competition with Indigo Buntings.

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JGK at: biojk@tamu-commerce.edu

## SIZE RELATIONSHIP BETWEEN ADULT MALE CAENOCHOLAX FENYESI (STREPSIPTERA: MYRMECOLACIDAE) AND ITS HOST, SOLENOPSIS INVICTA (HYMENOPTERA: FORMICIDAE)

#### Jerry L. Cook

Department of Biological Sciences Sam Houston State University Huntsville, Texas 77341-2116

Abstract.—A positive size correlation exists between the emerging adult male strepsipteran parasite Caenocholax fenyesi Pierce and its host Solenopsis invicta Buren. Normally, one male C. fenyesi emerges per host, but superparasitism is found in a small number of hosts. Caenocholax fenyesi emerges most commonly from larger hosts, but males are able to complete development in all sizes of worker S. invicta. A preference by C. fenyesi for larger hosts has implications that favor its use as a potential biological control agent for S. invicta.

There appears to be a positive correlation between the size of emerging hymenopterous parasitoids and their hosts (Mendel 1986). (1940) was one of the first to report that large parasitoids tend to develop from large hosts when he experimentally showed that Trichogramma evanescens Westwood had a range of sizes directly correlated to its corresponding host's size. Subsequently, parasitoids of several families of Hymenoptera have been reported to exhibit a similar relationship. These families include Ichneumonidae (Arthur & Wylie 1959), Pteromalidae (Assem 1971), Braconidae (Tillman & Cate 1993), Aphelinidae (Opp & Luck 1986) and Eulophidae (Corrigan & Lashomb 1990); in addition to the Trichogrammatidae demonstrated by Salt (1940). One common factor of these studies was that a single parasitoid usually developed from a specific host. Salt (1940) found that this positive size correlation is not always the case when dealing with superparasitism as evidenced in the superparasitism by T. evanescens which resulted in smaller adults. Salt also found that superparasitism is capable of producing apterous adult parasitoids, while wasps developing singly always produced well-developed wings.

The life history of male strepsipterans is somewhat similar to hymenopterous parasitoids. One difference in strepsipteran life history is that the first instar larva is responsible for host selection (Kathirithamby 1989); as opposed to host searching by the female in

Hymenoptera. After the host has been infected, larval and pupal development of male parasitoids in the two orders follow a similar pattern of endoparasitic developmental stages, pupation and the emergence of a free-living adult. Superparasitism is rare in many species of Strepsiptera (Kathirithamby 1989), but it does occur in some species, including at least one species in the family Myrmecolacidae (Kathirithamby 1992). Kathirithamby found that a *Pheidole* species in South Africa was commonly superparasitized by Stichotrema robertsoni Kathirithamby. In Caenocholax fenyesi, super-parasitism appears to be uncommon (Kathirithamby & Johnston 1992; Cook 1996). Although Kathirithamby & Johnston (1992) found that superparasitism rates sometimes reached over 10% in alate females of Solenopsis invicta, Cook (1996) found it to occur in less than one percent of stylopized S. invicta workers. Since superparasitism does occur, effects on the size of adult male C. fenyesi were observed in this study when more than one developed in a single host, as well as in the normal relationship of one parasitoid developing per host. The goal of this research was to determine if there is a positive size correlation between C. fenyesi and S. invicta.

The only known host of male *C. fenyesi* is the red imported fire ant, *S. invicta* (cf. Kathirithamby & Johnston 1992). Female *C. fenyesi* do not develop in *S. invicta*, but instead develop in an orthopteran host (Cook 1996) in a heteronomous relationship typical of the Myrmecolacidae. *Solenopsis invicta* has a polymorphic worker caste, with a gradient of worker sizes (Greenberg et al. 1985) that can all serve as hosts for *C. fenyesi* as reported by Cook (1996). Greenberg et al. (1985) found most workers (87%) in polygyne colonies in Texas to have head widths smaller than 0.72 mm. The current study examines the possibility that *C. fenyesi* tends to emerge from larger hosts than would be expected from random host utilization.

Host size, parasitoid density within a host and diet can all influence the biology of an adult parasitoid (Vinson & Iwantsch 1980). Studies have shown that, at least in hymenopterous parasitoids, larger adults have a greater fitness and therefore it is beneficial to the parasitoid to have a larger host (Salt 1940; Wylie 1967; Sandlan 1979; Opp & Luck 1986; Corrigan & Lashomb 1990; Tillman & Cate 1993). Therefore, this study examines whether more *C. fenyesi* were emerging from larger hosts and might therefore infer selection by *C. fenyesi*; or if emerging

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C. fenyesi utilized a host population that resembled the general size distribution of S. invicta.

#### MATERIALS AND METHODS

Mature Solenopsis invicta colonies were collected from Brazos County, Texas in May 1996. All ants for this study were taken from one of ten polygyne S. invicta colonies. Colonies were kept in the laboratory in 37 by 27 by 9 cm plastic shoe boxes with the inside walls coated with Fluon AD-1™ (Northern Products, Inc., Woonsocket, RI). Within the shoe box an artificial nest was provided which was made from a 150 by 15 mm plastic Petri dish that had approximately five cm of Labstone™ (Miles Inc. Dental Products, South Bend, IN) in the base. The upper portion of the Petri dish had two holes (diameter = 5 mm) to allow ants to go in and out of the nest. Colonies were fed mealworms (Tenebrio molitor larvae) daily. A 5% honey-water solution and water were provided ad libitum. An 8 cm dowel, kept upright by insertion into a rubber stopper, was added to the shoe box to aid in collecting stylopized ants (Cook 1996).

Stylopized ants were isolated in 35 by 10 mm Petri dishes. These ants were recognized by a behavior change that occurs prior to the emergence of the adult male strepsipteran. Stylopized ants climb to a perch and remain still for extended periods in a position resembling gaster flagging, until emergence occurs (Cook 1996). After emergence of the adult male strepsipteran, both host and parasite die within a few hours. Paired specimens, consisting of the ant host and its corresponding strepsipteran parasite, were placed in vials and preserved in 80% ethyl alcohol to be later measured. Head size was used as a representation of body size for both *C. fenyesi* and *S. invicta*. Head capsules were measured at the widest area of the head. Host and parasitoid head widths were recorded as corresponding pairs.

#### RESULTS

The head capsules of 226 worker *Solenopsis invicta* and the corresponding male *Caenocholax fenyesi* that emerged from these workers were measured (Fig. 1). These pairs were all single parasitoids emerging from a host. Three superparasitized hosts were also collected, two with two male *C. fenyesi* and one with three male *C. fenyesi*. The sizes

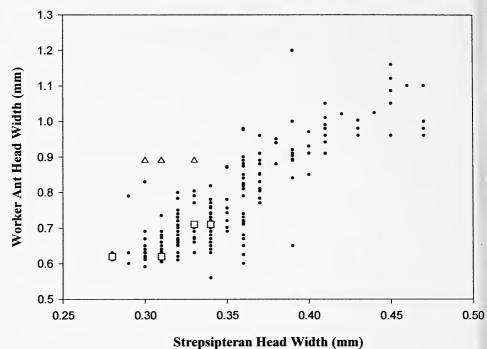


Figure 1. Distribution of head widths of adult male Caenocholax fenyesi emerging from workers of Solenopsis invicta. Dots represent single males emerging from a host; squares represent males emerging from hosts superparasitized by two strepsipterans; stars represent males emerging from the same host superparasitized with three strepsipterans.

of single male C. fenyesi were positively correlated with the sizes of their host ( $r^2 = 0.723$ , n = 226, P = <0.0001). The linear regression of this relationship gives the following model for one strepsipteran developing in a host: Strepsipteran head width = 0.151 + (0.259 x ant head width). Incidences of superparasitism were too few to allow statistical comparison between males from superparasitized hosts and single strepsipterans from a host. However, all male C. fenyesi that emerged from superparasitized hosts were fully developed adults.

Caenocholax fenyesi appears to develop more frequently in larger ants than would be expected in a sampling of the general polygyne ant population. The largest proportion (64%) of the general population ant workers in the study had head widths between 0.6 and 0.7 mm. Only 12% of this general worker population had head widths over 0.8 mm. These percentages are nearly identical to sizes found in Texas polygyne ant colonies by Greenberg et al. (1985). Stylopized ants were sig-

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nificantly larger than the general population (Rank Sum Test: P = <0.0001; T=13522, n (general) = 99, n (stylopized) = 226). Over 28% of stylopized hosts had head widths greater than 0.8 mm. Ants with head widths over 0.9 mm make up about 7% percent of the normal population, but over 17% of stylopized ants.

#### DISCUSSION

There is a strong positive correlation between the size of adult *Caenocholax fenyesi* males and size of the host in which they developed. This is not unexpected because a larger host would be expected to provide more nutrition. However, the effect of superparasitism on the size of resulting adult *C. fenyesi* is not as clear.

The frequency of superparasitism may be somewhat variable between colonies of S. invicta. In the study of Kathirithamby & Johnston (1992), superparasitism of worker S. invicta ranged from 1.6% to 8.5%. However, their rates were determined by dissection and the study did not report as to whether the sampling was random. It may be that some C. fenyesi from stylopized hosts do not develop into fully-formed, emerging adults or, possibly large amounts of variation in superparasitism exists between colonies from some geographical areas. Superparasitism represented 1.3% (3 of 229) of hosts from which male C. fenyesi emerged in the present study. Due to this low incidence, not enough emerging adult strepsipterans from superparasitized hosts were collected to analyze numerically. However, observations were made concerning the size of emerging adult male C. fenyesi that were collected from the three superparasitized hosts in this study. When two C. fenyesi males emerge from one host, their sizes are similar to what would be expected in a singly emerging strepsipteran. This was not expected, since results from single stylopization suggested that male C. fenyesi size is dependent upon the size of the host and its resources. If two parasites were required to develop in the same host, it would suggest that the developmental resource would be divided in half, or at least reduced. In the two observed cases in this study, size does not appear to decrease when two developing strepsipterans share a host. When three strepsipterans develop in the same host, their size appears to be reduced from what would be expected when only a single strepsipteran utilizes a host. However, these results are not outside the observed limits from single stylopization. More observations are needed to make definitive judgments, but the scarcity of superparasitism may make this difficult.

In over 2,000 observations from this study and Cook (1996), there has been only one observation of three *C. fenyesi* males emerging from a single host *S. invicta*.

Size has often been used in distinguishing strepsipteran species (Kifune & Hirashima 1980). The results of this study demonstrate that, at least in *C. fenyesi*, a large size variation exists, although not all strepsipteran species have hosts as variable in size as *S. invicta*. Ample variation in identical size hosts should still cause concern in using size as a valid taxonomic character for identifying strepsipteran species.

There may be implications for biological control based on the conclusion from this study that emerging strepsipterans appear to be more common in larger hosts than would be expected in a random sampling of the general population. This means that first instar strepsipteran larvae may prefer to parasitize either ant larvae or pupae that are large. These larger forms ultimately become major workers or alates. Stylopization may result in female alates not developing into queens. These ideas are supported by the findings of Kathirithamby & Johnston (1992), who found alates stylopized at rates between 41.2% and 55.6%, but no stylopized queens. Decreasing the number of viable alates would have a detrimental impact on the ability of S. invicta to found new colonies. The largest workers are the longest lived of the worker caste of S. invicta (Calabi & Porter 1989) and are more likely than smaller ants to forage for insect prey (Mirenda & Vinson 1981). Stylopization changes the behavior of workers to a point that they no longer contribute to the social structure of the colony, but instead live off of the colony and become a liability (Cook 1996). This would cause a direct negative impact on a colony with stylopized workers.

Another possible explanation for the results of this study is that if *C. fenyesi* does not select larger larvae and pupae to stylopize, then the strepsipterans developing in smaller ant workers may have a decreased ability to reach the adult stage. The only way to ascertain which of these proposed scenarios is true is to experimentally infect *S. invicta*. This has yet to be accomplished.

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JLC at: bio\_jlc@shsu.edu

#### LIGHT REFLECTANCE CHARACTERISTICS AND FILM IMAGE RELATIONS AMONG THREE AQUATIC PLANT SPECIES

J. H. Everitt, D. E. Escobar, C. F. Webster\* and R. I. Lonard\*\*

USDA/ARS Integrated Farming and Natural Resources Unit
2413 E. Highway 83, Weslaco, Texas 78596,
\*Region 15 Water Program, Texas Natural Resource Conservation Commission
1804 W. Jefferson, Harlingen, Texas 78550 and
\*\*Biology Department, University of Texas-Pan American
Edinburg, Texas 78539

Abstract.—Radiometric canopy light reflectance measurements were made on three aquatic plant species in Lake Texana near Edna in southeast Texas. Plant species studied included waterhyacinth (*Eichhornia crassipes*), American lotus (*Nelumbo lutea*) and hydrilla (*Hydrilla verticillata*). Reflectance measurements were made at the visible green (0.52-0.60  $\mu$ m), visible red (0.63-0.69  $\mu$ m), and near-infrared (NIR) (0.76-0.90  $\mu$ m) wavelengths. Reflectance values differed significantly (P=0.05) among the three species at all three wavelengths. Differences in reflectance were primarily attributed to variable foliage coloration and vegetative density; however, the NIR reflectance of hydrilla was also contributed to by a large percentage of the plant being below the water surface. Color-infrared (CIR) aerial photographs of the three species showed that they could be readily differentiated. Reflectance measurements were related to the image tonal responses of the plant species on CIR film.

The inaccessibility and often large expanses of many wetlands make ground inventory and assessment difficult, time consuming, expensive and often inaccurate (Scarpace et al. 1981). Consequently, remote sensing techniques employing aerial photography have been used extensively to inventory and assess wetlands (Carter 1982; Tiner 1997). The high resolution of aerial photography makes it particularly useful for dis-tinguishing among individual aquatic plant species (Seher & Tueller 1973; Carter 1982; Martyn 1985).

Light reflectance measurements have been used to differentiate between stressed and nonstressed crop plants (Gausman 1985) and to distinguish among individual crop, weed and wetland plant species (Gausman & Allen 1973; Gausman et al. 1981; Best et al. 1981). Reflectance measurements have also been used to distinguish between woody plant species (Gausman et al. 1977) and coastal zone plant species (Everitt et al. 1999) and related to their tonal responses on CIR aerial photographs.

Little information is available on the light reflectance characteristics of aquatic plant species. The objectives of this study were to: (1)

characterize the visible and NIR reflectance of three common aquatic plant species in a Texas lake and (2) evaluate large scale CIR aerial photography for remotely distinguishing among these plant species.

#### STUDY SITE

This study was conducted at Lake Texana near Edna in Jackson County, Texas. Edna is located approximately 35 km northeast of Victoria, Texas. Lake Texana was chosen as a study site because it supports large populations of aquatic plant species. Plant canopy reflectance measurements, aerial photographs and ground truth observations were conducted for this study.

#### METHODS AND MATERIALS

Radiometric plant canopy reflectance measurements were made on three common aquatic plant species: waterhyacinth (Eichhornia crassipes), American lotus (Nelumbo lutea) and hydrilla (Hydrilla verticillata). Waterhyacinth is a floating species, American lotus is an emersed species, and hydrilla is a submersed species. These species are abundant in Lake Texana and occur in lakes and streams throughout the southeastern United States. Reflectance measurements were made on 10 randomly selected plant canopies of each species with a Barnes modular multispectral radiometer (Robinson et al. 1979). Measurements were made in the visible green (0.52-0.60  $\mu$ m), visible red (0.63-0.69  $\mu$ m) and NIR (0.76-0.90 µm) spectral bands with a sensor that had a 15degree field-of-view placed approximately 1.0 to 1.5 m above each plant canopy. Measurements were made using a small boat. Field radiometric measurements were corrected to reflectance using a barium sulfate standard. All reflectance measurements were made between 1130 and 1400 h under sunny conditions on 30 September 1998. Overhead photographs were taken of plant canopies measured with the radiometer to help interpret reflectance data.

Kodak Aerochrome CIR (0.50-0.90  $\mu$ m) type 2443 film was used for aerial photographs. Color-infrared film is sensitive in the visible green (0.50-0.60  $\mu$ m), visible red (0.60-0.70  $\mu$ m), and NIR (0.70-0.90  $\mu$ m) spectral regions. Photographs were taken with a Hasselblad 70-mm camera equipped with a 80-mm lens and an orange (minus blue) filter. The camera had an aperture setting of f11 at 1/500 sec. Photographs were taken on 11 August 1998 at an altitude above ground level of 460 m and a scale of approximately 1:6000. All photographs (nadir) were obtained under sunny conditions using a Cessna 404 fixed-wing aircraft. The camera was mounted vertically in a camera port in the floor of the

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Table 1. Canopy light reflectance of three aquatic plant species at the green, red, and near-infrared wavelengths. Reflectance measurements were made with a Barnes modular multispectral radiometer at Lake Texana near Edna, Texas.

	Reflectar	nce (%) for three	e wavelengths
Plant species	Green	Red	Near-infrared
Nelumbo lutea (Willd.) Pers (American lotus) Eichhornia crassipes (Mart.) Solms	9.3 a¹	3.4 a	47.5 a
(Waterhyacinth)	4.3 b	1.7 b	42.3 a
Hydrilla verticillata (L. F.) Royle (Hydrilla)	3.0 с	1.6 b	15.3 b

Means within a column followed by the same letter do not differ significantly at the 5% probability level, according to Duncan's multiple range test.

aircraft. Field surveys were conducted at sites where aerial photographs were obtained. Aerial photographic prints were used to verify plant species and ecological parameters.

Visible green and red, and NIR reflectance data were analyzed using *ANOVA* techniques. Duncan's multiple range test was used to test statistical significance at the 0.05 probability level among means (Steel & Torrie 1980). Trade names are included for the benefit of the reader and do not imply an endorsement of or a preference for the product listed by the United States Department of Agriculture.

#### RESULTS AND DISCUSSION

Mean canopy light reflectance measurements for the three plant species at three wavelengths are given in Table 1. Reflectance values differed significantly (P=0.05) among the three species at the visible green wavelength, with American lotus having the highest reflectance and hydrilla the lowest. At the visible red wavelength, American lotus had higher reflectance than waterhyacinth and hydrilla. Differences in visible reflectance among the plant species was primarily attributed to differences in foliage color and subsequent plant pigments (Myers et al. 1983; Gausman 1985). American lotus had light green leaves, whereas waterhyacinth and hydrilla had dark green and deep dark green leaves, respectively. The darker green foliage (higher chlorophyll concentration) of waterhyacinth and hydrilla reflected less of the green light and absorbed more of the red light than the light green foliage (lower chlorophyll concentration) of American lotus (Gausman 1985).

American lotus and waterhyacinth had significantly higher (P=0.05) reflectance at the NIR wavelength than hydrilla (Table 1). Near-infrared reflectance in vegetation is highly correlated with plant density (Myers et al. 1983; Everitt et al. 1986). A qualitative analysis of the overhead

photographs of the three species showed that American lotus and waterhyacinth had greater leaf density and less gaps in their canopies than hydrilla. American lotus and waterhyacinth also had large leaves as opposed to the very small leaves of hydrilla. Both American lotus and waterhyacinth had > 90% cover with very little water showing in the background. Conversely, hydrilla had 50-60% cover with only the upper portion of the plants exposed at the surface; a large percentage of the plant biomass was approximately 1-2 cm below the surface of the water. The low NIR reflectance of hydrilla was attributed to both its more open canopy and to the water integrated with its canopy which absorbed a large percentage of the NIR light (Myers et al. 1983; Everitt et al. 1989).

American lotus, waterhyacinth and hydrilla could be distinguished in CIR aerial photographs (not shown) obtained on 11 August 1998 at Lake Texana. American lotus had a distinct pink image response, whereas waterhyacinth had a bright red image tone. Hydrilla had a dark brown color that could be easily differentiated. Mixed populations of American lotus and waterhyacinth had a orange-red color. All three species had similar CIR image responses at eight scattered locations in Lake Texana and could be readily separated at each location.

The pink image of American lotus was primarily attributed to its high visible green and moderately high visible red reflectance values, whereas the bright red image tone of waterhyacinth was attributed to both its low green and red reflectance values. The high NIR reflectance of both American lotus and waterhyacinth also contributed to their image responses. The dark brown color of hydrilla was primarily attributed to its very low NIR reflectance; however, its low visible green and red reflectance also influenced its image response. Martyn (1985) reported that surfaced hydrilla plants had a cinnamon-brown image tone in CIR aerial photos. The darker brown CIR image of hydrilla in this study was probably due to a large percentage of the plants being slightly below (1-2 cm) the water surface.

#### CONCLUSIONS

Results from this study showed that radiometric plant canopy light reflectance measurements at two visible wavelengths and one NIR wavelength varied greatly for American lotus, waterhyacinth and hydrilla. Differences in reflectance among the three plant species was related to variable foliage colors and plant canopy densities. The low NIR reflectance of hydrilla was also contributed to a large percentage of the plant being below the water surface. Color-infrared aerial photographs

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obtained of the three species showed that they could be readily distinguished. Reflectance measurements could be related to the CIR film tonal responses of the three species. Although the phenology of these species was not studied, the optimum time to acquire aerial photographs is probably from mid to late summer when the plants have reached peak foliage development (Everitt et al. 1980). The reflectance measurements assisted in the interpretation and understanding the association among the plant species reflectivity and color tonal responses on the CIR film. The ability to remotely distinguish among American lotus, waterhyacinth and hydrilla should enhance future remote sensing inventories of lakes and other waterways where these species occur.

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JHE at: jeveritt@weslaco.ars.usda.gov

#### THE NATURAL HISTORY OF THE ROATÁN ISLAND AGOUTI (DASYPROCTA RUATANICA), A STUDY OF BEHAVIOR, DIET AND DESCRIPTION OF THE HABITAT

### Thomas E. Lee, Jr., Kevin R. Rhodes, Jenny L. Lyons and Daniel K. Brannan

Department of Biology, Box 27868 Abilene Christian University Abilene, Texas 79699

Abstract.—The behavior of a population of the Roatán Island agouti (Dasyprocta ruatanica) of Honduras was examined. The population was isolated, semi-tame and approachable. Behavioral characters of the population were timed and an analysis of the time spent per behavior was conducted. The diet of D. ruatanica was found to have some similarities to that of the Central American agouti (Dasyprocta punctata), although this population's diet is augmented from agricultural food products. The ecological communities of Roatán are tremendously influenced by human activity. Consequently, indigenous habitat is fragmented and shrinking.

Considerable research has been conducted on the Central American agouti, *Dasyprocta punctata* (i.e., Smythe 1978; 1983; Janzen 1983; Hallwachs 1986; Smythe et al. 1996). However, Reid (1997) indicated that nothing was known about (*D. ruatanica*) the Roatán Island agouti. Notwithstanding, Goodwin (1942) does present a description of *D. ruatanica*. All other information about *D. ruatanica* is inferred from the studies on *D. punctata* by Reid (1997). This study presents a habitat description, examines the behavior, and feeding habitats of *D. ruatanica*. This study describes *D. ruatanica* in a heavily human impacted area to which the animals have become habituated. Considering the paucity of data on *D. ruatanica* however, it nevertheless expands the knowledge of this species.

#### **METHODS**

During the period from 14 March 1999 through 19 March 1999, *D. ruatanica* were observed and photographed at intervals throughout the day and night for periods of approximately 45 minutes to one hour each. Generally, one *D. ruatanica* was selected to be followed and observed for the entire period by one observer. On occasion, the behavior of more than one individual was observed because of its interactions with other *D. ruatanica*. The following types of behavior were recorded:

eating, sitting, standing, walking, running, sniffing, lead/following, scratching, digging, grooming, marking, muzzling and nursing. An average of the time spent exhibiting a specific behavior was taken from the field collected data, and the percentages calculated. Other behaviors were observed but not included as general behaviors. These include lying near brush piles or at the base of trees, raising hair on back, or a juvenile supporting itself on its mother's back.

#### RESULTS AND DISCUSSION

Habitat description.—The population of D. ruatanica observed in this study inhabits a small island (100 m in diameter) 30 meters off the middle south shore of Isla de Roatán (Roatán Island), Honduras. island is named Fantasy Island for the resort hotel on the island. Fantasy Island is connected to Roatán Island by a one-lane bridge. Much of the area of Fantasy Island is occupied by the resort hotel and the hotel grounds. All of the island's habitat has been modified greatly by human activity. There is a small hill on the island covered with several species of trees (Thrinax sp., Cocos nucifera, Swietenia sp., Casuarina equisetifolia, Pentaclethra sp.). Some ground cover (bamboo clumps) occurs on the hill below the tree canopy. Dasyprocta ruatanica utilizes the bamboo patches for sleeping. Dasyprocta ruatanica seem to prefer the tree covered hill, although, they use all of the island including sites under the hotel buildings. Fantasy Island has patches of mangrove (Rhizophora sp.) along its shore and mangrove islands occur between it and Roatán Island. Roatán Island has patches of low tropical scrub forest mostly in the eastern half of the Island. Most of the scrub forests that still exist are on mountains. Much of Roatán Island is currently under development for large houses and resort hotels.

With the presence of the hotel staff and guests, *D. ruatanica* have become habituated to people. *Dasyprocta ruatanica* on Fantasy Island can be approached to within two meters. *Dasyprocta ruatanica* on the Island of Roatán, are hunted for food and consequently run at the sight of humans. No *D. ruatanica* were observed on the Island of Roatán. The population of *D. ruatanica* studied has been present on Fantasy Island from at least 1995.

Dasyprocta ruatanica shares Fantasy Island with other vertebrates. These include Iguana iguana, Ctenosaura sp., Columba leucocephala, Rattus rattus, Artibeus phaeotis and Artibeus jamaicensus. The A.

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Table 1. The percentage of observed time spent on select behavior by Dasyprocta ruatanica.

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General Behavior Observed	Percent of Time Spent	Total % Time in Each General Behavior
Feeding Behavior		
Feeding	21.9%	
Sniffing	12.1%	
Digging	3.3%	37.3%
Social Behavior		
Lead/following	12.1%	
Scratching	4.4%	
Grooming	3.3%	
Marking	2.2%	22.0%
Ambulatory Behavior		
Sitting	23.1%	
Standing	7.7%	
Walking	8.8%	
Running	1.1%	40.7%

jamaicensus roost in the coconut palms on the island. Bats and other aboreal frugivores are important to *D. ruatanica* (as they are to *D. punctata*) because they drop unripe fruit to the ground that otherwise would not be available (Smythe et al. 1996). One notable invertebrate that lives on the island is the leaf cutter ant *Atta* sp. The leaf cutter ant colony dominates the crown of the hill on the island.

Behavior.—In this study, a range of D. ruatanica behaviors were observed and timed. The D. ruatanica were observed to spend a large part of their time sitting (23.1%) and feeding (22.0%). Dasyprocta ruatanica were observed picking up fruits, flowers and nuts. Dasyprocta ruatanica would feed, drop, carry then drop, or they would bury their food. Looking for food, which may also include the behaviors of walking, sniffing and digging, occupied 29.2% of the time recorded (Table 1). Digging was considered to be part of feeding because D. ruatanica were observed burying their seeds for storage. However, food storage would not seem critical on Fantasy Island because the food supply is augmented. The behavior of burying seeds was reported in D. punctata by Smythe (1978) and Smythe et al. (1996).

The social interactive behavior of muzzling was observed among *D. ruatanica*. The muzzling observed in *D. ruatanica* consisted of the animals approaching each other and rubbing rostrum to rostrum. Smythe (1983) indicated that odor is an important form of communica-

tion in *D. punctata*. *Dasyprocta punctata* use the perineum to mark trails, feeding and sleeping spots. Marking accounted for 2.2 % of the observation time in this study.

Conflict behavior was observed among *D ruatanica*. Symthe (1983) reported that when fleeing, *D. punctata* will erect the long rump hairs. This behavior presumably protects the fleeing agouti against bites. Fleeing with rump hairs erected was observed among *D. ruatanica*. Although, the behaviors of marking, fleeing and biting could be attributed to territorial behavior, as has been reported in *D. punctata*, by Smythe et al. (1996). Territorial boundaries were not observed among *D. ruatanica* during this study.

There were at least three pairs of mothers and juveniles on the island at the time of the study. The leading/following behavior they exhibited accounted for 12.1% of their time. The juveniles were about two-thirds of the size of the mother and had the same color. Some of the juveniles were observed trying to nurse, but usually the mother would back away when the juvenile attempted to nurse. On one occasion a juvenile was seen resting its front legs and half its body on its mothers shoulders for about 20 minutes.

Dasyprocta ruatanica was not observed to live in, or dig holes large enough to live within; similar behavior of D. punctata was reported by Smythe (1983). Smythe (1983) suggests that the holes are dug as a means to avoid carnivores. There are no native carnivore predators on the island. However, D. ruatanica may use the crawl spaces under the hotel as a substitute.

For an analysis of the time spent in each behavior, specific behaviors were lumped into three general behavior classes (Table 1). General behavior time was then analyzed to determine whether significant differences existed among them. A chi-square analysis of the data showed that D. ruatanica spent a significantly greater amount of time in ambulatory behavior than in social or feeding activities ( $X^2 = 5.952$ ; P = 0.051).

Diet.—Dasyprocta ruatanica was observed to feed on a wide variety of plants including coconuts (Cocos nucifera), hibiscus flowers, almonds (Terminalia sp.) and Pentaclethra pods. Pentaclethra pods were found on the forest flour with chew marks presumably from D. ruatanica.

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The only other known rodent that could have made the chew marks on Fantasy Island is *Rattus rattus*. *Dasyprocta ruatanica* also fed on rice, oranges and corn kernels that were the intended food for domestic chicken, turkey and peafowl. Janzen (1983) documented that *D. punctata* fed on the large, thick mature pods of *Hymenaea courbarii*. With the exception of coconuts, nothing that large or thick was in fruit on Fantasy Island at the time of this study. Moreover, Smythe et al. (1996) documented *D. punctata* fed on 34 different food items during a period of 18 months.

#### **CONCLUSIONS**

A possible bias exists in these data because the subjects may be isolated from other Roatán Island populations of *D. ruatanica* and the Fantasy Island population is semi-tame. In the rainforest habitat of Guyana the observer can only get a few seconds of observation time of *D. agouti* before the animal flees. On Roatán Island *D. ruatanica* are hunted and flee at the sight of humans. However, on Barro Colorado Island in Panama, *D. punctata* does not view humans as a threat and therefore this species can be observed (Smythe et al. 1996). Some behavioral differences between populations of agoutis seem principally due to whether or not they perceive humans as a threat. No one can say what the Roatán Island population behavior was akin to before human settlement. Perhaps their behavior was analogous to Galápagos Island animals, in that they were approachable, as the semi-tame *D. ruatanica* are on Fantasy Island.

Dasyprocta ruatanica is listed as threatened (Woods 1993). Habitat alteration is occurring on the Island of Roatán. On Fantasy Island, D. ruatanica and other wildlife species are not hunted and are encouraged to live on the hotel grounds. However, the population seems small and may even be isolated from other populations on Roatán. If habitat alteration continues, populations of D. ruatanica may become more fragmented and this could cause an overall population decline.

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TEL at: lee@biology.acu.edu

#### **GENERAL NOTES**

# NESTING OF ROSE-THROATED BECARD PACHYRAMPHUS AGLAIAE (PASSERIFORMES: INCERTAE SEDIS) AND CLAY-COLORED ROBIN TURDUS GRAYI (PASSERIFORMES: TURDIDAE) IN HIDALGO COUNTY, TEXAS

#### **Timothy Brush**

Department of Biology, University of Texas-Pan American 1201 West University Drive, Edinburg, Texas 78539

Many widespread tropical birds reach the northern edge of their range in northeastern Mexico or southern Texas. Some, such as Red-billed Pigeon (*Columba flavirostris*), have declined during the 20th century (Brush 1998), while others, such as Ringed Kingfisher (*Ceryle torquata*), have increased (Oberholser 1974). The current status of two tropical species, Rose-throated Becard (*Pachyramphus aglaiae*) and Clay-colored Robin (*Turdus grayi*), is poorly known in Texas.

Rose-throated Becards have bred in very small numbers in the Lower Rio Grande Valley (LRGV) of Texas from the 1940s to 1970s (Oberholser 1974; Gehlbach 1987). Clay-colored Robins have been reported almost annually in the LRGV since 1940 (American Ornithologists' Union 1998), but no nests have been described in the United States.

Territories and nests were sought, mainly during May-July 1999, at three locations in southern Hidalgo County, Texas: (1) Anzalduas County Park (hereafter Anzalduas), approximately 10 km SSE of Mission; (2) Santa Ana National Wildlife Refuge (hereafter Santa Ana), approximately 11 km S of Alamo; and (3) Bentsen-Rio Grande Valley State Park (hereafter Bentsen), approximately 6 km SW of Mission. Anzalduas is a developed park with many picnic tables and is heavily used by people on weekends. It has a very open, park-like understory, with an overstory typical of mature floodplain forest, dominated by native cedar elm (*Ulmus crassifolia*), Mexican ash (*Fraxinus berlandieriana*) and sugar hackberry (*Celtis laevigata*), with some planted live oak (*Quercus virginiana*) (names from Lonard et al. 1991). Santa Ana and Bentsen are dominated by subtropical thorn forest with numerous honey mesquite (*Prosopis glandulosa*), Texas ebony (*Pithecellobium flexicaule*), granjeno (*Celtis pallida*), brasil (*Condalia hookeri*) and many

Table 1. Nests of Rose-throated Becards (RTB) and Clay-colored Robins (CCR) in Hidalgo Co., Texas, during 1999. Both RTB nests hung from slender branches over open space. All CCR nests were placed in major branch forks or on top of horizontal branches. Fledglings identified as recent had incomplete tails and seldom flew.

Nest no.	Location	Tree species	Nest height	Outcome
RTB-1	Anzalduas	cedar elm	9.6 m	Being built by female, 8 May Completed by 3 June <sup>1</sup> Male first seen on 3 June Abandoned by 24 June
RTB-2	Anzalduas	Mexican ash	6.1 m	Being built, 24 June Completed by 2 July <sup>2</sup> Abandoned by 15 July
CCR-1	Anzalduas	live oak	4.9 m	Completed by 10 April Abandoned by 16 April
CCR-2	Anzalduas	Mexican ash	4.8 m	Being built on 18 May Complete, abandoned by 28 May
CCR-3	Santa Ana	Mexican ash	7.6 m	2 nestlings on 25 May 1 recent fledgling on 2 June
CCR-4	Anzalduas	live oak	4.2 m	1-2 nestlings on 15 June 1 fledgling on 24 June
CCR-5	Anzalduas	live oak	6.0 m	1 nestling on 3 August Outcome unknown

Since Rose-throated Becards continue to add nesting material during egg-laying and incubation (Rowley 1984), it is difficult to tell when a nest is completed.

other thorny trees and shrubs, and with smaller amounts offloodplain forest and marsh.

Observations made by the author and others earlier in the 1990s are also presented.

Rose-throated Becard.—No birds were seen during the summers of 1992-1996, or 1998. A subadult male was seen at Santa Ana, 30 July-3 October 1997 (Lockwood 1999). Birds were reported at four locations in 1999: a male at Bentsen on 24 April; single females, on 27 May at Laguna Atascosa National Wildlife Refuge (Cameron County) and at Bentsen on 26 June; and the nesting pair discussed below.

At Anzalduas, a female was first seen on 24 April 1999. At least one becard was present during May-August. An incomplete nest, never finished, was found there on 7 May. Two nests (RTB-1 and RTB-2) were completed at Anzalduas (Table 1), but the adults were never seen bringing food. On 12 May, a female Bronzed Cowbird (*Molothrus aeneus*) landed on RTB-1 but did not enter. Similarly, a female

<sup>&</sup>lt;sup>2</sup> Active Altamira Oriole nest about 7 m away in same tree.

Bronzed Cowbird landed on RTB-2 on 24 June, but was quickly chased off by the male becard.

In late July, after both RTB-1 and RTB-2 were abandoned, the becard pair approached inactive and active nests of Great Kiskadee (*Pitangus sulphuratus*), within 400 m of RTB-2, apparently looking for nesting material. They were chased by Couch's Kingbirds (*Tyrannus couchii*) and Altamira Orioles (*Icterus gularis*) from the territories or nests of the latter two species. However, the becard pair was never seen building another nest or making any further use of the earlier nests. At no time during the summer did the becards give alarm calls around nests, which would indicate nestlings or fledglings (Davis 1945).

Clay-colored Robin.—On 2 June 1992, a pair of Clay-colored Robins brought food, at least five times, to a nest hidden in a dense clump of Spanish moss (*Tillandsia usneoides*) at Anzalduas. Singing birds or pairs were seen during 1978 (Gehlbach 1987), 1994, 1996 and 1998 (Haynie 1995; Lockwood 1999) in at least one location/year in the LRGV.

Four nests were found at Anzalduas and one was seen at Santa Ana in 1999 (Table 1). At least two nests became active at Anzalduas, and CCR-4 produced at least one fledgling. CCR-5 was not checked regularly enough to determine whether it was successful. Both adults brought food to the active nests and chased Fox Squirrels (*Sciurus niger*) from within 30 m of the nest. Nestlings and fledglings were silent as they raised their heads to receive food from adults. Adults foraged mainly on moist ground in areas that had been recently watered and also in fruiting anacua (*Ehretia anacua*) and coma (*Bumelia celastrina*) trees.

Also in 1999, CCR-3 was built at Santa Ana, within 10 m of the edge of a temporary woodland pool. Adults foraged in nearby fruiting sugar hackberry and anacua and on the bare ground at the edge of the pool, which was dry much of the time that the nest was active. Anacua fruits and unidentified invertebrates were regularly brought to the nestlings. At least one nestling fledged, and it was seen regularly through 6 June.

A male sang regularly in the camping area at Bentsen from 2 June - 17 July 1999. A second bird was seen there on 24 June, ca. 150 m from the singing male, but despite intensive searches, no nests were found. The male at Bentsen always sang loudly, indicating possible unmated status, unlike the nesting Anzalduas and Santa Ana birds, which sang very softly most of the time.

Clay-colored Robin could be slowly increasing in the LRGV region,

given the records reported above. Continued urban growth in the LRGV region, producing park-like, watered habitat suitable for robin foraging and nesting may be contributing to its increase. Rose-throated Becard has become less common in Texas since the 1970s and may no longer maintain a stable breeding population. Suitable riparian forests are now very rare in the LRGV, due to habitat deterioration (Brush & Cantu 1998). Annual field work is needed to determine whether these observations of Rose-throated Becard and Clay-colored Robin are real trends or fluctuations at range-margins (Gehlbach 1981).

#### **ACKNOWLEDGMENTS**

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TB at: tbrush@panam.edu

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## VERTEBRATE REMAINS FOUND IN BARN OWL PELLETS FROM CROSBY COUNTY, TEXAS

#### Mark W. Lockwood and Clyde Jones

Natural Resources Program, Texas Parks and Wildlife Department
Austin, Texas 78744 and
Department of Biological Sciences and
The Museum of Texas Tech University
Lubbock, Texas 79409

Remains of vertebrates were identified from pellets cast by barn owls (*Tyto alba*) in a small, grain-storage bin on the Lockwood Farm 8.5 miles south of Lorenzo, Crosby County, Texas. Most of the area is devoted to the cultivation of crops, mostly of cotton, grain sorghum and wheat. Small stands of trees growing adjacent to farmsteads are scattered about the area. Junier brushland is the dominant vegetation along the escarpment of the Llano Estacado located about six miles south of the locality. Detailed descriptions of the geology, physiography, climate, soils and vegetation of the region were presented by Choate (1997).

Since at least 1990, barn owls have been year-around residents of the small, grain-storage bin on the Lockwood Farm. Broods of young owls were fledged each year; two broods were produced in 1997. On 26 July 1997, seven owls were observed in the roost; there were two adults and five chicks (one with emerging flight and contour feathers, four in downy plumages). On 26 December 1997, two adult owls were present in the roost.

Pellets were collected on 17 January-8 February 1993, 25 December 1995, 15 June 1996, 21 December 1997, 26 July 1997 and 26 December 1997. On each of the aforementioned dates, pellets were collected and placed in labeled plastic bags. Although pellets in various stages of disintegration were found scattered throughout the roost area, only entire intact pellets were collected. In order to extract the remains of vertebrates, each pellet was soaked in water for a few minutes and the bones were teased from the matrix. Remains of crania and mandibles from each pellet were placed in either vials or boxes and labeled in accordance with dates of collections. In an attempt to avoid the potential bias

Table 1. Numbers of individual vertebrates (percent of total in parentheses) recovered from barn owl pellets from Crosby County Texas, by date of collection. Peromyscus sp. = P. leucopus, P. maniculatus; Reithrodontomys sp. = R. megalotis, R. montanus.

	`	-	•			•				
Species	17 Jan (18	17 Jan-8 Feb 1993 (180 pellets)	25 De (84 p	25 Dec 1995 (84 pellets)	15 Jun 1996 (140 pellets)	21 Dec 1996 (133 pellets)	26 Jul 1997 (262 pellets)	26 Dec 1997 (188 Pellets)	Total (987 pellets)	ul llets)
Cryptotis parva	16	(5.80)	10	(5.18	5 (1.45)	3 (1.06)	4 (0.65)	26 (6.37)	64	(3.02)
Sylvilagus audubonii		1	1	(0.52)	I	I	ı	1	1	(0.05)
Cratogeomys castanops	31	(11.23)		1	L	I	ı	I	31 (	(1.46)
Geomys bursarius	4	(1.45)	2	(1.04)	6 (1.74)	4 (1.42)	I	2 (0.49)	18 ((	(0.85)
Chaetodipus hispidus		1	24	(12.44	41 (11.88)	89 (31.56)	51 (8.32)	47 (11.52)	252 (1	(11.90)
Dipodomys ordii		1		1	I	l	1 (0.16)	ı	1 (6	(0.05)
Baiomys taylori		1	21 (	(10.88)	I	50 (17.73)	24 (3.92)	98 (24.02)	193 (9	(9.12)
Neotoma micropus	_	(0.36)		1	ı	I	I	I	1	(0.05)
Onychomys leucogaster	4	(1.45)		ı	4 (1.16)	2 (0.71)	14 (2.28)	3 (0.74)	27 (	(1.28)
Peromyscus sp.	65	(23.55)	30	(15.54)	113 (32.75)	35 (12.41)	249 (40.62)	108 (26.47)	600 (2)	(28.34)
Reithrodontomys sp.	72	(26.09)	42 (3	(21.76)	164 (47.54)	71 (25.18)	236 (38.50)	74 (18.14)	659 (3	(31.13)
Sigmodon hispidus	76	(27.54)	62 (3	(32.12)	12 (3.48)	22 (7.80)	31 (5.06)	46 (11.27)	249 (1	(11.76)
Mus musculus	S	(1.81)	1	(0.52)	ı	6 (2.13)	I	4 (0.98)	)) 91	(0.76)
Rattus rattus	1	(0.36)		ı	ı	I	I	1	1	(0.05)
Unidentified birds	1	(0.36)		ı	ı	I	3 (0.49)	1	4	(0.19)
Total	276	276 (100.00)	193 (1)	193 (100.00)	345 (100.00)	282 (100.00)	613 (100.00)	408 (100.00)	2117 (100.00)	0.00)

of duplicate counting, only cranial parts recovered from the pellets were used to obtain the numbers of vertebrates reported herein.

Remains of at least 14 species of mammals were recovered from the barn owl pellets. Frequencies of occurrences of these prey species are listed in Table 1. The taxa of mammals listed are all known to occur in this region of the Llano Estacado (Choate 1997). However, the number of crania of Cryptotis parva extracted from the owl pellets (Table 1) is quite remarkable. Although reported previously from barn owl pellets collected in the county (Manning & Jones 1990), the least shrew has been considered as uncommon in Crosby County in particular and on the Llano Estacado in general (Choate 1997; Owen & Hamilton 1986). The presence of remains of Cratogeomys castanops in only the early sample (Table 1) is of considerable interest. Although known from adjacent counties, Choate (1997) reported no specimens of Cratogeomys from Crosby County. Based on the data available (Table 1), other species of rodents were preyed upon relatively frequently by the barn owls, such as Reithrodontomys sp., Peromyscus sp., Chaetodipus hispidus, Sigmodon hispidus and Baiomys taylori, respectively. Small rodents (Peromyscus sp., Reithrodontomys sp.) were major components of remains extracted from the owl pellets that were collected in June and July (Table 1). The relatively high incidence of remains of these rodents at these periods of the year may have been correlated with the presence of additional owls in the roost, as implied above, especially in 1997, when two broods of young owls were produced. Manning & Jones (1990) found numerous small rodents (Reithrodontomys montanus, Chaetodipus hispidus, Baiomys taylori) in barn owl pellets collected in February, April and May from southeastern Crosby County.

Crania of four unidentified passerine birds were extracted from owl pellets collected on 17 January-8 February 1993 and 26 July 1997, respectively. It has been noted that barn owls frequently take more birds during winter months than at other times of the year (Adams et al., 1986). However, Jones & Goetze (1991) reported remains of birds from owl pellets obtained at all seasons of the year in north-central Texas. Manning & Jones (1990) found parts of two passerines and one charadriiform bird in barn owl pellets collected in April in southeastern Crosby County.

Jorgensen et al. (1998) concluded that barn owls selected particular prey in that they took certain species of small mammals preferentially.

Adams et al. (1986), Manning & Jones (1990) and Goetze (1991) concluded that barn owls apparently are opportunistic predators and prey on species that are easiest to catch. Although data from this current study do not indicate that barn owls are other than opportunistic predators, there are needs to study predation by barn owls in the context of available prey species and corresponding habitat relationships. In some areas on the Llano Estacado, some small mammals, such as *Peromyscus* sp., *Reithrodontomys* sp., *Sigmodon hispidus*, *Chaetodipus hispidus* and *Baiomys taylori*, are most often associated with the vegetation along fencerows (Pesaturo et al. 1989; Choate 1997).

In this study, examination of a total of 987 barn owl pellets yielded cranial remains of 2,117 vertebrates (2,113 mammals, four birds), for an average of 2.14 remains per pellet. For the larger mammals, remains were found mostly in single pellets. However, for smaller mammals, remains of eight to ten individuals were found commonly in a single pellet. Each of the crania of birds was found in a single pellet.

Results of this study are in agreement with Errington (1930), who commented that barn owls are effective collectors of small mammals, and analyses of mammalian remains from pellets provide distributional data on some species that sometimes are difficult to obtain by conventional means of collecting. For example, for the monumental monograph on the mammals of the Llano Estacado, Choate (1997) examined more than 10,000 specimens of mammals from the area; a total of 568 specimens of *Reithrodontomys* sp. was available in scientific collections. A total of 659 crania of harvest mice was extracted from barn owl pellets collected from 17 January 1993 to 26 December 1997 (Table 1) during this current study.

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CJ at: cjones@packrat.musm.ttu.edu

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#### RANGE EXTENSION FOR RIO GRANDE CICHLID *CICHLASOMA CYANOGUTTATUM* (PISCES: CICHLIDAE) IN TEXAS

#### R. Trent Martin

6310 Overdale, Houston, Texas 77087

The Rio Grande cichlid *Cichlasoma cyanoguttatum* is the most northern species of the Cichlidae in North America, historically occurring as far north as the Rio Grande and Pecos River (Brown 1953; Hubbs 1991). This species has been widely introduced in Texas and is currently found in the Colorado, San Antonio, San Marcos, Guadalupe, San Gabriel and Comal rivers (Brown 1953; Hubbs et al. 1978; Hubbs et al. 1991). These introductions began in 1928 with the San Marcos Fish Hatchery of the U. S. Fish and Wildlife Service, which bred and distributed the Rio Grande cichlid from 1928 through 1941 or 1943 (Brown 1953).

The Rio Grande cichlid has also been used in the aquarium trade. Courtenay et al. (1979) state that this species was raised and distributed for the aquarium trade in Florida under the name "Texas bluespot" during the 1940's and 1950's. Currently, this species is a common item in Houston area pet shops, under the name "Texas Cichlid". Intentional releases of aquarium fish undoubtedly have expanded the range of the

Rio Grande cichlid. This paper documents the extension of the Rio Grande cichlid into Harris County, Texas.

Sims Bayou is a large stream flowing along the southern portion of Harris County, Texas. The lower capture site on Sims Bayou is at Hemingway Drive (latitude 29° 40' 09", longitude 95° 17' 49"), just upstream of the bayou's estuarine reach. The Bayou here is approximately 15 m wide. This section of the bayou is currently being rechannelized to be deeper, wider and lined with concrete blocks. The upper capture site on Sims Bayou is at its intersection with Martin Luther King Boulevard (latitude 29° 38' 44", longitude 95° 20' 14"). The bayou here is about 10-15 m wide and has a clay bottom. This stretch was also channelized in the past. This upper site lies immediately below a medium-sized municipal sewage treatment plant (Chocolate Bayou Wastewater Treatment Facilities, average discharge 7.0 million gallons per day). Sims Bayou primarily drains agricultural and residential lands.

Brays Bayou flows through the southern part of Houston, Harris County, Texas. The capture site on Brays Bayou is at its intersection with Interstate 45 (latitude 29° 42′ 44″, longitude 95° 18′ 41″). The bayou is 30-40 m wide at this point, with a clay bottom. It has been channelized in the past and chiefly drains commercial and residential property.

Both Sims and Brays Bayou form part of the Houston Ship Channel/Buffalo Bayou Tidal Segment No. 1007 of the San Jacinto River Basin. From personal observation and in comparing USGS Gaging Station records to State of Texas discharge permits, large municipal wastewater treatment plants contribute to a substantial portion of the base flow of both Sims Bayou and Brays Bayou.

From June 1998 through August 1999, approximately 200 Rio Grande cichlid were collected from the lower Sims Bayou site using a 1.5 m radius cast net. These fish ranged in size from 2 cm total length (TL) juveniles to 15 cm + TL adults. In February 1999, eight small ( $\approx 8$  cm TL) Rio Grande cichlid were collected from the upper Sims Bayou site with a 3.0 m seine. In May 1999, eighteen medium ( $\approx 8$ -12 cm TL) Rio Grande cichlid were collected from Brays Bayou with a 1.5 m radius cast net. The Texas Cooperative Wildlife Collection at Texas A&M University (TCWC) has no listing of the Rio Grande cichlid in

Texas as far northeast as Harris County (R. Kathryn Vaughan, pers. comm.). Likewise, the Nonindigenous Aquatic Species at US Geological Survey (Leo Nico, pers. comm.) and the Texas Memorial Museum at University of Texas (Allison Anderson, pers. comm.) has not recorded this species from Harris County or adjacent drainages, e.g. San Jacinto and Trinity rivers. Three specimens from Sims Bayou at Martin Luther King Boulevard are deposited in the Texas Memorial Museum (TMM 25754). Five specimens from Sims Bayou at Hemingway Drive are deposited in the TCWC at Texas A&M University (TCWC 10814.01).

Based on collections from the lower Sims Bayou site, Rio Grande cichlid is most prevalent in warm weather. While among the most common and persistent fish in warm weather, they altogether disappear in winter weather (beginning November-December), returning again in large numbers as the water begins to warm (beginning April-May). Although it is unknown why the Rio Grande cichlid exhibits this seasonal distribution, it is possible that they move to a thermal refuge from the cold water in the winter.

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RTM at: rtrentmartin@email.com

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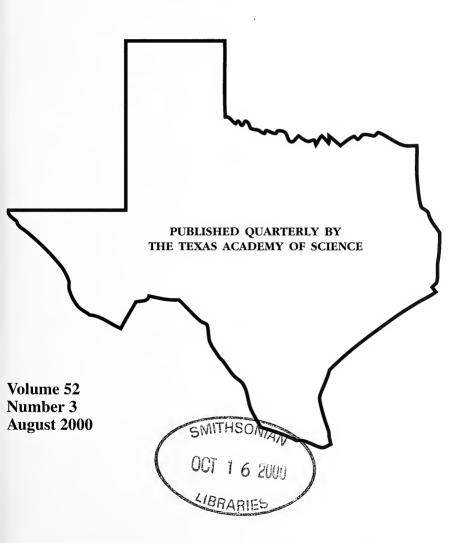
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# REPRODUCTION, GROWTH, SURVIVORSHIP AND ACTIVITY PATTERNS IN THE SOUTHWESTERN EARLESS LIZARD (COPHOSAURUS TEXANUS SCITULUS) (PHRYNOSOMATIDAE) FROM THE BIG BEND REGION OF TEXAS

#### Fred Punzo

Box 5F, Department of Biology, University of Tampa Tampa, Florida 33606

Abstract.—This study reports on various aspects of reproduction, growth and survivorship in a population of the southwestern earless lizard, *Cophosaurus texanus scitulus*, from Big Bend Ranch State Park in west Texas (U.S.A.) over a two year period. Testis enlargement began in mid-April. For females, the earliest presence of oviductal eggs was the first week of June. No females with oviductal eggs were found after 27 August. Yolked follicles were first observed in females collected on 10 April 1998 and 4 April 1999. Egg weight ranged from 13 - 35% of the total weight of a female with oviductal eggs. Mean clutch sizes ranged from 2.77 - 4.67 over the two year period. Females collected earlier in the season had larger fat bodies and higher levels of storage lipids. Males had higher growth rates than females in the juvenile-to-yearling age class and in the growth period between yearling and adult. Adult males were significantly larger than females. No bias in the sex ratio was found for any age class in 1998. In 1999, the sex ratio was skewed in favor of yearling males. Survivorship was low for males (juveniles, 12%; yearlings, 29%; adults, 24%) and females (14, 37 and 16%, respectively). Peak periods of activity were between 1000 - 1159 hr CST.

Previous research on the reproductive biology and other aspects of the natural history of the southwestern earless lizard (*Cophosaurus texanus scitulus*) includes studies on feeding habits and diets (Engeling 1972; Smith et al. 1987; Maury 1995), reproductive biology (Ballinger et al. 1972; Shrank & Ballinger 1973; Vitt 1977; Smith et al. 1987; Sugg et al. 1995), and general life history traits (Howland 1992). However, relatively few populations of this species have been studied in any detail. Any information that will help to assess intraspecific geographical variation in life history traits is essential for a more complete understanding of the relationships between environmental conditions and lizard life histories.

Cophosaurus texanus scitulus is a small to medium-sized phrynosomatid lizard. It is found in far west Texas, west of the Pecos, and extends into southern New Mexico and eastern Arizona (Bartlett & Bartlett 1999). It prefers open areas with sand and exposed rocks (Engeling 1972; Garrett & Barker 1987). This species has a short life

span, exhibits small clutch sizes, and produces a variable number of clutches during the breeding season (Sugg et al. 1995).

Field studies were conducted on the reproductive ecology, growth rates and survivorship in a population of *C. texanus* from west Texas in order to gather more information on these important parameters. The evolutionary processes that act on body size and growth rates have become increasingly interesting to evolutionary biologists (Stearns 1976; 1992; Roff 1992; Punzo 1998; 2000). In addition, differences in growth rates between the sexes have been shown to result in size dimorphism in many species of lizards which has been attributed to a variety of causes (Trivers 1976; Schoener & Schoener 1978; Anderson & Vitt 1990; Sugg 1992). These studies also argue that dimorphism in size is primarily the result of sexual selection. However, others have suggested that size dimorphism results from females having a higher investment in reproductive activities and as a result can allocate less energy toward growth (Downhower 1976; Carothers 1984).

The purpose of this paper is to describe studies on various aspects of the biology of *Cophosaurus texanus scitulus* from the Big Bend region of west Texas, including reproduction, growth, survivorship and activity patterns.

#### MATERIALS AND METHODS

The study site consisted of a sandy wash and surrounding flats at the bottom of Madera Canyon (MC) (29°7'30"N; 103°55'04"W). The entrance to this canyon is located directly off State Road 170, 5 km NW of Lajitas, Brewster County, Texas, at an elevation of approximately 900 m. This site, which is part of Big Bend Ranch State Park, is characterized by an abundance of exposed rocks, and lies within the creosote/lechuguilla/cactus association of the Chihuahuan Desert in the Big Bend region of Trans Pecos Texas (Punzo 1974; 1998). For a detailed description of the vegetation of this region the reader should consult Tinkham (1948), Warnock (1970) and Powell (1988). A 2.2 ha portion of the site was gridded with wooden stakes and thoroughly censused during March through September, in 1998 and 1999. Additional areas adjacent to the canyon walls were sampled at weekly intervals to assess the degree of migration into and out of the main study site. Voucher specimens have been deposited in the University of Tampa Vertebrate Collection.

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Cophosaurus texanus were abundant at this study site. The animals were observed through the use of binoculars while walking slowly through the area. All animals were captured with a noose over the two year period and provided with a unique identification number via toe clipping as described by Punzo (1982). Each lizard was sexed and given a unique paint mark to facilitate identification at a distance as well as upon recapture. Each animal was weighed to the nearest 0.01g using an Ohaus Model 31677 Port-O-Gram electronic balance, and measured its snout-vent length (SVL) to the nearest 0.1 mm with vernier calipers. Cloacal temperatures were recorded with a Schultheis quick-reading thermometer. The microhabitat, soil type, air temperature and time of capture were also recorded for each lizard. Time of capture data were used to determine diel periodicity. All animals were released within 6 hr at the original capture site. Population density was estimated from data on yearling and adult lizards.

Lizards were aged according to SVL. Previous observations on this population indicated that juveniles could be reliably aged on the basis of their small body size, and that there was no overlap in size between yearlings and adults of known age. Lizards were classified as juveniles if their SVL was less than 45 mm. Snout-vent lengths for adult males and females were > 50 mm. Lizards of intermediate size were aged based on their size at first capture, date of first capture, or subsequent growth history if available.

Individual growth rates were calculated from the first capture of a lizard to its final capture in the same active season, based on the increase in SVL (in mm/day), and from the last capture of a lizard in 1998 to its first capture in 1999 (Howland 1992). SVL was chosen as the index of body size due to the high within-individual variation in mass attributed to the reproductive condition of females that has been previously reported for this lizard as well as other species (Ballinger et al. 1972; Vitt 1977; Dunham & Reznick 1987; Howland 1992).

Survivorship was measured for three age classes as described by Howland (1992): juveniles (lizards in their first season); yearlings (lizards in their second active season; approximately one year old); and adults (lizards two years or older). The number of animals of age x+1 recaptured in 1999 that were initially marked at age x in 1998 divided by the total number of lizards marked at age x in 1998 was used to

estimate survivorship. In this approach, the time of hatching is considered age 0, and mortality of eggs between oviposition and hatching is ignored.

Additional samples of males and females were collected weekly during the two year period for anatomical examination. A total of 160 males and 203 females were killed, frozen, returned to the laboratory, and autopsied for reproductive condition. This population is contiguous with several others which allowed for the migration of additional lizards into the study population over subsequent years. Testis weight and length were recorded for males. For females, data were recorded for the number, weight and size of vitellogenic follicles, oviductal eggs, corpora lutea and corpora adiposa. The simultaneous occurrence of vitellogenic follicles with either oviductal eggs or corpora lutea were taken as evidence for the production of multiple clutches by a single female (Vitt 1977). Since previous work has indicated that approximately four weeks are required to produce a clutch of eggs in this species (Ballinger et al. 1972), females that possess vitellogenic follicles or oviductal eggs on two capture dates separated by at least four weeks can provide further evidence for multiple clutches. These data were used to estimate clutch frequency, average clutch size, and age and size at first reproduction.

After removal of the eggs and follicles, the fat bodies were removed, and along with the remaining carcasses, weighed on a Sartorius 215 analytical balance to the nearest 1.0 mg. Fat bodies and carcasses were freeze dried and reweighed to the nearest 0.1 mg. Male and female lizards used for lipid analyses were collected from three sample periods during 1999: early (19-25 June), middle (16-23 July) and late (19-23 Quantitative lipid extraction (total lipids) was conducted August). separately for vitellogenic follicles, individual oviductal eggs, fat bodies and carcasses according to the procedure described by Congdon & Gibbons (1989). Follicle and egg lipids were not included as a part of storage lipids for females (Ballinger et al. 1972; Howland 1992). The statistical tests used in the analysis of data follow the procedures described by Sokal & Rohlf (1995). For variables influenced by body size, analysis of covariance (ANCOVA) was employed with SVL as the Adjusted least squares means were then compared with covariate. t-tests.

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Table 1. Testis weight (g) for *Cophosaurus texanus* males during the reproductive season. Values represent means  $(\pm SD)$ . Numbers in parentheses beneath the testes weights represent the sample size for that year.

		Mean Testis Weight (g)		
Month	n	1998	1999	
March	12	0.076 (.008) (7)	0.078 (.007) (5)	
April	31	0.134 (.006) (18)	0.132 (.007) (13)	
May	24	0.154 (.011) (10)	0.149 (.008) (14)	
June	27	0.127 (.009) (16)	0.129 (.010) (11)	
July	30	0.085 (.002) (17)	0.091 (.004) (13)	
August	22	0.066 (.004) (10)	0.071 (.003) (12)	
September	14	0.047 (.005) (7)	0.049 (.002) (7)	

#### **RESULTS**

In males, testis enlargement began in mid-April and regression was completed by mid-August in both years (Table 1). Males attain maturity at a size of 50-52 mm SVL, about 11 months after hatching. For females, the earliest presence of oviductal eggs was 5 June in 1998, and 3 June in 1999. No females with oviductal eggs were found after 27 August. The highest frequency of gravid females occurred during two periods in 1998, and three periods in 1999. In 1998, 23 females with oviductal eggs were collected between 11-17 June, and 19 females between 7-13 August. For 1999 the data were as follows: 29 females between 6-12 June, 33 between 6-15 July and 17 between 12-18 August. Thus, oviposition in *C. texanus* from this site occurs from early June to late August.

The smallest female in either year that contained oviductal eggs had a SVL of 51 mm. In 1998 and 1999, 14 and 9 females, respectively, were reproductive at body sizes ranging from 51.5 - 53.5 mm SVL. A total of 24 females were reproductive at approximately 11.5 months after the initial appearance of hatchlings. On the basis of body size (SVL) and dates of capture, females attain sexual maturity at a SVL of 51-53 mm, and an age of 11-11.5 months.

Table 2. Mean clutch sizes and snout-vent lengths (SVL) for females of *Cophosaurus texanus* (1998 and 1999) from west Texas. Values in parentheses represent  $(\pm SD)$ . Sample data with different superscripts differ (Mann-Whitney-Wilcoxon Z-tests, P < 0.02); those with the same superscripts do not differ (P > 0.05)

	Number	Clutch Size	S V L (mm)
1998			
June	34	4.67 (0.38) <sup>a</sup>	58.83 (2.92) <sup>a</sup>
July	26	4.21 (0.18) <sup>a</sup>	59.53 (3.27) <sup>a</sup>
August	43	3.07 (0.22) <sup>b</sup>	59.33 (2.79) <sup>a</sup>
1999			
June	47	4.43 (0.44) <sup>a</sup>	58.47 (3.04) <sup>a</sup>
July	31	3.86 (0.31) <sup>a</sup>	58.89 (2.85) <sup>a</sup>
August	29	2.77 (0.17) <sup>b</sup>	58.97 (2.68) <sup>a</sup>

The mean clutch sizes for *C. texanus* ranged from 3.07 - 4.67 in 1998, and from 2.77 - 4.43 in 1999 (Table 2). It can be seen that clutch sizes decreased as the breeding season progressed, and this decrease was significant from July to August. Seventeen recaptured lizards showed evidence for multiple clutches in 1998, and 12 in 1999. The simultaneous presence of vitellogenic follicles and oviductal eggs suggested that these lizards would have produced two clutches.

With respect to the reproductive condition of the females, the ovaries of immature females contained 8-21 translucent white follicles ranging in size from 0.25 - 1.2 mm in diameter. Yolked follicles were first observed in females collected on 10 April in 1998, and on 4 April in 1999. It is possible that yolking could occur earlier at this site since oviductal eggs were present in some females as early as 17 and 20 April, in 1998 and 1999, respectively. Yolked follicles were found in females throughout the reproductive season. None were observed after 5 August in 1998, and 11 August in 1999.

Although the precise length of time required for follicular development is not known, it is most likely about 30 days, since the first large proportion of females containing oviductal eggs occurred in the May sample for both years, one month after the occurrence of a high proportion of females with yolked follicles. The occurrence of yolked follicles

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Table 3. Total lipid levels and fat body wet masses for *Cophosaurus texanus* during 1999. Adults and yearlings are lumped within each group. Values represent means  $\pm SD$ . Numbers in parentheses represent sample sizes. Statistical analyses are based on comparisons of adjusted least squares means on log - transformed data using *t*-tests with significance level adjusted for multiple comparisons. For intrasexual comparisons, values with different superscripts differ significantly (P < 0.01) whereas others do not (P > 0.05). Sampling periods for lizards used in these analyses: early (19-25 June); middle (16-23 July); late (19-23 August).

	Fat body	Fat body lipids (g)		Carcass lipids (g)	
Sample period	Females	Males	Females	Males	
Early	$0.043 \pm .03^{a}$ (21)	$0.036 \pm .01^{a}$ (8)	$0.161 \pm .03^{a}$ (21)	0.159 ± .01 <sup>a</sup> (8)	
Middle	$0.037 \pm .01^{a}$ (36)	$0.033 \pm .02^{a}$ (21)	$0.174 \pm .04^{a}$ (36)	$0.161 \pm .02^{a}$ (21)	
Late	$0.018 \pm .01^{b}$ (19)	$0.025 \pm .003^{\text{ b}}$ (28)	$0.067 \pm .01^{b}$ (19)	$0.158 \pm .02^{a}$ (28)	

in females containing oviductal eggs occurred in 24 cases in 1998, and 18 cases in 1999. The size of the follicles ranged from 0.007 - 0.023g in weight, and from 2.1 - 3.2 mm in diameter. Since this represents only 12% of the maximum follicular size attained in this population, it is reasonable to conclude that follicular development was only recently initiated in these females.

Oviductal eggs were present in 31% (31 out of 100) and 49% (50 out of 103) of the reproductive females in 1998 and 1999, respectively. These eggs ranged from 9-14.3 mm in length, and 4.6-7.5 mm in width, with a mean weight of 0.307g (0.209-0.426g). The egg weight ranged from 13-35% of the total weight of a female with oviductal eggs. The smallest oviductal egg weight was less than the largest follicle weight. However, the largest egg weight recorded for females over the entire study period was markedly heavier than that for the largest follicle weight (0.248g). In smaller females (< 58 mm, SVL), the average weight of eggs deposited before June (0.265g  $\pm$  .024 SD) was significantly smaller (p < 0.05) than the average egg weight (0.316g  $\pm$  .017) laid by larger females (> 59 mm) during the same period. The difference in the average weight of eggs laid by smaller females (51-55 mm) after the middle of June (0.339g  $\pm$  .021) did not differ significantly (P > 0.1) from the egg weight of larger females (0.331g  $\pm$  .016).

The levels of storage lipids and lipid components (fat body and carcass lipids) for females (1999) showed significant differences among the sampling periods (ANCOVA: F = 12.13, P < 0.001; F = 10.76, P < 0.001; F = 11.84, P < 0.001, respectively) (Table 3). Larger fat

Table 4. Mean sex-specific growth rates (in mm/day) for various age classes of *Cophosaurus texanus* from west Texas. Values represent means  $\pm$  SD. Data pooled from 1998 and 1999. Sample sizes shown in parentheses. For intrasexual comparisons of age classes, growth rates with different superscripts indicate significant differences (Tukey's HSD test: a - 0.05). Asterisks indicate age classes exhibiting intersexual differences in growth rates (t-tests: all P < 0.05).

	Mean growth rates (mm/day)			
Age class	Females	Males		
Juvenile	$0.149 \pm .009 (15)^{a}$	$0.115 \pm .011 (11)^{ab}$		
Juvenile-to-yearling*	$0.134 \pm .012 (22)^{a}$	$0.159 \pm .015 (9)^{a}$		
Yearling*	$0.073 \pm .015 (35)^{b}$	$0.121 \pm .008 (19)^{a}$		
Yearling-to-adult*	$0.022 \pm .005 (10)^{c}$	$0.063 \pm .002 (15)^{bc}$		
Adult	$0.010 \pm .001 (20)^{c}$	$0.006 \pm .003 (27)^{c}$		

bodies were observed in females earlier in the season perhaps attributable to the incomplete development of follicles. No significant differences in the variation of storage lipids or major lipid components were found for males between sampling periods (P > 0.05). Females collected during the early sampling period had significantly higher levels of storage lipids (F = 7.71, P < 0.03) and carcass lipids (ANCOVA: F = 8.06, P < 0.02) than males. No significant differences were found in the late sampling period (P > 0.05). Since yearlings and adults exhibited no significant differences in lipid levels for any sample period, they were lumped for all other lipid comparisons.

In females (1999), fat body wet mass was significantly correlated with carcass lipids (F = 37.22, P < 0.001,  $r^2 = 0.71$ ), fat body lipids (F = 601.23, P < 0.001,  $r^2 = 0.88$ ), and storage lipids (F = 506.67, P < 0.001,  $r^2 = 0.84$ ). Similar patterns were observed for males (F = 29.66, P < 0.001,  $r^2 = 0.92$ ; F = 544.76, P < 0.001,  $r^2 = 0.79$ ; F = 447.21, P < 0.001,  $r^2 = 0.87$ , respectively). The correlations between egg lipids and egg wet mass (F = 3.99, P < 0.05,  $r^2 = 0.42$ ), and dry mass (F = 33.84, P < 0.001,  $r^2 = 0.39$ ), were significant. The carcass lipids accounted for 27% of the variation in total egg lipids within a clutch (F = 3.87, P < 0.05,  $r^2 = 0.27$ ). Lipid levels in eggs were significantly higher during the early sample period than in the middle or late samples (ANCOVA, with SVL as covariate: P < 0.05). Egg dry mass from the late sample period was significantly lower than that of the early period (P < 0.01) or the middle period (P < 0.02).

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The mean sex-specific growth rates (in mm/day) for the various age classes of C. texanus are shown in Table 4. In this population, males had higher growth rates than females in the juvenile-to-yearling age class in both 1998 and 1999 (ANOVA: F = 9.32, P < 0.01; F = 7.88, P < 0.02, respectively) and in the growth period between yearling and adult (F = 11.01, P < 0.01; F = 6.99, P < 0.02). The data also indicated that male growth rates were highest during the juvenile-to-yearling and yearling age classes. Growth rates typically declined with increasing age as well.

Adult males were significantly larger than females (Z=12.07, P<0.001). The mean SVL for females in August was 59.33 mm (1998) and 58.97 mm (1999) (Table 2), with a maximum of 64 mm. For males, the values were 63.72 mm ( $\pm$  3.01SD) in 1998, and 66.03 mm ( $\pm$  2.97) in 1999, respectively, with a maximum of 72 mm. No bias in the sex ratio was observed for adults and yearlings in 1998 ( $X^2=3.07$ , P>0.05) and 1999 ( $X^2=5.36$ , P>0.1).

No bias was found for the sex ratios of juveniles ( $X^2 = 1.93$ , P < 0.1), yearlings ( $X^2 = 3.01$ , P > 0.15), or adults ( $X^2 = 2.23$ , P > 0.1) in 1998. In 1999, the sex ratio was skewed in favor of yearling males (1.6 : 1;  $X^2 = 8.04$ , P < 0.01), but not in other age classes. Survivor-ship for males between 1998 and 1999 was 12% for juveniles, 29% for yearlings and 24% for adults. For females, the values were 14%, 37%, and 16%, respectively.

Only adults and yearlings were tabulated for density estimates. The gridded area encompassed 2.2 ha and contained 193 marked individuals (88 lizards/ha) in 1998.

Cophosaurus texanus was strongly diurnal in its diel periodicity (Table 5). The peak time of activity at this site was between 1000 - 1159 hr (Central Standard Time, CST). No lizards were collected between 1900 - 0759 hr. No significant differences in activity were found between the sexes or age classes. In addition, activity patterns showed no significant seasonal shifts (G = 1.87, P > 0.05).

Incidental observations indicated that only those lizards whose home ranges were located close to the boundaries of the gridded study site exhibited any movements into or out of this area. Twenty-one adult (12 males; 9 females) and 7 yearling lizards traveled in various directions

Table 5. Daily activity patterns for *Cophosaurus texanus* from west Texas. Time intervals represent CST.

Time interval		1998		1999	
	n	Percent frequency	n	Percent frequency	
0700 - 0759	0	0	0	0	
0800 - 0859	7	4.9	2	0	
0900 - 0959	13	9.0	10	8.8	
1000 - 1059	42	29.2	31	27.4	
1100 - 1159	31	21.6	29	25.8	
1200 - 1259	19	13.1	11	9.7	
1300 - 1359	11	7.6	7	6.2	
1400 - 1459	4	2.8	9	8.0	
1500 - 1559	6	4.2	8	7.1	
1600 - 1659	3	2.1	0	0	
1700 - 1759	6	4.2	3	2.7	
1800 - 1859	2	1.3	3	2.7	
1900 - 1959	0	0	0	0	
2000 - 2059	Ö	0	Ö	0	

over distances ranging from 35 to 90 m. No directional bias was noted in these movement patterns.

#### **DISCUSSION**

The Madera Canyon (MC) population of C. texanus exhibited similar clutch sizes and growth rates when compared to populations from Rosillos Ranch (RR) and Grapevine Hills (GVH), approximately 40 km further south, in Big Bend National Park (BBNP) as reported by Howland (1992), and from Elephant Butte (EB), New Mexico (Sugg et al. 1995). Body sizes were somewhat smaller and adult survivorship was lower. However, the MC population exhibited smaller clutch sizes, lower clutch frequencies, and delayed reproduction when compared to times reported for populations of this species from central and west-central Texas (Johnson 1960; Ballinger et al. 1972; Engeling 1972). Although Howland (1992) suggested that C. texanus from BBNP may lay up to three clutches per season, and Ballinger et al. (1972) suggested a maximum of five clutches for a population from San Angelo, Texas, additional studies are needed to determine the reproductive potential of C. texanus more precisely. Nonetheless, the data available for reproduction in C. texanus indicates that this species is characterized by early sexual maturation and reduced longevity. Selection should favor animals that expend a large amount of reproductive

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energy earlier in life (Congdon et al. 1982; Stearns 1992).

The smaller clutch size and growth rates exhibited by the MC, RR and GVH, and EB populations may be due to lower annual precipitation levels at these locations. The larger clutch sizes reported from central and west-central Texas are associated with habitats that experience 1.5 - 2.5 times the mean precipitation levels that characterize lowland habitats at BBNP (Medellín-Leal 1982; Punzo 1991) and MC (Punzo 1997). It is well known that precipitation has a pronounced effect on the amount of vegetation and the abundance of arthropods available as food to lizards (Dunham 1978; Pianka 1986; Fellers & Drost 1991). Increased food intake would increase the amount of energy available for reproduction and other activities.

At MC, the mean clutch sizes decreased significantly from July to August. Howland (1992) reported a significant decrease in clutch size for *C. texanus* from GVH (from a mean of 3.7 in early July, to 2.82 in mid-August). However, females from the RR population did not show any concomitant decrease. Ballinger et al. (1972) reported no significant difference in clutch size over the breeding season in *C. texanus* from San Angelo (SA), Texas.

The results of this study showed that males had higher growth rates than females in the juvenile-to-yearling age class. This is in agreement with studies on populations of *C. texanus* from other localities (Johnson 1960; Ballinger et al. 1972; Engeling 1972; Howland 1992) as well as other species of desert lizards (Pianka 1986). Females most likely begin to allocate increasing energy sources to reproduction as soon as they become sexually mature thereby decreasing the amount of energy allocated toward growth. Sugg et al. (1995) showed that female growth in the EB population of *C. texanus* decreases at an earlier age than males suggesting that females devote more energy to reproduction earlier in life. They found that the energy content of eggs accounted for 63-90% of the difference in adult size between the sexes.

At the MC site, females can attain sexual maturity at minimum body sizes (SVL) from 51-53 mm, and at an age of 11-11.5 months. At the GVH site, the smallest female with oviductal eggs was 52 mm SVL, and 6 females were reproductive at 53-55 mm (Howland 1992). Several females at the GVH and RR sites were reproductive at 12 months of age. Thus, the lizards at MC appear to reach maturity a little earlier in

the season than those animals further south in BBNP. In the SA population, reproductive females ranged in size from 50-56 mm SVL (mean: 60 mm) (Ballinger et al. 1972). In view of the relatively short life span of *C. texanus*, sexual selection may have acted to increase male growth rates as well as size, resulting in sexual dimorphism (Sugg 1992; Sugg et al. 1995). Previous studies have shown a relationship between large size and the mating success of males in several species of lizards (Trivers 1976; Cooper & Vitt 1989; Anderson & Vitt 1990). The increased growth and size of males may also be related to their defense of territiories. It has been shown in other species of lizards that larger males are usually more successful at establishing and defending territories than smaller males (Clarke 1965; Stamps 1983).

The sum of fat body lipids and carcass lipids represents storage lipids (Shrank & Ballinger 1973; Derickson 1976). Females from MC collected early in the season exhibited significantly higher levels of storage lipids and carcass lipids when compared to males. These levels decreased later in the season when reproduction was complete. Schrank & Ballinger (1973) observed a depletion of the fat bodies of C. texanus females from central Texas during the early summer months and suggested that these lipids are used during early reproduction. Hahn & Tinkle (1965) were among the first to experimentally demonstrate the importance of stored lipids in corpora adiposa to the production of the first clutch of eggs in the side-blotched lizard, *Uta stansburiana*. Large corpora adiposa were present in females early in the season before follicular development, followed by a decrease during the middle of the summer, and another increase in post-reproductive females later in the season. Similar depletion patterns have been reported for other populations of C. texanus (Johnson 1960; Ballinger et al. 1972; Howland 1992) as well as other species of lizards (Vitt & Cooper 1985: Ramirez-Pinilla 1991; Flemming 1993; Van Wyk 1994).

The low values for survivorship in juveniles, yearlings and adult males and females indicates a high rate of mortality for these lizards. They are commonly preyed upon by roadrunners (*Geococcyx californianus*), long-nose leopard lizards (*Gambelia wislizenii*), and a number of snakes including the western coachwhip, (*Masticophis flagellum*), striped whipsnake (*M. taeniatus*), and long-nosed snake (*Rhinocheilus lecontei*) at the MC site. Even higher mortality rates were reported by Engeling (1972) for *C. texanus* from Comal County, Texas. The relatively low abundance of arthropods at this site may also contribute to

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mortality as well (Punzo 1997).

No bias in the sex ratio was observed for any age class of *C. texanus* in 1998 from MC. In 1999, the sex ratio was skewed in favor of yearling males (1.6:1). A bias toward juvenile females was reported for a population of *C. texanus* from GVH in 1981 but not from the RR site (Howland 1992). No bias was observed for any age class in 1982. Smith et al. (1987) reported a bias toward adult males in a population of *C. texanus* from Arizona and male: female sex ratios of 53:50 (1.6:1) and 42:40 (1.05:1) for yearlings and juveniles, respectively.

Cophosaurus texanus at the MC site were strongly diurnal which is in agreement with data from other populations (Cagle 1950; Degenhardt 1966; Smith et al. 1987). Between 0800-0859 hr, they begin to emerge from their shelter sites within rock crevices and under rocks and other surface debris. They prefer open sandy areas with scattered vegetation and occupy this habitat with other ground-dwelling species including two teiid lizards, the western whiptail, Cnemidophorus tigris and the little striped whiptail, C. inornatus. Future studies should analyze the types of interactions between these ground-dwelling lizard species to assess the degree of competition and resource partitioning.

In summary, *C. texanus scitulus* populations in west Texas exhibit short life spans, early maturity, relatively large clutch size, and low population densities. Their growth rates are lower than those reported for *C. texanus* from central Texas. *Cophosaurus texanus scitulus* exhibits a seasonal decline in clutch and egg size as well as in the depletion of storage lipids, suggesting that this population is limited by available resources.

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FP at: fpunzo@alpha.utampa.edu

## TRAP-REVEALED MICROHABITAT USE BY SMALL MAMMALS IN MONOCULTURE GRASSLANDS

#### S. S. Davis, R. B. Mitchell and S. Demarais

Department of Range, Wildlife and Fisheries Management Texas Tech University, Lubbock, Texas 79409-2125

Abstract.—This study was conducted to determine if microhabitat differences in canopy cover of weeping lovegrass (Eragrostis curvula) monoculture grasslands influenced presence of small mammals. Canopy cover of weeping lovegrass was measured at 12 trapping grids of 100 traps each. Traps were pooled and separated into four categories (0 to 25%, 26 to 50%, 51 to 75% and 76 to 100%) based on percentage of weeping lovegrass canopy cover at each trap location. First captures of small mammals were analyzed using the chi square test statistic. Four species, cotton rat (Sigmodon hispidus; n = 100), western harvest mouse (Reithrodontomys megalotis; n=173), hispid pocket mouse (Chaetodipus hispidus; n=28), and deer mouse (*Peromyscus maniculatus*; n=41) were captured in sufficient numbers for statistical comparison. Captures for P. maniculatus and C. hispidus differed from the expected distribution of captures ( $X^2$ , 3 df,  $P \le 0.01$ ) with more captures in open trap sites and fewer captures in sites with increased cover. In contrast, captures for S. hispidus and R. megalotis differed from the expected distribution  $(X^2, 3 df, P \le 0.001)$  with more captures in densely vegetated trap sites and fewer captures in open trap sites. Canopy cover appears to influence small mammal microhabitat selection during the spring in weeping lovegrass monocultures. Land management decisions which affect microhabitat characteristics may impact small mammal community structure.

Researchers have developed several hypotheses seeking to explain coexistence of sympatric small mammalian species in the Southwestern United States. Microhabitats have long been thought to influence diversity of small mammals (Price 1978). Brown (1973) suggested that heteromyid rodents partitioned food resources based on seed size and body size. Weeks (1997) suggested foraging time as a possible niche partitioning mechanism. Jorgensen (1996) suggested abiotic factors such as substrate play a role in structuring mammalian communities. Additionally, habitats with reduced canopy cover and vertical structure could increase predation risks for some species and impact mammalian community structure (Kotler 1989).

Much of the work on small mammal communities has been done in heterogeneous habitats. Little is known about small mammal distributions in relatively homogeneous habitats (Smith et al. 1975). The Conservation Reserve Program (CRP) has provided an opportunity to study small mammals in relatively homogeneous, monoculture grassland habitats. Hall (1992) found 11 rodent species in CRP grasslands in the Texas Southern High Plains suggesting species richness in monoculture

grasslands may be as high as that of native grasslands.

Conservation Reserve Program grasslands often have monotypic substrates and are seeded to monoculture stands which provide a uniform habitat and food supply. Consequently, it is of special interest to attempt to determine how numerous small mammalian species can coexist in a monoculture habitat given the lack of heterogeneity of the plant community. This study examines the hypothesis that small mammal species use different microhabitats during the spring in monoculture grasslands based on canopy cover.

#### STUDY AREA AND METHODS

The study site was located approximately 4 km south and 12 km east of Tahoka in Lynn County, Texas. The 132 ha site which was formerly cropland was seeded to a monoculture of weeping lovegrass (*Eragrostis curvula*) in 1989. Historic vegetation at the site was shortgrass plains dominated by blue grama (*Bouteloua gracilis*), buffalograss (*Buchloe dactyloides*) and sideoats grama (*Bouteloua curtipendula*).

Although the plant community on this site was essentially a monoculture of weeping lovegrass, some invasive plants colonized the area. A limited number of other grass species occupied small isolated pockets within thin stands of weeping lovegrass, including Johnsongrass (Sorghum halepense), threeawns (Aristida spp.) and squirreltail (Sitanion hystrix). Forbs such as kochia (Kochia scoparia) and Texas blueweed (Helianthus ciliaris) also occupied open and disturbed sites.

The climate of Lynn County is subhumid, and characterized by low annual precipitation, a high rate of evaporation and high average wind velocity (Mowery & McKee 1959). Average annual precipitation is 51 cm and varies greatly. Typically, 70% of the annual precipitation falls from May to September. The period from January to May is characterized by strong prevailing west and southwest winds. Soils were loams and sandy loams, specifically Amarillo loam (Aridic Paleustalfs) and Portales fine sandy loam (Aridic Calciustolls) (Mowery & McKee 1959). Eolian sands from adjacent cotton (Gossypium hirsutum) fields occurred along the east, south, and west perimeters of the site.

Twelve 8.8 ha plots were established in a grid pattern with centrally located trapping grids comprised of 10 rows and 10 columns of traps (100 traps/grid) with a 10 m station interval. One Sherman live trap was placed at each trap site. Trapping occurred for five consecutive nights in March 1996, totaling 500 trap nights/grid, and 6000 trap nights during the five-night study.

Traps were baited with oats (*Hordeum vulgare*) and set each afternoon and checked the following morning. Captured animals were removed from traps, weighed, sexed and identified to species using Davis & Schmidly (1994). Large-eared species were marked with a small metallic ear tag, and small-eared species were toe-clipped for later identification. All animals were released at the capture site after processing.

Microhabitat structure was quantified at each of the 1200 trap sites by a modified line intercept technique (Canfield 1941). Canopy cover was determined by centering a 2 m transect pole at each trap site oriented to a random azimuth, and placing a second 2 m transect pole perpendicular to the first (Jorgenson et al. 1995). Canopy cover was recorded as the number of decimeters of weeping lovegrass intercepting each transect pole. Canopy cover classes of 0 to 25, 26 to 50, 51 to 75 and 76 to 100% were used as a quantifiable measure to differentiate habitat types at each trap site and habitat use in this monotypic grassland.

Due to low capture rates, chi square requirements for number of observations per cell were not met at the individual plot level. Therefore, data were pooled across plots for analysis. Chi square analysis (3 df) was used to test the hypothesis that the distribution of captures within the four cover categories (0 to 25, 26 to 50, 51 to 75 and 76 to 100%) did not differ from the expected capture distribution of equal number of captures within each category. Results were considered statistically significant at  $P \leq 0.05$ .

#### RESULTS

Mean canopy cover ( $\pm$  standard error) for the study area was 85.4  $\pm$  10.2% for weeping lovegrass, 5.1  $\pm$  2.6% for other grasses and 2.0  $\pm$  1.0% for forbs. Canopy cover measurements revealed 208 trap sites with 0 to 25% cover, 373 trap sites with 26 to 50% cover, 307 trap sites with 51 to 75% cover and 312 trap sites with 76 to 100% cover. In 6000 trap nights during March 1996, 351 unique captures of eight small mammal species were recorded. Sufficient captures for statistical analysis occurred for R. megalotis (n=173), S. hispidus (n=100), P. maniculatus (n=41) and C. hispidus (n=28). Captures of Baiomys taylori (n=8), Onychomys leucogaster (n=6), Dipodomys ordii (n=2) and Spermophilus spilosoma (n=1) were not sufficient for statistical analysis.

The distribution of observed captures differed from the expected distribution for P. maniculatus ( $X^2 = 12.72$ , 3 df, P = 0.005) and C. hispidus ( $X^2 = 11.32$ , 3 df, P = 0.01). Captures for both species were higher at sparsely vegetated trap sites and lower at densely vegetated

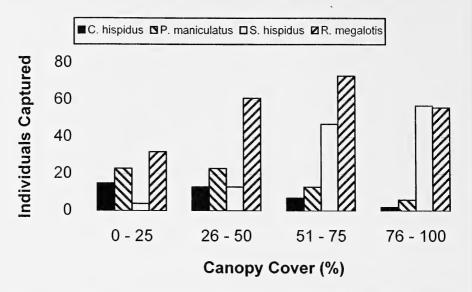


Figure 1. Number of individual Chaetodipus hispidus, Peromyscus maniculatus, Reithrodontomys megalotis and Sigmodon hispidus captured within four levels of grass canopy cover in a weeping lovegrass CRP grassland in Lynn County, Texas during March 1996.

trap sites (Figure 1). Average weeping lovegrass canopy cover at successful trap sites for *P. maniculatus* and *C. hispidus* was 35.8 and 29.5%, respectively.

Captures for *S. hispidus* and *R. megalotis* were higher at densely vegetated trap sites and lower at sparsely vegetated trap sites. The observed capture distribution differed from the expected distribution for *S. hispidus* ( $X^2 = 65.55$ , 3 df, P < 0.001) and *R. megalotis* ( $X^2 = 16.00$ , 3 df, P = 0.001) (Figure 1). Average weeping lovegrass canopy cover at successful trap sites for *S. hispidus* and *R. megalotis* was 69 and 56.4%, respectively.

#### **DISCUSSION**

Microhabitats often differ not only in the physical presence and structure of perennial plants, but also in seed abundance, soil texture, soil density and other attributes (Price & Waser 1985). Holbrook (1978) noted that subdivision of the habitat by vegetational type was an important mechanism of coexistence in four *Peromyscus* species. In this study, substrates across all plots were essentially the same, with similar soils present on all plots. Likewise, both food source (weeping lovegrass and its seeds) and cover type (weeping lovegrass) were constant

across plots. Consequently, hypotheses suggesting niche partitioning mechanisms based on substrate, food source and cover type apparently do not explain microhabitat use in monoculture stands of weeping lovegrass. Additionally, the morphology of weeping lovegrass was atypical of the historic vegetation in the region, and may provide subsequent support for niche partitioning of indigenous small mammals based on microhabitat variation in grassland monocultures.

Morphological adaptations such as locomotion have been correlated with microhabitat use (Price 1978; Price & Brown 1983; Kotler 1989). Quadrupedal species typically use a more closed canopy and bipedal species are associated with open canopies (Price & Waser 1985; Jorgensen et al. 1995). Although not all four species studied were from the same genera, all use quadrupedal locomotion. Consequently, it seems unlikely that locomotion plays a significant role in structuring this community, because two species were captured more often in microhabitats with closed canopies and two species were captured more often in microhabitats with open canopies. However, in the closed canopy sites and others, R. megalotis often climbs to obtain seeds from graminaceous species and other plants. Sigmodon hispidus prefers to travel within a runway system, and likely consumes a much higher percentage of green vegetation than seeds, as compared to the other species. Chaetodipus hispidus must occasionally dust bathe, obtains seeds and forage from the ground, and sometimes digs for food, all of which would be easier in a more open canopy. These subtle differences in locomotion and foraging likely influenced the capture of each species in different microhabitats at the site.

It is apparent from these data that during spring S. hispidus and R. megalotis use sites which are densely vegetated with higher grass canopy cover, whereas P. maniculatus and C. hispidus use sites which are more open with lower grass canopy cover (Figure 1). In addition to the differences discussed above, other possible explanations for coexistence of each species within more open or closed microhabitats could be differences in foraging microhabitats (Price & Waser 1985), differences in time of foraging (Weeks 1997), or seasonal variation in canopy cover and microhabitat use, none of which were examined during this study. Another possible explanation is that within each cover microhabitat there exists both a microhabitat generalist and microhabitat specialist (Ribble & Samson 1987). Whatever the mechanisms, this study supports the hypothesis that microhabitat canopy cover may play a role in the structure and diversity of small mammals within monoculture grassland systems.

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RBM at: rob.mitchell@ttu.edu

#### SMALL MAMMALS OF THE POST OAK SAVANNAH IN EAST-CENTRAL TEXAS

#### Kenneth T. Wilkins and David R. Broussard\*

Department of Biology and Graduate School Baylor University, Waco, Texas 76798-7388 \*Current address: Department of Zoology and Wildlife Science Auburn University, Auburn, Alabama 36849

Abstract.—Inventory of the terrestrial vertebrate fauna at two Texas Parks and Wildlife Department sites during 1994-1995 presented the opportunity to examine the diversity of the small-mammal community of the Post Oak Savannah vegetational region. Sampling documented only slightly more than half of the expected species of rodents and insectivores at these sites in relation to the biodiversity documented in the literature for Freestone and Limestone counties. Many of the species present occurred at densities much lower than previously found in similar habitats elsewhere in the region. A westward extension of range is also reported for *Oryzomys palustris*, the marsh rice rat, in Freestone County.

The Post Oak Savannah is one of 10 vegetational areas traditionally recognized in Texas (Gould 1975). This region encompasses some 3.4 million hectares of gently rolling to hilly terrain. This narrow zone, whose east-west extent is generally less than 100 km wide, trends south-westwardly from the Red River of northeastern Texas to its southern-most extent in Wilson, Goliad and Victoria counties. It is bounded to the west by the Blackland Prairies and to the east by the Pineywoods and the Gulf Plains and Marshes regions. Floristically, the Post Oak Savannah is considered an ecotone between the adjacent vegetational regions. Dominant trees of the uplands are post oak (*Quercus stellata*), blackjack oak (*Q. marilandica*), with little bluestem (*Schizachyrium scoparius*), Indiangrass (*Sorghastrum nutans*) and switchgrass (*Panicum virgatum*) being the dominant grasses in woodland clearings (Gould 1975).

The Post Oak Savannah is included within the Texan biotic province, which includes no endemic vertebrates (Blair 1950). Rather, the vertebrate (and, therefore, mammalian) fauna of the Post Oak Savannah in Texas is an assemblage of species characterizing adjacent vegetational regions and biotic provinces. To date, few papers have been published concerning the mammalian fauna of the Post Oak Savannah. Treatments of eastern Texas mammals by McCarley (1959) and Schmidly (1983) constitute the primary literature resources on mammals of this region. Grant et al. (1985) described the structure of a small-mammal communi-

ty in an old field in the Post Oak Savannah in Brazos County. Recent studies at two sites near the western edge of the Post Oak Savannah in Limestone and Freestone counties enable this study to contribute additional observations of the distributions and ecological affinities of small mammals in the Post Oak Savannah.

#### **DESCRIPTION OF STUDY SITES**

From August 1994 through September 1995, field surveys were conducted to develop inventories of terrestrial vertebrates at two Texas Parks and Wildlife Department sites. Fairfield Lake State Recreational Area (FLSRA) is located approximately 7 mi northeast of Fairfield in Freestone County. Fort Parker State Park (FPSP) is located in Limestone Co., approximately 7 mi south of Mexia. These sites are approximately 35 miles from each other.

Fairfield Lake State Recreational Area.—FLSRA comprises 1,460 acres (590 ha) at the southern end of Fairfield Lake, an impoundment of Big Brown and Little Brown creeks and associated tributaries of the nearby Trinity River. Elevations in the park range from approximately 93 m at lake level to approximately 119 m. Upland soils are fine sandy loams and loamy fine sands, whereas lower-lying areas along drainages are less-well-drained clay loams and sandy loams.

Five primary terrestrial habitats were sampled for small mammals at FSLRA. Three of these are woodlands. Tree species dominating the "creekbottom woodlands," sampled along Big Brown and Little Brown creeks, include water oak (*Quercus nigra*), American elm (*Ulmus americana*), box elder (*Acer negundo*), hackberry (*Celtis laevigata*) and pecan (*Carya illinoiensis*); the understory includes coralberry (*Symphoricarpos orbiculatus*) and green brier (*Smilax bona-nox*). A distinctively different forest, the "oak woodland", characterizes the drier, higher elevations. Post oak dominates this woodland, with juniper (*Juniperus virginiana*), winged elm (*Ulmus alata*), black hickory (*Carya texana*), white ash (*Fraxinus americana*), blackjack oak and water oak present to lesser degrees. Loblolly pine (*Pinus taeda*) distinguishes the third type of forest present at FLSRA, the "pine woods." Other woody species present are those of the oak woodland, which surrounds the pine woods.

Sampling was done in two non-woody situations. "Grasslands" present in openings in the oak woodlands were dominated by little

bluestem with occasional Indiangrass. The "marsh" was a low-lying, intermittently flooded grassy area near the southern shore of the lake. Gibson (1996) offers a thorough floristic description of the plant communities at FLSRA.

Fort Parker State Park.—Topographic relief within this 1,485-acre (600-ha) site is approximately 33 m, rising from 126 m at the surface of Lake Fort Parker to approximately 159 m in the limestone bluffs region at the southern edge of the part. The lake was produced by damming of the Navasota River in 1933. Soils at this site range from loamy fine sands in the uplands to loam and clay loam in the lowlands associated with the Navasota River and Baines Creek (unpublished soils maps for Limestone County, U.S. Natural Resources Conservation Service).

Two primary terrestrial habitats, woodland and grassland, were recognized for purposes of small-mammal studies. "Wooded habitats" were sampled in the vicinity of Lake Springfield (a small spring-fed lake to the north of Lake Fort Parker) and, to the south of Lake Fort Parker, in the bluffs area and along a streambed. At the higher elevations in the bluffs, the woods comprise stands of large bur oak (*Quercus macrocarpa*) and large hackberry trees; nearer the lake's edge, the dominant woody vegetation was water oak, willow (*Salix*) and juniper. In drier settings with sandier soils, post oak and blackjack oak were common. The predominant vegetation in "grasslands" sites was little bluestem grass. Depending on the site, species interspersed with little bluestem included dewberry (*Rubus riograndis*), sandbur (*Cenchrus* sp.), prickly pear cactus (*Opuntia* sp.) and an array of forbs.

# METHODS AND MATERIALS

Terrestrial small mammals (i.e., rodents and insectivores) were trapped along transects consisting of 30-50 Sherman live-traps set at approximately 5 m intervals in a straight line. Traps were set in the late afternoon, baited with rolled oats and left overnight. Field workers returned within 2-3 hours after sunrise on the next morning to retrieve the traps. This procedure was performed approximately monthly in most habitats in both FPSP and FLSRA. Though many animals were released at the point of capture after identity was determined and recorded, several individuals of each species were prepared as voucher specimens (as skin-and-skull specimens or preserved in alcohol); these were deposited into the collection of vertebrates in the Department of

Biology, Baylor University in Waco, Texas. MacAbee traps and Victor harpoon traps were set according to sign to obtain voucher specimens of pocket gophers and moles, respectively. Presence of other species of mammals was gleaned from study of feces, tracks, sightings, gnawings and other assorted sign.

#### RESULTS

#### FAIRFIELD LAKE STATE RECREATIONAL AREA:

During 13 months of field work, a sampling effort of 2,448 trapnights was conducted (Table 1). Overall, eight species of rodents and one species of insectivore totaling 36 individuals were captured: hispid cotton rat (Sigmodon hispidus, 12 individuals in all habitats), marsh rice rat (Oryzomys palustris, 7 individuals), cotton mouse (Peromyscus gossypinus, 6 individuals), white-footed mouse (Peromyscus leucopus, 3 individuals), pygmy mouse (Baiomys taylori, 3 individuals), eastern wood rat (Neotoma floridana, 2 individuals), deer mouse (Peromyscus maniculatus, 1 individual), fulvous harvest mouse (Reithrodontomys fulvescens, 1 individual) and least shrew (Cryptotis parva, 1 individual). The overall relative abundance of small mammals was 1.47 animals/100 trapnights of sampling effort.

Creekbottom woodlands.—Eleven individuals representing five species of rodents were caught in this habitat during 568 trapnights of effort for a relative abundance of ground-dwelling small mammals of 1.94 individuals/100 trapnights (Table 1). Cotton mice (six individuals) were the most abundant rodent species in this habitat. One white-footed mouse and one deer mouse were trapped here. The one rice rat (skin-and-skull specimen BU1359), trapped during February 1995, was a reproductively inactive, adult female captured near the edge of a temporary pool of water that resulted from a recent heavy rainfall. Two cotton rats were trapped in this habitat.

Oak woodland.—The only identified specimen of small mammal trapped here was a white-footed mouse. This habitat was remarkable for the virtual absence of understory or ground cover other than leaf litter and occasional fallen tree trunks and limbs. Capture of only one rodent in 567 trapnights yielded a relative abundance of 0.18 individuals/100 trapnights.

Table 1. Results by habitat of small-mammal trapping at Fort Parker State Park, Limestone County, Texas, and Fairfield Lake State Recreational Area, Freestone County, Texas, September 1994 - September 1995. For each species sampled, the number of individuals caught is followed by relative abundance (number caught / 100 trapnights).

	Fort	Fort Parker State Park	Park		Fairfi	Fairfield Lake State Recreational	Recreational	Area	
Effort, Species and Totals	Grasslands	Grasslands Woodlands	Totals	Bottomland Forest	Upland Post Oak	Upland Post Oak Pineywoods	Grassland	Marsh	Totals
Trapnights	879	936	1,815	568	567	460	628	220	2,448
Species Hispid cotton rat	10 / 1.14		10 / 0.55	2 / 0.36			2 / 0.32	8 / 3.64	12 / 0.49
Taylor's pygmy mouse	10 / 1.14		10 / 0.55				1 / 0.16	2 / 0.91	3 / 0.12
White-footed mouse	2 / 0.23	21 / 2.24	23 / 1.27	1 / 0.18	1 / 0.18	1 / 0.22			3 / 0.12
Deer mouse	8 / 0.91	6 / 0.64	14 / 0.77			1 / 0.22			1 / 0.04
Cotton mouse				6 / 1.06					6 / 0.25
Eastern wood rat		2 / 0.21	2/0.11	1 / 0.18		1 / 0.22			2 / 0.08
rectoma fioriaana Fulvous harvest mouse Reithrodontomys filmscen	7 / 0.80		7 / 0.39					1 / 0.45	1 / 0.04
Marsh rice rat Orwannes nalustris	c			1 / 0.18				6 / 2.73	7 / 0.29
House mouse  Mus musculus  Mus musculus		1/0.11	1 / 0.06						
Mus muscutus Least shrew Cryptotis parva							1 / 0.16		1 / 0.04
Totals	37 / 4.20	30 / 3.21 67 / 3.69	67 / 3.69	11 / 1.94	1/0.18	3 / 0.65	4 / 0.64	17 / 7.73 36 / 1.47	36 / 1.47

Pine woods.—The catch of small mammals in the pine woods comprised one eastern wood rat, one deer mouse and one white-footed mouse. The relative abundance of small mammals in this restricted parcel of habitat was only 0.65 rodents/100 trapnights. The understory here was sparse and ground cover consisted of little more than pine needles.

Grassland.—An effort of 628 trapnights in this habitat yielded two cotton rats, one pygmy mouse and one least shrew. The relative abundance of small mammals in the grassland habitat was 0.64 individuals/ 100 trapnights. This habitat was notable for the great abundance of imported fire ants (Solenopsis invicta); during most sampling periods, at least 80% of the traps became infested with fire ants.

Marsh.—Nearly half of the total number of small mammals trapped (17 of 36 animals) came from limited efforts (only 220 of the total 2,448 trapnights) in a marshy area near the southern end of the lake. The catch was four species comprising eight cotton rats, six rice rats, two pygmy mice and one fulvous harvest mouse for a relative abundance of 7.73 rodents/100 trapnights. Five rice rats (skin-and-skull specimens BU 1355-1358, 1360) were caught in this intermittently flooded tall-grass field during late fall and winter (November 1994 - January 1995). Testes were abdominal in the three male Oryzomys palustris; the two females showed no signs of reproductive activity.

Other species of mammals.—Two species of fossorial mammals occurred at FLSRA. Mounds of pocket gophers, Geomys breviceps, were seen in many of the grassy sandy upland areas; two specimens were collected with MacAbee traps in the pipeline right-of-way, a grassy opening in the oak woodland. The eastern mole (Scalopus aquaticus) was rather widely distributed within FLSRA as evidenced by the presence of their tunnel systems in well-drained and moist grasslands, pine woods, oak woodland and creekbottom woodlands.

Considerable evidence demonstrated the presence of beavers (*Castor canadensis*) in FLSRA along waterways and in adjacent wooded habitats. Along Big Brown Creek and nearby temporary pools of water, numerous saplings of Texas ash and water oak had been felled by gnawing. Tree squirrels of two species were sighted frequently in most wooded areas of FLSRA. Gray squirrels (*Sciurus carolinensis*) were more abundant in creekbottom woodlands (e.g., Big Brown Creek),

whereas fox squirrels (S. niger) predominated in the upland oak woodlands. White-tailed deer (Odocoileus virginianus) were abundant throughout the park.

### FORT PARKER STATE PARK:

A sampling effort of 1,871 trapnights was conducted in FPSP. Overall, 67 specimens of seven species of mammals (all rodents) were captured: white-footed mouse (23 individuals in all habitats), deer mouse (14 individuals), hispid cotton rat (10 individuals), pygmy mouse (10 individuals), fulvous harvest mouse (7 individuals), eastern wood rat (2 individuals) and a house mouse (*Mus musculus*, 1 individual). The overall relative abundance of small mammals was 3.69 animals/100 trapnights of sampling effort (Table 1).

Grassland.—Five species of rodents were caught along the grassland transects. Cotton rats and pygmy mice were captured at equal abundances (10 individuals each). Three other species were encountered in the grasslands: deer mouse (8 inviduals), fulvous harvest mouse (7 individuals) and white-footed mouse (2 individuals). The relative abundance of ground-dwelling small mammals in this habitat was 4.20 individuals/100 trapnights. Fire ants were present in most traps in this habitat during most of the sampling interval.

Woodlands.—Four species of rodents were captured in the woodlands at FPSP. White-footed mice were most abundant (70% of the captures), followed distantly by deer mice (6 individuals), two eastern woodrats and a house mouse. The relative abundance of small mammals in the woodlands was 3.21 rodents/100 trapnights.

Other species of mammals.—Pocket gophers and eastern moles are fossorial species occurring rather widely at FPSP. Beavers and nutria (Myocastor coypus) were present along waterways and in adjacent wooded habitats. In woodlands along the Navasota River, numerous saplings (mostly willow) had been felled by gnawing. Gray and fox squirrels were sighted in most wooded areas of FPSP.

## **DISCUSSION**

This work revealed direct or indirect evidence of 14 species of rodents and insectivores within the boundaries of FLSRA and 13 species of rodents and insectivores within FPSP (Table 2). Among these is one

Table 2. List of small-mammal species recorded at Fairfield Lake State Recreational Area (FLSRA) and Fort Parker State Park (FPSP) in relation to species expected in Freestone County and Limestone County (Schmidly 1983; Davis & Schmidly 1994).

si s	Southern short-tailed shrew Least shrew			1	Linestone Co.
uaticus linensis r		×	××		××
linensis	Eastern mole	×	×	×	×
			,	;	;
	Eastern gray squirrel Eastern fox squirrel	<b>~</b> ×	<b>~</b> ×	××	××
olans	Eastern flying squirrel		×	:	: ×
	Baird's pocket gopher	×	×	×	×
'ns	Hispid pocket mouse		×		×
7	American beaver	×	×	×	×
is	Marsh rice rat	×			
fulvescens	Fulvous harvest mouse	×	×	×	×
	Eastern harvest mouse		×		×
nus	Plains harvest mouse		×		×
SX	Cotton mouse	×	×		×
	White-footed mouse	×	×	×	×
niculatus I	Deer mouse	×	×	×	×
	Northern pygmy mouse	×	×	×	×
Sigmodon hispidus His	Hispid cotton rat	×	×	×	×
a	Eastern woodrat	×	×	×	×
	Norway rat		×		×
	Roof rat		×		×
S	House mouse		×	×	×
Microtus pinetorum Wo	Woodland vole		×		×
J	Common muskrat		×		×
Myocastor coypus Nut	Nutria		×	×	×
Totals		14	24	13	24

species (marsh rice rat) collected at FSLRA not previously documented as far west as Freestone County; the nearest published records for *Oryzomys palustris* are Anderson County to the east, Brazos County to the south and Hunt County to the northeast (Wilkins 1990; Davis & Schmidly 1994). Additionally, the capture of an eastern woodrat (*Neotoma floridana*) at FLSRA represents a county record for Freestone County within the broader distribution of this species as mapped in Davis & Schmidly (1994).

Species expected but not encountered.—Documented counts in this study are short of the approximately 24 species of rodents and insectivores that potentially occur in Freestone and Limestone counties (Davis & Schmidly 1994). A number of mammalian species not encountered within FLSRA and FPSP should be expected there on the basis of the primary habitats. For example, the southeastern short-tailed shrew, Blarina brevicauda, occurs in habitats that seemingly are represented in these parks. McCarley & Bradshaw (1953) reported this species in a variety of habitats including densely wooded floodplains, pine-oak uplands and grassy situations. Schmidly (1983:47) found them in these and other situations: "... in mixed hardwood-pine forests in traps placed adjacent to or under old logs and in the leafy cover and humus of the forest floor in lower-slope hardwood-pine, upper-slope pine-oak and flatland hardwood-pine habitats which, during the winter months, are often damp or wet."

No flying squirrels (*Glaucomys volans*) were observed, though FLSRA and FPSP are within their known geographic range and suitable wooded habitat is present. Two semiaquatic rodents, nutria (*Myocastor coypus*) and muskrats (*Ondatra zibethicus*), were not documented from FLSRA, although their prefered habitats (marshes, rivers, lakes, assorted drainages) certainly are present. Visual sightings document the presence of nutria, but not muskrats, at FPSP.

The eastern and plains harvest mice (*Reithrodontomys humulis* and *R. montanus*, respectively) occur in grasslands and various field-type situations. Both are known from Freestone County where they have been taken in reclaimed strip-mine areas with light cover and lacking tall grass (Schmidly 1983). *Reithrodontomys montanus* is also known from highway rights-of-way supporting sparse stands of bluestem, bermuda grass and Johnson grass (Wilkins & Schmidly 1980). In the native blackland prairie of Hunt County, Texas, *R. humulis* occurs in dense

stands of little bluestem, Indiangrass and eastern gama grass (*Tripsacum dactyloides*) (cf. Wilkins 1991).

Chaetodipus hispidus, the hispid pocket mouse, is most common in situations with friable, sandy substrate that permits digging. Vegetation covering such soils can be quite varied (e.g., roadsides with moderate-to-dense grassy cover; heavy stands of broomsedge and weeds associated with scattered stands of woody vegetation) yet still be suitable for this species (Schmidly 1983). Such habitat tolerances suggest that this mouse might occur in both parks.

The woodland vole, *Microtus pinetorum*, occurs in an array of ecological settings. Schmidly (1983:205-206) listed the following: "poorly drained, wet places in old fields with a dense growth of grasses and weeds growing on sandy soils and moist deciduous woodlands with a heavy layer of leaves and humus," grassy marshes; under a tangle of old grass and blackberry bushes near the edge of a swamp; ecotones between old fields and pine stands; tall grassy fields at lake's edge; grassy highway rights-of-way. Extensive sampling might well reveal this species as a member of the small-mammal community at both parks.

Comparisons with other localities.—Comparisons of relative abundance values for selected rodent species at FLSRA and FPSP that also occur in similar habitats at Clymer Meadow, a blackland-prairie preserve in Hunt County (Wilkins 1995), demonstrate relatively low population densities at the former sites. In the grasslands habitats of Clymer Meadow, cotton rats occurred at 7.6 individuals/100 trapnights, some 6 to 23 times the densities at FPSP and FLSRA, respectively (Table 1). Fulvous harvest mice occurred in grassland habitats at FPSP and FLSRA at densities of approximately half that at Clymer Meadow (1.4 individuals/100 trapnights). A similar trend holds for woodlands: relative densities of white-footed mice at FPSP and FLSRA were 50% and 4%, respectively, that at Clymer Meadow (4.5 individuals/100 trapnights). For eastern wood rats, relative densities at Clymer Meadow (1.8 individuals/100 trapnights) were approximately 9-fold greater than at FPSP and FLSRA.

Possibly the lower-than-expected biodiversity and population abundance values in this study are related to the moderately (FPSP) to highly (FLSRA) impacted condition of so much of the parks' habitats from historic and current uses (farming, grazing, former homesites and

townsites, recreation, etc.). High densities of white-tailed deer at FSLRA further contribute to habitat degradation. Additionally, imported fire ants abound within these parks, particularly in the grassland areas and, to a lesser extent, in the woodlands. Such fire ants are known to negatively impact ground-nesting birds and terrestrial small mammals (Smith et al. 1990; Allen et al. 1995; Lechner & Ribble 1996). In recent decades, imported fire ants have become a significant biogeographic force operating on both private and public lands with an array of land usage patterns.

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KTW at: Ken Wilkins@Baylor.edu

# MAPPING OF ANTISENSE INHIBITION SITES IN THE LEADER REGION OF BROME MOSAIC VIRUS RNA 3

# Janel Schulte and Christopher Kearney

Department of Biology, Baylor University P.O. Box 93788, Waco, Texas 76798

Abstract.—Antisense oligonucleotides (ASOs) are used as therapeutic agents in medicine and antisense genes are used in agriculture to modulate the gene expression in transgenic crops. One mechanism of antisense action is by interfering with ribosome function and blocking translation. In this study, the inhibition of translation caused by a series of ASOs was tested, using RNA 3 of the multipartite brome mosaic virus as a model system. RNA 3 hybridized to a series of ASOs was used as a template for *in vitro* translation using rabbit reticulocyte lysates. Using 25mers, it was found that ASOs complementary to the AUG site, the cap region, and the first 5' stem loop provided strong inhibition. In order to more exactly map these inhibitory sites, a series of 15-20mers were tested. These were less inhibitory than the 25mers, but the AUG site was again one of the most susceptible sites. Only certain ASOs targeted to the 5' stem loop were effective, one of which "clamped" two stem flanking regions together, perhaps stabilizing the stem loop. This study demonstrated the efficient inhibition of *in vitro* translation of this plant virus by relatively short antisense sequences, and suggested avenues of investigation for the control of plant viruses in transgenic plants.

For each gene in a DNA double helix, only one strand codes for useful information, while the other strand is the complement to the first strand. The sequence of the mRNA coded for by a gene is termed the "sense" version, while the complementary version is termed "antisense." It was realized fairly early that a short synthesized segment of antisense DNA could be used to disrupt RNA function. This was first demonstrated with retroviruses in cell culture (Zamecnik & Stephenson 1978). These antisense oligonucleotides (ASOs) and antisense genes (expressing longer antisense mRNAs) have since been tested as novel therapeutic tool against cancers, viral infections and genetic disorders in human cells (Agrawal 1996; Temsamani & Guinot 1997), as well as against plant mRNAs (Smith et al. 1988) and plant viruses (Nelson et al. 1993) for agricultural purposes.

Antisense has been shown to operate by either of two mechanisms to control mammalian or bacterial gene expression. First, antisense binding can sterically block the ribosome and/or translation factors from binding to the 5' end of mRNA and initiating translation. Some naturally occurring control systems are based on this mechanism, such as the control the expression of the transposase of IS10 in bacteria (Ma &

Simons 1990) or the N-myc gene in human cells (Krystal et al. 1990). Synthesized ASOs designed to bind to the initiation codon and block translation have been effective against coxsackievirus B3 (Yang et al. 1997) and the E2 gene of papilloma viruses (Cowsert et al. 1993). A second mechanism involves the destruction of double stranded RNAs in the cell by ribonuclease H (RNase H). The binding of mRNA by ASOs induces the cleavage of the mRNA at the binding site. A natural system exemplifying this is bacteriophage lambda and the *cII* gene (Krinke & Wulff 1987). This RNase mechanism has served as the focus of much work to design therapeutic ASOs (e.g., Duroux et al. 1995).

In plant systems, artificial control of gene expression has been attributed to a variety of mechanisms. Partial or full viral genes in sense orientation have been used quite successfully to control viral replication (Beachy et al. 1993), suggesting a protein-based control of the virus. However, many examples of antisense and other noncoding sequences have been shown to inhibit plant viruses and cellular mRNAs, prompting the proposal of a gene-silencing mechanism (Kooter et al. 1999).

In this study, brome mosaic virus (BMV) RNA was used as a model system to investigate the inhibition of plant viral RNA translation by antisense. An in vitro translation system was chosen, rabbit reticulocyte lysates, to test the inhibition of translation by ASOs in the absence of RNase. With the variety of antisense mechanisms proposed, little work has been conducted on the potential of antisense to inhibit plant viral translation directly. One previous study has demonstrated such inhibition in vitro (Crum et al. 1988), but a precise survey of the mRNA sites most susceptible to inhibition has not been reported, as it has for many cellular mRNAs. The genome of BMV is divided into three RNAs (RNAs 1, 2 and 3) plus a subgenomic RNA (RNA 4). RNA 3 serves as the mRNA for a movement protein which aids the cell-to-cell movement of the virus and is a determinant for host specificity (Mise et al. 1993). The present study sought to map the sites of inhibition of in vitro translation of the movement protein by hybridizing a series of test ASOs to the 5' leader sequence of BMV RNA 3.

# MATERIALS AND METHODS

The native secondary structure of BMV RNA 3 was estimated using the RNAfold program, version 1.2.1 (Vienna RNA package). Using this proposed structure, ASOs were designed by the authors and synthesized by Life Technologies, Inc. Per reaction, 2  $\mu$ L of diethylpyrocarbonate-treated water containing 250 ng of commercially prepared

BMV virion RNA (a mix of RNA 1,2, 3 and 4; Promega, Inc.) and 10 units of RNasin (Promega, Inc.) was incubated at 65°C for 5 min to denature the RNA, followed by incubation at 37°C for 5 min to recreate a native RNA conformation. Two  $\mu$ L of this RNA was aliquoted for incubation with each ASO (1  $\mu$ L), for a final ASO concentration of 3  $\mu$ M. These were incubated at 37°C for 30 min to allow hybridization of the ASO to BMV RNA 3.

In vitro translation was performed with rabbit reticulocyte lysates (Amersham Pharmacia Biotech, Inc.) using company protocols. Biotinlysine-tRNA was included in the reaction mix to label the protein products for subsequent detection. A 22  $\mu$ L aliquot of translation mix was added to each 3  $\mu$ L sample of hybridized RNA/ASO and incubated for 1-2 hrs at 30°C. The resulting protein products were separated on a 10% SDS-polyacrylamide gel. In vitro translation reactions were denatured at 100°C for 4 min in SDS buffer (2% SDS, 10% 2-mercaptoethanol, 100 mM Tris (pH 6.8), 20% glycerol (final concentrations)) before electrophoresis. The proteins were transferred to a nylon membrane (Immobilon-P; Millipore, Inc.) by electroblotting.

Detection of proteins was performed with ECL chemiluminescence reagents (Amersham Pharmacia Biotech, Inc.). Streptavidin-horseradish peroxidase (HRP) conjugate was first bound to the biotinylated proteins on the membrane. HRP-sponsored breakdown of luminol was detected with autoradiography film. Densitometry was performed on the resulting images using the NIH Image program. The percent inhibition by each ASO was determined by dividing the pixel density of each imaged ASO band by that of the "No ASO" control sample for each gel, multiplying by 100, and subtracting this value from 100.

# **RESULTS**

A test system was established by examining the inhibition of translation of BMV movement protein from RNA 3 by various ASOs in an *in vitro* translation assay. BMV RNA, containing all four RNAs naturally found in virions, was allowed to anneal with the ASO. An *in vitro* translation mix, consisting of a lysate of rabbit reticulocytes, was then added to the annealed RNA/ASO. The ability of the ASO to inhibit translation *in vitro* was measured as the amount of movement protein produced by the RNA with or without the ASO present, as measured by the amount of luminescence detected on autoradiography film. An example experimental run is shown in Fig. 1. A native translatable mRNA remaining in the rabbit reticulocyte lysate preparation routinely

No

5-SL

5-SL

AUG

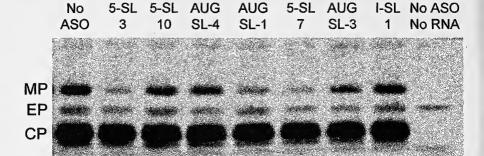


Figure 1. Example data of luminescence autoradiography. ASOs were hybridized to BMV RNA and in vitro translated in rabbit reticulocyte lysates containing biotin-labeled lysine-tRNA. Following electrophoresis and blotting, the protein products were visualized by luminescence after treatment with streptavidin-horseradish peroxidase and luminol. An example autoradiograph is presented here. Lane 1: Positive control translation with no ASO. Lanes 2-8: Test translations with indicated ASOs. Lane 9: Negative control translation with no BMV RNA template or ASO. MP, viral movement protein (expression targeted by antisense); CP, viral coat protein; EP, protein endogenous to in vitro translation system.

produced an endogeneous protein in the absence of BMV RNA, as seen in Lane 9. When BMV RNA was added, the coat protein product from RNA 4 always produced a large lower band which was not noticeably affected by any of the ASOs against RNA 3 (Lanes 1-8). The movement protein product from RNA 3 was seen as a dark band just above the lysate's endogenous protein band. The uniformity of the endogenous protein and coat protein bands served as indicators that the translation efficiency and BMV RNA integrity were uniform from sample to Densitometry analysis was performed on each movement protein band in the autoradiograph. Percent inhibition by antisense was determined by comparing the pixel density of the imaged band of each test sample to that of the "No ASO" band (Lane 1) for each experimental run.

Initially, 5-SL-3, which is a 25mer ASO complementary to the 5'terminus (Fig. 2), was chosen to test the system. It was found that this ASO greatly inhibited movement protein production (83.6% reduction from "no ASO" control) but did not noticeably affect the production of coat protein. The replicase proteins produced by RNAs 1 and 2 were present in too low a quantity to accurately quantify. The inhibition by 5-SL-3 (and ASOs used in another BMV study by the authors) was quite consistent in several replications, prompting the use of single or duplicate runs for tests of the other ASOs in this study. Values for duplicate runs are reported as averages.

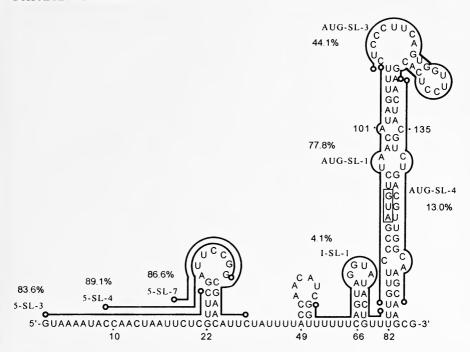


Figure 2. Sites of complementarity to 25mer ASOs on the 5' leader sequence of BMV RNA 3. The numbers beside the dotted nucleotides represent the distance from the 5' terminus in nucleotides (nts.). The AUG translation initiation codon is boxed at nt. 91. Each ASO is represented as a heavy line with a circle at each end. The name of the ASO and its percent inhibition relative to the "no ASO" control translation is listed by each ASO. Complete inhibition (no translation products) would yield a percentage inhibition value of 100%

A series of 25mer ASOs was then designed and tested. In Fig. 2 it can been seen that three sites on the 5' end of BMV RNA 3 appeared to be most susceptible to antisense inhibition of *in vitro* translation. ASOs 5-SL-3, 4 and 7 were very effective (83.6%, 89.1% and 86.6% reduction) and targeted either the 5' terminus or the 5'-most stem loop. The third effective site was the AUG, which is bound by AUG-SL-1 (77.8% reduction). Interestingly, AUG-SL-4 did not strongly inhibit translation (13.0% reduction), even though it would be expected to disrupt the AUG stem-loop as well as the effective AUG-SL-1 does.

To define these inhibitory sites more precisely, a series of 15-20mer ASOs was designed and tested (Fig. 3). In general, these shorter ASOs were less effective than the 25mer ASOs. However, the AUG site still proved to be sensitive to inhibition, in this case by AUG-SL-2 (54.0% reduction), a 15mer version of AUG-SL-1. Each portion of the 5'-most stem loop was examined by a series of 15-16mer ASOs, 5-SL- 5, 6, 8

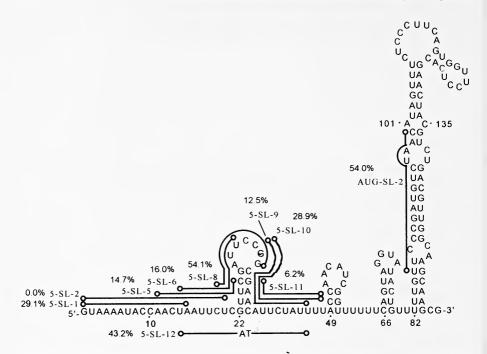


Figure 3. Sites of complementarity to 15-20mer ASOs on the 5' leader sequence of BMV RNA 3. Labeling as in Fig. 2.

and 9. 5-SL-8 provided greater inhibition (54.1%) than the other ASOs in this series (12.5 - 16.0%). This ASO covered the stem loop most completely, while the other ASOs covered a portion of the stem loop and a portion of the flanking sequences. When the less inhibitory 5-SL-9 (12.5%) was lengthened to produce 5-SL-10, more inhibition was seen (28.9%). The ASO, 5-SL-12, was designed to "clamp" the 5'-most stem loop together by binding the flanking regions, with a 2-base bridge between the sections complementary to the flanking region. With 17 bases of complementary, this ASO was one of the strongest inhibitors (43.2%) among the shorter ASOs. The 25-mer, 5-SL-3, which bound the 5'-terminus, was highly inhibitory (Fig. 1), so shortened versions binding to the 5' terminus were tested. The 20mer, 5-SL-2, had no inhibitory effect (0.0%), but the 15mer, 5-SL-1 did inhibit translation (29.1%).

#### **DISCUSSION**

In this study, 25mer ASOs targeted to both the 5' terminal region and the AUG translation initiation codon region were highly inhibitory to in

vitro translation. A 25mer ASO targeted upstream of the AUG loop (I-SL-1) and one complementary to the strand opposite the AUG on the AUG stem loop (AUG-SL-4) were not effective. Both of the cap and AUG regions have been successfully targeted in previous studies on cellular mRNAs. A 15mer ASO targeted to the first 5' nucleotides (nts.) of the mRNA was found to inhibit in vitro translation in rabbit reticulocyte lysates (RRL), but a 12mer ASO centered on the AUG site was not effective (Bertrand et al. 1989). The same results were obtained with a 17mer ASO for the cap site and a 16mer for the AUG site for β-globin mRNA with RRL (Boiziau et al. 1991). However, another study of  $\beta$ -globin mRNA with RRL found that the AUG site was optimal for *in vitro* translational inhibition with ASOs (Jaroszewski et al. 1993). An ASO centered on the AUG site was also the most effective ASO at inhibiting translation of ICAM-1 protein in cell culture (Chiang et al. 1991). In examining the 5'-most gene (the replicase gene) in tobacco mosaic virus (TMV), two 51mer ASOs were found to inhibit in vitro translation in RRL (Crum et al. 1988). These ASOs bound TMV nts. 7-58 or nts. 64-115 (the replicase AUG is at nt. 69). However, when these sequences were included as transgenes in Nicotiana tabacum (L.) cv. Xanthi nn, only the nt. 7-58 sequence, which did not include the AUG site, was effective at conferring resistance to TMV in the transgenic tobacco.

In an effort to more precisely map the sites most sensitive to ASO inhibition, a series of 15-20mer ASO was tested in this study. As expected, AUG-SL-2, which is complementary to the AUG region, was one of the two most inhibitory ASOs. However, the other most inhibitory ASO (5-SL-8) was not complementary to the cap but rather to the 5' stem loop. Among the next most effective short ASOs, one also bound the 5' stem loop (5-SL-10) and the other bound the cap region (5-SL-1). Thus, this 5' stem loop may play an important role in regulating translation. It should be asked whether this stem loop's structure enhances or inhibits translation; i.e., whether these inhibitory ASOs are acting by stabilizing or destabilizing the stem loop. ASO 5-SL-12 would be expected to stabilize this stem loop, since it has complementary sequence on either side of this structure and should prevent its dissociation. This ASO was inhibited well considering its short length (17 bases of complementarity), suggesting that the 5' stem loop needs to be melted to allow translation to begin.

The relationship between 5' secondary structure and translation inhibition has support from other studies. The 5' leader of TMV was pro-

posed to stimulate translation due to its lack of secondary structure (Sleat et al. 1988). As well, deletions were made in the 5' leader of alfalfa mosaic virus RNA 3 to make a series of mutants; mutants lacking the 5' stem loop of this RNA had greater *in vitro* translation rates than wild type sequence. This indicated that the secondary structure of this region serves as a negative regulator of translation rather than a positive one. Thus, in seeking to inhibit translation of viral RNAs in plants via viral transgenes, it may be optimal to seek to stabilize 5' secondary structures to artificially enhance their natural inhibitory function.

The sequence occurring at the 5' end of all BMV RNAs is also important for viral replication initiation (Pogue & Hall 1992a). stem-loop at the 5' end of (+) RNA has its complement in the 3' end of the (-) RNA, and it is this (-) stem-loop which affects replication. A portion of this stem-loop (nts. 21-31 in RNA 3) appears to be homologous to the ICR2 sequence of tRNA, which acts as a promoter region occurring within the sequence of the transcript and serves the same function in the virus (Pogue et al. 1992b). The stem-loop predicted in these previous studies includes nts. 1-51. The stem-loop of Figs. 2 and 3 of the present study would represent only the distal half of this longer stem-loop (nts. 22-38). Mutations were made in the proximal half of the stem-loop which would be expected to destabilize the (-) stem-loop; these led to a drastic reduction in replication but not in translation (Pogue et al. 1992b). It may be that the distal half of the (+) stem-loop is the critical element for BMV translation, along with the initiator AUG site. This is supported by a study of rubella virus (Togaviridae), an RNA virus somewhat related to BMV. It was found that a 5' (+) stemloop (nts. 15-65) was important for translation, but not for replication and that the complementary 3' (-) stem-loop was critical for replication (Pugachev & Frey 1998). Thus, it could be postulated that the short 5' (+) stem loop of Figs. 2 and 3 is an important element for translation in BMV, and perhaps in related RNA viruses, while the 3' (-) stem-loop is critical for replication. This sequence would serve two purposes and, if derived originally from tRNA, must have been modified in the (+) form to attract translation factors, since tRNAs are not translated. Following initiation of translation, it may be critical that this stem-loop be melted, since ASO 5-SL-12, which is expected to stabilize this stemloop, was effective at inhibiting translation.

This study has demonstrated that ASOs targeted to the 5' terminal or AUG portions of BMV RNA 3 effectively inhibit translation *in vitro*. Other studies using *in vitro* translation with RRL have demonstrated that

ASOs need not act through an RNase effect, but may inhibit translation directly (Boiziau et al. 1991; Chiang et al. 1991). Targeting secondary structures is a common strategy in designing ASOs to enhance RNase degradation (e.g., Lima et al. 1997). Such an approach may also have potential for inhibiting translation *in vitro* and *in vivo*.

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CK at: Chris Kearney@baylor.edu

# THE MUTAGENIC EFFECTS OF ANTHRACYCLINES IN THE BACTERIUM SALMONELLA TYPHIMURIUM: INDUCTION OF TRANSITION MUTATIONS WITH DAUNOMYCIN

# William J. Mackay, Lisa A. Phelps, Anthony A. Cauchi\* and Lance T. Weaver\*

Department of Biology & Health Services Edinboro University of Pennsylvania Edinboro, Pennsylvania 16444 \*Department of Biology, Angelo State University San Angelo, Texas 76909

Abstract.—This study examined the mutagenic effects of the anthracycline daunomycin in a new set of *Salmonella* strains. Results show that daunomycin can induce base-substitution transition mutations in *Salmonella typhimurium*. Specifically, frameshift mutations (TA98) were induced 44.6-fold, AT to GC transition mutations (TA7001) were induced 7.3-fold and GC to AT transition events (TA7004) were induced 7.6-fold. Thus, it appears that daunomycin can induce a wide array of mutational events which include frameshifts and base-substitution mutations.

Anthracycline antibiotics (primarily daunomycin and adriamycin) are widely used and effective antitumor agents which exhibit antineoplastic activity in experimental tumors as well as in human malignant diseases (reviewed by Hortobagyi 1997). These compounds arrest tumor cell growth by interacting with cellular DNA. However, tumor resistance and the adverse effects of these compounds (acute myelosuppression and cardiotoxicity) hinder the clinical use of anthracyclines. In spite of extensive investigation, the mechanism for selective cell cytotoxicity of these drugs is not precisely understood. In particular, daunomycin has been shown to be a strong intercalator in DNA with the A-ring and amino sugar moieties having important interactions with the minor groove (Skladanowski & Konopa 1994). In addition, other mechanisms for daunomycin cytotoxicity have been proposed: e.g., superoxide formation, crosslinking of DNA strands, DNA alkylation and lipid peroxidation (Taatjes et al. 1997; Fenick et al. 1997). Daunomycin also produces protein-associated DNA cleavage in mammalian cells and inhibits the catalytic action of topoisomerase II, a target for several classes of unrelated anticancer drugs (Pommier 1995). Furthermore, daunomycin can also induce frameshift mutations in prokaryotic (McCann 1976; Pak et al. 1979; Anderson et al. 1980) and eukaryotic cells (Hannan & Nasim 1978).

It is important to fully characterize the mutational spectrum and

Table 1. Ames Salma	onella strains.
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Strain	Genotype	Mutation Detected
TA7001	hisG1775 Δara9 Δchl1004 (bio chlD uvrB chlA) galE503 rfa1041/pKM101	A:T to G:C
TA7004	hisG9133 Δara9 Δchl1004 (bio chlD uvrB chlA) galE503 rfa1044/pKM101	G:C to A:T
TA98	his $D3052~\Delta ara9~\Delta chl1008~(bio~chlD~uvrB~gal)$ rfa $1004$ /pKM101~(-1~C)	frameshift

mutagenic specificity of daunomycin as a first step to better understand the antitumor and cardiotoxicity effects of this compound. The use of bacterial mutation assays in genetic toxicology is now firmly established both for fundamental studies in mutagenesis and carcinogenesis, and for screening chemicals and environmental samples for genotoxic properties. The most general used and validated bacterial reverse-mutation assay is the Salmonella Mutagenicity Assay (Maron & Ames 1983). original Ames tester strains identified mutagens which reverted point mutations in the his operon of Salmonella typhimurium. Although the Salmonella Mutagenicity Assay has been widely used to screen chemicals for potential genotoxicity, it was not designed to yield information about the precise nature of the his+ revertants that were obtained. Recently, a new set of Salmonella strains was generated to identify specific base-substitution events (Gee et al. 1994; 1998). Since each strain can only revert by a single mutational event, it is not necessary to further classify or sequence the resulting revertants in order to know the specific mutation that has occurred.

Previous studies have shown that daunomycin is mutagenic in Salmonella typhimurium (i.e., this compound elicited a positive response with the Ames tester strain TA98) (Maron & Ames 1983; Thomas 1987). TA98 is a tester strain that detects compounds, which induce frameshift mutations. The present study was initiated to examine if daunomycin could induce mutations in addition to frameshift events. This study demonstrates that daunomycin can induce AT to GC and GC to AT transition mutations in Salmonella typhimurium.

# MATERIALS AND METHODS

Bacterial strains.—The strains and their genotypes used in this study are listed in Table 1. TA98 detects frameshift mutations (Maron &

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Ames 1983). TA7001 and TA7004 are base-substitution specific strains, which carry a target missense mutation in the *hisG* gene. The latter two strains revert to a prototrophic *his*<sup>+</sup> phenotype via a specific base-substitution event (TA7001, AT to GC, and TA7004, GC to AT) (Gee et al. 1994; Gee et al. 1998).

Chemicals.—Daunomycin (daunorubicin hydrochloride) and ampicillin were obtained from Sigma Chemical Co. (St. Louis).

Mutation assays.—The his reversion assays (triplicate assays were conducted for each concentration of daunomycin) followed a modified version of the traditional Ames "plate-incorporation" test (Maron & Ames 1983) and utilized a pre-incubation step in order to increase the sensitivity of the strains to daunomycin. Briefly, a Salmonella culture for each strain (TA98, TA7001 and TA7004) was grown overnight to stationary phase in Growth Medium (Oxoid Broth, UnipathOxoid, Basingstoke, UK) in the presence of 25  $\mu$ g/mL ampicillin in an environmental shaker (250 rpm at 37°C- New Brunswick Scientific, Edison, NJ). 110  $\mu$ L (approximately 5 X 10<sup>7</sup> cells) of the overnight culture was exposed to varying amounts of daunomycin (60 µg/mL to 960 µg/mL) for 30 minutes in a shaking incubator (250 rpm) at 37°C. 100 µL was plated onto minimal agar plates that contained 2% glucose, 0.05 mM L-histidine, and 0.005 mM biotin. These selective plates were incubated at 37°C, and the number of his+ revertants were scored after 48 hr. For each strain, a zero control was included in order to estimate the number of spontaneous his<sup>+</sup> revertants in each experiment. The total number of viable cells in each experiment was determined by plating serial dilutions onto nonselective plates (LB plates). Mutation frequency is expressed as the average number of his<sup>+</sup> revertants on selective plates divided by the total number of viable cells (determined by the number of colonies on the non-selective plates).

# RESULTS AND DISCUSSION

This report is a first step in a long-term study toward understanding the role of sequence context in both quantitative (i.e., hotspots) and qualitative (i.e., adduct conformation) aspects of mutations induced by anthracyclines. Previous studies indicated that daunomycin induced frameshift mutations in the bacterium *Salmonella typhimurium* (cf. McCann 1976; Maron & Ames 1983). This study investigated the possibility that daunomycin can also induce base-substitution events.

Strain	Spontaneous Mutation Frequency (S)	Induced Mutation Frequency (I) (120 μg/mL daunomycin)	Induction Fold (I/S)
TA7001	3.0 (± 1.2) X 10 <sup>-8</sup>	2.2 (± 0.3) X 10 <sup>-7</sup>	7.3
TA7004	1.1 ( $\pm$ 0.1) X 10 <sup>-7</sup>	8.4 ( $\pm$ 1.0) X 10 <sup>-7</sup>	7.6
TA98	$1.0~(\pm~0.3)~{\rm X}~10^{-7}$	$4.5~(\pm~0.4)~{\rm X}~10^{-6}$	44.6

Table 2. Mutation frequencies of Ames Salmonella stains.

Recently, a set of *Salmonella* strains was generated to identify specific base-substitution events (Gee et al. 1994; Gee et al. 1998; Table 1 this study). Specifically, TA7001, AT to GC, and TA70004, GC to AT, detect base-substitution transition events. In this report, both strains were exposed to varying amounts of daunomycin and monitored the number of  $his^+$  revertants on selective minimal glucose plates (see Materials and Methods for details of this assay).

Prior to this study, it was not known if daunomycin could induce base-substitution mutations. Thus, one could not rule out the possibility that these events might occur in the cytotoxic range in Salmonella. This study expanded the mutational analysis of daunomycin using the Salmonella Mutagenicity Assay by varying the concentration of this antineoplastic compound into the cytotoxic range, and performed serial dilutions for all concentrations of daunomycin tested (60µg/mL to 960µg/mL). These diluents were then plated onto nonselective plates (LB plates). It was possible to calculate the total number of viable cells during each experiment. Thus, it was possible to calculate a mutation frequency for each daunomycin concentration tested for each strain (in triplicate). Mutation frequency is expressed as the average number of his<sup>+</sup> revertants on selective plates divided by the total number of viable cells, which were determined by counting the number of colonies on the non-selective plates. In summary, this study was able to investigate daunomycin mutagenicity in the cytotoxic range in addition to the subtoxic, or traditional mutagenic range.

The results of this study are summarized in Table 2. The highest observed induced mutation frequency for all three *Salmonella* strains occurred at a daunomycin concentration of 120  $\mu$ g/mL (maximally induced mutation frequencies are listed in Table 2, Column 3). A 90% killing of *Salmonella* cells was observed at the highest tested daunomycin concentration of 960 $\mu$ g/mL. TA98 was highly mutagenic in this

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assay. This strain exhibited a 44.6-fold increase in mutation frequency in the presence of daunomycin, and these results confirmed earlier reports which demonstrated that daunomycin induced frameshift mutations in the traditional *Salmonella* Mutagenicity Assay (i.e., TA98 tested "positive" with daunomycin) (McCann 1976; Maron & Ames 1983). Interestingly, both base-substitution Ames strains (TA7001, AT to GC and TA7004, GC to AT) were clearly mutagenic in the presence of daunomycin ( $\chi^2$ : P < 0.005). Specifically, AT to GC transition mutations (TA7001) were induced 7.3-fold and GC to AT transition events (TA7004) were induced 7.6-fold.

Several biochemical analyses suggest that the interaction(s) between daunomycin and DNA are complex in nature. The primary mode of action (antitumor effect) of daunomycin appears to be the intercalation of the aglycone portion of the compound between adjacent DNA base pairs, and this activity results in topoisomerase-induced DNA strand breaks (Liu 1989; Pommier 1995). Free radical formation by daunomycin is another important mechanism of action (Taatjes et al. 1997; Fenick et al. 1997), and is more closely associated with cardiotoxic effects than antitumor effects. Myocardial tissue is relatively deficient in antioxidant defense mechanisms and is therefore susceptible to free radical damage (reviewed by Gewirtz 1999).

The mutational spectra of two carcinogens, namely activated benzo-[a]pyrene and activated dibenz[a,j]anthracene, can be quite complex and depend on both sequence context and adduct conformation (Rodriguez and Loechler 1993; Gill et al. 1993). In fact, it has been reported that the major adduct of (+)-anti-B[a]PDE can induce different base-substitution mutations (i.e., GC to AT, GC to TA, etc.), and it has been hypothesized that the induced mutational event is dependent on the conformation of the adduct when bypassed during DNA replication (Shukla et al. 1997; Kozack & Loechler 1999; Kozack et al. 1999). Although the molecular basis of the interaction(s) between daunomycin and the DNA helix are not yet fully understood, the mutational analyses reported here also suggest that this interaction(s) might indeed be very complex, and the role of sequence context in daunomycin-induced mutagenesis is under current investigation.

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WJM at: bamackay@erie.net

# A FOSSIL MARINE TURTLE FROM EAST CENTRAL LOUISIANA

# David C. Parris, James L. Dobie and A. Bradley McPherson

Bureau of Natural History, New Jersey State Museum PO Box 530, Trenton, New Jersey 08625-0530 211 South Spanish Oak Trail Kerrville, Texas 78028-2026 and Department of Biology, Louisiana Centenary College Shreveport, Louisiana 71104

Abstract.—A turtle carapace fragment from the well known Tunica Hills Locality, West Feliciana Parish, Louisana is referable to the marine genus *Caretta*. Attributed to the Citronelle Formation, its best comparisons are to a member of the genus from the Yorktown Formation at Lee Creek Mines, North Carolina (Latest Hemphillian). It indicates close proximity of the Pliocene coastline to the locality, now 150 km inland.

The numerous fossils from the Tunica Hills of West Feliciana Parish, Louisiana have long attracted the attention of amateur and professional paleontologists (Manning & MacFadden 1989). Despite problems of age interpretation, the thousands of individual specimens are a significant record of Cenozoic life in the Gulf Coastal region. The specimen reported here, a fossil of a marine turtle, is unprecedented, however, and adds a new dimension to interpretations, with possibilities of marine paleoenvironment and/or Tertiary epochs represented. Despite some uncertainties of provenience and interpretation, the significance of these aspects calls for placing the specimen on record.

SYSTEMATIC PALEONTOLOGY

ORDER TESTUDINES Linnaeus FAMILY CHELONIIDAE Gray GENUS *CARETTA* Rafinesque *Caretta* sp.

(Figure 1)

Referred specimen.—CCVC 1553, Centenary College Vertebrate Collection, now in Vertebrate Paleontology Collection at Louisiana State University.

*Provenience*.—surface collected along Tunica Bayou; believed to have been derived from Citronelle Formation.

Inferred age.—Early Pliocene, equivalent to Latest Hemphillian.

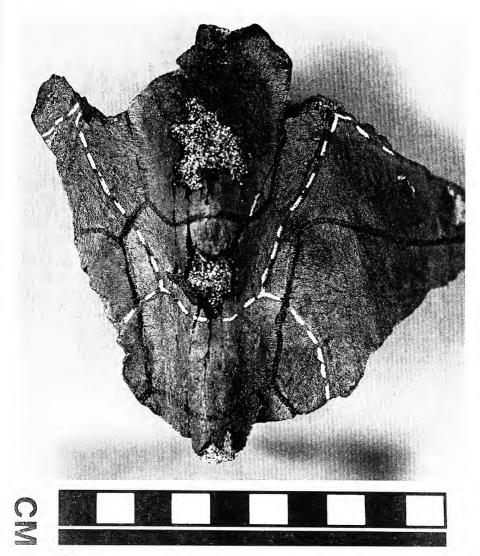


Figure 1: Dorsal view of CCVC 1553, Caretta sp., from Tunica Hills Locality, West Feliciana Parish, Louisiana.

Description.—Specimen consists of two sequential neurals and portions of adjacent costals, presumably the third and fourth (Figure 1). Profile of curvature low, with projecting prominences along midline, giving appearance of median ridge or keel. The neurals are generally elongate "coffin" shaped (six-sided) in dorsal view, but the posterior margins have convex (caudad) curvature, as do the posterior margins of the intervening sulci. Based on comparisons to more complete carapaces, the entire length of the carapace was originally 700-800 mm.

Especially interesting is the passage of one sulcus over the anterior portion of what is believed to be the fourth neural, although such positions of sulci may be subject to considerable individual variation. There is vermiculate sculpture but it is inconspicuous, visible only under magnification.

Identification is based on comparisons to all available genera of marine turtles (and large freshwater genera) of Cretaceous to Recent age, pleurodire and cryptodire. The general shape and proportions of the neurals, however, matches only the turtles of the Family Cheloniidae, which includes all living sea turtles (except the highly specialized *Dermochelys coriacea*). Detailed comparisons were thus made to the four genera of living cheloniids.

The estimated carapace size of the Louisiana specimen would be unusually great for *Eretmochelys* or *Lepidochelys*, reasonable for *Chelonia*, and probably of subadult dimensions for *Caretta*, based on comparisons to the extant species. The overall neural shape is similar to all recent taxa of Cheloniidae except *Lepidochelys*. However the posterior neural curvature (convex; caudad) is found only in *Chelonia* and *Caretta* among specimens compared during this study. Knob-like projections on the neural series are distinctive to *Lepidochelys* and some young specimens of *Caretta*. The sulcus positions seen in the Lousiana specimen are found only in some subadults of *Caretta*.

To summarize, the genus *Caretta* has the greatest resemblance to CCVC1553, more than to any other cheloniid. The shape of the neurals, including the posterior curvature, is very similar. Subadults of the living species are similar in size and at least some of them have sulcus positions like those of this specimen (Zangerl 1958). Young specimens may have knobbed and/or keeled midlines, and one Pliocene species is keeled throughout life. This latter species, to be named and described by G. Zug (pers. comm.), is notably found at the Lee Creek Mines of North Carolina in the Yorktown Formation (Latest Hemphillian).

Other Tertiary records of *Caretta* lack specimens with knobbed or keeled midlines (Portis 1890; Rothausen 1986). Dodd & Morgan (1992) discussed specimens from the Bone Valley Formation of Florida which may be conspecific with the North Carolina and Louisiana specimens, but reported no carapace material directly comparable.

# LOCALITY AND STRATIGRAPHY

In Cenozoic coastal plain deposits, derivation, reworking and redeposition are possibilities. As with many such coastal plain fossils, notably those found in streams rather than *in situ*, there may be doubts concerning the specimen's provenance. Fortunately, the matrix adhering to the specimen, the identity of the species, and the age determinations for other fauna from the locality are quite consistent. Although the Tunica Hills localities are well known, and have been described often, the uniqueness of the specimen justifies review in order to allay suspicions regarding its origins.

The matrix adhering to the specimen is a white quartz sand with calcareous cementation, which has weathered to a rust red color in more exposed portions. Although only moderately indurated it appears to have completely encased the specimen quite well, assuring its durability despite reworking. This matrix is consistent with lithologies given for the Citronelle Formation, beginning with its original description by Matson (1916), although more calcareous than is usually the case. It is speculated that the fossil was encased in a concretionary mass of sand in the Citronelle Formation cemented by carbonate, which assured its durability despite reworking.

# DISCUSSION

The morphology of the specimen suggests age correlation to the Yorktown Formation at Lee Creek Mine, latest Hemphillian of North Carolina. This is totally consistent with other Pliocene fossils from Tunica Bayou, the very age-diagnostic horses. Manning & MacFadden (1989), noted that all Pliocene vertebrates from the parish were from a twenty meter distance along Tunica Bayou. The turtle described here was within thirty meters distance from the Pliocene horse specimens.

The fauna of the Tunica Hills localities is otherwise essentially devoid of marine-adapted taxa of any kind, and supposedly represents terrestrial environments, of the late Cenozoic. This one specimen, however, indicates that sometime during the latest Hemphillian a coastal plain environment with closer marine proximity may have been the case.

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DCP at: dparris@museum.sos.state.nj.us

# INFLUENCE OF WATER DYNAMICS AND LAND USE ON THE AVIFAUNA OF BASIN WETLANDS NEAR RIVIERA IN SOUTH TEXAS

#### Milton W. Weller and Doris L. Weller

Department of Wildlife & Fisheries Sciences
Texas A&M University, College Station, Texas 77843
Present Address:
P. O. Box 280, Sanibel, Florida 33957-0280

Abstract.-Monthly surveys of 16 basin wetlands east of Riviera in south Texas were used to assess the importance of these wetlands to waterbirds throughout the year and to develop a conservation plan. Fifty-two bird censuses were made from October 1994 to December 1998. Mean water indices for the 16 wetlands varied dramatically and sizes of individual water areas ranged from dry to four times the mean. Of the 103 bird taxa observed, only 44 had frequencies suitable for analysis. Six waterbird groups dominated the avifauna: 15 species (34%) of waterfowl (Anatidae) of diverse feeding techniques and foods; five diving piscivores (11%) of water columns; one diving herbivore (0.5%); seven surfacefeeders (16%) that selected invertebrates, fish, or their remains by swimming or aerial diving; eight wading piscivores (18%); and nine taxa (20.5%) of shorebirds that used mudflats or sheetwater. Species richness and abundance were positively correlated with water area. Nesting was uncommon even among resident species, seemingly due to reduced shoreline vegetation essential for nest concealment and rearing. Recommended conservation measures include: reduced modification of individual wetlands, a regional effort to maintain diversity of basin sizes and water regimes useable by mobile birds, upland management aimed at reducing soil erosion into wetlands, and fenced watering areas to reduce turbidity.

Although south Texas is characterized by flat terrain and low rainfall, there are numerous depression or basin wetlands between Kingsville and Raymondville, and between the Laguna Madre and Highway 281 (Brown et al. 1977; McAdams 1987). These South Texas Plains are formed of Pleistocene river silts and loess deposits with wind-blown sand areas nearer the coast. Most basin wetlands result from wind action ("deflation swales" of Brown et al. 1977), and most undergo dramatic variation in duration of water (hydroperiod), flooding season and water depth. Wetland density varies greatly within the region from year-to-year and averaged 2.2 wetlands/km<sup>2</sup> (0.39 mi<sup>2</sup>) during the posthurricane period of the early 1980s (McAdams 1987). Because mean annual rainfall at Kingsville (just north of the study area) is only 70.1 cm (27.6 inches) and mean evaporation is at least twice that (Bomar 1995:230), many shallow wetlands filled by heavy rains dry quickly. However, tropical storms may bring torrential rains, with records of 42.9 cm (16.9 in.) in 24 h in 1980 and a mean of 134.1 cm (52.7 in.) in 1958. Resultant water in larger and deeper basins may last through

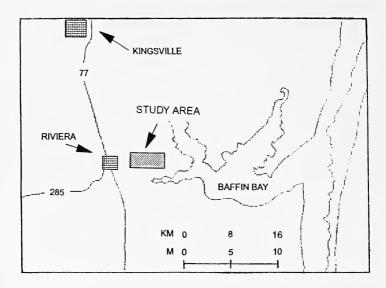


Figure 1. Location of the study area east of Riviera, Texas (Lat. N27 $^{\circ}$  29 $^{\circ}$ , Long. W97 $^{\circ}$  81 $^{\circ}$ ).

several dry years; hence, they are sometimes called "hurricane lakes." Localized but heavy "seabreeze" showers that parallel the coast (Bomar 1995:189) form an additional rainfall influence on water levels, and conditions at individual wetland sites may not reflect regional rainfall data.

Uplands surrounding the study-area wetlands were heavily grazed or intensively farmed, with some dispersed housing development. Nevertheless, these wetlands are widely known for attracting waterbirds throughout the year. Understanding when and how they are used by birds in light of human activity is essential to the development of a preservation strategy. Objectives of the study were to: (1) determine seasonal bird-species composition and abundance in a representative group of basin wetlands; (2) relate waterbird species diversity and abundance to size and wetland features; (3) and develop recommendations for preserving maximal bird habitat with minimal impact on land use.

#### STUDY AREA

Originally, most of the South Texas Plains were brushland dominated in drier upland by mesquite (*Prosopsis glandulosa*) sites and retama

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(Parkinsonia aculeata) in lowlands (Correll & Johnson 1970), but coastal areas once had extensive grassland patches (Jones 1982) separated as part of the Gulf Prairies and Marshes (Gould 1975). The study area represented the more open areas, now farmed or grazed, but with some residual brush patches. It lies between two arms of Baffin Bay in the Riviera loess sheet (Brown et al. 1977) east of Riviera, in Kleberg County, Texas (Fig.1) (Lat. N27° 29', Long. W97° 81'). Wetlands were selected within view of the road because of problems of access on private property. Vehicular travel along county roads was light and was a disturbance to birds only when wetland basins flooded into the road After several exploratory surveys, 16 wetlands were selected along a route with good road access and wetland visibility. Initially, all had some water in a basin with identifiable hydrophytes. These basins seemed typical of other wetlands in the area, ranging from ca 1 to 34 ha when not flooded beyond their normal basins. The longest inter-wetland distance was about 6.7 km, and nearest-neighbor distance averaged 420 m, thus allowing easy inter-wetland flights by birds. Distance of the wetlands from one arm of the hypersaline Baffin Bay was about 2.5 km, a modest flight for coastal species in search of food or fresh water.

Variation in size of individual wetlands was influenced by basin location and watershed size. Several wetlands flooded quickly in response to rainfall and dried almost as rapidly, whereas others were relatively more stable and may have reflected a near-surface aquifer during drier periods (Baker 1971). These dramatic variations in water presence, size, and depth changed them from habitats for water-dependent bird communities to habitats used mainly by meadow birds and livestock. All wetlands were totally dry during several low-rainfall periods except for three with areas deepened for livestock use in dry periods. Although these "dugouts" attracted a few waders in search of fish during drying periods, their influence on bird populations was negligible.

Only two wetlands were neither grazed nor tilled, which was reflected in the presence of persistent herbaceous vegetation like tule (*Scirpus californicus*) and cattail (*Typha domingensis*), or areas of less persistent but important annual plants such as smartweeds (*Polygonum* sp.) and sedges (*Carex* sp. and *Scirpus* sp.), grading into perennial sesbania (*S. drummondi*) shrubs. One "shrub-scrub" wetland (No.11 in Table 1) was covered with retama 3-6 m (9.8-19.5 ft), and others had only marginal willows (*Salix* sp.) or snags. Several wetlands had yellow lotus

Table 1. Mean and range in size of wetlands (ha), mean bird-species richness and abundance per survey, and cumulative bird-species richness and number observed at each of 16 wetlands (n = 52).

Wetland	Si	ze	Bird F	Richness	Bird N	lumber	Cumulative	Cumulative
No.	Mean	Range	Mean	Range	Mean	Range	Richness	Number
1	5.4	0-15.2	8.8	0-18	77.9	0-299	57	3977
2	1.1	0-3.5	3.8	0-19	32.3	0-528	45	1646
3	1.4	0.3-4.6	2.7	0-11	10.9	0-57	34	570
4	0.9	0-4.1	1.2	0-6	12.3	0-250	19	641
5	2.1	0-6.1	3.7	0-14	20.1	0-141	38	1046
6	31.9	0-88.6	8.6	0-25	131.4	0-529	62	5519
7	6.0	0-17.7	7.4	0-22	99.1	0-516	61	5055
8	0.7	0-3.5	0.7	0-8	4.9	0-80	16	254
9	1.9	0-7.1	2.6	0-19	19.7	0-359	36	1026
10	0.9	0-3.0	0.6	0-4	1.2	0-13	16	60
11	1.3	0-6.1	0.1	0-2	0.9	0-25	2	47
12	11.4	0-45.6	6.1	0-3	107.8	0-708	60	5606
13	6.5	0-23.0	4.5	0-1	83.3	0-837	48	4332
14	1.7	0-5.1	2.2	0-9	17.0	0-167	32	885
15	7.4	1-18.9	8.1	0-2	65.7	0-272	59	3418
16	4.3	0-11.1	3.2	0-9	19.4	0-91	39	1010

(Nelumbo lutea) beds that persisted despite deep flooding or extreme drought, and many had the small blue water lily (Nymphaea elegans) that bloomed even in heavily grazed meadows after flooding. Other wetlands commonly lacked central vegetation except for periods following germination of annuals on mudflats. Twelve wetlands were grazed at some time during the study, and sedges and smaller bulrushes at their edges were impacted quickly by cattle or horses. Margins of five wetlands were plowed periodically, one directly through the wetland when drying was timed with field activities.

## **METHODS**

Fifty-two bird censuses were made at about monthly intervals from October 1994 to December 1998. Observation time at each wetland varied with water and vegetation conditions as well as the number and diversity of birds, but typically varied from 5 to 30 min by two observers using one to several vantage points. Numbers of each species were counted when possible or estimated when flocks were large or active, but accuracy was no doubt influenced by water levels in relation to the vegetation. However, total numbers of birds changed so dramatically

that errors in estimates of individual species were not a serious problem in comparing use among wetlands, nor in assessing seasonal or annual changes in use.

Despite the visibility from observation sites, most calidrid sandpipers could only occasionally be identified to species, and were analyzed as "peeps." No attempt was made routinely to separate Greater Yellowlegs (Tringa melanoleuca) from Lesser Yellowlegs (Tringa flavipes), Short-billed Dowitchers (Limnodromus griseus) from Long-billed Dowitchers (Limnodromus scolopaceus), or Double-crested Cormorants (Phalacrocorax auritus) from Neotropic Cormorants (Phalacrocorax brasilianus). Species common to wet-meadow edges like cattle egret (Bubulcus ibis), Long-billed Curlew (Numenius americanus), or Black-bellied Plover (Pluvialis squatarola) were tallied only when within the wetland basin. Ubiquitous species like eastern meadowlark (Sturnella magna), Mourning Dove (Zenaida macroura), Great-tailed Grackle (Quiscalus mexicanus) and Red-winged Blackbird (Agelaius phoeniceus) were not tallied.

Water levels in the study wetlands were below basin maxima when the study started in the fall of 1994, but all had significant water coverage due to rains of September 1994. Because of personal experience with the magnitude of change in water volume in these ponds, water-depth gauges were considered impractical. Initially, total basin area of each wetland was determined from aerial photographs and topographic maps by using a dot-grid. Initial water coverage of the basin was recorded in ha as water index 4 (to allow estimates up or down), and that water area used as a basis for approximating wetland area at each observation day. Census-day wetland areas were calculated by multiplying the water index by the area per index value for each wetland. Thus, bird-area relationships reflect the dynamics of these wetlands as based on each survey day rather than by single measures per season or mean for the entire study period (see summary of published data in Weller 1999).

Although depths could not be assessed regularly, fence posts, topography and bird use suggested that basin water depths ranged from totally dry to about 2.43 m (8 ft) at flood stage in the larger wetlands. Due to their original formation and subsequent wind erosion, most basins sloped gradually from pond margins to center. Thus, as wetlands dried, shore areas dried to mudflats and then wet-meadows of grasses and forbs-

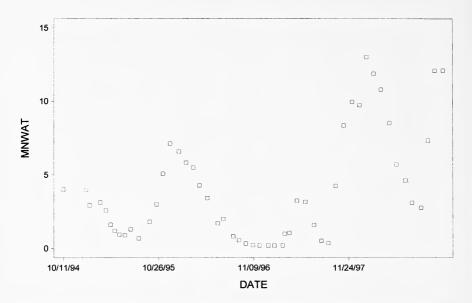


Figure 2. Mean water indices (MNWAT) of all 16 wetlands from October 1994 through December 1998. Although typically wet in late fall and winter, this was not true in 1996. Peaks were neither regular nor equal, creating diverse wetland sizes at various times of the year.

dependant on grazing history and timing. Unvegetated shorelines and flats with relatively steeper slopes often dried so rapidly that they were not attractive to mudflat birds.

#### RESULTS

Wetland characteristics.—Mean water indices for the 16 wetlands varied dramatically by survey over the four years of observation (Fig. 2). Using these indices to calculate area of water on each survey date as described above, size of individual basin water areas in hectares ranged from zero to four times the mean (Table 1, Fig. 3; an example of water dynamics in one wetland over the entire study period). Water area was generally related to regional rainfall, but spotty even on this small scale. Rainfall records for the nearest site with adequate data for this time frame (Corpus Christi) showed no significant correlation with wetland water indices at the study area.

Dominant avian taxa and their ecological characteristics.—Although 103 bird species were observed, only 44 taxa had frequencies over 15

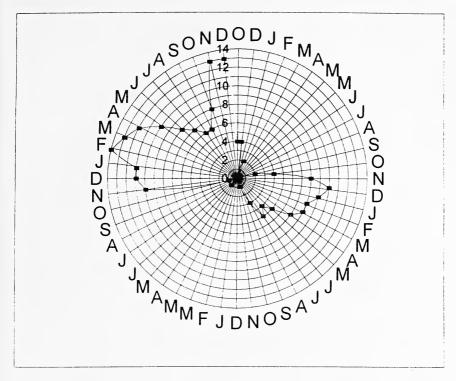


Figure 3. Example of change in water area of an individual wetland (No. 2) by approximately monthly readings from October 1994 (top right) through December 1998 (top left) (consecutive double letters indicate readings at the beginning and end of the same month during drought periods [one March and one May]). Water ratings from 0 to 14 are shown in upper center. The initial rating was an arbitrary 4; thereafter it varied to as high as 13. Note wet winter months in three of four years, but with considerable variation in duration of the water.

which had potential for statistical analysis (Table 2). As commonly separated by taxonomy, locomotion and/or feeding guilds, six waterbird groups dominated the avifauna. Waterfowl (family Anatidae) constituted 15 species(34%), of which eight favored surface swim-feeding in shallow water, and seven regularly dived for underwater foods such as invertebrates. Diving picivores of somewhat deeper water numbered five species (11%): two cormorants, Pied-billed Grebe, Least Grebe, Eared Grebe. There was only one diving herbivore (0.5%), the American Coot, although Fulvous Whistling-Duck, Canvasback, Ringnecked Duck and Redhead probably also used these limited resources. Seven surface feeders that fed by swimming or aerial diving on invertebrates, fish, or their remains (16%) included American White Pelican, Wilson's Phalarope, two gulls and four terns. Eight species were

Table 2. Frequency of occurrence, numbers and mean numbers per sighting of 44 taxa of wetland birds observed more than 15 times on 16 study wetlands on 52 dates, arrayed by frequency of sightings. Scientific names from American Ornithologists' Union (1998) and Texas Ornithological Society (1995). Species status codes in parenthesis after name: (1) permanent resident, (1a) resident but more common in summer, (1b) resident but less common in summer, (2) summer only, (3) spring and fall migration periods only, (4) spring and fall migration periods plus winter. An appended list includes wetland birds observed in lesser incidence (number in parenthesis).

Species or Taxon Status code in ( )	Frequency of Occurrence	Total Number Observed	Mean Number /Sighting
American Coot (1b) Fulica americana	215	7,355	34.2
Northern Shoveler (4) Anas clypeata	210	4,474	21.3
Mottled Duck (1a) Anas fulvigula	146	690	4.7
Pied-billed Grebe (1b) Podilymbus podiceps	146	493	3.4
Blue-winged Teal (4) Anas discors	143	2,148	15.0
Black-necked Stilt (1a) Himantopus mexicanus	139	896	6.5
Ruddy Duck (4) Oxyura jamaicensis	117	1,298	11.1
Cormorants (1)	113	690	6.1
Double-crested Cormorant Phalacrocorax as	uritus		
& Neotropic Phalacrocorax brasilianus			
Yellowlegs (3)	113	270	2.4
Greater Yellowlegs Tringa melanoleuca and			
Lesser Yellowlegs Tringa flavipes			
Killdeer (1a) Charadrius vociferus	111	220	1.9
Gadwall (4) Anas strepera	90	2,401	26.7
Great Blue Heron (1) Ardea herodias	86	103	1.2
Laughing Gull (1a) Larus atricilla	86	324	3.8
Dowitcher (4)	82	1,160	14.2
Short-billed Dowitcher Limnodromus griseus	5		
& Long-billed Dowitcher Limnodromus scol	opaceus		
Lesser Scaup (4) Aythya affinis	82	638	7.8
Northern Pintail (4) Anas acuta	79	1,566	19.8
Snowy Egret (1a) Egretta thula	78	554	7.1
Great Egret (1) Ardea alba	78	277	3.6
Black-bellied Whistling-Duck (1a)	69	757	10.9
Dendrocygna autumnalis			
White-faced Ibis (1a) Plegadis chihi	69	323	4.7
Green-winged Teal (4) Anas crecca	56	448	8.0
American Wigeon (4) Anas americana	54	864	16.0
Peeps (3) Calidris sp.	45	1,062	23.6
Ring-necked Duck (4) Aythya collaris	42	184	4.4
Long-billed Curlew (1a) Numenius americanus	42	207	4.9
White Ibis (1) Eudocimus albus	41	186	4.5
American Avocet (1a) Recurvirostra americana		308	7.7
Fulvous Whistling-Duck (2) Dendrocygna bicol		444	12.0
Cattle Egret (1a) Bubulcus ibis	32	403	12.6
Least Grebe (1a) Tachybaptus dominicus	31	340	10.9
Ring-billed Gull (1a) Larus delawarensis	31	115	3.7
Black-bellied Plover (4) Pluvialis squatarola	28	63	2.3
Forster's Tern (1) Sterna forsteri	28	93	3.3
Redhead (4) Aythya americana	26	79	3.0
Black Tern (2) Chlidonias niger	25	200	8.0
Little Blue Heron (1a) Egretta caerulea	25	39	1.6

Table 2. cont.

Species or Taxon Status code in ( )	Frequency of Occurrence	Total Number Observed	Mean Number /Sighting
Tricolored Heron (1) Egretta tricolor	23	30	1.3
Wilson's Phalarope (3) Phalaropus tricolor	22	586	26.6
Least Tern (2) Sterna antillarum	22	101	4.6
Eared Grebe (4) Podiceps nigricollis	21	47	2.2
Bufflehead (4) Bucephala albeola	19	103	5.4
American White Pelican (1)			
Pelecanus erythrorhynchos	18	587	32.6
Canvasback (4) Aythya valisneria	17	84	4.9
Gull-billed Tern (2) Sterna nilotica	16	43	2.7

Species observed fewer than 16 times out of 52 monthly surveys; listed in order of incidence of sightings (parenthesis); not used in statistical analyses but included in species richness.

Belted Kingfisher Ceryle alcyon (13)

Roseate Spoonbill Ajaia ajaia (13)

Cinnamon Teal Anas cyanoptera (10)

Common Moorhen Gallinula chloropus (10)

Common Snipe Gallinago gallinago (10)

Greater White-fronted Goose Anser albifrons (10)

Spotted Sandpiper Actitis macularia (10)

Stilt Sandpiper Calidris himatopus (10)

Canada Goose Branta canadensis (7)

Green Heron Butorides virescens (7)

Pectoral Sandpiper Calidris melanotos (7)

Black Skimmer Rynchops niger (6)

Buff-breasted Sandpiper Tryngites subruficollis (6)

Caspian Tern Sterna caspia (6)

Northern Harrier Circus cyaneus (6)

Willet Catoptrophorus semipalmatus (6)

American Pipit Anthus rubescens (5)

Solitary Sandpiper Tringa solitaria (5)

Horned Grebe Podiceps nigricollis (4)

Wood Stork Mycteria americana (4)

Dunlin Calidris alpina (3)

Snow Goose Chen caerulescens (3)

Herring Gull Larus argentatus (2)

Hudsonian Godwit Limosa haemastica 2)

Marbled Godwit Limosa fedoa (2)

Sanderling Calidris alba (2)

Sandhill Crane Grus canadensis (2)

Whimbrel Numenius phaeopus (2)

Anhinga Anhinga anhinga (1)

Common Goldeneye Bucephala clangula (1)

Black-crowned Night-Heron Nycticorax nycticorax (1)

Least Bittern Ixobrychus exilis (1)

Masked Duck Nomonyx dominica (1)

Northern Waterthrush Seiurus noveboracensis (1)

Osprey Pandion haliaetus (1)

Peregrine Falcon Falco peregrinus (1)

Reddish Egret Egretta rufescens (1)

Sora Porzana carolina (1)

wading picivores (18%) that fed regularly along wetland shallows or edges (ibises, egrets, herons), and nine shorebird taxa (20.5%) used mudflats or sheetwater.

Seasonality of bird use.—Of the 44 common taxa (Table 2), 23 use the area only part of the year: a few species for breeding (see status codes in Table 2), some only during migration, and some for wintering as well as migration. Twenty-one taxa were permanent residents, but populations were not stable; some were more common in winter (American Coot), and some increased in numbers during the breeding season (Black-necked Stilt). Others were relatively stable throughout the year, but their distribution by wetland seemed related more to water conditions and food than to other breeding requirements such as nest sites (e.g., Great Blue Heron).

Nesting was uncommon even among resident species but was documented (nests and/or young) for Killdeer, Pied-billed Grebe, Least Grebe, Black-necked Stilt and Mottled Duck. Based on older and flying young, nesting was probable in Black-bellied Whistling-Duck, and pair behavior suggested that Fulvous Whistling-Duck, Blue-winged Teal and Northern Shoveler may have attempted to nest. Some coastal summer residents used the area because all ponds were within 3 km of Baffin Bay, but probably nested on the coast and fed in freshwater wetlands (Least Tern, Gull-billed Tern and Forster's Tern). Herons, egrets and cormorants used the area regularly but seemingly did not nest. A few solitary nests of Red-winged Blackbirds were noted in roadside shrubs, and Great-tailed Grackles were seen carrying nest material in one wetland when tules and cattails were flooded, but nests or young were never observed.

Bird species richness.—After pooling peeps and deleting several passerines mentioned above, taxon richness ranged from 0 to 28 species per pond per census, and 3 to 47 species per census (Table 1). As would be expected, there was a significant difference in richness among the 16 ponds over the 52 surveys (Kruskal-Wallis ANOVA, KW = 263.63, P=0.0000). The influence of wetland size (water area in ha) on richness for each survey was reflected in a Linear Regression (data transformed Log+1 to correct for zeros;  $R^2=0.6001$ , t=35.01, df=818, P=0.0000) (Fig. 4). Although richness did not differ significantly among months, summer months were lowest, and species composition varied seasonally due to the multiple uses mentioned earlier.

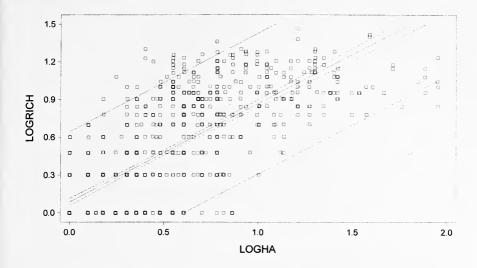


Figure 4. Bird-species richness (LOGRICH)-water area (LOGHA) relationship for all taxa observed at 16 ponds. Data were log transformed [x+1] to correct for zero readings. Solid line is fitted; inner dotted lines represent 95% confidence interval for fitted line and outer dotted lines represent 95% predicted interval.

Bird numbers.—There was great variation in bird abundance by survey day, season, and cumulative total numbers per wetland basin (Table 1). Resident birds that probably established territories for much of the year when water conditions were suitable (Black-necked Stilt, Mottled Duck, Killdeer) or during spring (American Avocet, Blackbellied Whistling-Duck) typically were seen as pairs or singles, and hence smaller mean numbers per sighting (Table 2). Birds that moved freely from pond to pond to feed socially or in mixed-species flocks on fish (e.g., American White Pelican, cormorants), foliage and seeds (ducks, American Coot), or invertebrates (Northern Shoveler, Ruddy Duck, Lesser Scaup, Wilson's Phalarope, peeps) were in larger flocks (Table 2). Number of birds differed significantly by wetland as would be expected (KW=249.20, P=0.0000), and numbers (log transformed) also were related to wetland area ( $R^2=0.6237$ , t=36.8, df=818, P=0.0000) (Fig. 5). Despite larger flocks during migration and in winter, mean numbers did not differ significantly by month, due probably to year-to-year irregularity in water regimes.

Habitat influences on bird use at the cluster level.—This study area constitutes a cluster or selected block from within a larger area of diverse wetlands. Movements of individuals and small groups from

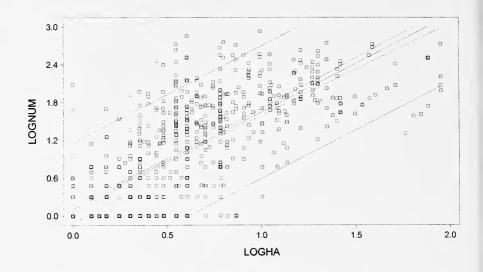


Figure 5. Bird-numbers (LOGNUM)-water area (LOGHA) relationship for all taxa observed at 16 ponds. Data were log transformed [x+1] to correct for zero readings.

wetland to wetland were commonly observed, especially when disturbed by farming activity or potential predators. To examine the influences of the cluster as a whole, mean data for all 16 wetlands for given survey days or for the study period of 52 monthly surveys were used. A Linear Regression of mean species richness on water index was significant ( $R^2$ =0.3143, t24.79, df=51, P=0.0000) but the nature of this index stresses size over water availability and bird distribution within the cluster. A Linear Regression of mean species richness for survey date and the number of wetlands with water ( $R^2$ =0.5579, t=5.69, df=51, P=0.0000) (Fig. 6) better reflects the extremes of wetlands that dried completely (water index 0) and eliminated most wetland bird use. Total numbers by survey date also were related to the number of wetlands containing water ( $R^2$ =0.3934, t=7.54, df=51, P=0.0000) (Fig.7).

Habitat influences at the individual wetland level.—Within this cluster, individual wetlands provided different habitats for different species at any given survey, and often for different species at different times due to the water regime, vegetation and bird needs (e.g., migration stopovers). Chi-square analysis of the distribution of the 44 common taxa (Table 2) indicated that some species were found in certain wetlands more commonly than expected. Coots and Pied-billed Grebes were associated with large and open water, and Killdeers with low water

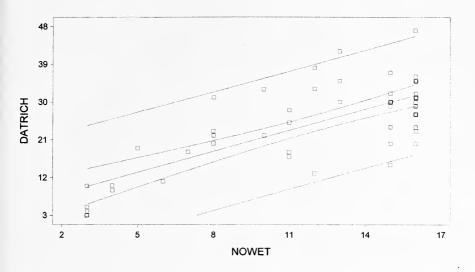


Figure 6. Relationship of bird species richness by survey day (DATRICH) to number of wetlands containing water (NOWET).

levels and smaller areas. Failure of the data to show other observed relationships presumably was due to the fact that basins were shallow and dynamic, so that each wetland provided a diversity of microhabitats as water levels changed in relation to vegetation and exposed substrates, thereby attracting different species at different water stages. For example, the largest wetland had, at different times, the highest frequency of usage (far above expected in a Chi-square analysis) for bird species with as diverse habitat requirements as American White Pelicans, cormorants, Ruddy Ducks, Canvasback, Great Egret, peeps, dowitchers and American Pipit due to different water levels and dryness of mudflats.

Feeding patterns.—Observed feeding behavior during the study demonstrated the flexibility of some species or guilds and the rather static behavior of others in food use. Although presence or abundance of potential waterbird foods were not assessed during this study, some foods (e.g., fish) were conspicuous by the presence of users such as American White Pelican, cormorants and herons. Early in the study, these birds frequented several ponds and were seen with large fish in their bills; after the first drying of the larger and deeper wetlands, such fish-feeders were rarely seen or seen only at loafing sites. Reestablish-

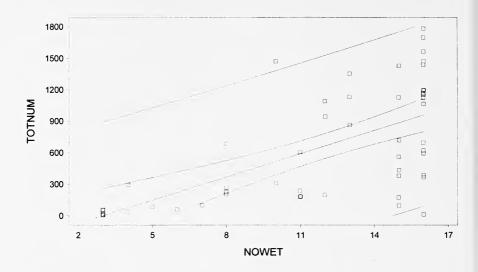


Figure 7. Relationship of bird abundance by survey day (TOTNUM) to number of wetlands containing water (NOWET).

ment of fish populations took several years and floods, probably because ponds dried again too quickly for fish reproduction and survival. The presence of small fish was evidenced by flocks of Least Terns and Black Skimmers feeding during low water levels.

Mixed species feeding flocks of American White Pelicans, cormorants, and grebes were induced when fish were abundant; typically, the former two fed together, whereas grebes fed near or among them opportunistically. When water depths declined in one wetland to a depth of only a few cm, Snowy Egrets concentrated with Least Terns and Black Skimmers to feed. Presumably the fish were small because cormorants and larger egrets and herons were rare.

Pied-billed grebes also fed in association with shovelers, herons and egrets which apparently served as "beaters" by disturbing aquatic foods which were subsequently captured underwater by the grebe. Wilson's Phalaropes sometimes followed Northern Shovelers, and the latter followed still larger birds such as herons to capture invertebrate prey they disturbed. Cattle Egrets typically used cattle or horses as "beaters" in much the same way but in wet meadow as well as in drier sites. Interspecies feeding associations also included coots, American Wigeon and Gadwall, presumably feeding on submergent vegetation. Coots

dived for the deep-water food items and subsequently were harassed for food by the non-diving ducks.

### **DISCUSSION**

Patterns of waterbird use over time are more complex at this latitude than in northern areas where freezing prevents winter use and species and numbers change mainly with migration. Temperature is not limiting at this site, but the dynamics of water dictate suitability of wetland areas for feeding, resting or nesting. Most species can be found year-round, but warm-temperate and subtropical species used these wetlands mainly during summer for nesting or perhaps nonbreeding habitat. During migration, they provide feeding areas for many northern birds that follow coastal flight corridors, and wintering is common.

These south Texas wetlands are similar in size and depth to those of the Prairie Pothole Region of the United States and Canada. Both are in semiarid regions and are characterized by dynamic water regimes, but water data recorded during this study suggested that water levels are even more variable. Irregularity of water during the growing season and high ambient temperatures that dried mudflats reduced the quality of the site for many emergent plant species. Thus, sparsely vegetated shorelines and open water resulted, with plant heights often further reduced by intensive grazing. There also was a conspicuous absence of submergent plants such as milfoil and pondweeds, but lotus tubers seemed to survive drought in the deeper wetlands.

Extensive mudflats were extremely important to a wide variety of the bird species observed there, and are a product of years of upslope erosion due to wind and water, and increased sedimentation exacerbated by grazing and tillage (see review in Weller 1996). High turbidity also was common to the larger of these wetlands due to wind, water and livestock action. In a short-term view, such siltation probably induced eutrophication and higher invertebrate productivity as evidenced by concentrations of shorebirds on mudflats along larger wetlands and in the bottoms of drying shallow wetlands. But ultimately, sedimentation will fill in these shallow basins, as evidenced by some that never exceeded a half-meter in depth even at flood stage, and dried to nearly flat and uniform substrates. Clearly, upslope management is vital to their long-term survival.

Thus, the role of these wetlands for breeding birds is dependent on seasonality, persistence and predictability of water, nesting and rearing cover, and lasting food supplies or access to adjacent sources. In typical rainfall years, these wetlands remain wet mainly during non-breeding periods, and are suitable for migrant and wintering ducks. The lack of waterbird breeding presumably is related to the historic patterns of wetland dry-down that would have reduced breeding success, and populations shifted to areas where they were more successful. However, studies by McAdams (1987) on waterfowl breeding and by Briggs & Everett (1983) on waterbird use of similar ponds in the region during post-hurricane years demonstrated a positive response to flooding that persisted for several years. American Coot, Common Moorhen, Piedbilled Grebe commonly nest in Texas coastal wetlands farther north where rainfall is greater, seasonal river overflows influence wetland water regimes, or water levels are managed (Cottam & Glazener 1959; Weller et al. 1996), and use of brackish marshes for nesting by Mottled Ducks (Stutzenbaker 1988). Wintering birds of those same well-watered and vegetated ponds also were more characteristic of species favoring submergent as well as emergent vegetation (e.g., White & James 1978; Weller et al. 1996).

Much of the regional species richness observed here was due to use of different wetland types, sizes and depths for different purposes, sometimes by the same species. However, it was a dynamic situation; larger wetlands that were deeper provided more stability on average, but as water levels gradually declined, the gently sloped basins had extensive shallow feeding areas suitable for diverse shorebirds and waders. Whether wet or dry, large open areas that might be interpreted as unattractive often are used as daytime rest and overnight roost areas for birds that feed elsewhere: American White Pelicans, Snow Geese, White-fronted Geese, Sandhill Cranes, Fulvous Whistling-Duck, Blackbellied Whistling-Duck and several diving ducks.

Specific water-index values as used in this study were not statistically correlated with better or worse conditions for most bird usage, probably because such changes influenced only small portions of individual wetlands. Black-bellied Plovers and Killdeer typically used drier areas than did Buff-breasted Sandpipers and peeps, and both used much drier sites than dowitchers that needed deep and soft mud for probing. Variability in water regimes is therefore vitally important in relation to temporal patterns of migration. For example, a basin that dried quickly

did not hold shorebirds or other mud probe-feeders as long as flats that remained muddy; for this reason, flat basins were better than steeper basins because the rate of drying was slower and more uniform.

Areas that dried in mid- to late-winter had low species richness and numbers, but they were used intensively by migrants in spring and fall after even modest rainfall. Fish-eating birds used several shallow ponds for loafing, with cormorants usually on snags or mudflats and American White Pelicans in water or on shorelines. Feeding by cormorants occurred in ponds deep enough for diving, and American White Pelicans used only the largest and deepest wetland for feeding when fish were present. Despite general relationships between richness or numbers and size of wetlands (Figs. 4 and 5, respectively), diversity must be considered comparatively. A small drying wetland often is much more attractive to a diversity of species than a large flooded one. However, the larger wetland may have a great abundance of a few species, but at drying, may have parts of it extremely species-rich. These microhabitats often are short-lived, and may occur seasonally at many ponds, thus requiring short-term analyses to compare wetland conditions and their attractiveness to single or multiple species.

## IMPLICATIONS FOR CONSERVATION OF BIRD HABITAT

Past drainage ditches have impacted some of the more shallow wetlands in this area by speeding drying, but larger wetlands would have been costly to drain because of the rolling landscape. Moreover, most landowners valued wetlands for livestock watering, as shown by areas deepened to hold water in the dry years ("dugouts"). Use of mobile pumps for small-scale irrigation resulted in water depth changes in some wetlands. More shallow areas adjacent to farm fields often were tilled during dry periods, and wind and water erosion were common, resulting in the potential of significant modification of wetlands (Briggs 1982). However, periodic flooding prevented continuous cropping. Further unregulated housing development would be harmful because of likely pressures to reduce flooding.

Despite lack of breeding habitat for many birds, these wetlands were heavily used by nonbreeding birds all year. Thus, they should be recognized for their contribution to the conservation of diverse species of regional, national, and international importance. Several authors have indexed bird use of wetlands to assess the dominance or relative impor-

tance of each species in the bird assemblage (Boyer & Psujek 1977; Briggs & Everett 1983) or identify key wetlands within a complex. Bird use in this study was indexed to compare different wetlands to provide conservation personnel with assessment methodology for wetland acquisition or other protective strategies. Simple ranks were established on the basis of mean data per survey for richness, abundance, maximal or cumulative richness and numbers per wetland. All rankings identified the same six wetlands but ordered them differently. Similar ranking results from Kruskall-Wallace ANOVA of richness or numbers per wetland, and identified generally the larger wetlands with more persistent water regimes because of their actual use over time rather than assessment of habitat suitability. These rankings selected larger (over 5 ha) wetlands that amounted to 68.7 ha or 81 percent of the total 85 ha of wetland basins in the study area (see size data in Table 1).

Because of the geomorphic setting, this cluster of wetlands can be viewed as a unit hydrologically as well as biologically with a need to preserve various types and sizes of wetlands for the diverse functions of many species of mobile birds. Unlike the large land holdings characteristic of most of south Texas, wetlands of this cluster tend to be individually owned. Thus, conservation approaches must appeal to individuals with different economic and aesthetic values. They are vulnerable to loss because of ever-changing regulations concerning small wetlands on agricultural land. There is sufficient interest in this area for birding that a regional or county plan for preservation might be possible, and could be led by county extension information specialists with a local group of cooperators. But because not all wetlands are likely to be protected, evaluation and prioritization for birds is an essential tool in formulating such a conservation strategy.

To provide habitat for breeding as well as resting and feeding, a larger number of these wetlands should be protected from intensive grazing or farming that increases chances of soil erosion and increased turbidity with subsequent sedimentation. Fencing part of each wetland during late fall, winter and early spring would allow the development of emergent vegetation. This is not to imply that uniformity of management for every wetland is good, as a variety of land uses seems to diversify shoreline food resources and minimize invasion by woody shrubs and trees.

While some conservationists encourage preservation of areas with the

greatest bird species richness or significant breeding habitats, this small cluster of multi-function wetlands is unique, and has survived without dedicated funds or any legal conservation designation. Perhaps this is because they do serve society in a variety of ways, and in part because they have built-in protection due to their geomorphic setting. They are well worth preserving.

### ACKNOWLEDGMENTS

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MWW at:mwwdlw@msn.com

### **GENERAL NOTES**

# MORTALITY OF PRAIRIE STREAM FISHES CONFINED IN AN ISOLATED POOL

### Kenneth G. Ostrand and Derek E. Marks

Department of Range, Wildlife and Fisheries Management Texas Tech University, Lubbock, Texas 79409 Present addresses:

Sam Parr Biological Station, Center for Aquatic Ecology Illinois Natural History Survey, 6401 Meacham Road Kinmundy, Illinois 62854 and

Wetland Resources, Inc., 9505 19th Avenue S.E., Suite 106 Everett, Washington 98208

Prairie stream fishes confined to isolated pools during periods of drought are subject to highly variable abiotic conditions, which may cause physiological stress and ultimately death (Tramer 1977; Matthews et al. 1982; Mundahl 1990). There have been few opportunities to observe natural fish mortality while it was occurring in situ, because of water depth, turbidity and chance timing. Unless mortality is observed as it occurs, casual factors become difficult to identify. Of the few instances in which fish mortality was observed, abiotic factors such as high temperature (Huntsman 1942; 1946; Bailey 1955; Matthews et al. 1982; Mundahl 1990), high salinity (Barlow 1958) and low dissolved oxygen (Tramer 1977) were identified as causal factors. In each of these cases, abiotic variables were beyond physiological tolerable limits for fish. Independently, single abiotic variables can cause mortality, but when the effects of several abiotic variables are combined fish mortality may also occur (Rutledge & Beitinger 1989). This study reports an instance of in situ fish mortality as it was occurring and identifies abiotic factors related to that caused mortality.

On 21 July 1998 at 1330 hrs, on the North Fork of the Double Mountain Fork of the Brazos River in Garza County, Texas, mortality of cyprinids trapped in an isolated pool was observed as it occurred. To determine whether lethal physical and chemical conditions existed, several variables were measured in this pool and in five neighboring pools that showed no signs of ongoing mortality. Variables measured were dissolved oxygen (mg/L), temperature (°C), conductivity (mS), pH (using a model 60 and 85 YSI meters; Yellow Springs Instruments), turbidity (NTU; using a LaMOTTE portable turbidity meter; LaMOTTE

Instruments), ammonia (mg/L; using a Hanna portable ammonia meter; Hanna Instruments), maximum depth (cm), and volume ( $m^3$ ) in each pool prior to sampling fish. Live fish were sampled with one seine haul that covered the length and breadth of each pool. Live fish were enumerated, identified and returned to the pool. Dead fish were collected after seining and preserved in 10% buffered formalin. Bonferroni *t*-tests were used to examine differences (P < 0.006) in abiotic variables between the pool that experienced mortality and those pools that did not.

One hundred percent of the smalleye shiner (*Notropis buccula*) (n = 6), 93% of the plains minnow (*Hybognathus placitus*) (n = 125) and 82% of the red shiner (*Cyprinella lutrensis*) (n = 14) were collected dead from the pool in which fish mortality was observed. Conversely, 100% (n = 1) of the plains killifish (*Fundulus zebrinus*) and 100% (n = 3) of the green sunfish (*Lepomis cyanellus*) were captured alive from the pool in which ongoing mortality was observed. Within the five pools, in which no mortality was observed, a total of 89 plains minnow, n = 100% 58 smalleye shiner, eight red shiners and 47 plains killifish were collected. Dissolved oxygen, pH, ammonia, maximum depth and volume differed between the pool with mortality and the five neighboring pools that showed no signs of mortality (Table 1).

Since the advent of Shelford's (1913) Law of tolerance, ecology has recognized the importance of maximum and minimum abiotic conditions limiting the presence or success of organisms. Thus, fish live within a range of abiotic variables that encompass tolerable physiological limits. Even though temperatures were high, they were not lethal. Temperatures within all pools (33.1 to 33.8°C) were below the critical thermal maxima of the red shiner (38.9°C), plains minnow (40.0°C) (Matthews & Hill 1979) and smalleye shiner (37.2°C; Ostrand 2000). In addition, pH (7.13 to 7.83) in all pools was below lethal levels for the cyprinids observed dying (Echelle et al. 1972; Matthews & Hill 1979; Ostrand 2000). However, dissolved oxygen (0.17 mg/L) and ammonia (10.81 mg/L) were at lethal levels within the pool that had ongoing fish mortality. Plains minnow, smalleye shiner, and red shiner lose equilibrium and may die at dissolved oxygen levels lower than 1.2-1.5 mg/L (Matthews & Hill 1979; Ostrand 2000) and ammonia levels in excess of 10 mg/L (sensu Horne & Goldman 1994). Synergistic effects may become increasingly important as pools shrink, particularly when fish density increases as pool volume decreases due to water evaporation (Capone & Kushlan 1991). The formation of isolated pools coupled with their volume and fish density could impact fish survival via competitive inter-

Table 1. Abiotic variables measured 21 July 1998 on the North Fork of the Double Mountain Fork of the Brazos River in Garza County, Texas within isolated prairie stream pools. N is sample size and value is the measured abiotic variable within the pool in which no fish mortality was observed. An asterisk denotes significantly (P < 0.006) different environmental variables.

Abiotic variable	Pool w	ith mortality	Poo	ols with no	mortality	P-value
	n	Value	n	$\overline{x}$	SE	
Dissolved Oxygen (mg/L)	1	0.17	5	4.37	0.93	0.0054*
рН	1	7.13	5	7.59	0.06	0.0012*
Ammonia (mg/L)	1	10.81	5	1.87	0.74	0.0001*
Maximum depth (cm)	1	34.29	5	13.20	2.94	0.0010*
Volume (m <sup>3</sup> )	1	1.80	5	0.36	0.15	0.0003*
Temperature (°C)	1	34.70	5	34.84	0.27	0.3176
Conductivity (mS)	1	1.34	5	1.60	0.10	0.0370
Turbidity (NTU)	1	119.00	5	318.32	185.01	0.1710

actions and altered habitats due to overcrowding (Matthews 1998). However, the pool in which observed mortality occurred had a greater volume and a lower or equivalent density of fish than those that did not.

Since, the abundance of cyprinids throughout Texas have undergone a general reduction from the 1950's (Anderson et al. 1995) and since the Texas Water Development Board (1990) has proposed several additional water developmental projects on the upper Brazos River the impact of abiotic variables on fish species survival within isolated pools should be of considerable importance. Differential mortality of species, particularly the loss of cyprinids, can potentially reduce local diversity (Matthews 1987; Matthews et al. 1982; Rutledge & Beitinger 1989) by impacting colonization rates and reproductive success (Capone & Kushlan 1991) and will most likely be exacerbated by current and future water use trends. Water scarcity is chronic in many parts of the world, and droughts have become worse in places like the southwestern United States, where growing human populations demand more water withdrawal from streams (Matthews 1998). As water development projects (e.g., Red River Chloride Control Project; Lake Alan Henry, Texas) continue, further restrictions in the geographic ranges of species and local extinction may become more prevalent (Wilde & Ostrand 1999), particularly if flows are reduced and fishes are subject to prolonged confinement in isolated streambed pools.

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KGO at: ostrand@uiuc.edu

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# A NEW COLORATION IN THE BROADHEAD SKINK, *EUMECES LATICEPS* (SAURIA: SCINCIDAE)

# Toby J. Hibbitts, John H. Malone\*, James R. Dixon and Kelly J. Irwin

Texas Cooperative Wildlife Collections
Department of Wildlife and Fisheries Sciences
Texas A&M University, College Station, Texas 77843
\*Present address:
Department of Zoology, University of Oklahoma
Norman, Oklahoma 73019

The discovery of an undescribed color morph of *Eumeces laticeps* was made at the Temple-Inland Experimental Forest, located 7 km E and 4 km S of Spurger, Tyler County, Texas (30°38'45.0"N 94°04'36.0"W). Lizards were collected in 1995-1997 using 168 drift fences with two funnels and two buckets per fence, totaling 672 traps distributed among three 24 ha study plots.

Typical juvenile *E. laticeps* in the study population are deep black above with five dorsal stripes. The lines are greenish-white anteriorly, but posteriorly they may be light blue or blue-white, and extend to about one-third the length of the tail. Their tails are deep blue and slightly ultramarine below (Fig.1). In males the stripes lose their distinctness and the dorsal color becomes more or less uniform brown or olive by sexual maturity. During the breeding season head color turns orangered and jowls become enlarged. Adult females retain more of the juvenile pattern and coloration, with the light stripes remaining discernible in all but the very largest specimens. The ground color becomes lighter and usually is olive and the stripes are not as distinct as in juveniles. The blue tail color is lost in adult males, but the tail of some adult females retains a bluish cast (Taylor 1935; Conant & Collins 1998).

Juvenile E. laticeps of the undescribed color morph exhibit a uniform cinnamon-brown (Smithe 1975:Color 33) dorsal coloration, but retain the blue tail. Thus, they differ from normal individuals by their complete lack of stripes (Fig.2). The adults of both sexes are uniform cinnamon-brown. All but the largest females were easily distinguishable from the normal colored individuals due to the absence of stripes. Males were more difficult to discern due to the uniform adult coloration.

Five percent (24) of 494 E. laticeps at this site exhibit the cinnamon-brown pattern. The mean snout-vent length (SVL) of E. laticeps with the cinnamon-brown pattern was 67.3 mm (n = 25, SD = 20.3). The

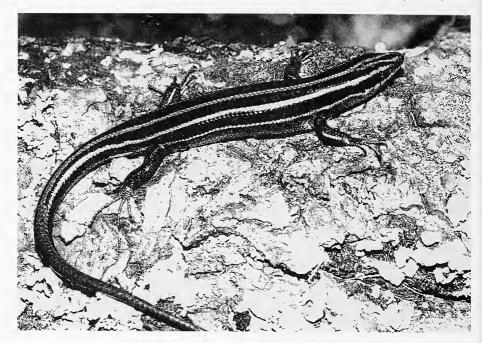


Figure 1. Eumeces laticeps juvenile with normal coloration.

mean SVL of all normal patterned *E. laticeps* captures was 77.4 mm (n = 499, SD = 21.9). The cinnamon-brown skinks were distributed throughout the study site. The first cinnamon-brown morph found was a juvenile captured on 29 August 1995 and several more specimens were found in 1995, 1996 and 1997. Seven cinnamon-brown individuals were recaptured and appear to have normal growth rates as exhibited by the species. Voucher specimens of the cinnamon-brown skinks were collected and deposited in the Texas Cooperative Wildlife Collections (TCWC) at Texas A&M University (TCWC 72114, 72115, 78699-78702, 78705-78708).

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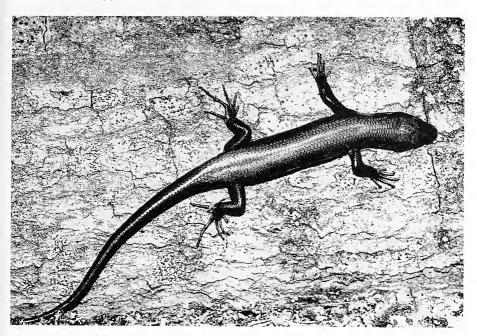


Figure 2. Eumeces laticeps juvenile with cinnamon-brown coloration.

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TJH at: tobyh@tamu.edu

# UTILIZATION OF FISH AS A FOOD ITEM BY A MOUNTAIN LION (*PUMA CONCOLOR*) IN THE CHIHUAHUAN DESERT

# Philip L. McClinton\*, Susan M. McClinton\* and Gilbert J. Guzman

Department of Biology, Sul Ross State University
Alpine, Texas 79832 and
Texas Parks and Wildlife Department,
4200 Smith School Road, Austin, Texas 78744
\*Current Address:
P. O. Box 1431, Ft. Davis, Texas 79734

Post-ingestion samples (scats, pellets) commonly have been used to determine individual food items used by wild animals. Such studies have proved valuable in learning the degree to which mountain lions (*Puma concolor* Linnaeus) use specific prey bases (Anderson 1983;

Leopold & Krausman 1986; Waid 1990). Diets of mountain lions in the Chihuahuan Desert region of North America have included numerous items (McBride 1976; Leopold & Krausman 1986; Waid 1990). Varied diets in other regions within the southwest deserts demonstrate the ability of mountain lions to adapt to environmental conditions to maximize food sources (Anderson 1983). While most studies indicate that mountain lions consume primarily deer (*Odocoileus* spp.) in non-arid regions (Anderson 1983), desert-adapted mountain lions supplement their diet of deer with various other vertebrates when densities of deer are low (Leopold & Krausman 1986). For example, collared peccary (*Tayassu tajacu*) were shown to be the top prey item of mountain lions on Big Bend Ranch State Ranch from 1993-1997 (McClinton & McClinton 1997).

No published literature was found reporting fish consumption by mountain lions in the desert southwest. Fish, however, have been reported as diet components in other areas (Hansen 1992). Young (1946) reported that mountain lions are successfully trapped by using fish as bait. However, a comprehensive critical review of literature on mountain lions by Anderson (1983) does not mention fish as occurring in any of the 17 studies reviewed for the publication.

# COLLECTION SITE AND METHODS

On 18 May 1995, the junior author collected scat from the bank area directly above the Rio Grande on the Ocotillo Unit of the Las Palomas Wildlife Management Area, a Texas Parks and Wildlife Department property adjacent to Ruidosa, Texas, in southern Presidio County. The dried scat was estimated to be about 7-10 days old when collected. The scat was collected in conjunction with an on-going TPWD study on mountain lions (and their food habits as determined from scat analysis) in Big Bend Ranch State Park. After collection, the scat was transported to Sul Ross State University (SRSU) for examination and identification of contents.

The dry scat was softened in water for approximately 24 hours. It was then washed with water through a series of screens (diameter of sieve mesh, 0.074 to 1.682 mm). The moist, undigested residues were spread to a depth of approximately 5 mm on absorbent paper toweling and segregated into individual food items for identification. Hair, bone and scales were identified by direct comparison with specimens housed in the SRSU vertebrate collection. A reference collection of items identified was retained for inclusion in the vertebrate collection at SRSU.

### RESULTS AND DISCUSSION

Post-ingestion sample analysis revealed that the many bones and cycloid scales (Moyle & Cech 1988) found in the scat were identical to those of carp (*Cyprinus carpio*) examined from the SRSU vertebrate collection. These items comprised approximately 15% of the volume; the remaining volume was composed of collared peccary hair. Carp has not been reported as a food item for mountain lions in North America before this study.

Many scenarios may explain the presence of carp scales and bones in the examined scat. The exact scenario is not known; however, the presence of scales in the scat indicate that mountain lions can be resourceful when choosing food items in the Chihuahuan Desert region.

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PLM at: tpbio@brooksdata.net

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# NOTEWORTHY RECORDS OF MAMMALS FROM THE ORINOCO RIVER DRAINAGE OF VENEZUELA

Thomas E. Lee, Jr., Burton K. Lim\* and John D. Hanson

Department of Biology, Box 27868
Abilene Christian University, Abilene, Texas 79699
\*Center for Biodiversity & Conservation Biology
Royal Ontario Museum, 100 Queen's Park
Toronto, Ontario, Canada M5S 2C6

The most thorough documentation of mammals found in Venezuela resulted from the Smithsonian Venezuelan project with field work occurring between 1965 and 1968 (Handley 1976). One area that was sparsely sampled, however, was the transition region from savanna to rainforest south of the Orinoco River (Ochoa et al. 1988; 1993). This paper presents noteworthy records of small mammals from a collecting trip to the Llanos region in central Venezuela and includes range extensions for six taxa (*Pteronotus gymnonotus*, *Lonchorhina orinocensis*, *Lonchophylla thomasi*, *Mus musculus*, *Rhipidomys leucodactylus* and *Sylvilagus floridanus*).

Mammals reported in this paper were collected in July and August 1997 from five localities within the Orinoco River drainage basin. The habitat of locality 1 was a mix of riparian forest along the Orinoco River with granite outcrops and forested hills within savanna (Amazonas; Pozon, 50 km NE of Puerto Ayacucho, 6° 3'N, 67° 25'W). In addition, because it was the rainy season, much of the savanna and all of the riparian forests were flooded. Locality 2 was a cattle ranch on the Llanos (Bolivar; Hato La Florida, 35 km ESE of Caicara, 7° 34'N, 65° 52'W). The habitat was savanna with many small creeks and swamps. There were also granite outcrops and isolated mountains that were forested. Locality 3 was gallery forest in savanna near a river about 50 m acros's and bordering a ranch (Bolivar; 20 km S of Guaniamo, 6° 26'N 66° 7'W). Locality 4 was the only collecting site north of the Orinoco River (Guarico; Rio Orituco 10 km W of Chaguaramas, 9° 24'N, 68° 28'W). This site consisted of extensive (mixed-grain crop) cultivated fields with a secondary growth riparian forest, in conjunction with a major highway and bridge. Locality 5 was an old secondary growth forest near the Caura River (Bolivar; 3 km E of Puerto Cabello del Caura, 7° 10'N, 64° 50'W). Some trees in this locality were large with buttressed roots and there were epiphytes in the canopy. There were, however, cultivated fields in the vicinity with many roads and trails in the area. Voucher specimens were deposited in the Abilene Christian University Natural History Collection (ACUNHC) and the Royal Ontario Museum (ROM).

Pteronotus gymnonotus Natterer.—Handley (1976) reported this species from localities north of the coastal mountains in northwestern Venezuela, with most captures in dry areas. One lactating female (ROM 107925) was caught (at locality 2) over a pond in riparian forest south of the Orinoco River.

Lonchorhina orinocensis Linares & Ojasti.—Records of this species are documented from near the border with Colombia in southwestern Venezuela (Handley 1976). This study provides documentation of specimens from south central Venezuela (locality 2) about 200 km. east of the most proximate recorded locality. Five specimens (ROM 107911, ROM 107912, ACUNHC 302, ACUNHC 375 and ACUNHC 376) were collected from an isolated igneous rock outcrop within savanna. Four were pregnant with embryo crown-rump (CR) measurements ranging from 7 to 11mm and the fifth female was lactating. The outcrop contained several large crevices in which L. orinocensis roosted with Micronycteris megalotis, Saccopteryx bilineata and Peropteryx macrotis. An additional 15 specimens of L. orinocensis were collected at locality 1 in savanna mixed with patches of forest and rock outcrops. Of the 11 females, all were pregnant, with embryo CR ranging from 6 to 20mm.

Lonchophylla thomasi Allen.—One male (ROM 107906) was caught (locality 3) about 150 km north of the most proximal documented locality in Venezuela (Handley 1976). Eisenberg (1989) reported a disjunct distribution for this species, south of the Orinoco River and west of the Andes in Colombia and Panama. The animal was caught in riparian habitat along a river that was about 50 m across. A second male (ACUNHC 395) was collected from locality 5 in an agriculturally disturbed rainforest. This species is usually associated with evergreen forests (Handley 1976).

Rhipidomys leucodactylus Tshudi.—A non-pregnant female (ROM 107873) was collected at locality 1, 300 km NNW of the next most proximal record in southern Venezuela (Handley 1976). The animal was trapped in a small (7 m tall) tree that was growing over an igneous rock outcrop. There was a stream on one side of the rock outcrop and flooded llanos on the other side. This is the first documentation of R.

leucodactylus from riparian habitat in the Llanos. Previous records are from primarily moist, evergreen forests (Handley 1976).

Mus musculus Linnaeus.—All the recorded localities for this species are in association with the large cities and towns long the Caribbean coast of Venezuela or in the adjacent mountains (Handley 1976; Eisenberg 1989). A pregnant female (ACUNHC 272) with four embryos (CR =13) was collected about 70 km south of the coastal mountains in the Llanos (locality 4). It was caught in a farm house which was next to the Orituco River.

Sylvilagus floridanus Allen.—The previously reported range for this species in Venezuela was north of the Orinoco River (Eisenberg 1989). This study documents the existence of the species southeast of the Orinoco River (locality 1). One male (ROM 107864) was shot on a dry grassy hill in the Llanos by a local hunter. An additional specimen (ACUNHC 266) was found as a roadkill north of the Orinoco River (Guarico; 10 km NE of Las Mercedes, 9° 11'N 66° 20'W). There were also several sightings along Highway 12 between Las Mercedes and Puerto Ayacucho in the savanna.

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TEL at: lee@biology.acu.edu

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# EVALUATING GERMINATION PROTOCOLS FOR CHINESE TALLOW (SAPIUM SEBIFERUM) SEEDS

Warren C. Conway, Loren M. Smith and James F. Bergan\*
Department of Range, Wildlife and Fisheries Management
Texas Tech University, Lubbock, Texas 79409 and
\*The Nature Conservancy of Texas
Corpus Christi, Texas 78403

Chinese tallow (Sapium sebiferum L. Roxb) is an exotic deciduous tree that was introduced from China into the United States in the mid-1800s (Scheld et al. 1984; Bruce et al. 1997). In Texas, tallow is expanding its range (NRCS 1993) and is a dominant component in coastal ecosystems (Scheld et al. 1984; Bruce et al. 1997). Rapid growth rates (2-3 m per yr) (Scheld & Cowles 1981) combined with large annual seed loads (4,500 kg seeds per ha per yr) (Scheld et al. 1984) make tallow control difficult (Conway et al. 1999). Because of. these characteristics in concert with various seed dispersal mechanisms (i.e., via water and birds) (Conway 1997), once naturalized, tallow woodlands are potentially self-perpetuating (Cameron & Spencer 1989). If control efforts were to focus upon germination/establishment phases rather than post-naturalization, tallow control could be more effective. However, tallow germination requirements have not been published (Bonner 1974; Conway 1997). Germination has been obtained in greenhouse studies, but germination requirements were not documented (Cameron et al. 2000). The objective of this study was to examine tallow seed germination rates following various treatments developed to mimic natural conditions.

Ripe, mature, undamaged Chinese tallow seeds, not whole fruits, were collected along the mid-Texas coast during September, October and November 1995 from randomly selected tallow trees (n=15). Seeds were collected at The Nature Conservancy of Texas' Mad Island Marsh Preserve (28° 6'N 95° 8'W) in Matagorda County and the Brazoria National Wildlife Refuge (29° 10'N 95° 8'W) in Brazoria County. Seeds were placed in paper bags and stored at 20°C under dark dry conditions (Bonner 1974; Conway 1997). Seed viability-tetrazolium tests were not conducted because viability has been shown to be high in this species (i.e., 88-98%) (Bruce 1993).

Tallow seeds were exposed to three experimental regimes: (1) soaking in a continual cold water rinse for 6, 20, 48, or 72 h (Wester 1991,

Table 1. Means and standard errors of percent germination for Chinese tallow seeds exposed to soaking, soaking and chilling, and control treatments during seed germination experiments performed under laboratory conditions in February 1997.

Treatment $(n = 3 \text{ for each})$	Mean % $(\overline{x})$	SE
Control	1.67	1.67
6 hr Soak w/o Chilling	0.00	0.00
20 hr Soak w/o Chilling	3.33	1.67
48 hr Soak w/o Chilling	3.33	1.67
72 hr Soak w/o Chilling	10.00	2.89
6 hr Soak w/Chilling	3.33	1.67
20 hr Soak w/Chilling	3.33	3.33
48 hr Soak w/Chilling	3.33	1.67
72 hr Soak w/Chilling	3.33	3.33

1995); (2) soaking in a continual cold water rinse treatment for 6, 20, 48 or 72 h and then chilling in a refrigerator (4.5°C) for 7 days to simulate winter storm events; and (3) a control, with no soaking nor chilling. Soaking and cold stratification regimes are commonly used to stimulate germination of woody plants (Wester 1991). For each treatment within each regime, three replicated 10 cm sterilized Petri dishes (i.e., three dishes for 6 h soaking, three dishes for 20 h soaking, etc.) each sown with 20 tallow seeds were used. Each dish was lined with a single sheet of Whatman No. 1 filter paper and irrigated with 5 mL of distilled water (Zhu & Mallik 1994). Dishes were placed into a Stults Scientific 100% humidity germination chamber. Temperature/light was maintained at 30°C for 16 h light and 25°C for 8 h dark (Zhu & Mallik 1994) for 35 days (Conway 1997). Percent germination was calculated for each dish. Percent data were then arcsine transformed to meet parametric assumptions (Zar 1996). Analysis of variance (ANOVA) was used to examine differences in percent germination among treatment regimes.

Percent germination did not vary (F = 1.18; 8, 18 df; P = 0.359) among the soaking, soaking and chilling, and control treatments. Percent germination ranged from 0% germination for seeds exposed to continual cold water rinse for 6 h to 10% for seeds soaked for 72 h, but not chilled (Table 1).

Tallow seeds were difficult to germinate under simulated natural conditions. Although not significant, highest germination rates were observed for seeds exposed to 72 h of a continual cold water rinse. Conversely, low germination rates (1.67%) were observed for control treatments. Low germination rates for control treatments may indicate that tallow seeds require some soaking treatment for germination to be

initiated. Seed imbibition is commonly required to initiate seed germination via water uptake and tissue rehydration in many species (Wester 1995). However, future investigations of tallow seed respiration processes, hormonal activity and responses to varying light and temperature regimes are needed.

Although germination was low, if tallow seed production is compared with seed germination rates, successful establishment still occurs, via high seed production. A single tallow seed weighs approximately 0.167g and if established tallow woodlands produce up to 4,500 kg seeds per ha per yr (Scheld et al. 1984), then 26.9 million seeds per ha per vr would be produced. Therefore a 2% germination rate (representative of germination rates for the control treatment of this study) would still account for over 500,000 germinated seeds per ha per yr. If these data are extrapolated to a landscape level, where tallow covers 30-40,000 ha in Brazoria County (NRCS 1993), 16.2-21.6 billion seeds per yr may germinate in Brazoria County alone. Because coastal Texas often experiences winter storm events, tallow seeds are likely exposed to flooded conditions, which may in turn enhance germination rates. Even low germination rates are problematic when evaluated in terms of potential annual seed load combined with potential seedling survival at a landscape level. Consequently, future efforts should focus on (1) preventing tallow establishment in areas where it is presently absent and (2) delineating and documenting specific germination requirements for this species under field conditions.

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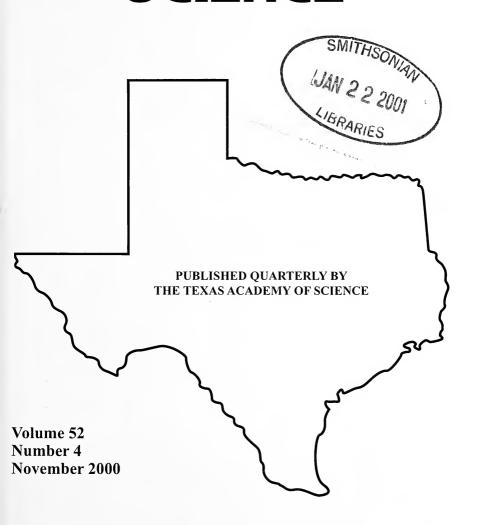
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# A REVIEW OF 20TH CENTURY RANGE EXPANSION AND POPULATION TRENDS OF THE GOLDEN-FRONTED WOODPECKER (MELANERPES AURIFRONS): HISTORICAL AND ECOLOGICAL PERSPECTIVES

#### Michael S. Husak and Terry C. Maxwell

Department of Biological Sciences, Mississippi State University Mississippi State, Mississippi 39762 and Department of Biology, Angelo State University San Angelo. Texas 76909

Abstract.—Within the United States, the overall breeding range of golden-fronted woodpeckers (*Melanerpes aurifrons*) appears to have expanded northward and westward during the last century. These distributional changes have occurred concurrently with population fluctuations in regions of historical occupation and changes in regional habitat. To date, there exist no comprehensive review of these changes in the literature. For that reason, a review of historical and recent literature, as well as breeding bird atlas data, was conducted to determine when and where changes in golden-fronted woodpecker geographic distribution have occurred. North American Breeding Bird Survey data were reviewed to determine regional population fluctuations in species densities, especially as they relate to geographic distribution. It was determined that between 1900 and 2000, golden-fronted woodpeckers expanded their breeding range within the United States about 300 km westward in Texas and about 400 km north and northwest into the Texas panhandle and southwestern Oklahoma. Periods of most noticeable expansion were concentrated during the 1930s, 1950s and 1980s. Data available in the literature on regional changes in habitat characteristics were used to speculate on mechanisms involved in range expansion and population trends.

The golden-fronted woodpecker (*Melanerpes aurifrons*) is a sedentary species of temperate and tropical Middle America. Occupying a variety of both xeric and mesic environments, golden-fronted woodpeckers are found in habitats ranging from arid scrub to tropical deciduous forest. Within its limited U.S. range, this species is most closely associated with mesquite (*Prosopis glandulosa*) brushlands and riparian corridors (Husak & Maxwell 1998).

The 7th edition of the A.O.U. Checklist of North American Birds (AOU 1998), describes golden-fronted woodpeckers as "Resident from southwestern Oklahoma and north-central Texas south through central Texas (west to the Big Bend region), Mexico..., Guatemala..., Belize..., El Salvador... and Honduras..." The 1st edition (AOU 1886) describes this species' range as south Texas southward, suggesting considerable range expansion to the north and west during the 1900s. Many large-scale accounts of golden-fronted woodpecker distribution are vague, conflict-

ing, and fail to address historical changes. While some detailed regional accounts of range expansion such as Sutton (1967) and Baumgartner & Baumgartner (1992) for Oklahoma and Pulich (1988) for north Texas do exist, no previous accounts have addressed range expansion of this species within the United States comprehensively or in reference to historical events.

The goal of this paper was to address the following questions: (1) When and to what extent has the golden-fronted woodpecker extended its breeding range within the United States during the 20th century? (2) Of apparent range expansion, how much can be supported by evidence of actual expansion and not just a reflection of increased knowledge of local avifauna? (3) What factors have contributed to range expansion? Also of interest was overall and regional 20th Century population trends and their potential relationship to range expansion.

#### **METHODS**

A literature review of historical and recent records of breeding golden-fronted woodpeckers was conducted. Included in this review were regional records from *American Birds*, *Field Notes* and *North American Birds*, unpublished nesting records from the Texas Breeding Bird Atlas Project (TBBAP; collected 1987-1992) and Oklahoma Breeding Bird Atlas Project (OBBAP; collected 1997-1999), and regional surveys and field guides. For atlas data, only "Confirmed" and "Probable" records were considered. Breeding Bird Survey (BBS) data (Sauer et al. 1999) were used to address regional population trends. Known breeding ranges of golden-fronted woodpeckers for four time periods within the 20th century (1900, 1940, 1970 and 2000) were mapped at the county level, except for the large counties of the Trans-Pecos region of Texas where more detailed distributional limits were warranted.

#### RESULTS AND DISCUSSION

Early descriptions of the breeding distribution of golden-fronted woodpeckers limit the species to South Texas, ranging north only to the Guadalupe River and San Antonio, Bexar County, Texas (Ridgeway 1885; AOU 1886). However, by 1900, further avifaunal surveys expanded this species range into Central and west-central Texas (Ridgeway 1914). In particular, Lloyd (1887) found golden-fronted woodpeckers as far west as Reagan County, Texas, in the Concho Valley region, and Hasbrouck (1889) found breeding individuals as far north as Eastland

County, Texas. Figure 1a illustrates the known distribution of golden-fronted woodpeckers as of 1900. In the subsequent 100 years, golden-fronted woodpeckers have apparently extended their breeding range about 300 km westward into the Trans-Pecos region of Texas (to Presidio County), about 400 km northwest into the Panhandle region of Texas (to Potter County), about 280 km north into the southwestern corner of Oklahoma (to Greer County), and 175 km eastward, primarily into the East Texas Tallgrass Prairie region (to Ellis County).

Changes 1900-1940.—During this time period, the known breeding distribution of golden-fronted woodpeckers extended primarily northward (about 100 km) to Young County, Texas and northwest (about 350 km) to the Palo Duro Canyon region of the Texas panhandle (Stevenson 1942; Fig. 1b of this study). This range expansion appears to be real and not simply the result of lack of knowledge regarding avifaunal distribution at the turn of the century. Pre-1900 regional surveys by Lloyd (1887) and Hasbrouck (1889) report well-defined boundaries for this species to the north and west (surveys covered areas well beyond the reported range of the species). Furthermore, Ragsdale (1890) specifically looked for golden-fronted woodpeckers breeding to the north, northwest, and northeast of these boundaries and was unsuccessful (although wintering individuals were observed as far north as Young County, Texas). Neither McCauley (1877) nor Strecker (1910) encountered golden-fronted woodpeckers in their early surveys of the upper Red River or Panhandle region.

Changes 1940-1970.—By 1970, golden-fronted woodpeckers had expanded another 180 km northward into north-central Texas and southwestern Oklahoma (Fig. 1c). Distributional changes during this time are well documented. Although Wolfe (1956) makes no mention of golden-fronted woodpeckers in Texas counties along the Red River, Oberholser (1974) reported breeding specimens collected from Hardeman and Wilbarger counties of Texas. First reported in Oklahoma in 1954, golden-fronted woodpeckers were found breeding in Harmon County, Oklahoma in 1958 (Sutton 1967). By 1967, golden-fronted woodpeckers were regular breeders in Harmon, Jackson, Greer and Tillman counties of Oklahoma (Sutton 1967) and by 1970 were reported as post-breeding season visitors in Beckham, Comanche, Roger Mills, Caddo, Ellis and Custer counties, Oklahoma (Baumgartner & Baumgartner 1992). Oberholser (1974) also extended the breeding distribution westward to Val Verde and Crocket counties, Texas, and southeastward to Lavaca County, Texas.

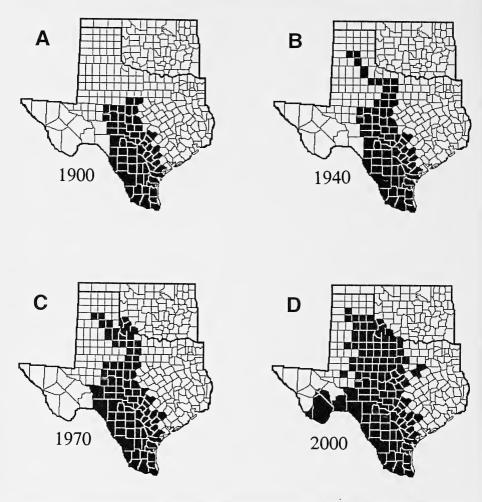


Figure 1. Breeding range of golden-fronted woodpeckers in Texas and Oklahoma for (a) 1900 (following Ridgeway 1885, AOU 1886, Lloyd 1887, Hasbrouck 1889, Ragsdale 1890); (b) 1940 (following Ridgeway 1914, Simmons 1925, Bent 1939, Stevenson 1942); (c) 1970 (following Wetmore 1948, Sutton 1967, Wauer 1973, Oberholser 1974); and (d) 2000 (following Pulich 1988, Baumgartner & Baumgartner 1992, Rappole & Blacklock 1994, TOS 1995, Peterson & Zimmer 1998, Oklahoma Breeding Bird Atlas Project, Texas Breeding Bird Atlas Project).

Changes 1970-2000.—During the last 30 yrs, golden-fronted woodpeckers expanded their breeding range 250 km west into the Trans-Pecos region, slightly north in the Texas panhandle to Potter County, Texas, and about 75 km east to Ellis County, Texas (Fig. 1d). No further expansion has been noted in Oklahoma (Grzybowski 1986; Baumgartner & Baumgartner 1992; OBBAP).

Within the Trans-Pecos region, expansion was relatively rapid. During the 1970s, golden-fronted woodpeckers were considered uncommon visitors to the Big Bend region. However, following the first confirmed breeding by this species in 1982 (Williams 1982a), populations in Big Bend National Park and Alpine, Brewster County, Texas rapidly increased during the late 1980s (Lasley & Sexton 1988). By 1996 golden-fronted woodpeckers were considered common residents in local riparian woodlands (Wauer 1996). Individuals originally observed near Presidio, Presidio County, Texas in 1991 (Lasley & Sexton 1992a) and 1992 (Lasley & Sexton 1992b) currently represent the western most confirmed breeding population in Texas. Individuals have become common along Alamita Creek, Presidio County (Lasley & Sexton 1992b). Possible breeders have been observed as far west as Ruidosa, Presidio County, Texas (TBBAP) but await further confirmation. Thornton (1951) did not mention golden-fronted woodpeckers in his survey of northern Terrell County, Texas, and, no recent surveys appear to have been published for the county. However, R. Dawkins (pers. com.) has observed and banded breeding individuals at Independence Creek, Terrell County since 1993.

Populations in the Trans-Pecos region, although very localized, appear to have the potential for continued expansion. Individuals have been reported wandering outside of the breeding season in all other Trans-Pecos counties except El Paso County (Peterson & Zimmer 1998).

Following several winter appearances in the preceding 35 years (Williams 1982b), golden-fronted woodpeckers were first reported breeding in Midland County, Texas in 1983 (Williams 1983), and they have apparently continued to breed locally since that time (TBBAP). Local distribution of golden-fronted woodpeckers has also increased in the Panhandle region. Of note is the westward expansion to Lubbock County, Texas (Williams 1973a; 1973b; TBBAP) and observations of possible nesting in Bailey County, Texas near the New Mexico border (TBBAP). Although Pulich (1988) questioned the breeding of golden-fronted woodpeckers east of Palo Pinto County in north-central Texas, TBBAP data indicate nesting east to Parker County, Texas. The observed breeding in Ellis County (TBBAP) further expanded the range of this species into the East Texas Tallgrass Prairie region.

Compared to other regions, eastward expansion has been minimal. Root (1988) suggested that increased precipitation in eastern Texas effec-

Table 1. Regional population trends of golden-fronted woodpeckers for 1966-1998 based on Breeding Bird Survey data (Sauer et al. 1999).

	1966-1998		
BBS Region	Trend <sup>a</sup>	P	$N^{\mathrm{b}}$
United States	-1.6	0.13	77
Texas	-1.6	0.16	75
Oklahoma	-25.0	0.28	2
South Texas Brushlands	-1.9	0.06	30
East Texas Prairie	-16.6	0.71	3
Osage Plain-Cross Timbers	-1.0	0.44	6
Edward's Plateau	-0.8	0.63	20
Red Rolling Plains	6.3	0.02	15

<sup>=</sup> Percent change per year.

tively prevents further range expansion by golden-fronted woodpeckers. Of greater importance may be the closely related red-bellied woodpecker (*M. carolinus*), which competes with golden-fronted woodpeckers for resources (Selander & Giller 1959) and which is the most common woodpecker species throughout forested east Texas (Shackelford & Conner 1997). Its presence may effectively limit eastward expansion by golden-fronted woodpeckers, a factor warranting further field study.

Regional population trends.—Overall, BBS data (1966-1998) indicate no significant changes for U.S. populations (Sauer et al. 1999; Table 1). Likewise, most physiographic regions have had minimal changes, although slight negative trends are evident for many regions. The apparent decrease in East Texas Tallgrass Prairie is probably due to small sample size (n=3) from an area that the golden-fronted woodpecker has only recently invaded. The increase in the Red Rolling Plains region is indicative of the localized expansion in the Texas panhandle.

The observed range expansion during the last few decades, and the lack of significant declines in regional populations suggest that overall golden-fronted woodpecker numbers have increased in the United States during the past three decades. However, prior to the initiation of BBS routes in 1966, golden-fronted woodpeckers may have experienced sharp declines in numbers, as has been suggested anecdotally in the literature. During the 1930s, golden-fronted woodpeckers were targeted by hunters

b = Number of Breeding Bird Survey routes included in analysis.

and utility crews because of damage the birds inflicted upon utility poles and fence posts (Bent 1939). It is unclear to what extent the species was affected or to what degree populations have rebounded in subsequent decades. Furthermore, Oberholser (1974) suggested dramatic population declines in south and central Texas during the 1960s following habitat loss resulting from large-scale removal of mesquite trees on rangeland. Again, degree of population-wide impact is unknown.

Potential reasons for range expansion.—Why the range of any species changes may be due to a number of factors ranging from habitat changes (loss or gain), to interspecific competition, to introduction to new areas (Jackson & Davis 1998). Golden-fronted woodpecker breeding range expansion probably involves a number factors, but the following are proposed as the most significant factors.

- (1) MESQUITE TREE DENSITY. During the past century, mesquite trees have increased in density throughout the southwestern U.S.A., including the once expansive grasslands and savannahs of Texas and Oklahoma (Bogusch 1952; Johnston 1963). This increase in potential nesting and foraging resources has undoubtedly allowed goldenfronted woodpeckers to move into once uninhabitable regions of western Texas, the Texas Panhandle, and prairie and cross timber regions of Oklahoma and Texas.
- (2) URBAN DEVELOPMENT. Towns in semi-arid regions, with their landscaped yards and man-made reservoirs, have provided new expanses of a variety of tree species. Many of the regional breeding records (especially the Texas panhandle) are located in or near such urban developments.
- (3) UTILITY POLES AND FENCE POSTS. Installation of utility poles and fence posts over the last 150 yr has provided nesting and foraging sites in areas void of trees. Even early descriptions of golden-fronted woodpecker breeding ranges note the use of telegraph poles in areas of sparse woody vegetation (e.g., Ragsdale 1890), which begs the question "Did pre-1900 range descriptions already reflect a recently altered range?"
- (4) LACK OF INTERSPECIFIC COMPETITORS. The only other resident woodpecker found in much of the area recently invaded by golden-fronted woodpeckers (except to the east) is the ladder-backed wood-

pecker (*Picoides scalaris*), a considerably smaller species that appears to occupy a different niche than that of the golden-fronted woodpecker (Short 1982; Husak 2000). The lack of competing species in areas of recently increased tree density or utility pole installation may have permitted easy range expansion.

- (5) Post-breeding Nomadism. Golden-fronted woodpeckers are well known for late summer and winter wandering at considerable distances from their breeding range in Texas and Oklahoma (Texas Ornithological Society 1995; Husak & Maxwell 1998). More extreme distances include New Mexico, Michigan (AOU 1998), and possibly Florida (Stevenson & Anderson 1994). This habit may predispose golden-fronted woodpeckers for finding suitable habitats not previously occupied. Many of the areas expanded to during the 1980s and 1990s were sites golden-fronted woodpeckers were previously recorded as winter vagrants for several years.
- (6) DROUGHT INDUCED DISPERSAL. Periods of most noticeable expansion by golden-fronted woodpeckers were concentrated during the 1930s, 1950s and 1980s. While this may be an artifact of publication time, it is worth noting that these are also decades of prolonged moderate to extreme drought in Texas. It has previously been observed that drought may significantly reduce resources for birds, thus initiating increased dispersal by members of populations to new areas in search of food (Takekawa & Bessinger 1989). Unfortunately, no data are currently available regarding factors influencing dispersal by this species, but the potential influence of droughts should be considered.

It will be interesting to monitor this species in the following decades to determine if this expansion will persist and/or continue to increase.

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MSH at: msh2@ra.msstate.edu

#### AVIAN DYNAMICS OF A CHIHUAHUAN DESERT CREOSOTEBUSH (*LARREA TRIDENTATA*) COMMUNITY IN NUEVO LEON, MEXICO

Armando J. Contreras-Balderas, Juan A. Garcia-S., Jose I. Gonzalez-R. and Antonio Guzman-V.

Laboratorio de Ornitología, Facultad de Ciencias Biológicas Universidad Autónoma de Nuevo León Apartado Postal 425, San Nicolás de los Garza Nuevo León, México 66450

Abstract.—This study examined the avifauna of a Chihuahuan Desert creosotebush community in the state of Nuevo León in northeastern Mexico from May 1995 to April 1996. Based upon foraging strategies, the 48 species recorded were grouped into seven functional groups (or guilds) which were further divided into 12 subgroups. The diversity of foraging strategies was found to be high in this area of Nuevo León and is compared with other localities in the Chihuahuan Desert. Due to its richness and number of functional subgroups, this area represents an important site in the avian diversity of the Chihuahuan Desert.

Resumen.—El presente trabajo, estudia la avifauna de una comunidad de Gobernadora (*Larrea tridentata*) en el Desierto Chihuahuense en el estado de Nuevo León, NE de México, de mayo de 1995 a abril de 1996. Basado en las estrategias de forrajeo, se registran 48 especies incluidas en siete grupos funcionales subdivididos en 12 subgrupos. La diversidad de estrategias de forrajeo, fue encontrada alta en esta área de Nuevo León, y es comparada con otras localidades del Desierto Chihuahuense. Esta área, representa un sitio importante del Desierto Chihuahuense, tanto por su riqueza, como por el numero de subgrupos funcionales presentes.

Many studies have examined the structure and temporal changes of avian communities in desert habitats of North America. Tomoff (1974) examined the diversity of avian species in the desert scrub of the Sonoran Desert of southwestern Arizona. Based upon the diversity of breeding bird species, the study recognized seven distinct subgroups of foraging categories. Thiollay (1979) studied the structural dynamics and seasonal changes of birds in two different sites in the Bolson de Mapimi in the Chihuahuan Desert of northern Mexico. He studied low plain (playa) habitat and that of mountain slopes (cerro). Granivorous birds were three times more abundant in the playa site than insectivores during the summer, but decreased to one-half during the winter. In the shrubby habitats (cerro), insectivorous birds exhibited the same overall density as granivores during the breeding season. In contrast, Landres & MacMahon (1980) examined the community organization of avifauna in an oak woodland habitat in Sonora, Mexico. They recognized only five foraging strategies and reported that species within different strategy groups were most often separated by food-site and perch height.

Blake (1984) examined seasonal changes of bird communities of a creosotebush habitat in southern Nevada and found that foliage insectivores were more abundant, although fluctuations in abundance were likely for a variety of different factors, including resources, climate, etc. Thiollay (1985) examined the differences between two species of insectivores exhibiting different foraging strategies in the Chihuahuan Desert of northern Mexico. Babb-Stanley & Verhulst-R. (1992) analyzed the avifauna in the southern limit of the Chihuahuan Desert in Durango and reported the presence of 92 species of birds in six different foraging strategies. Insectivores were the most abundant with 27 species. Garcia-Salas et al. (1995) compared the distribution of 66 species of birds from eight additional Chihuahuan Desert localities with those from Cuatrocienegas.

Recently Contreras-Balderas et al. (1997) examined the ecological and seasonal distribution of the avifauna of the Cuatrocienegas region of Coahuila and reported the presence of 87 species of birds. Finally Garcia-Salas et al. (1997) examined the trophic structure of birds in the creosotebush communities of Cuatrocienegas and reported the presence of 31 species in five foraging strategies. Insectivores comprised the major feeding group accounting for 65% of the permanent residents, 71% of the summer residents and 100% of the migrant species.

This current study was undertaken to examine the avifauna of a creosotebush community located in the eastern margin of the Chihuahuan Desert near the municipality of García in the state of Nuevo León, México.

#### STUDY SITE

García is located approximately 35 km northwest of Monterrey in the state of Nuevo León, México. The study site (25°54'48" N and 100°44'27" W) is arid with an annual precipitation of 200-400 mm and exhibits an annual average temperature of 18-20°C (mean of colder months of December-January 13-14°C, and hottest month of July 28-29°C). The elevation at the study site ranges from 595 to 685 m. This site is located in the extreme east central margin of the Chihuahuan Desert. The vegetational community is dominated primarily by the creosotebush *Larrea tridentata*, intergrading with Tamaulipan thorn scrub species, that are not typical of the Chihuahuan Desert. Other species of plants present are: anacahuite (*Cordia boisieri*), black brush (*Acacia rigidula*), ocotillo (*Fouqueria splendens*), coyotillo (*Karwinskia humoldtiana*), beargrass (*Yucca sp.*), lechugilla (*Agave lecheguilla*), leather stem (*Jatropha dioica*), tree cholla (*Opuntia imbricata*), desert

christmas cactus (*Opuntia leptocaulis*), candelilla (*Euphorbia antisiphylitica*), hedgehog cactus (*Echinocereus* sp.) and several species of cactus (*Opuntia microdesys* and *Opuntia* sp.).

#### METHODS AND MATERIALS

This study began in May 1995 and extended through April 1996. The study site was visited 24 (two/month) times during this time period. Censusing techniques followed those of Skirvin (1981); they began at sunrise and were continued for approximately four hours. Species were registered following the systematic nomenclature of the A.O.U. (1998). Observed species were categorized as permanent resident (present year around), summer resident (recorded only during summer), winter resident (present only in autumn or winter) and transient (present only once during the year). Assignment of species to foraging strategies followed the criteria of Ehrlich et al. (1988) but was modified for some species.

#### RESULTS AND DISCUSSION

Based upon foraging strategies, the 48 species observed and recorded during this study were divided into seven functional groups. These are carrion, prey, insectivorous, granivorous, nectivorous, omnivorous and frugivorous. The prey group was subdivided into two subgroups (high patrol and swoops) and the insectivorous group subdivided into five subgroups (swoops, ground glean, aerial, bark glean and foliage). This results in the seven foraging strategies being divided into 12 functional subgroups. These foraging strategy groups/subgroups are given in Table 1 and the species assigned to each group are also given. The residency of each species is also given.

Table 2 lists the number of species in each of the 12 foraging groups/subgroups for each season of the year. Table 3 gives the number of species present along with the number of foraging groups/subgroups during each season of the year. Four foraging strategy groups exhibited no change during the course of this study. These were carrion/high patrol, prey/high patrol, insectivorous/bark glean and frugivorous/ground glean (Table 2). These four groups are dominated by species which were permanent residents (Table 1). The number of species in the eight remaining groups are given in Table 2 and Figure 1. The results of Figure 1 reveal that those foraging strategies including insectivorous, granivorous and omnivorous birds included the larger number of species but exhibit minimal change in numbers of species from season to season. In general, these eight foraging groups/subgroups are

Table 1. Avian species and their respective foraging stratigies for the Creosotebush (*Larrea tridentata*) community in García, Nuevo León, México. The residency is given for each species. Three species marked with an asterisk (\*) were not observed during all seasons of this study, but are considered permanent residents based upon data from other studies and observations.

Carrion/high patrol

Turkey vulture (Cathartes aura)

Permanent resident

Prey/high patrol

Red-tailed hawk (Buteo jamaicensis)

Permanent resident

Prey/swoops

Great horned owl (Bubo virginianus)

Permanent resident

Insectivorous/swoops

American kestrel (Falco sparverius) Loggerhead shrike (Lanius ludovicianus) Winter resident Winter resident

Insectivorous/ground glean

Long-billed curlew (Numenius americanus)
Cactus wren (Campylorhynchus brunneicapillus)

Rock wren (Salpinctes obsoletus)
Bewicki wren (Thryomanes bewickii)
Northern mockingbird (Mimus polyglottos)
Blue grosbeak (Guiraca caerulea)

Brewer's sparrow (Spezella breweri) Black-throated sparrow (Amphispiza bilineata) Grasshopper sparrow (Ammodramus savannarum) Transient
Permanent resident
Permanent resident
Permanent resident
Permanent resident
Summer resident
Transient
Permanent resident

Winter resident

Insectivorous/aerial

Lesser nighthawk (Chordeiles acutipennis)
Least flycatcher (Empidonax minimus)
Flycatcher (Empidonax sp.)
Says's phoebe (Sayornis saya)
Ash-throated flycatcher (Myiarchus cinerascens)

Great-crested flycatcher (Mytarchus tyrannulus)
Eastern kingbird (Tyrannus tyrannus)

Barn swallow (Hirundo rustica)

Summer resident Winter resident Winter resident Winter resident Summer resident Winter resident Transient

Summer resident

Insectivorous/bark glean

Ladder-backed woodpecker (*Picoides scalaris*) Verdin (*Auriparus flaviceps*)

verdin (Auriparus Jiavice)

Permanent resident Permanent resident

Insectivorous/foliage glean

Ruby-crowned kinglet (Regulus calendula)
Black-tailed gnatcatcher (Polioptila melanura)
Blue-gray gnatcatcher (Polioptila caerulea)
White controls (Virus caircins)

White-eyed vireo (Vireo griseus)
Bell's vireo (Vireo hellii)

Bell's vireo (Vireo bellii).
Orange-crowned warbler (Vermivora celata)
Nashville warbler (Vermivora ruficapilla)
Townsend's warbler (Dendroica townsendi)
MacGillivray's warbler (Oporornis tolmiei)
Wilson's warbler(Wilsonia pusilla)

Wilson's warbler(Wilsonia pusilla) Scott's oriole (Icterus parisorum) Winter resident
Permanent resident
Winter resident
Transient
Summer resident
Winter resident
Transient
Transient
Winter resident
Transient
Summer resident

Granivorous/ground glean

Scaled quail (Callipepla squamata)
Inca dove (Columbina inca)
White-winged dove (Zenaida asiatica)
Mourning dove (Zenaida macroura)
Pyrrhuloxia (Cardinalis sinuatus)

Permanent resident Permanent resident Transient Permanent resident Permanent resident

#### Table 1 cont.

F

Lazuli bunting (Passerina amoena) Brown towhee (Pipilo fuscus) Brown-headed cowbird (Molothrus ater) House finch (Carpodacus mexicanus)	Summer resident *Permanent resident Summer resident *Permanent resident
Nectivorous/hover & glean Black-chinned hummingbird ( <i>Archilochus alexandrei</i> )	Winter resident
Omnivorous/ground glean Common raven ( <i>Corvus corax</i> ) Great-tailed grackle ( <i>Quiscalus mexicanus</i> )	Permanent resident *Permanent resident
Frugivorous/ground glean Curve-hilled thrasher (Toxostoma curvirostre)	Permanent resident

characterized by the lack of permanent resident species. Four of these (prey/swoops, insectivorous/swoops, insectivorous/aerial and nectivorous/hover and glean) exhibit no permanent resident species (Table 1). The percent permanent residency in the remaining four groups are insectivorous/foliage (9%), omnivorous/ground glean (50%), insectivorous/ground glean (56%) and granivorous/ground glean (67%).

The results of this study differ in the number of functional groups/subgroups of foraging strategies when compared to other studies conducted in different regions of the Chihuahuan Desert. Tomoff (1974) reported 3/7, Thiollay (1979) 5/10, Babb-Stanley & Verhulst-R. (1992) 6/0 and Garcia-Salas et al. (1997) 5/0 respectively. The results of this study at García in Nuevo León recognizes seven functional groups (or guilds) with 12 subgroups. In comparison, Blake (1984) reported 47 species of birds in only four groups/seven subgroups from a creosote-bush community, but in another desert of Nevada. This increase in the overall number of foraging strategies observed at García is important in understanding the role of the scrub community in providing this diversity of foraging strategies.

An examination of Table 3 reveals that there is little change in the number of species as well as numbers of groups/subgroups of foraging strategies from season to season.

It is important to understand the role of the creosotebush in determining the high diversity of foraging strategies observed in this area of the Chihuahuan Desert. Upon examination, the increase in foraging strategies observed at García is a result of the diversity of insectivorous species. Five different subgroups (swoops, ground glean, aerial, bark glean, foliage) of foraging strategies were observed. Considering that the creosotebush represents a uniformly stable plant community, it would

1

1

5 2 5

4

0

1

1

Insectivorous/aerial Insectivorous/bark glean Insectivorous/foliage

Granivorous/ground glean

Omnivorous/ground glean

Frugivorous/ground glean

Nectivorous/hover & glean

	Spring	Summer	Autumn	Winter
Carrion/high patrol	1	1	1	1
Prey/high patrol	1	1	1	1
Prey/swoops	0	1	0	0
Insectivorous/swoops	0	1	1	2
Insectivorous/ground glean	8	5	7	5

3 2 6

8

1

2

Table 2. Number of species of each group/subgroup of foraging strategies for each season.

Table 3.	Number of species,	groups and	subgroups	of foraging	strategies	present	during
each s	season of study.						

	<u>Species</u>	<u>Groups</u>	Subgroups
Spring	33	7	10
Spring Summer	29	7	12
Autumn	30	6	11
Winter	26	7	12

appear unlikely that this area would exhibit such an unusually high variation in its production of insects. It would then appear that the increased variation of foraging strategies of insectivorous birds is dependent upon the variation in the productivity of the desert ecosystem rather than being specifically dependent upon the Creosotebush, or the presence of other species besides *Larrea*. This proposal is supported by the observed foraging strategies of three of the remaining six primary foraging groups. The nectivorous species was observed to be present only following periods of rain and the omnivorous and frugivorous species were observed migrating to and from areas of Tamaulipan scrub which is located near the study site. Additionally, this area is unusual in having several species that are more typical of the Tamaulipan; these are the Blue grosbeak, Bell's vireo, MacGillivary's warbler and Lazuli bunting.

#### Conclusions

Based upon foraging strategies, the 48 species of birds recorded from the creosotebush community in Nuevo León were grouped into seven functional groups which were further divided into 12 subgroups. This

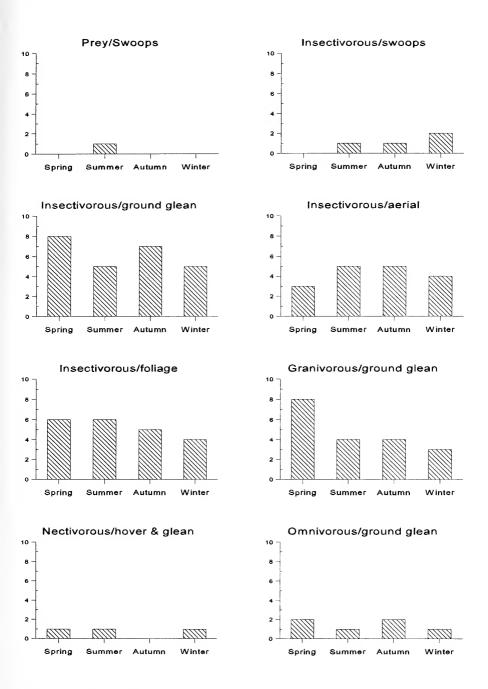


Figure 1. Seasonal changes of number of species of birds in select foraging strategies of the creosotebush community.

diversity of foraging strategies is high when compared to other regions of the Chihuahuan Desert and is attributed to insectivorous species. While the exact nature of the basis for this increased diversity of insectivorous foraging strategies remains unclear, it appears to be dependent upon variation in productivity of the desert ecosystem rather than upon the creosotebush *Larrea tridentata*.

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#### CONSERVATION OF ISLA SOCORRO, MEXICO: THE IMPACT OF DOMESTIC SHEEP ON THE NATIVE PLANT COMMUNITIES

## S. Alvarez-Cárdenas, P. Galina-Tessaro, A. Castellanos and A. Ortega-Rubio

Centro de Investigaciones Biológicas del Noroeste Apartado Postal No. 128, La Paz, 23090 Baja California Sur, México

Abstract.—The impact of feral sheep on the natural resources at Isla Socorro, the largest of the four Pacific islands that constitute the Revillagigedo Archipelago Biosphere Reserve off the northwest coast of Mexico, has the potential to severly damage this ecosystem. The feral sheep population, which is concentrated on the southern part of the island, was determined through surveys during 1988 and 1990. Direct counts indicate the present population size to be well over the carrying capacity of the island's ecosystems. As a result of the high population density and the overgrazing, there is severe erosion on 30% of the island. If the feral sheep of Isla Socorro are not totally removed in the very near future, this Biosphere Reserve and the world's natural heritage will likely lose all endemic species on this island.

Resumen.—El impacto del borrego feral sobre los recursos de la Isla Socorro, la más grande de las cuatro islas que constituyen la Reserva de la Biosfera del Archipiélago de Revillagigedo alejada de las costas del Noroeste de México, tiene el potencial de dañar severamente este ecosistema. La población del borrego feral, la cual se concentra en la parte sur de la isla, fue determinada por medio de censos durante 1988 y 1990. Los conteos directos indican que el tamaño de la población es mayor que la capacidad de carga del ecosistema isleño. Como resultado de la alta densidad poblacional y el sobrepastoreo, hay erosión severa en el 30% de la isla. Si el borrego feral de la Isla Socorro no es totalmente removido en un futuro inmediato, esta Reserva de la Biosfera y la herencia natural mundial perderán probablemente todas las especies endémicas de esta isla.

Isla Socorro is the largest of the four islands that constitute the Revillagigedo Archipelago Biosphere Reserve in the Mexican Pacific Ocean (Fig. 1). Given its large number of endemic plants and animals (Levin & Moran 1989; Brattstrom 1990; Ortega et al. 1992) Isla Socorro is a priority area for the conservation of the biological diversity of Mexico (Ortega et al. 1992) and of the world (IUCN 1980; ICBP 1992). About 33% of all vascular flora (Levin & Moran 1989), two species and eight subspecies of birds, and one species of reptile are endemic (Brattstrom 1955; 1990). The island lacks native mammals and amphibians. There are three introduced mammalian species: the domestic cat (*Felis catus*), the house mouse (*Mus musculus*) and the sheep (*Ovis aries*). The sheep introduced on the island in 1869 (Hanna 1926) are now considered feral.

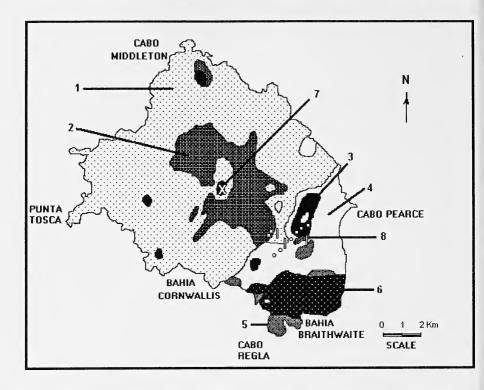


Figure 1. Location of Isla Socorro and vegetation types: (1) Mixed scrub, (2) Forest (Ficus/Guetarda/Ilex), (3) Ficus forest, (4) Eroded areas and Human disturbed areas, (5) Induced prairie and grassland, (6) Deciduous scrub (Croton/Opuntia), (7) Highland prairie, (8) Psidium forest. "X" denotes the location of Monte Evermann (1040 m).

At present, the biodiversity of this insular ecosystem is in extreme danger because of several factors. These are soil erosion, the loss of vegetation and habitat fragmentation. About 30% of the island's surface area is totally denuded of vegetation (Ortega et al. 1992; 1995) from soil losses varying from 30 to 90 metric tons/ha/year (Maya-Delgado et al. 1994). The erosion is a product of torrential precipitation from annual hurricanes. Of the natural vegetational associations on the island, the guayabillo scrub (*Psidium socorrense*) is most severely affected. Its distribution is restricted to only two reduced areas (León de la Luz et al. 1994).

Loss of the vegetational covering and the consequent erosion are the result of sheep trampling and overgrazing. This herbivore has been introduced on different islands, causing changes to the habitat composition and structure (Wood et al. 1987; Ebenhardt 1988; Van Vuren & Coblentz 1989).

In spite of the effects produced by the sheep on the island, little is known about their distribution (Villa 1960: Levin & Moran 1989; Brattstrom 1990) and abundance (Brattstrom & Howell 1956; Villa 1960; Brattstrom 1990; Wehtje et al. 1993).

This study presents information on the distribution and relative abundance of the non-native sheep on the island, the estimated carrying capacity of habitat, and the effect of these feral sheep on native vegetation and soils.

#### STUDY AREA

Isla Socorro is 400 km southwest of Baja California de Sur, Mexico (18°47'N, 110°57'W) (Fig. 1). The island is about 110 km² (Wehtje et al. 1993) and has a maximum elevation of 1,140 m (Johnston 1931). Island landscape varies widely with canyons and volcanic lava flows covered with short and dense vegetation descending from abrupt cliffs into the sea. The terrain is rugged in the northern half, whereas the southern half is dominated by medium elevations, hillocks and plains. The climate is arid-tropical with mean annual temperature of 24.6°C, varying between 5.0°C in winter to 36.1°C in summer. The mean annual precipitation is 404.7 mm; most of which occurs in summer (Coria-Benet 1994). There are no freshwater sources, except for condensation, runoff and water caught in depressions and some caves after rains.

The vascular flora of the island includes 117 species (Levin & Moran 1989) distributed in eight vegetational associations (Fig. 1) (León de La Luz et al. 1994). Three associations are the most conspicuous: (1) Mixed scrub, covering an area of 8,400 ha is composed mainly of perennial herbaceous shrubs (Dodonea viscosa) and stocky trees (Guetarda insularis and Prunus serotina); (2) Forest, covering 1,600 ha, includes the arboreal communities represented by associations of zapotillo/ guayabillo (Bumelia socorrensis/Psidium galapageium) and fig trees (Ficus cotinifolia) with or without guayabillo; (3) Deciduous scrub (1,120 ha) which has a dominant component of Croton masonii as reported by León de La Luz et al. 1994. In the southern half there are eroded surfaces (about 4,620 ha) with small patches of perennial vegetation. This part of the island is characterized by plains coveredwith grasses, forest vegetation patches and eroded zones of a number of scarce species (Maya-Delgado et al. 1994).

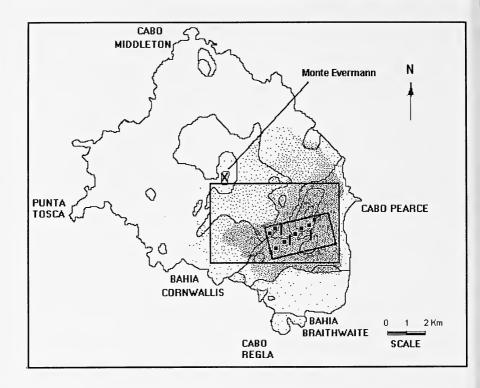


Figure 2. Feral sheep distribution area, and sampled sites for animal counts and carrying capacity estimates. Dots represent distribution and abundance of sheep. "X" denotes the location of Monte Evermann (1040 m).

#### MATERIALS AND METHODS

Distribution and abundance of sheep.—The distribution of sheep was determined during surveys in different topographic zones and vegetational associations (Fig. 2) during April, July and September 1988, and January and February 1990. Individuals, tracks, bedding sites, fecal pellets and vocalizations of sheep were recorded.

During winter 1990 (January-February), the number of sheep in the southern portion of the island was counted in an area of 600 ha. The area was divided in three sections and for three consecutive days, each of the sections was searched with binoculars by an observer located at a strategic high point. A observational "sweeping" of each area, with three repetitions of 20 minutes each, was made from each observation point. All counts were made from 1700 to 1800 h due to the fact that sheep concentrate in pastures during this time period. These observation periods were short to avoid significant displacement of animals and overcounting or repeated counting for the same individual.

The number of sheep in the sampled areas was determined by adding the averages of the three repetitions at each of the observation points over the three days (n=27, and nine man hours of observation). Results were extrapolated to 2,400 ha of similar habitat on the southern portion of the island.

Carrying capacity.—In the same areas where sheep counts were conducted, the habitat carrying capacity was calculated. The accessible vegetational biomass potentially usable as food by sheep was calculated in three representative sites: (1) a forest area, (2) an open grassland and (3) an area with open grassland and forest patches. In each site, a 400 m randomly placed line transect with ten 1 m² circular sampling areas separated by 40 m was marked. A double-sample system was used. First, after a training period, visual estimates of the weight of each plant species present in the sampled areas was made (Pechanec & Pickford 1937). Later in two areas of each transect, a total cut of the vegetation was made to determine its dry weight (Pechanec & Pickford 1937). From the relationship between the dry weight and the estimated weight, a correction factor was obtained to adjust all the estimated values of the plant species. This methodology has been successful in measuring the available biomass for other herbivores (Gallina 1990).

In grassland zones, all herbaceous and grass species were counted and weighed in each sampled area. In the forest zones, all browse material in each sample area below 1.5 m height (maximum foraging height of sheep) was counted.

The habitat carrying capacity (CC) for the winter was calculated using the formulae CC = (ps) (fs) / (cs) (ts) (Rasmussen & Ffolliott 1981; Gallina 1990), taking into account the average of the biomass values (ps), and using a use factor of 0.15 (fs) and a total consumption for sheep of 46.42 kg/individual/month (cs); these values were used by Patton (1992) for domestic sheep. The foraging time for this season (ts) was estimated as four months.

#### RESULTS

Distribution and abundance.—Except for the northern part of the island, feral sheep were observed from sea level to 1,140 m at the Evermann Volcano Peak in all vegetational associations (Fig. 2). On the southwestern part of the island, sheep sightings were rare. The primary zone used by sheep was in the southeastern area between 200-500 m elevation. Herds concentrated and grazed in grassland areas, in open

areas of fig forest and zapotillo/guayabillo forest, and in the mixed scrub. Sheep were observed on the southern and eastern volcano slopes between 500 and 700 m elevation, but their number were less than those of the lower sections.

A total of 389 sheep were recorded in the 600 ha plot. This results in an estimated density of  $0.64 \pm 0.30$  animals/ha. With the extrapolation to 2,400 ha with equivalent habitat in the south-southeast portion of the island, the estimated population of the island was approximately 1,550 sheep.

Biomass and carrying capacity.—The available plant biomass as potential forage for sheep during the winter was 1,003 kg/ha ( $\bar{x}$  334). The highest biomass value was obtained in the open grassland with forest patches 453 kg/ha, the open grassland site was 370 kg/ha, and the site located inside the forest had the lowest biomass quantity at 180 kg/ha. The grasses are mainly *Eragrostis* sp., and they constituted the greater part of the biomass, followed by the herbs *Argemone* sp. and *Nicotiana stocktonii* (Table 1).

Based on estimates of plant biomass in the sample sites, the area would support 0.27 animals/ha. This result implies that the 600 ha sampled could support a total of 160 sheep. The estimate for 2,400 ha of the south-southeast portion of the island would support about 650 sheep in the winter, which is considered the critical season of the year.

#### **DISCUSSION**

The main ecological problem at Isla Socorro is the loss of biodiversity caused by the introduction of exotic species and the collateral effects of their presence (Ortega-Rubio & Castellanos 1995). Domestic sheep have had a severe impact on the vegetation because of intense overgrazing. Their density greatly exceeds the carrying capacity of available habitat. All these habitats on the island could support 650 animals without habitat deterioration. However, the estimated population is about 1,550 animals. This is over 240% of the estimated carrying capacity, causing in consequence, a severe loss of the vegetative cover.

Although these data on sheep numbers and distribution are similar those recorded in the literature (Villa 1960; Levin & Moran 1989; Brattstrom 1990), this study reported that there is no movement of sheep toward the north half of the island. In the norther part of the island, there is very dense vegetation, but the crab (*Gecarcinus planatus*) is abundant. It is diurnal or nocturnal in habit and notably aggressive possibly limiting sheep numbers.

Table 1. Importance of available plant species as potential forage for feral sheep, in the main sheep distribution area on Isla Socorro during winter 1990.

Species		Biomass kg/ha%	% of Biomass
Eragrostis sp.*	AH	391.33	39.00
Argemone sp.	AH	238.00	23.72
Cotiana stocktonii	AH	161.17	16.06
Mitracarpus hirtus	AH	49.96	4.97
Chamaesyce thymifoila	PH	35.80	3.56
Rhynchelitrum repens*	PH	32.36	3.22
Opuntia sp.	SH	28.00	2.79
Cynodon dactylon*	PH	18.71	1.86
Psidium galapageium	TR	16.80	1.67
Boerhavia coccinea	AH	8.19	0.81
Chamaesyce hyssopifolia	PH	7.59	0.75
Dactyloctenium aegyptium*	AH	7.22	0.72
Cenchrus ciliaris*	PH	5.83	0.58
Galium mexicanum	AH	1.57	0.15
Elytraria imbricata	AH	0.42	0.04
Chamaesyce hirta	PH	0.35	0.03

<sup>\*</sup>Grasses, Annual Herbs = AH, Perennial Herbs = PH, Shrubs = SH, Trees = TR.

The present work is the first that has been done in systematic fashion to obtain a record of sheep density on Isla Socorro. Knowing that sheep are scarce outside of the area studied in the south of the island, the total estimated population should not exceed 2,000 animals for the whole island.

This estimate is similar to reports of Brattstrom & Howell (1956) and Wehtje et al. (1993), but differs with the report of Villa (1960) who believed there was a population of 5,000 animals and Brattstrom (1990) who felt there were only 1,000 animals. Those authors based their data on visual sightings without any systematic transect counts.

The sheep have adversely affected the fig and guayabillo forests on the southern half of the island. There are few trees and many of them are dying of old age. There are relatively few new growth areas or saplings and a great number of dead trees still standing (León de la Luz et al. 1994). Levin & Moran (1989) attributed the deterioration of these plant associations directly to sheep.

Not only has the natural vegetation been affected, but also the native fauna. Terrestrial endemic birds of Isla Socorro were widely distributed on the island and most species were relatively abundant at the end of the 19th Century (Grayson 1872; Anthony 1898; Stone 1986). Now, the loss of vegetative cover has caused changes in the distribution and density of the endemic birds (Ortega et al. 1992; Ortega-Rubio &

Castellanos 1995; Ortega et al. 1995). Endemic birds are rare in areas lacking low vegetation, whereas, they are relatively abundant in areas where there are no sheep; therefore, there are several vegetational strata (Rodríguez-Estrella et al. 1994). The density of birds is directly related to plant cover, plant density and vertical stratification. In well vegetated area there are more nesting sites, food and better protection (Morse 1985; Martin 1988; Petit et al. 1988). The decrease in numbers of endemic birds on Isla Socorro, especially those species that forage in the lower strata of vegetation (e.g. *Thryomanes sissonii*, *Parula pitiayumi graysoni*, *Pipilo erythrophtalmus socorroensis*) appears directly related to the scarcity of this strata (Rodríguez-Estrella et al. 1994). Most shrubs and trees are lacking leaves up to the height limit that sheep can reach (1.50 m) to forage.

Although the introduction of domestic ungulates to insular ecosystems has been a common practice in many parts of the Western hemisphere (Wood et al. 1987), their presence has been far more negative than positive. There has been substantial degradation of vegetation on most islands studied (Ebenhard 1988). Loss of vegetational cover is also the main cause of the severe erosion on the island. At Isla Socorro, sheep represent an imminent danger to the existing biological diversity. It is imperative that corrective measures be taken to reverse the deterioration of vegetative cover and soil loss. The total eradication of the feral sheep population on the island appears necessary. Failure to remove the sheep only increases the risk of losing all endemic species of the flora and fauna on Isla Socorro.

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AO-R at: aortega@cibnor.mx

# RODENT HABITATS IN A CHIHUAHUAN DESERT/DESERT PLAINS GRASSLAND ECOTONE

#### Eric E. Jorgensen, Stephen Demarais and Tony Monasmith

USEPA, P. O. Box 1198
Ada, Oklahoma 74820,
Department of Wildlife and Fisheries
Mississippi State University
Mississippi State, Mississippi 39762 and
4714 39th Street, Lubbock, Texas 79414

Abstract.—Habitat associations and diversity of rodents were studied in four vegetation types associated with a Chihuahuan Desert/desert plains grassland ecotone in southern New Mexico during spring 1993 and 1994. Vegetation types included two types of draw (i.e., wide bottomed vegetated drainage) (Rhus and grass) and two types of upland (Acacia and mesa relic) characterized by occurrence of unique shrub and/or grass species. In 14,400 trap nights 1,314 individuals of 16 species were collected. Of these, 13 demonstrated associations with vegetation types that remained constant even as rodent populations increased 99% from 1993 to 1994. Only a population increase of 1,309% for *Perognathus flavus* in grass draws was sufficient to cause a vegetation type x year interaction. Diversity measurements also remained consistent among vegetation types. This suggests that within the ecotone studied, vegetation types support consistent rodent populations when compared to other nearby vegetation types. Ecotones are probably important reservoirs of biodiversity within landscapes. Whereas the prevailing view of ecotones is that they comprise edges or regions of intergradation between habitats, data from this suggest that (for rodents) ecotones such as those investigated may be more appropriately understood to comprise a patchwork of adjacent discrete habitats. Under this view, habitats within ecotones can be identified and classified on their own merit without specific reference to the biomes that border the ecotone.

Rodents have been studied intensively in North American deserts. Studies have emphasized community organization (e.g., Brown & Kurzius 1989), distribution and natural history (e.g., Bailey, 1931; Hall & Kelson 1959; Findley et al. 1975). On occasion, this work is complemented by local observations of species presence such as those by Dice (1930; 1942) and Blair (1941) in southern New Mexico.

With increasing use of desert areas by humans, proactive management regimes will be needed to preserve rodent populations and diversity on their merit and as important constituents of food webs. These management regimes will require dependable information on habitat associations, especially within uncommon, dynamic habitats such as ecotones. Lack of such information has long been identified as an area of deficiency (De Vos 1975; Gibbons 1988; Edwards et al. 1996).

Variation in distribution and abundance is the norm for rodents (e.g., Brown & Kurzius 1989). What remains uncertain is how well habitats maintain their species composition given stochastic variation in population. This question would appear to be pertinent to any management regime directed toward rodents or one that relies on them as biological indicators.

Quantitative studies typically avoid ecotones (Risser 1993) because they occur across spatial and temporal scales (Gosz 1993). Ecotones usually are considered dynamic systems under the influence of succession or climate (Webb et al. 1987; Montana et al. 1990; Neilson 1991). This study documents association with vegetation types and diversity of rodents in a Chihuahuan Desert/desert grassland ecotone in southern New Mexico.

#### STUDY AREA

This research was conducted in four vegetation types associated with a Chihuahuan Desert/desert plains grassland ecotone near the northern limit of the Chihuahuan Desert. Sites were located within a 200 km² area, on the western edge of the Otero Mesa in the Tularosa Valley of southern New Mexico.

Topography was formed by erosion of the mesa. Within the ecotone were two types of wide-bottomed draw that carry water only during intense rainfall. Grass draws had little shrub cover. *Rhus* draws were distinct because of their heavy growth of shrubs, especially *Rhus microphylla*. Flanking the draws were uplands comprised of mesa relics and *Acacia* uplands. Mesa relics were grass covered with virtually no shrub cover. *Acacia* uplands were dominated by *Acacia neovernicosa*.

#### **METHODS**

Habitat measurements.—Habitat characteristics (representation of substrate constituents, grasses, forbs and shrubs) were measured with the line transect technique of Jorgensen et al. (2000). Briefly, transects comprised two poles, each two m long, oriented perpendicular to each other to a random azimuth and centered on each trap site. Thus in each trapping-grid, 360 linear m of transect were sampled.

The experimental design was a one-way analysis of variance (ANOVA) for completely random designs. Rank transform was conducted because

Table 1. Total and species specific shrub cover (%), point diversity (no. per 400 cm transect), and height (cm) in four vegetation types in a Chihuahuan Desert/desert plains grassland ecotone in southern New Mexico, 1993-94. Values accompanied by the same superscript in the same row are not significantly different.

		Vegetation type					
	Draws		Ul	plands			
Group	Rhus	Grass	Acacia	Mesa Relic	SE	P	
Acacia neovernicosa	0.0 b	0.0 b	17.9 <sup>a</sup>	0.0 b	1.30	0.000	
Atriplex canescens	1.3 <sup>a</sup>	0.1 b	0.0°	0.0 <sup>c</sup>	0.11	0.000	
Flourencia cernua	5.8 a	0.3 b	0.1 <sup>c</sup>	0.0 <sup>c</sup>	0.46	0.000	
Koberlinia spinosa	0.0 b	0.0 b	0.3 <sup>a</sup>	0.0 <sup>b</sup>	0.02	0.000	
Larrea tridentata	0.4 b	0.0°	1.4 <sup>a</sup>	0.1 <sup>c</sup>	0.15	0.000	
Parthenium incanum	0.0 b	$0.0^{\mathrm{b}}$	1.9 <sup>a</sup>	0.0 b	0.16	0.000	
Prosopis glandulosa	0.6 a	$0.0^{\mathrm{b}}$	0.0 b	$0.0^{ \rm b}$	0.05	0.000	
Rhus microphylla	10.2 a	0.2 bc	0.2 b	$0.0^{c}$	0.75	0.000	
Tiquilia greggii	0.0 b	0.0 b	0.5 a	0.0 <sup>b</sup>	0.07	0.002	
Ephedra sp.	0.0 <sup>c</sup>	0.0°	0.7 <sup>a</sup>	0.1 <sup>b</sup>	0.07	0.000	
Shrub Coverage	16.9 <sup>b</sup>	0.7°	23.9 a	0.5 <sup>c</sup>	1.75	0.000	
Point Diversity	$0.8^{b}$	0.0 c	1.4 a	0.0 <sup>c</sup>	. 0.10	0.000	
Height	115.8 a	49.8 b	73.3 b	14.2 <sup>c</sup>	7.08	0.000	

the data were not normal and were heteroscedastic. Significant treatment F tests ( $\alpha$ =0.05) were followed by multiple comparisons with the LSD test ( $\alpha$ =0.05).

Rodent sampling.—The locations of five trapping grids per vegetation type (20 total) were randomly selected. At each, a 0.58 ha, 3 by 30 trap-grid, with 10 m trap spacing was randomly placed. One Sherman folding aluminum live trap, baited with rolled oats, was placed at each trap site for four nights. Grids were trapped in a random sequence each spring during 1993 and 1994.

The response variable for each species was minimum number of animals known alive (MNA) over each 0.58 ha grid, adjusted to number of animals per ha. Rank transform was conducted for vegetation type and year effects and multiple comparisons were conducted on ranks with the LSD test. Vegetation type x year interactions were analyzed with untransformed data, because interactions cannot be analyzed with rank transforms (Hora & Conover 1984). This increased the potential for Type 1 error. Nonetheless, it is believed that this sample size was large enough to produce a robust analysis of interaction (Cohen 1965; Conover 1980; Johnson & Wichern 1992).

This study measured  $\alpha$  diversity relations for rodents (species

Table 2. Grass cover (%) in four vegetation types in a Chihuahuan Desert/desert plains grassland ecotone in southern New Mexico, 1993. Values accompanied by the same superscript in the same row are not significantly different.

	Vegetation type					
	Draws		Uplands			
Taxon	Rhus	Grass	Acacia	Mesa Relic	SE	P
Aristida sp.	0.0 b	0.2 b	3.8 a	1.6 a	1.08	0.000
Bouteloua curtipendula	0.1 bc	0.2 <sup>c</sup>	3.7 <sup>a</sup>	2.6 <sup>ab</sup>	1.69	0.005
Bouteloua eriopoda	0.1 <sup>c</sup>	0.3 <sup>c</sup>	2.0 b	15.4 <sup>a</sup>	3.30	0.000
Bouteloua gracilis	2.9 <sup>a</sup>	5.6 a	0.0 b	3.8 <sup>a</sup>	2.11	0.003
Bouteloua hirsuta	0.0 b	0.0 b	0.0 ab	3.2 <sup>a</sup>	1.04	0.043
Distichlis stricta	1.3 b	6.3 <sup>a</sup>	0.0 <sup>c</sup>	0.0 <sup>c</sup>	2.58	0.000
Erioneuron pulchellum	$0.0^{0}$	0.0 b	0.3 <sup>a</sup>	0.0 <sup>b</sup>	0.15	0.000
Hilaria mutica	11.8 <sup>b</sup>	27.8 <sup>a</sup>	0.0 <sup>c</sup>	0.0 <sup>c</sup>	4.19	0.000
Muhlenbergia sp.	0.2 b	0.1 <sup>b</sup>	0.2 b	2.6 a	0.71	0.005
Panicum hallii	0.0 <sup>b</sup>	$0.3^{a}$	0.0 b	0.0 ab	0.18	0.048
Panicum obtusum	2.3 <sup>a</sup>	1.2 b	0.0 b	0.0 b	0.77	0.002
Setaria leucopila	1.4 <sup>a</sup>	0.2 b	0.0 b	0.0 b	0.87	0.003
Sporobolus cryptandrus	0.0 <sup>b</sup>	$0.0^{b}$	0.0 b	0.5 a	0.07	0.000
Sporobolus airoides	2.6 a	16.8 <sup>a</sup>	0.0 b	0.1 <sup>b</sup>	4.74	0.000
Ŝtipa neomexicana	0.0 <sup>b</sup>	0.0 b	0.0 b	2.3 <sup>a</sup>	1.03	0.007

richness, Simpson's index, Shannon's index, Brillouin's index) using BIODIV 4.1 (Baev & Penev 1993). Measures were analyzed with the same statistical model as were the rodent species data previously described. Diversity data were not transformed as they conformed to the assumptions of normality and heteroscedasticity.

#### **RESULTS**

Vegetation.—Sixteen shrub species were documented, with Ephedra sp. determined only to genus (Table 1). No shrub species were associated with grass draws or mesa relic uplands. Shrub coverage of these vegetation types was < 1%. Eight shrub species and Ephedra sp. were associated with only one vegetation type.

Fifteen grass species were documented, with *Aristida* sp. and *Muhlenbergia* sp. determined only to genus (Table 2). At least four grass species were associated with each vegetation type. Eight grass species and *Muhlenbergia* sp. were associated with only one vegetation type.

Rodents.—During this study 1,314 individual rodents from 16 species were captured in 14,400 trap nights. Relative abundance of all species combined increased by 99% (P=0.000) from 1993 to 1994. Increases were documented for Dipodomys merriami (55%, P=0.014), Chaetodipus hispidus (196%, P=0.011), Perognathus flavus (526%, P=0.001),

Table 3. Relative abundance (number/ha) of rodents in 4 vegetation types (*Rhus* and grass draws, *Acacia* and mesa relic uplands) and probability of equal abundance across vegetation types (*P*) in a Chihuahuan Desert/desert plains grassland ecotone in southern New Mexico, spring 1993-94. Values accompanied by the same letter in the same row are not significantly different.

			Vegeta	tion type			
		Dra	aws	Up	lands		
Species	Yr	Rhus	Grass	Acacia	Mesa Relic	SE	P
Dipodomys	93	2.4 b	0.0°	14.1 <sup>a</sup>	0.0 °	4.1	0.000
merriami	94	5.5 <sup>b</sup>	1.0°	$7.2^{a}$	2.4 <sup>c</sup>	6.5	
Dipodomys	93	11.4 <sup>a</sup>	2.4 b	1.4 b	1.0 b	4.0	0.000
ordii	94	14.8 <sup>a</sup>	3.8 b	0.7 <sup>b</sup>	3.1 b	5.6	
Dipodomys	93	0.0	0.0	0.0	0.3	0.1	0.134
spectabilis	94	0.0	0.0	0.0	0.3	0.1	
Chaetodipus	93	0.7 b	$0.0^{\rm b}$	1.0 a	0.0 b	0.4	0.016
intermedius	94	0.0 b	0.0 b	1.7 <sup>a</sup>	0.3 b	0.5	
Chaetodipus	93	0.3 <sup>b</sup>	1.7 <sup>a</sup>	0.7 b	2.4 <sup>a</sup>	1.3	0.017
hispidus	94	2.8 b	7.2 a	0.3 <sup>b</sup>	4.8 a	3.8	
Perognathus	93	2.4 a	2.1 a	1 O b	4.1 <sup>a</sup>	2.4	0.002
flavus	94	11.4 a	27.5a	1.0 b	10.3 a	12.6	
Perognathus	93	0.3 b	1.0 <sup>b</sup>	12.7a	7.6 <sup>a</sup>	5.4	0.000
flavesens	94	1.4 b	$2.1^{\circ}$	8.9 <sup>a</sup>	8.6 a	5.2	
Peromyscus	93	8.9 a	4.1 <sup>b</sup>	69b	0.0 c	5.0	0.000
leucopus	94	22.7 <sup>a</sup>	4.1 b	10.0 b	0.0 °	9.2	0.000
Peromyscus	93	4.8 a	$0.0^{b}$	3.4 a	0.0 b	2.1	0.001
maniculatus	94	9.6a	7.2 b	8.9 a	2.1 b	7.0	0.001
Peromyscus	93	$0.3\mathrm{b}$	$0.3^{\rm b}$	8.9 a	0.0 b	2.4	0.000
eremicus	94	0.7 b	0.0 b	8.3 a	0.0 b	2.2	0.000
Reithrodontomys	93	8.3 a	6.9 a	9.6 <sup>a</sup>	4.1 b	7.2	0.000
megalotis	94	32.0 a	17.2 a	14.8 a	2.4 b	16.6	0.000
Sigmodon	93	1.0 a	1.0 a	0.0 b	0.0 b	0.5	0.006
hispidus	94	5.5 a	3.8 a	0.0 b	0.0 b	2.3	0.000
Neotoma	93	1.0 b	0.3 b	5.8 a	0.0 0.0 b	1.8	0.000
albigula	94	0.0 b	0.0 b	4.8 <sup>a</sup>	0.0 0.0 b	1.8	0.000
Neotoma	93	0.0 0.7 <sup>a</sup>	0.0 b	0.0 b	0.0 b	0.2	0.004
	93	1.7 a	0.0 b	0.0 b	0.0 b		0.004
micropus	94 93	0.7	0.0	0.0	1.7	0.4	0.241
Onychomys						0.8	0.241
arenicola	94	1.7	0.3	1.4	1.7	1.3	0.542
Onychomys	93	0.0	0.0	0.3	0.0	0.1	0.543
leucogaster	94	0.3	0.3	0.7	0.3	0.4	
All Species	93	43.3	20.3	66.4	21.3	37.8	
	94	110.4	74.6	78.8	36.8	75.2	

Peromyscus maniculatus (339%, P=0.001) and Reithrodontomys megalotis (230%, P=0.005) (Table 3). Association with vegetation types were consistent across years, evidenced by the measurement of a only a single vegetation type x year interaction.

Notably, five species reached high relative abundances in only a single vegetation type; *C. intermedius*, *P. eremicus* and *N. albigula* on *Acacia* uplands and *D. ordii* and *N. micropus* in *Rhus* draws. The only

Table 4. Diversity of rodents, measured by 4 indices, in 4 vegetation types (*Rhus*, Grass, *Acacia*, Relic) over 2 years (Y1, Y2), and probability of equality (*P*, 32 *df*), in a Chihuahuan Desert/desert plains grassland ecotone in southern New Mexico, spring 1993-94. Values accompanied by the same letter in the same row are not significantly different.

Vegetation type		Vegetat	ion type			
	Dra	ws	Up	olands		
Index	Rhus	Grass	Acacia	Mesa Relic	SE	P
Richness	8.2 a	5.7 b	8.3 <sup>a</sup>	5.0 b	0.74	0.000
Simpson	5.21 <sup>a</sup>	3.93 <sup>b</sup>	5.50 <sup>a</sup> 1.84 <sup>a</sup>	3.33 b	0.562	0.001
Shannon	1.78 <sup>a</sup>	1.47 b	1.84 <sup>a</sup>	1.29 b	0.140	0.001
Brillouin	1.50 a	1.17 <sup>b</sup>	1.58 a	1.00 b	0.125	0.000

Vegetation x year		Dra	ws			Upla	nds		
Interaction	Rh	nus	Gr	ass	Aca	acia	Mesa	Relic	
	Y1	Y2	ΥI	Y2	Υl	Y2	Y1	Y2	P
Richness Simpson	7.4 4.69	9.0 5.74	5.0 3.78	6.4 4.08	7.4 4.99	9.2 6.00	3.8 2.76	6.2 3.89	0.92 0.88
Shannon Brillouin	1.66 1.34	1.90 1.65	1.37 1.00	1.56 1.35	1.73 1.48	1.94 1.68	1.11 0.83	1.47 1.18	0.92 0.91

significant vegetation type x year interaction occurred in grass draws: P. flavus had higher abundance during 1994 than in 1993 (F-test P=0.011, LSD test (=0.05).

Each vegetation type supported at least one species that increased in abundance between 1993 and 1994 (Table 3). Rhus draws supported high numbers of P. flavus, P. maniculatus and R. megalotis, that increased, and D. ordii, P. leucopus, S. hispidus and N. micropus that did not. All of the species that were abundant in grass draws increased. Acacia uplands supported high numbers of D. merriami, P. maniculatus and R. megalotis, that increased, and C. intermedius, P. flavescens, P. eremicus and N. albigula that did not. Mesa relics supported C. hispidus and P. flavus, that increased, and P. flavescens that did not.

Rhus draws and Acacia uplands had greatest diversity according to all indices (Table 4). Over all vegetation types, richness increased from a mean of 5.9 species per 0.58 ha in 1993 to 7.7 per 0.58 ha in 1994 (P=0.001). Despite these increases, there is no suggestion of vegetation type x year interaction (Table 4) for diversity measures. Therefore, richness did not change between vegetation types, but it increased uniformly in all vegetation types studied.

#### **DISCUSSION**

Nine of 16 shrubs, 13 of 15 grasses, and five of 13 rodent species clearly were associated with specific vegetation types. It is most interesting to note that rodent species' associations with vegetation type remained constant, even in the face of a near doubling in overall rodent numbers, and year-to-year single species increases as large as 339% (i.e., *P. maniculatus*). The large increase for *P. flavus* in grass draws (1309%) is thought to be biologically significant. Under appropriate climatic conditions grass draws may be the most suitable vegetation type for this species.

Between the 1993 and 1994 collections a fire burned much of the area, including five study plots (two grass draws, one *Rhus* draw, two mesa relic uplands). Analysis for changes in relative abundances of rodents attributable to the fire was negative, however power was low because few plots were impacted directly. Nonetheless, it is thought that affects caused by the fire do explain some of the response observed for *P. flavus*. Abundant local rainfall, following the fire, flowed unimpeded off of burned slopes, moving seeds and disturbing soil. Dense patches of annuals were observed in 1994 within burned draws even though these patches did not show up in the sampling. Grass cover was much reduced in grass draws and decadent old growth was replaced by newly sprouting grasses. Although changing vegetation may be a necessary condition (Rosenzweig et al. 1975) for impacts on rodent populations to be observed, local climatic effects are also critical (Cloudsley-Thompson 1975; Crawford & Gosz 1982).

The consistent associations with vegetation type exhibited by rodent species carried over into comparisons based upon diversity indices. Alpha diversity changed within vegetation types (year effect) but not between vegetation types (Table 4). This is consistent with a result observed 40 km to the north over the same sampling period as this research, where diversity measures held constant among vegetation types (Jorgensen 1996) over a period of substantial decline in rodent abundance (Jorgensen 1996; Jorgensen et al. 1998).

Some of the vegetation types studied did not correspond to vegetation types found in the adjoining Chihuahuan Desert and desert plains grasslands. For instance, *Acacia* uplands and *Rhus* draws were unique to the ecotone. It has been recognized that ecotones consist of habitat mosaics (Gosz 1993). It has not been clearly recognized that these mosaics are

comprised of stable, discrete, habitats in part derived from adjoining biomes and in part endemic only to the ecotone. Thus, ecotones are important reservoirs of landscape biodiversity; they support habitats and species not found in adjoining biomes. Gradients within the ecotone might best be characterized by a shift in relative frequency of discrete habitat types.

Patterns in relationships of rodents associated with vegetation types in this Chihuahuan Desert/desert plains grassland ecotone were stable and easily detected. This suggests that previous research focused upon gradients can also be viewed in another light. In seeking to define gradients (which by their very nature are unstable), stability within the gradient may have been overlooked. The stable regions observed in this study are consistent with mosaics (Gosz 1993) and patches (Wiens 1985).

The regions of stability in Chihuahuan Desert plant and animal communities are examples of McAuliffe's (1994) spatial scales that incorporate considerable spatial complexity that have been neglected. Although the precise location of mosaic boundaries may shift over time, discrete relatively stable "core" habitats seem to persist (Goldberg & Turner 1986; Montana et al. 1990; Turner 1990; Gosz 1993; Martinez & Fuentes 1993). For management, areas of stability within the mosaic will be of primary interest. Unstable boundary regions will be under the influence of climate, weather, etc. (Neilson 1991) that are beyond management control.

In general, previous work has been conducted at a scale too coarse for management-directed, comparative ecological work. It is typical to recognize broad associations, especially with reference to elevation (e.g., Lowe 1961; Whittaker & Niering 1965; Moir 1979; Henrickson & Johnston 1986). Many investigators recognize the unique characteristics of finer grained landscapes. The practices of ecosystem management and landscape ecology will require detailed knowledge of these finer grained areas if they are to become effective management tools.

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# FISHES OF THE RICHLAND CREEK WILDLIFE MANAGEMENT AREA OF EAST TEXAS

# Frances P. Gelwick, Brian D. Healy, Nikkoal J. Dictson and Jim Cathey\*

Department of Wildlife and Fisheries Sciences
Texas A&M University, 2258 TAMUS
College Station, Texas 77843-2258
\*Gus Engeling Wildlife Management Area
Texas Parks and Wildlife Department, Rt. 1 Box 27
Tennessee Colony, Texas 75861

Abstract.—The aquatic habitats of the Richland Creek Wildlife Management Area in central east Texas were surveyed in April, May, June and August of 1998 and 1999. A total of 49 species from 15 families was documented, including a juvenile grass carp (Ctenopharyngodon idella). These records serve to establish the initial database for fishes in this management area as a part of the conservation program of the Texas Parks and Wildlife Department.

Wildlife Management Areas (WMAs) are often acquired by Texas Parks and Wildlife Department as partial mitigation against loss of wildlife habitat resulting from reservoir construction, and can contain important aquatic habitats such as natural streams and wetlands. Fiftyseven of the more than 80 species of fishes (17 families) that are known to occur in the Trinity River drainage in this region of Texas (Hubbs et al. 1991; Travis et al. 1994) are confirmed in nearby Gus Engling WMA (Telfair 1997). These WMAs are managed for numerous consumptive and nonconsumptive outdoor activities including hunting, fishing, birdwatching and research. Land for Richland Creek WMA (RCWMA) was acquired in 1987 and 1988. It is located along an ecotone separating the Post Oak Savannah and Blackland Prairie ecological regions. RCWMA includes 1945 ha in Freestone and Navarro counties (North Unit), and 3641 ha in Freestone County (South Unit). These lie within the Trinity River drainage and a large portion lies within the floodplain. RCWMA contains Alligator Creek, Tehuacana Creek and several oxbow lakes. Carroll Lake Slough and associated diked wetland compartments (C1, C2, Triangle) have control structures to allow manipulation of natural water flow that results from rainfall and flooding. Compartment 2 could be filled with water pumped from Richland Chambers Reservoir. In order to gauge the effects of changes in land use and to make informed management decisions, baseline data on fishes were collected as part of a long-term monitoring program. This study reports the results of the initial fish surveys in RCWMA in 1998 and 1999.

#### **METHODS**

Fishes were sampled across representative habitats in RCWMA in April, May, June and August of 1998 and in these months in 1999. Sampling gear included a backpack electrofisher, seines, gill nets and a boat-mounted electrofisher. Gear was selected to efficiently collect the species and size classes of fish that might be present in each habitat. A boat mounted electrofisher and experimental gill nets (38.1 m in total length and 1.8 m depth) were used in lentic habitats to sample larger fish. Gill nets were comprised of five different panels of equal length with graduated mesh of 7.6 cm, 6.4 cm, 5.1 cm, 3.8 cm and 2.5 cm, and were fished for four hours. For shallow open-water sites, seines of 7.4 mm mesh, 1.2 m depth and lengths of 2.4 m, 3 m, and 4.5 m were A 1.2 m by 4.5 m bag seine was used in the open wetland habitats. Sampling effort continued in each available microhabitat until no new species were captured in three successive passes with the gear. Tissue samples and whole specimens of smaller fishes were collected, and within 24 h were placed in an ultracold (-70°C) freezer, and then into frozen tissue collections of the Texas Cooperative Wildlife Collections (TCWC) at Texas A&M University in the Department of Wildlife and Fisheries Sciences, College Station. Voucher specimens of each species were preserved in formalin, transferred to ethanol, for deposit in the TCWC. All captured fish were recorded, but specimens > 150 mm total length (TL) of species previously captured and vouchered at a site during a survey year were weighed, measured and released.

#### RESULTS AND DISCUSSION

Forty of the 57 species previously confirmed in collections from nearby Gus Engling WMA (Telfair 1997) were collected in RCWMA (Table 1). Red shiner, inland silversides (*Labidesthes sicculus*), white bass (*Morone chrysops*), yellow bass (*M. mississippiensis*) and bantam sunfish (*Lepomis symmetricus*) were newly added to the list of species confirmed in this area. Collections of silverband shiner (*Notropis schumardi*), bigmouth buffalo (*Ictiobus cyprinellus*), striped bass (*M. saxatilis*), and one grass carp (*Ctenopharyngodon idella*) added and confirmed four new species to the checklist, and newly confirmed one family (Percichthyidae). Eighty species and 16 families are now of probable or possible occurrence in the RCWMA, and 66 species are confirmed.

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The nonnative striped bass were likely transients from stocks introduced for sportfishing into Richland Chambers Reservoir, but the collection of a 155 mm TL grass carp in April 1999 is problematic. Grass carp 150-200 mm TL are stocked, but since 1992, only sterile triploids are stocked legally into Texas ponds and reservoirs, and only as permitted by Texas Parks and Wildlife Department (Howells 1994). Because grass carp are not produced in Texas hatcheries (Ott & Henson pers. comm.), it might have been illegally released. Grass carp eggs and larvae have been found in the Trinity River (Howells 1994) and active spawning by grass carp was observed during this study in the upper reaches of the San Jacinto River. Such evidence suggests that natural reproduction by grass carp and survival of offspring might have resulted from legal or illegal release of diploids, or rare fertility of triploids.

The Carroll Lake and Slough system contained 41 species (13 families) (Table 1). Six of the species in the Carroll Lake and Slough system were not found in other habitat types. Diked wetlands in the North Unit each contained 24 species (nine families), for a combined total of 27 species (10 families). Alligator Creek contained a subset of species found in the Carroll Lake and Slough. Several species were confirmed at only one site, despite three or four separate collections at each. Freckled madtom (*Noturus nocturnus*) was collected only in Tehuacana Creek, and spotted bass (Micropterus punctulatus) only in the old Richland Creek channel. Ribbon shiner (Lythrurus fumeus) and grass pickerel (Esox americanus) only in Hillside Slough, and the grass carp (Ctenopharyngodon idella) in Snag Lake. Total relative abundance was greatest for red shiner (Cyprinella lutrensis), the western mosquitofish (Gambusia affinis), and threadfin shad (Dorosoma petenense) (Table 1). Creek chubsucker is state-listed as a species of special concern, in large part because it is at the edge of its range in this area of Texas. Neither creek chubsucker (Erimyzon oblongus), nor lake chubsucker (E. sucetta) were collected. Both species may potentially occur (Hubbs et al. 1991; Travis et al. 1994; Telfair 1997), but are associated with vegetation, and creek chubsuckers may require gravel shoals for spawning (Robison & Buchanan 1988). Such habitats are uncommon in RCWMA. However, both species are confirmed in nearby Gus Engling WMA, where perennial sandy-bottom streams with gravel shoals occur.

The importance of temporal dynamics was indicated by species' variable occurrences (Table 1). Although 41 species were collected in each year, 16 were collected in only one year, eight only in 1998, and eight only in 1999.

Table 1. Mean abundance of fish in collections at Richland Creek Wildlife Management Area in April, May, June and August of 1998 and 1999. Total relative abundance (RA) and years in which a species was collected are indicated.

				Nort	North Unit				S	South Unit			To	Total
		Alligator	Carroll	Carroll	Dike	Diked Wetlands		Tehuacana	Rich.Ck Hillside	Hillside	Lost	Snag		
Family	Species	Creek	Lake	Slough	C 1	C 2	Triangle	రే	(old ch)	Slough	Lake	Lake	<b>₽</b>	Years
Lepisosteidae	Lepisosteus oculatus	0	-	5	3	0	0	0	-	-	0	0	<0.01	1998-99
	Lepisosteus osseus	0	0	7	7	-	_	0	0	0	0	7	< 0.01	1999
	Lepisosteus spatula	0	0	0	7	0	0	0	0	0	0	0	< 0.01	1998
Amiidae	Amia calva	0	0	_	7	0	9	0	-	0	0	0	< 0.01	1998-99
	Dorosoma													
	cepedianum	3	3	8	42	3	m	-	Ξ	_	10	<b>∞</b>	0.04	1998-99
	Dorosoma petenense		282	32	104	27	41	7	7	8	49	56	0.14	1998-99
Cyprinidae	Ctenopharyngodon													
	idella		0	0	0	0	0	0	0	0	0	_	<0.01	1999
	Cyprinella lutrensis	•	30	12	13	15	235	24	51	0	171	368	0.21	1998-99
	Cyprinella venusta	0	0	0	0	0	0	3	4	0	0	0	<0.01	1998
	Cyprinus carpio		7	7	15	7	9	0	_	7	_	0	0.01	1998-99
	Lythrurus fumeus		0	0	0	0	0	0	0	∞	0	0	< 0.01	1998-99
	Notemigonus													
	crysoleucas		0	12	10	_	Π	2	0	0	7	0	0.01	1998-99
	Notropis shumardi		0	0	-	0	0	0	0	0	0	0	< 0.01	1998
	Notropis texanus		0	3	2	19	24	_	7	4	7	_	0.03	1998-99
	Opsopoeodus emiliae		<b>∞</b>	∞	12	10	4	_	0	9	4	0	0.01	1998-99
	Pimephales vigilax		3	3	3	-	19	18	9	_	17	53	0.03	1998-99
Catastomidae	Carpiodes carpio		0	0	7	0	0	0	0	0	0	0	<0.01	1999
	Ictiobus bubalus		23	9	13	0	0	0	30	3	0	0	0.03	1998-99
	Ictiobus cyprinellus		0	0	_	0	0	0	0	0	0	0	< 0.01	1999
Ictaluridae	Ameiurus melas		0	0	7	S	_	0	_	Ξ	-	0	< 0.01	1998-99
	Ameiurus natalis		0	_	0	0	0	0	0	0	7	0	<0.01	1998
	Ictalurus furcatus		_	0	-	0	0	0	0	0	_	0	<0.01	1998-99
	Ictalurus punctatus	0	0	_	-	0	0	0	0	0	0	_	<0.01	1998-99
	Noturus nocturnus		0	0	0	0	0	7	0	0	0	0	<0.01	1999
Esocidae	Esox americanus		0	0	0	0	0	0	0	m	0	0	<0.01	1998

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Alligator Carroll Carroll Diffeed Wetlands  Alligator Carroll Carroll Diffeed Wetlands  annus  edoderus  edoderus  edoderus  edoderus  carons  Oreek Lake Slough C1 C2 Triangle  edoderus  originatis  originatis					Norti	North Unit				S	South Unit			Ĭ	Total
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e Aphredoderus           sayanus         0         0         1         0         0         0         0         0         0         2         0         0         4         0	ramily	Species	Creek	Lake	Slough	2	7 0	Iriangle	ž .		Slough	Lake	Lake	₹	Years
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Gambusia affinis         91         10         18         26         38         103         85         127         155         177         117           Labbidesthes sicculus         0         0         0         0         0         0         3         3         0	Cyprinodontidae		0	0	_	0	0	0	9	0	7	0	4	< 0.01	1998-99
Labidesthes sicculus         0         0         0         0         0         0         3         0           Menidia beryllina         0         10         13         13         16         21         0	Poeciliidae	Gambusia affinis	91	10	18	56	38	103	82	127	155	177	117	0.21	1998-99
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mississippiensis         6         15         4         0         0         1         0         0           Morone saxatilis         0         1         0         <	Percichthyidae	Morone chrysops	0	0	7	_	0	0	0	0	0	0	-	< 0.01	1998-99
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dae         Lepomis cyanellus         0         3         3         3         4         0         8         2         4           Lepomis gulosus         0         3         8         9         12         2         79         7         4         5         0           Lepomis marginatus         0         0         0         1         0		Morone saxatilis	0	0	· –	0	0	0	· C	· C	o	0	0	0.0	1998
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Lepomis microlophus         0         10         3         0         1         0		Lepomis megalotis		16	15	Ξ	_	0	4	7	7	2	0	0.01	1998-99
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punctulatus         0 <th< td=""><td></td><td>Micropierus</td><td></td><td>,</td><td>(</td><td>•</td><td>•</td><td>,</td><td>,</td><td></td><td>,</td><td></td><td></td><td></td><td></td></th<>		Micropierus		,	(	•	•	,	,		,				
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nigromaculatus     3     14     27     8     30     10     0     1     5     10     6       Etheostoma     1     0     4     0     5     20     0		Pomoxis													
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		grunniens	0	0	0	_	0	0	0	-	0	0	_	<0.01	1998-99

With the exception of Carroll Slough, other habitat types held few adult-sized game species such as channel catfish (*Ictalurus punctatus*), largemouth bass, and both black and white crappies (*Pomoxis nigromaculatus* and *P. annularis*). However, the occurrence of juvenile-sized fishes indicated that certain life stages are more likely to use these habitats temporarily (for spawning, foraging and shallow, low-velocity refuges), before returning to main streams on the waning flow or next flood (Turner et al. 1994). Thus, repeated surveys across seasons and years would increase the likelihood that all fish species and life stages using RCWMA are documented.

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FPG at: fig0697@acs.tamu.edu

# REPRODUCTION IN THE LONGNOSE SNAKE, RHINOCHEILUS LECONTEI (SERPENTES: COLUBRIDAE)

## Stephen R. Goldberg

Department of Biology, Whittier College Whittier, California 90608

Abstract.—Reproductive tissue was examined from 275 museum specimens of *Rhinocheilus lecontei* from the southwestern United States and northwestern Mexico. Males follow a seasonal testicular cycle with sperm produced July-October; regressed testes were present March-July. Males with recrudescent testes were present April-August. Breeding presumably occurs in spring although sperm was present in the vasa deferentia during all months examined. Females with enlarged ovarian follicles (> 12 mm length) or oviductal eggs were present April-July. Mean clutch size for 20 R. *lecontei* females was  $6.1 \pm 2.1$  SD, range = 3-11. Clutch size of 11 appears to be a maximum clutch size record. The reproductive cycle of R. *lecontei* fits the aestival (summer) and postnuptial category of Saint Girons (1982) in which sperm are stored throughout the winter in the vas deferens.

The longnose snake, *Rhinocheilus lecontei*, ranges from southwest Idaho and southeast Colorado to central Baja California, San Luis Potosí and southern Tamaulipas, México; from central Texas to southern California from below sea level to 1650 m where it inhabits deserts, prairies, shrubland and tropical habitats (Stebbins 1985). There are only anecdotal accounts of reproduction in this species (Conant & Downs 1940; Klauber 1941; Woodin 1953; Wright & Wright 1957; Shannon & Humphrey 1963; Lardie 1965; Dixon 1967; Vitt 1975; Tennant 1984; Degenhardt et al. 1996; Hammerson 1999). Fitch (1970) summarized clutch sizes for *R. lecontei* and Medica (1975) reported on the biology of this species. The purpose of this study is to provide information on the seasonal ovarian and testicular cycles of *R. lecontei* from a histological examination of museum specimens and to provide information on clutch sizes.

### MATERIALS AND METHODS

A sample of 275 specimens of R. lecontei (64 females, mean Snout-Vent Length, (SVL) =  $580 \text{ mm} \pm 46.4 \text{ SD}$ , range = 509-713 mm; 211 males, SVL =  $566 \text{ mm} \pm 85.9 \text{ SD}$ , range = 361-771 mm) from Arizona, California, Nevada, New Mexico, Texas, Utah and México was examined from the herpetology collections of Arizona State University, Tempe (ASU), The Natural History Museum of Los Angeles

County (LACM) and The University of Arizona, Tucson (UAZ). Snakes were collected 1941-1991. Counts were made of enlarged ovarian follicles (> 12 mm length). The left testis and vas deferens were removed from males; the left ovary was removed from females for histological examination. Tissues were embedded in paraffin and sectioned at 5  $\mu$ m. Slides with tissue sections were stained with Harris' hematoxylin followed by eosin counterstain. Testes slides were examined to determine the stage of the male cycle; ovary slides were examined for the presence of yolk deposition (secondary yolk deposition sensu Aldridge 1979a). Because some of the specimens were road kills, not all vasa deferentia were available for histological examination due to damage. In two cases ovaries of road-killed females were damaged and counts of clutches were not possible. Number of specimens examined by reproductive tissue were: testis = 211, vas deferens = 92, ovary = 64. Since the R. lecontei samples were from a wide geographic area, there is the possibility that reproductive data from large samples of this species from geographic or taxonomic subpopulations may vary from the pattern described herein. There was a geographic bias to the samples with 56% coming from Arizona and 27% from California.

Material examined.—The following specimens of Rhinocheilus lecontei were examined: ARIZONA: COCHISE COUNTY, (ASU 3180, 3678, 15720, UAZ 4766, 28570, 41436, 43068, 44282, 45474); GRAHAM COUNTY, (UAZ 35769, 42286); MARICOPA COUNTY, (ASU 173, 1265. 1404, 1417, 1790, 1791, 3066, 3825, 8822, 9083, 13481, 13912, 14180, 14181, 14183-14186, 14188, 14191-14197, 14199, 14201, 14202, 14204, 14206, 14207, 22405, 22410, 22592, 23237, 23496, UAZ 26113); MOHAVE COUNTY, (UAZ 26097, 34136, 44863); PIMA COUNTY, (ASU 3826, 3837, 3846, 13869, 13870, 13913, 22783, 23113, 23902, 24320, 26377, 26498, 28379-28381, 28398, 28588, 28589, 28596, 29490, 29499, 29673, 29674, UAZ 4487, 4503, 4506, 4508, 4511, 4515, 4523, 4733, 4736, 4737, 4745, 4748, 4749, 4761, 4763, 4768, 26086, 26095, 26096, 26104, 26106-26108, 26137, 26139, 26144, 26145, 26149, 30725, 31609, 42277, 45885, 45993, 46156, 48594, 48861, 49124, 50295); PINAL COUNTY, (ASU 3836, 8896, 13911, 22769, 22865, 23235, 23596, 26370, 26372, 26376, 26380, 26382, 26384-26386, 26485, 28015, 28016, 28026, 28028, 28029, 28031, 28033, 28036, 29500-29503, 29507, 29508, 29510, 29543, UAZ 4517, 42999); SANTA CRUZ COUNTY, (ASU 3679, UAZ 4493); YAVAPAI COUNTY, (ASU 13898, UAZ 36192); YUMA COUNTY, (ASU 15853, 23584).

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CALIFORNIA: IMPERIAL COUNTY, (LACM 20809, 64302, 102607, 102608); INYO COUNTY, (LACM 102611); KERN COUNTY, (LACM 20783, 20788, 102612, 102614, 102618, 123791); LOS ANGELES COUNTY, (LACM 20795, 20798); RIVERSIDE COUNTY, (LACM 20757, 20759, 20766, 52476, 52479-52482, 102622, 102627, 102630-102632, 102636, 102638, 102639, 102641-102643, 102645, 102646, 102648, 102653, 102655, 102656, 102660-102666, 102669, 102672-102675, 102677, 102702, 115893, 115894, 122104, 123792; SAN BERNARDINO COUNTY, (LACM 20773, 27879, 102678-102680, 102683, 102684, 102686, 102689, 102691); SAN DIEGO COUNTY, (LACM 20802, 20803, 27877, 102693-102695, 102697-102699).

NEVADA: CLARK COUNTY, (LACM 133934); NYE COUNTY, (LACM 27887, 126207, 126209, 133945, 133947, 133954-133956, 133960, 133967-133970, 133973, 133980).

NEW MEXICO: BERNALILLO COUNTY, (LACM 2646); HIDALGO COUNTY, (LACM 133933); SOCORRO COUNTY, (UAZ 4567); QUAY COUNTY, (LACM 20819).

TEXAS: BREWSTER COUNTY, (LACM 102749); LLANO COUNTY, (LACM 67024, 67025); TOM GREEN COUNTY, (LACM 20810, 20811); VAL VERDE COUNTY, (LACM 135264).

UTAH: WASHINGTON COUNTY, (LACM 102708).

MÉXICO: CHIHUAHUA (UAZ 39201), COAHUILA (UAZ 37747), SINALOA (UAZ 4554, 13651, 37746), SONORA (UAZ 9614-9617, 9619, 26153, 26155, 31427, 35723, 36394, 36395, 39842, 45122); BAJA CALIFORNIA (LACM 36576, 102711).

### RESULTS AND DISCUSSION

Testicular histology was similar to that reported by Goldberg & Parker (1975) for the colubrid snakes *Masticophis taeniatus* and *Pituophis catenifer* (= *P. melanoleucus*). In the regressed testes, seminiferous tubules contained spermatogonia and Sertoli cells. In recrudescent testes, there was renewal of spermatogenic cells characterized by spermatogonial divisions; primary and secondary spermatocytes were typically present. Some spermatids were occasionally seen. In testes undergoing spermiogenesis, metamorphosing spermatids and mature sperm were present. Males undergoing spermiogenesis were found July to October, regressed testes were found March-July. Recru-

Month	n	Regressed	Recrudescence	Spermiogenesis
March	6	6	0	0
April	49	48	1	0
May	53	52	1	0
June	45	39	6	.0
July	24	4	17	3
August	27	0	15	12
September	6	0	0	6
October	1	0	0	1

Table 1. Monthly distribution of conditions in seasonal testicular cycle of *Rhinocheilus lecontei* from examination of 211 museum specimens. Values shown are the numbers of males exhibiting each of the three conditions.

descent testes occurred April-August (Table 1). Mating is believed to occur in spring after emergence from winter inactivity (Collins 1993). Because spermiogenesis occurs during late summer and autumn, there is the potential for some mating to occur during this time although it has not been reported.

The smallest reproductively active male measured 361 mm SVL and was collected in Pinal County, Arizona 14 April (ASU 23235). It had regressed testes but sperm was present in the vasa deferentia. Another male (LACM 133954) close to that size (394 mm SVL) that was collected 20 July from Nye County, Nevada contained recrudescent testes; sperm were present in the vasa deferentia. Males below 361 mm SVL were excluded from the study to avoid the possibility of including immature males in the analysis of the testicular cycle.

Vasa deferentia of the following *R. lecontei* males contained sperm: 3/3 (100%) March; 25/25 (100%) April; 29/29 (100%) May; 19/19 (100%) June; 6/7 (86%) July; 7/7 (100%) August; 2/2 (100%) September indicating the potential for inseminating females during all of these months.

Females with enlarged follicles or oviductal eggs were found April-July (Table 2). Females with early yolk deposition (secondary vitellogenesis sensu Aldridge 1979a) occurred April-June. Fitch (1970) and Degenhardt et al. (1996) suggested that more than one clutch may be produced by some females in the same year. However, histological examination indicated that none of the ovaries of *R. lecontei* females

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Table 2. Monthly distribution of conditions in seasonal ovarian cycle of *Rhinocheilus lecontei* from examination of 64 museum specimens. Values shown are the numbers of females exhibiting each of the four conditions.

Month	n	Inactive	Early yolk deposition	Enlarged follicles > 12 mm length	Oviductal eggs
March	1	1	0	0	0
April	21	12	2	6	1
May	17	6	2	9 a	0
June	13	7	1	3	2
July	4	3	0	0	1 b
August	5	5	0	0	0
September	3	3	0	0	0

<sup>&</sup>lt;sup>a</sup> Includes one road-killed snake with coagulated yolk, follicles could not be counted.

with oviductal eggs were undergoing yolk deposition for a second clutch of eggs.

Clutch sizes for 20 R. lecontei females (oviductal eggs or enlarged follicles > 12 mm length) had a mean of 6.1  $\pm$  2.1 SD, range = 3-11. This value may be slightly higher than what actually occurs since clutch sizes for 17/20 (85%) of females (Table 3) came from counts of enlarged follicles (> 12 mm length), some of which may not have completed development. Seven clutches (mean =  $6.0 \pm 1.5$  SD, range 3-7) were from California females; 12 clutches (mean =  $5.7 \pm 1.9$  SD, range 3-9) were from Arizona females. One clutch of 11 came from Washington County, Utah. There was no significant difference between Arizona and California clutch data sets (t = 0.39, df = 17, P = 0.70). The mean clutch size of 6.1 reported herein is close to the average of 6.4 (range = 4-9) reported by Fitch (1970). One female with 11 eggs apparently is a new maximum clutch record for R. lecontei. Linear regression analysis revealed there was no significant positive correlation (r = 0.21, P = 0.36) between female body sizes (SVL) and clutch sizes for 20 R. lecontei (Table 3). This may reflect the snakes being collected in different years and from different geographic areas. The smallest reproductively active female (7 oviductal eggs) measured 509 mm SVL and was from Riverside County, California (LACM 115894). Females smaller than 500 mm SVL were excluded from the study to prevent the inclusion of immature females in the analysis of the ovarian cycle. The smallest reproductively active male R. lecontei measured only 361 mm

b Includes one road-killed snake with squashed oviductal eggs which could not be counted.

Table 3. Clutch sizes for 20 *Rhinocheilus lecontei* (estimated from counts of yolked follicles > 12 mm length or oviductal eggs) from museum specimens.

Date	SVL	Clutch (mm)	Locality size	Source
19 April	534	7	Pinal Co., AZ	ASU 26380
19 April	571	4 <sup>a</sup>	Pima Co., AZ	UAZ 4745
24 April	624	6	Maricopa Co., AZ	ASU 14195
24 April	633	8	Maricopa Co., AZ	ASU 14194
24 April	656	6	Riverside Co., CA	LACM 102677
25 April	586	5	Pima Co., AZ	UAZ 49124
27 April	518	5	Pima Co., AZ	UAZ 48594
6 May	645	7	Riverside Co., CA	LACM 102660
7 May	605	11	Washington Co., UT	LACM 102708
10 May	582	7	Riverside Co., CA	LACM 102664
10 May	593	9	Pima Co., AZ	UAZ 4749
14 May	633	7	San Bernardino Co., CA	LACM 102686
15 May	520	3	San Diego Co., CA	LACM 102697
18 May	552	5	Riverside Co., CA	LACM 102630
19 May	565	8	Pima Co., AZ	UAZ 4733
28 May	525	_ b	Pinal Co., AZ	ASU 29507
2 June	582	4 <sup>a</sup>	Pima Co., AZ	UAZ 26108
4 June	530	4	Pinal Co., AZ	ASU 26384
10 June	713	5	Yuma Co., AZ	ASU 15853
14 June	592	3	Mohave Co., AZ	UAZ 34136
16 June	509	7 <sup>a</sup>	Riverside Co., CA	LACM 115894
1 July	557	- c	Pinal Co., AZ	ASU 28026

<sup>&</sup>lt;sup>a</sup> Oviductal eggs, all other females contained enlarged follicles.

SVL which may suggest males mature at a younger age than females.

The presence of reproductively inactive females of mature size during the months females are reproductively active indicates that not all females breed each year. Forty nine percent (27/55) of adult females from April-July were undergoing yolk deposition, contained enlarged follicles (> 12 mm length) or oviductal eggs.

The reproductive cycle of *R. lecontei* fits the aestival (summer) and postnuptial category of Saint Girons (1982) in which sperm are stored throughout the winter in the vas deferens. This pattern appears common

<sup>&</sup>lt;sup>b</sup> Road-killed snake with coagulated yolk, follicles could not be counted.

<sup>&</sup>lt;sup>c</sup> Road-killed snake with squashed oviductal eggs that could not be counted.

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in other colubrid snakes from western North America and has been reported in: *Masticophis lateralis*, *Masticophis taeniatus*, *Pituophis catenifer*, *Arizona elegans*, *Lampropeltis zonata* and *Lampropeltis pyromelana* (Goldberg 1975; Goldberg & Parker 1975; Aldridge 1979b; Goldberg 1995; Goldberg 1997).

In view of the extensive geographic range (Stebbins 1985) occupied by *R. lecontei*, additional studies on the reproductive biology of populations other than Arizona and California will be needed before the amount of variation from the reproductive pattern described herein can be determined.

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SRG at sgoldberg@whittier.edu

# POLYMORPHIC NATURE OF CRANIAL FLUORESCENCE IN THE FOX SQUIRREL (SCIURUS NIGER) FROM TEXAS AND OKLAHOMA

# Kimberly D. Spradling, Bonnie L. Blossman-Myer and Frederick B. Stangl, Jr.

Department of Biology, Midwestern State University Wichita Falls, Texas 76308

Abstract.—Congenital erythropoietic porphyria (CEP) is a rare pathological condition in man and some domestic animals. Reported diagnostic features include skin lesions, shortened life span, darkened bones and fluorescent skeletal tissues. Literature sources report the condition is characteristic of fox squirrels (Sciurus niger), and that the species suffers no ill effect of the disease. Examination under an ultraviolet light of 157 skulls and associated mandibles of S. niger from Texas and Oklahoma revealed that skeletal fluorescence is a polymorphic feature that affects only about 70 percent of examined specimens. Sexes did not vary in frequency of occurrence of this phenomenon, although adult specimens were significantly more likely to fluoresce than those of younger age classes. Among other findings are the purple fluorescence of nestling and juvenile specimens, in contrast to the orange coloration of older age classes. These results suggest several potential avenues for future study relative to the biogeography and genetics of this phenomenon in S. niger.

Congenital erythropoietic porphyria (CEP) is a rare pathological condition that has been documented in humans and some domestic species of mammals. Onset of the diagnostic skin lesions and hemolytic anemia occurs in humans shortly after birth or early in childhood, and life expectancy is limited to a few years. The disease results from a defect in heme biosynthesis and the resulting porphyrins are distributed in the urine, feces, and tissues such as spleen, blood, bones and teeth. Deposition of these porphyrins imparts both a dark color to bone and a fluorescent quality is reported for the skeletal and other affected tissues of diseased individuals when exposed to long-wave ultraviolet light (Schmid et al. 1954; 1955; Bloomer et al. 1993).

The unusual dark pink to dull reddish hue of the skull and post-cranial skeleton of specimens of the fox squirrel (*Sciurus niger*) is a distinctive feature that has long been noted by naturalists and has even been used by some biologists (Jones et al. 1983; Lowery 1974) as a means to distinguish the skull of this species from that of the morphologically similar skull of the gray squirrel (*S. carolinensis*). Turner's (1937) documentation of porphyria as the causative factor of this phenomenon

in S. niger was followed up in more detail by Levin & Flyger (1973) and Flyger & Levin (1977). Laboratory studies by these workers of the urine, blood and bone marrow demonstrated the presence of CEP in their series of fox squirrels, as well as an absence of the condition in a comparable series of gray squirrels.

Flyger & Levin (1977) noted that *S. niger* is asymptomatic for pathological aspects of CEP, which prompted them to suggest the species as a laboratory animal model for future studies of this physiological condition, but perhaps because of the rarity of the disease in humans, there has been no answer to their call for follow-up investigations of the species in this role. However, this study has been cited as the basis for a series of statements in the literature (Caire et al. 1989; Fitzgerald et al. 1994; Flyger 1999; Koprowski 1994) proclaiming CEP and the resulting fluorescent qualities of bones and teeth as characteristic of the fox squirrel.

The impetus for the present study was provided by a recent mammalogy laboratory exercise conducted by the third author, which unexpectedly demonstrated that a large proportion of fox squirrel skulls did not fluoresce when exposed to ultraviolet light. This study details the regional polymorphic aspect of this unusual feature in *S. niger*, and describes the frequency of fluorescence between sexes and relative age groups.

## METHODS AND MATERIALS

Skulls of Sciurus niger deposited in the Collection of Recent Mammals of Midwestern State University comprised the basis for this investigation. This study was restricted to those specimens from Oklahoma and Texas because sample sizes from other states were judged insufficient to characterize any regional polymorphism for porphyria. Individual specimens were recorded as to gender (when known), specific locality (state and county), date of collection and relative age classes. Cranial dimensions and degree of ossification were useful age class indicators, as were the following dental criteria: nestlings (no visible formation of tooth crowns in alveoli); juveniles (incomplete battery of erupted molars - at least M3 not yet erupted); subadults (erupting or newly erupted P4, and all molars erupted); adults (full dental complement exhibiting wear on occlusal surface of all cheek teeth). Statistical

analyses of the association of these parameters with the incidence of fluorescence were performed using the NCSS 97 statistical package (Hintze 1997).

Specimens were examined under a portable, battery-operated, long-wave ultraviolet lamp Model ML-49 (manufactured by UVP Incorporated, P.O. Box 1501, San Gabriel, California 91778). Fluorescence was treated as a qualitative character, although intensity and cranial distribution of the phenomenon varied considerably, and these features were recorded for each specimen.

An initial concern was that sometimes-harsh curatorial treatment or length-of-storage of specimens might have had a negative effect on the fluorescent property of some skulls. To address the issue of possible postmortem change, a skull of known fluorescent properties was placed in a solution of water and sodium carbonate ("soda ash"; sometimes used to soften persistent connective tissue for ease of removal from skeletal preparations) and boiled for 5 min, followed by 8 h in a drying oven at 80°C. The dried skull fluoresced as before. Similarly, length-of-storage of specimens appeared to have no effect, for some of the fluorescent specimens date back to the founding of MWSU collection in the early 1950s.

#### **RESULTS**

Of the 157 skulls and associated mandibles examined in this study, 108 exhibited some degree of fluorescence. Sites most commonly found to be fluorescent were the palatal complex, frontals and parietals of the skull, and the masseteric fossa of the mandible. Among the upper and lower dentition, the incisors and premolars were most commonly observed to react under UV light. Incidence of fluorescent skulls per examined specimens (arranged by state and county) are as follows: OKLAHOMA (6/6): Bryan Co., 1/1; Commanche Co., 2/2; Cotton Co., 1/1; Pontoco Co., 1/1; Rogers Co., 1/1. TEXAS (97/151): Archer Co., 1/3; Baylor Co., 0/3; Bowie Co., 1/1; Brown Co., 1/1; Clay Co., 2/5; Collin Co., 0/1; Cooke Co., 1/1; Ellis Co., 1/1; Fannin Co., 1/2; Grayson Co., 1/1; Haskell Co., 0/1; Jack Co., 1/1; Kimble Co., 9/12; Knox Co., 2/3; Milam Co., 1/1; Montague Co., 17/22; Stephens Co., 1/1; Sutton Co., 1/1; Tarrant Co., 2/2; Wichita Co., 50/77; Wilbarger Co. 1/8; Young Co., 3/3.

Table 1. Statistical analyses for frequency of occurrence by sex and relative age category of cranial and mandibular fluorescence among 157 specimens of *Sciurus niger* from Oklahoma and Texas.

Parameters (n)	No. normal skulls (No. fluorescent skulls)	Frequency of fluorescence
Specimens of known sex (146)		
Males (79)	25 (54)	0.68
Females (67)	18 (49)	0.73
Specimens of unknown sex (11)	6 (5)	0.45
Age classes *		
Nestlings (8)	3 (5)	0.63
Juveniles (3)	2 (1)	0.33
Subadults (37)	17 (20)	0.54
Adults (109)	27 (82)	0.75
Pooled age classes **		
Adults (109)	27 (82)	0.75
Subadults and younger (48)	22 (26)	0.54

<sup>\*</sup> ANOVA: P < 0.05

Cranial and mandibular materials of subadult and adult specimens reflected an orange to reddish color under the UV lamp. Fluorescence among the two younger age categories was a distinctive dull purple color, which was restricted to the frontal-parietal complex of the skull and upper incisors, although the palate of one nestling also fluoresced.

Frequency of fluorescence between age categories was significant, with adult-aged specimens significantly more likely to exhibit fluorescence than specimens of younger age (Table 1). Juveniles represented the smallest sample (one of three specimens of this age category exhibited fluorescence), and slightly more than half of the subadult specimens fluoresced. Adults, comprising nearly two-thirds of the total sample, exhibited a significantly greater frequency of fluorescence than the pooled number of individuals representing younger age categories.

#### DISCUSSION

Fluorescent coloration and bone pigmentation.—Porphyrin compounds and heme pigments deposited in the calcified tissues produce the dark coloration reported of skeletal materials in *Sciurus niger*. How-

<sup>\*\*</sup> Two-tailed t-test; P < 0.01.

ever, bone coloration was found to be a poor indicator of fluorescence, for some paler materials fluoresced brightly, whereas some darker materials failed to evince fluorescence. The observed lack of fluorescent characteristics may be the result of deposition of intermediates of porphyrin biosynthesis, such as the partially oxidized "porphomethenes" characterized by Mauzerall & Granick (1958). These intermediates, which would contribute to bone-darkening features, are purported to exhibit no fluorescence (Schwartz et al. 1980).

Color and regional distribution of fluorescent pigments in materials used in this study contrast somewhat with the findings of Levin & Flyger (1973), who reported that their freshly dissected skeletal materials of *S. niger* fluoresced a "brilliant crimson" over the entire surface of the skull. Perhaps areas of lesser deposition of porphyrins lose fluorescent properties soon after death, while regions of heavier deposition remain fluorescent for at least decades.

Color changes from the crimson of fresh material (Levin & Flyger 1973) to the orange of MWSU museum specimens of the two older age categories may be attributed to pH changes incurred in the museum preparatory, preservation and storage processes, for Schwartz et al. (1980) observed that porphyrins fluoresce orange in acidic solutions and appear red under alkaline conditions. However, no mention exists in earlier studies of this purple coloration unique to specimens of the two younger age categories, which leads one to believe that the specimens reported by Flyger & Levin (1977) were adults. This distinctive purple fluorescence of nestling and juvenile fox squirrels likely represents the differential fluorescent qualities of a precursor product to porpyrin I, although laboratory verification is warranted.

Geographic aspects.—The study by Levin & Flyger (1973) is responsible for subsequent inferences in the literature that all members of Sciurus niger are characterized by congenital erythropoietic porphyria, and that skeletal tissues of the species fluoresce diagnostically under ultraviolet light. The bases for these assumptions presumably were the widely separated (but not specifically detailed) geographic localities from which their samples were procured: Maryland and a commercial squirrel farm in Palestine, Texas. However, results reported in this study suggest that these conclusions were premature. Levin & Flyger (1973) never stated their total sample size, although from the varying number

of specimens subjected to their hematological and biochemical assays, it is deduced that no more that 15 animals of unknown age were at their disposal. Their remarks on the fluorescent nature of skeletal tissues suggest that the phenomenon occurs in all animals, although it is not clear whether all specimens were tested for fluorescence.

This study clearly demonstrates the polymorphic nature of cranial and dental fluorescence in the fox squirrel in Texas and Oklahoma. Nine of the 12 counties represented by two or more specimens produced both fluorescent and non-fluorescent specimens, and no discernable pattern of distribution of the phenomenon was evident. However, the species is usually abundantly represented in systematic mammal collections, and the examination of holdings from across a wide range of the species' distribution will aid in determination of the geographic scope of this polymorphism.

Age class variation.—The high frequency of occurrence of fluorescence among nestling-aged specimens deserves some clarification. This sample is comprised of three litters (n=1,2,5), and each of the five fluorescent specimens were siblings of the largest litter. With such a limited series, sampling error cannot be discounted, although a genetic basis sufficiently simple to be readily demonstrated by breeding studies seems plausible.

The authors are reluctant to offer an explanation for the significantly greater occurrence of fluorescence among adult-aged specimens, especially given the limited samples of the two younger age categories (Table 1). The younger age classes for most species are commonly underrepresented in systematic collections (Stangl & Jones 1987), and concerted efforts to address this age bias is warranted before addressing any possible differential fitness topics.

Medical implications.—The observed polymorphism for bone fluorescence in S. niger is based on museum specimens subjected to a series of environmental variables during the course of procurement and preparation, and not on living tissues. Nevertheless, findings from this study suggest the need to address the potential utility of S. niger as an animal model for porphyria research, as first proposed by Flyger & Levin (1977). If CEP is indeed the normal physiological condition in the fox squirrel, then fluorescence may not be an accurate indicator of the

condition in this species (porphyrins deposited in tissues other than bone in some animals). If skeletal fluorescence proves to be a reliable indicator of CEP, then the fox squirrel could prove useful in determining the genetic basis for this condition.

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FBS at: stanglf@nexus.mwsu.edu

# RHIZOSPHERE ACTIVITY OF CATTAIL (TYPHA LATIFOLIA) AND SPIKE RUSH (ELEOCHARIS TUBERCULOSA) INHABITING AN OIL SPILL

### Chris A. Allen and Stephen C. Wagner

Department of Biology, Stephen F. Austin State University Nacogdoches, Texas 75962

Abstract.—The esterase activity and populations of soil microorganisms inhabiting cattail (Typha latifolia) and spike rush (Eleocharis tuberculosa) stands growing in a crude oil and brine spill within the Roy E. Larsen Sandyland Sanctuary, Silsbee, Texas were investigated. Rhizosphere and non-rhizosphere soil samples were analyzed for populations of total bacteria, gram-negative bacteria, fungi and actinomycetes, and soil esterase activity. No significant differences among population numbers were found within the microorganism categories. Esterase activity was significantly higher in soils collected outside the contaminated site than within. Twenty-four potential crude oil degraders were isolated from the soil samples and screened for growth on diesel, pentane and anthracene and tolerance to NaCl. Twelve and five isolates utilized diesel and pentane, respectively, as sole carbon sources while none utilized anthracene. All, 23, 17 and 10 of the isolates were found to be tolerant of 0.85, 5, 10 and 15% salt, respectively. Although microbial populations were not affected by the spill, microbial activity was significantly reduced within the impacted site. This may hinder the ability of hydrocarbon degraders indigenous to the spill site to decompose the oil contaminants.

Contamination of the environment by xenobiotic compounds continues to be a worldwide problem. In the U.S. alone, 300 million metric tons of waste is generated annually (Bollag & Bollag 1995). Chemical industries release 2.5 billion pounds of this material into natural surface and underground water sources or simply onto the land. Spills involving crude oil and various hydrocarbons are major contributors to such pollution (U.S. EPA 1993).

Many bacteria and fungi that are capable of degrading petroleum hydrocarbons have been isolated from contaminated sites (Fan & Krishnamurthy 1995). Some of these organisms can degrade crude oil (Atlas & Bartha 1973; Benoit & Wiggers 1995). Others can metabolize crude oil constituents such as benzenes, toluene, ethylbenzene and xylenes (Fulthorpe et al. 1996; Weiner & Lovley 1998) and polycyclic aromatic hydrocarbons (PAH) (Tongpim & Pickard 1996; Wetzel et al. 1997).

The impact of hydrocarbon contamination on microbial populations has also been investigated. Kastner et al. (1998) found higher polycyclic

aromatic hydrocarbon degraders in a hydrocarbon-contaminated site when compared to an adjacent, non-contaminated site. Hood et al. (1975) also found higher numbers of hydrocarbon degraders in an oil-field salt marsh compared to a pristine area.

The Roy E. Larsen Sandyland Sanctuary abuts State Highway 92, 6.5 miles northeast of Silsbee in Hardin County, Texas. Approximately ten acres of this preserve was impacted by an accidental crude oil/brine spill in 1982 resulting in a significant reduction in plant life within this area. Since the spill occurred, two plant species, cattail (*Typha latifolia*) and spike rush (*Eleocharis tuberculosa*), have colonized the area. The combination of the spilled crude oil and brine together with the emergence of the two plant species offers the opportunity to study the impact of the spill on microbial communities associated with the plants. This report describes an initial study of the effect of the contamination on microorganisms inhabiting the site.

The two objectives of this study were to: (1) assess the impact of the spill on rhizosphere and non-rhizosphere microbial populations and activity; and (2) characterize microbes inhabiting the spill site in terms of petroleum hydrocarbon utilization and salt tolerance.

#### MATERIALS AND METHODS

Sample collection.—Soil samples were collected from the oil/brine-contaminated site and adjacent, non-contaminated areas. The oil/brine-contaminated site is located on an access road approximately 150 m south of Highway 92, 6.5 miles northeast of Silsbee in Hardin County, Texas. The adjacent non-contaminated areas were distinguishable from the spill site because they contained many plants (Fagus, Magnolia, Myrica, Pinus and Quercus) not found within the spill site, indicating that these areas were not impacted by the oil spill. Rhizosphere (roots and soil adhering to the roots) and non-rhizosphere (soil shaken from the roots) soil samples of cattail and spike rush were removed from 20 by 20 cm plots randomly selected at least 30 m within the contaminated field and placed in plastic bags at 4°C for transport and storage. Because spike rush was not found outside the spill site, only cattail stands were randomly selected and sampled from adjacent, non-contaminated areas that were at least 30 m away from the contaminated area's perimeter.

Soil populations and enzyme activity.—Soil samples were enumerated

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for total bacteria, gram-negative bacteria, fungi and actinomycetes using standard dilution plate count methods (Zuberer 1994). Diluted soils were plated with a Model D automated spiral plater (Spiral Systems, Inc.) on yeast extract peptone agar (YEP) for total bacteria, YEP supplemented with crystal violet (4 mg/L) (YEPCV) for gram-negative bacteria, glycerol casein agar (GC) for actinomycetes and potato dextrose agar (PDA) for fungi (Goodfellow 1968; Williams & Wellington 1982; Fuhrmann 1994). The YEP, YEPCV, and GC were supplemented with cycloheximide (0.05 g/L) to inhibit fungi while the PDA was supplemented with streptomycin (0.03 g/L) to inhibit bacteria. After incubation at 28°C, colonies were counted on each of the plates using a Quebec colony counter.

The soil samples were also analyzed for enzyme activity using an esterase assay that incorporates fluorescein diacetate (FDA) as the substrate (Schunrer & Roswall 1982). This assay is highly correlated with respiration and is a general indicator of microbial metabolic activity.

Petroleum degrader characterization.—Crude oil degraders inhabiting rhizosphere soil samples from rush and cattail plots in both oil-contaminated and non-contaminated areas were isolated by an enrichment technique (Focht 1994) using Bushnell-Haas broth (Bushnell & Haas 1941) supplemented with crude oil (0.2 g/L). Isolated colonies were maintained on tryptic soy agar slants at 4°C prior to testing for carbon utilization.

Twenty-four isolates from the procedure described above were screened for utilization of anthracene, diesel and pentane as sole carbon sources (Madsen 1997). Each strain was grown in YEP broth and diluted to approximately 10<sup>5</sup> colony forming units (cfu)/mL with phosphate buffer (0.1 M, pH 7.2). Test tubes containing 4.5 mL Bushnell-Haas medium supplemented with yeast extract (10 mg/L) and one of the carbon sources (1000 mg/L) were inoculated with 0.5 mL of each cell suspension (final concentration of 10<sup>4</sup> cfu/mL). The inoculated tubes were incubated on a reciprocal shaker (150 rpm) for 20 days (28°C). Visible turbidity (optical density > 0.1 @ 600 nm as measured with a Milton Roy Spectronic 21D spectrophotometer) indicated growth on the carbon source (an increase in cell number from (10<sup>4</sup> to 10<sup>8</sup> cfu/mL).

The isolates were also tested for tolerance to four different concentrations of salt (0.85, 5, 10 and 15% w/v). One hundred micro-

Table 1. Effect of crude oil and brine contamination on microbial populations and esterase activity of soils collected from cattail (Typha latifolia) and spike rush (Eleocharis tuberculosa) inhabiting the Roy E. Larsen Sandyland Sanctuary.

Plant	Location	RS c	p SN	RS	RS <sup>c</sup> NS <sup>d</sup> RS NS RS NS	RS	NS	RS	SS NS	RS	RS NS
E. tuberculosa	Contaminated	0.9	4.8	5.4 4.6	4.6	5.1 4.7	4.7	5.4	4.6	5.4 4.6 12.6	14.7
T. latifolia	Contaminated	5.9	6.3	5.4	5.1	5.4	5.4 3.9	4.9	4.9 4.8	11.5	8.6
T. latifolia	Non-Contaminated 6.2	6.2	5.6	5.7	5.0	6.2 4.5	4.5	5.0	4.6	30.5	36.7
T. latifolia	Non-Contaminated	6.2	5.6	5.7	5.0	6.2	4.5		5.0	5.0 4.6	5.0 4.6 30.5 36.7

log ctu/g (dry weight)
μmol fluorescein diacetate hydrolyzed h/g (dry weight)
Rhizosphere soil

d Non-rhizosphere soil

Table 2. Analysis of variance of microbial enumeration data collected from the Roy E. Larsen Sandyland Sanctuary.

Microbial Group	Effect	DF Effect	MS Effect	DF Error	MS Error	F Value	p-level
Total Bacteria	1	5	1.1899	16	0.7043	1.6896	0.1942
Gram-negative Bacteria	teria 1	5	0.7406	16	0.5307	1.3954	0.2782
Actinomycetes	1	5	2.1729	16	2.1484	1.0114	0.4429
Fungi	-	5	0.2689	16	0.5143	0.5228	0.7556

 $\alpha = 0.05$ 

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liters of an overnight culture grown in YEP broth were transferred to test tubes containing 5 mL YEP media supplemented with one of the four salt concentrations. Inoculated tubes were incubated at room temperature on a shaker (150 rpm) for 7 days. Tolerance to a salt concentration was indicated by visible turbidity as described above.

Statistical analysis.—An analysis of variance (ANOVA) was performed comparing microbial numbers and FDA hydrolysis (Statistica, StatSoft, Inc., Tulsa, OK). When significant differences were found from this ANOVA, the following planned comparisons were performed: contaminated vs. non-contaminated soil from the cattail stands; contaminated rush vs. contaminated cattail soil; rhizosphere vs. non-rhizosphere soil within the contaminated cattail stands; and rhizosphere vs. non-rhizosphere soil within the contaminated rush stands. Because these planned comparisons were not orthogonal, alpha was adjusted to 0.0125.

#### RESULTS AND DISCUSSION

Soil populations and enzyme activity.—Total bacteria numbers ranged from 4.8-6.2 log cfu/g soil; gram-negative bacteria, 4.6-5.8 log cfu/g soil; fungi, 4.6-5.4 log cfu/g soil; and actinomycetes, 3.9-6.2 log cfu/g soil (Table 1). There were no significant differences between treatments in terms of any microbial parameters tested (Table 2). Additionally, populations of total bacteria, gram-negative bacteria, fungi and actinomycetes within the contaminated zone were not significantly different from those outside the spill area. These data suggest that contamination of the site by crude oil and brine had a minimal impact on microbial populations within the site. Similarly, Cobet & Guard (1973) found that a bunker fuel spill did not impact microbial populations on three San Francisco beaches. On the other hand, Wright et al. (1997) reported less hydrocarbon degraders than non-degraders in oil-contaminated samples in a salt marsh. Others have found that oil contamination increased microbial populations (Hood et al. 1975; Horowitz & Atlas 1977).

The fact that numbers of rhizosphere microorganisms of plants within the contaminated zone were not significantly different from those outside suggests that the spill did not impact microbial populations. However, the oil/brine spill could have been more detrimental on microbial populations if the cattail and spike rush plants were absent. The plant species growing within the oil-impacted zone may be helping to lessen the

Table 3. Analysis of variance of pair-wise comparisons for soil esterase activity of soils collected from cattail (Typha latifolia) and spike rush (Eleocharis tuberculosa) inhabiting Roy E. Larsen Sandyland Sanctuary.

Source of Variation	df	Mean Square	F Value	p-level
T. latifolia Rhizosphere vs. Non-Rhizosphere	1	5.44	0.059	0.811
E. tuberculosa Rhizosphere vs. Non-Rhizosphere	-	8.82	960.0	0.761
E. tuberculosa vs. T. latifolia	-	36.00	0.390	0.541
Contaminated vs. Non-Contaminated T. latifolia	,	1361.16	14.766	0.001
Error	16	92.24	I	1

Table 4. Response of microorganisms isolated from cattail (Typha latifolia) and spike rush (Eleocharis tuberculosa) to diesel or pentane as sole carbon sources and to various levels of NaCl.

Plant Type  E. uberculosa  E. uberculosa  T. latifolia  T. latifolia  T. latifolia	0.5	Growin on rightrocaroons	TOCALDONS	N.	Naci Iolerance	
E. tuberculosa E. tuberculosa T. latifolia T. latifolia T. latifolia		Diesel	Pentane	2%	10%	15%
E. tuberculosa T. latifolia T. latifolia T. latifolia T. latifolia	-	-	0	-	-	0
T. latifolia T. latifolia T. latifolia	4	-	_	4	4	· C
T. latifolia T. latifolia	4	-	-	4	- 4	10
T. latifolia	4			. 4	. ~	10
: F	S	4	. 0	4	-	۰-
Non-Contaminated 1. tatifolia Non-Khizosphere 6	9	4	7	. 9	· vo	. ~

Number of isolates that showed growth (optical density > 0.1 @ 600 nm) in media with hydrocarbons as sole carbon sources or at each concentration of NaCl.

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effects of the crude oil and brine on the microbes. There is increasing evidence that plants enhance the survival of degrader organisms and also shorten the time needed for these organisms to acclimate to the contamination and thus start degrading the pollutants. For example, the plant rhizosphere supports large numbers of microbial species at high population numbers that may be capable of stepwise transformation of xenobiotics (Crowley et al. 1997).

Soil esterase activity in soils, as determined by FDA hydrolysis, ranged from 9.8 to 36.4 FDA  $\mu$ mol/h/g (Table 1). Enzyme activity in *T. latifolia* samples from outside the contamination site was threefold higher than that of soils taken from within the site. Pairwise comparisons revealed that total microbial activity was significantly higher in non-contaminated cattail samples than samples taken within the contaminated site (Table 3). The lower levels of microbial activity within the contaminated site could be caused by the crude oil and brine. This circumstance might increase the longevity of the pollutants in the spill zone because microorganisms capable of degrading the oil are not actively breaking down organic compounds. Similarly, Schunrer & Roswall (1982) reported greater soil esterase activity in non-contaminated soil.

One could argue that the population data indicates that the oil spill impacted the adjacent non-contaminated sample sites. However many of the plant species found at these sites are normally found in American beech and southern magnolia forests that are typical of this part of east Texas. The presence of these plant species suggests that the oil brine spill did not impact these sites. The fact that esterase activity was significantly higher in samples from these sites compared to those from the oil/brine-impacted area provides further support for this argument.

Petroleum degrader characterization.—Of the 24 isolates, twelve and five utilized diesel and pentane, respectively, as a sole source of carbon; four of the five pentane degraders also utilized diesel (Table 4). None of the isolates screened were able to use anthracene as a sole carbon source. Because, anthracene is more difficult to break down than pentane and diesel due to its complex multiple ring structure (Cole 1994) it is not surprising that no anthracene degraders were isolated. Also, diesel and pentane would more closely resemble hydrocarbons commonly found in crude oil (Benoit & Wiggers 1995).

Diesel degraders were isolated from both oil-contaminated and non-contaminated soils and from both rhizosphere and non-rhizosphere samples from both plant species. This indicates that hydrocarbon degraders are present within and outside of the spill zone and that one particular plant species did not favor hydrocarbon degraders. In a similar investigation, Benoit & Wiggers (1995) reported that five strains of bacteria were able to utilize diesel while four utilized mineral oil.

A Gordona strain was capable of metabolizing mid-distillates and light distillates (Rhee et al. 1998). Others found microorganisms capable of degrading some of the complex hydrocarbons found in crude oil and diesel. Tongpim & Pickard (1996) isolated three Rhodococcus strains that were able to utilize anthracene; one isolate was able to degrade 2-methylanthracene and 2-chloroanthracene. Fungi have been found to break down lengthy hydrocarbon chains (Fan & Krishnamurthy 1995) as well as benzene, toluene, ethylene, and xylene (MacDonald & Rittman 1993).

All isolates tolerated 0.85% salt while 23 out of 24 tolerated 5% salt (Table 4). Seventeen of the isolates grew in the presence of 10% salt while 10 isolates were found to be tolerant of 15% salt. Eleven of the 17 isolates found tolerant of 10% salt were isolated from soils collected within the contaminated area with five from rush soil and six from cattail soil. Two isolates tolerant of 15% salt were isolated from contaminated rush samples and four from contaminated cattail samples. The remaining four isolates tolerant of 15% salt were from outside the contaminated area. These results suggest that the microflora within the contamination site have adapted to the effects of the spill. These organisms may be able to carry out biodegradation of the oil contaminants under the site's salinity conditions. Similarly, Ashok et al. (1995) found that PAH-degrading bacteria isolated from soil located near an oil refinery grew in media containing 7.5% NaCl.

#### **CONCLUSIONS**

Though the contamination at Roy E. Larson of the site by crude oil and brine had adversely affected the local fauna and flora, the impact on microbial communities was found to be less detrimental as judged by total counts. Both *Eleocharis* and *Typha* are supporting a viable population of microbes that could be capable of metabolizing the crude oil. Indeed, diesel and pentane degraders were isolated from non-contaminated as well as oil-contaminated areas. About half of these isolates were also tolerant of high salt concentrations.

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Because significantly lower levels of esterase activity were found within the oil-contaminated area, the ability of the microbial populations to break down the oil may be hindered. This may mean that indigenous microorganisms may not be able to remediate the oil spill under present conditions or may do so at lower rates than desired. Conditions at the site may have to be altered in order to stimulate hydrocarbon degraders to remediate the oil spill, warranting further research of this site.

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SCW at: swagner@sfasu.edu

## DIFFERENTIATION OF BOURBON WHISKIES USING GAS CHROMATOGRAPHY AND CLUSTER ANALYSIS

Michael M. Looney and Amy C. Ford
Chemistry Department, Schreiner College, CMB 5964
Kerrville, Texas 78028

Abstract.—The practice of counterfeiting liquors in drinking establishments is common today. In order for a forensic scientist to help prevent this from occurring, a database of chromatographs must be compiled of all the spirits available. In this study, five brands of bourbon were analyzed and cluster analysis was applied to the gas chromatographic data. The cluster analysis showed the least expensive brand and the most expensive brand to have the greatest differences, but the three other brands formed one large third group. These groupings showed that one can distinguish one brand of bourbon from another. This study should prove useful to consumers and distillers to help in the detection of counterfeit liquors.

There have always been attempts to adulterate spirits, for instance by blending high-quality distillates with ethanol made from a cheaper raw material, by adding synthetic volatile components to natural alcohol or by misleading labeling of the variety and origin of the raw material used. The practice in drinking establishments of placing an inexpensive brand of liquor into a well-known expensive bottle is quite common. To prevent this, research must be performed and a database compiled of each of the varieties of liquors. Then, using a portable gas chromatograph, a forensic scientist could go to an establishment and test the sample on site. This counterfeiting seems minimal, but it is costly for the major distilleries. Thus chemists need a simple method for distinguishing among various types of aged whiskeys. One such aged whiskey is bourbon. Chemists over the years have been attempting to analyze the differences in brands of bourbons, using various methods.

Gas chromatography (GC) is generally the method of choice for alcoholic beverage analyses, but the complete profile is quite complex and difficult to interpret. The volatile components, other than ethanol, are contained in what is known as the fusel oil fraction. Fusel oil is a collective term applied to the alcoholic fraction of whiskey, or high wine, with a higher boiling point than the ethanol-water azeotrope. Fusel oil usually includes n-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol in various concentrations. Individual components of fusel oil are not usually used to distinguish bourbons. This study was carried out to distinguish among bourbons using GC analysis of the fusel oil components combined with hierarchical cluster

analysis.

Capillary GC with a flame ionization detector has proven to be very effective in separating fusel oils and the resulting chromatograms show excellent separation of the individual components in a variety of whiskeys (Kahn et al. 1968). This study set the standard for the separation of the major fusel oil compounds rapidly and directly. Advantages of Kahn's method are that no prior treatment of the sample is necessary, concentrations of all major fusel oil components can be measured individually, and only a small sample is required.

In a similar study, a well-known brand of Scotch whiskey was compared to various less expensive brands using GC data and cluster analysis (Saxberg et al. 1978). The results showed that the samples of the well-known brand completely separated from the less expensive samples. By building up a GC library, a forensic laboratory could use cluster analysis to distinguish among various whiskey samples. This method could easily be practiced by a mobile laboratory equipped with a portable GC and laptop computer.

Through the years the efficiency and resolution of GC has improved due to the development of capillary columns. These columns using supports such as carbon graphite (Carbopack B) as the adsorbent and Carbowax (Carbowax 20M) as the single liquid phase have been very useful for determining fusel oils (Martin et al. 1981). It has been shown (Di Corcia et al. 1979) that a 5% concentration of Carbowax 20M gives the optimum resolution of methanol, ethanol, n-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol.

Statistical pattern recognition analysis (cluster analysis) of the various whiskeys has been shown to be useful in analyzing fusel oil components, contained in sour mash/bourbon, blended and scotch whiskeys (Wilson et al. 1991). This method involved a single direct injection procedure for separating and quantitatively determining methanol and fusel oil in whiskey using temperature programming with a GC. The data was then analyzed using a statistically based pattern recognition program, Pirouette, to determine whether the fusel oil fraction and methanol concentrations of four whiskeys could be used to distinguish one type of whiskey from another. The results showed that analysis of the fusel oil fraction is sufficient to classify whiskeys as scotch, blended or bourbonsour mash.

Other studies have also shown that pattern recognition can be applied

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to GC data in the analysis of alcoholic beverages. Varieties of wine and grapes (Rapp et al. 1978) have been differentiated, wine has been classified as to geographic origin (Kwan & Kowalski 1980), French and German grape brandies have been distinguished (Schreier & Reiner 1979), and Venetian white wines have been classified (Scarponi et al. 1982; Moret et al. 1984a; Moret et al. 1984b).

The current study performed on bourbon whiskies is patterned after the techniques of Saxberg et al. (1978) and Wilson et al. (1991), this research team developed a technique that provided a distinct separation of five known bourbon brands. Using a GC and cluster analysis, data proved to be distinct among the various brands. But, even though the results answered the question at hand, much more research is needed on alcoholic products to compile the database needed to lessen the likelihood of counterfeiting.

### METHODS AND MATERIALS

Sample collection.—Five bourbon whiskey bottles were purchased from a major liquor store in Kerrville, Texas. The five whiskey samples are as follows: Kentucky Deluxe (KD), Ten High (TH), Old Taylor (OT), Jim Beam Black (JB) and Knob Creek (KC). The particular samples were chosen because they were Kentucky bourbons and they showed a broad range in price from \$0.29 to \$1.08 per ounce. The selection of these five brands in no way represents an endorsement of these products; their selection was based entirely upon cost per unit volume.

Sampling method.—Unopened bottles of the five bourbon whiskeys were kept at room temperature. When each sample was ready to be tested, the bottle was shaken, the seal was broken and 20 mL was poured into a labeled 50 mL Erlenmeyer flask. The flask was immediately sealed and the bottle resealed. The samples were removed from the flask using a 10  $\mu$ L Supelco S.G.E. syringe. The sample was then injected into the gas chromatograph.

Sample analysis.—The samples were analyzed using a Buck Scientific (Model 610) gas chromatograph which was equipped with a "split/splitless" capillary injector (operated in split mode) and a flame ionization detector (FID). The column of choice was an Alltech Heliflex® capillary column; Phase: AT-1; 25 meters by 0.25 millimeter I.D. and 0.2 micrometer film thickness with a range of -60°C to 350°C. Helium was used as the carrier gas with a head pressure of 18 psi and

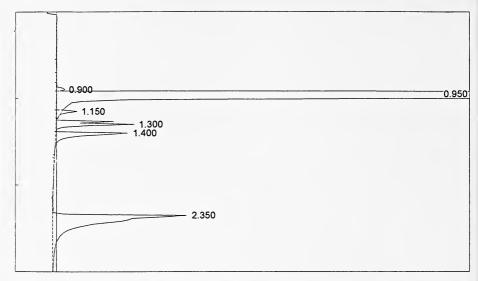


Figure 1. A gas chromatograph for a typical bourbon whiskey. The six peaks correspond to methanol (0.900), ethanol (0.950), 1-propanol (1.150), 2-methyl-1-propanol (1.300), 2-methyl-1-butanol (1.400) and 3-methyl-1-butanol (2.350).

a flow rate of 4 mL/min. The flame gases were air and hydrogen having head pressures of 22 psi and flow rates of 20 mL/min. The initial oven temperature was set at 30°C, which was held for 5 minutes then, increased to 70°C at a rate of 2°C per minute. This made the total run time 25 minutes.

Peak Simple for Windows was used on the IBM compatible computer for instrument control and data collection. Five samples of each bourbon were analyzed, the samples being obtained as described above. Microsoft Excel and Pirouette (version 2.7)(Infometrix, Seattle, WA) software were also used for data analysis on IBM compatible computers.

### RESULTS AND DISCUSSION

The resulting chromatograms of the bourbons were visually compared to one another to determine which peaks occurred in all of the bourbons. Figure 1 shows a typical chromatogram of a bourbon whiskey labeled with retention times. The six peaks correspond to methanol, ethanol, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol. Visual comparison of chromatograms is very difficult when complex samples are analyzed; thus, eight peaks that showed the greatest similarity were selected for analysis. One easily identifiable peak,

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Table 1. Relative retention times and normalized peak areas of bourbon samples.

RRT:	9.335	10.000	12.013	13.628	14.731	15.080	24.569	25.784
Sample								
KD1	0.000	997.481	0.316	0.000	0.000	0.230	0.000	1.972
KD2	0.000	998.066	0.199	0.125	0.000	0.177	0.000	1.287
KD3	0.000	998.245	0.129	0.123	0.000	0.157	0.000	1.130
KD4	0.000	991.893	0.103	0.103	0.000	0.169	0.000	1.164
KD5	0.000	998.287	0.126	0.118	0.000	0.157	0.000	1.198
TH1	0.169	989.170	0.577	0.490	1.936	0.000	7.567	0.000
TH2	0.173	987.549	0.941	0.714	2.587	0.000	7.984	0.000
TH3	0.182	989.065	0.655	0.709	2.137	0.000	6.933	0.000
TH4	0.180	988.887	0.578	0.511	2.127	0.000	7.567	0.000
TH5	0.170	990.019	0.454	0.867	1.807	0.000	6.592	0.000
OT1	0.000	990.590	0.000	0.417	1.476	0.000	7.399	0.000
OT2	0.139	991.724	0.318	0.687	1.146	0.000	5.986	0.000
OT3	0.149	991.647	0.209	0.490	1.253	0.000	6.171	0.000
OT4	0.159	991.758	0.264	0.426	1.267	0.000	5.790	0.000
OT5	0.105	990.427	0.348	0.455	1.502	0.000	6.917	0.000
JB1	0.120	987.380	0.785	0.318	1.631	0.000	9.767	0.000
JB2	0.188	991.165	0.494	0.643	1.102	0.000	6.040	0.000
JB3	0.162	991.859	0.405	0.556	1.221	0.000	5.547	0.000
JB4	0.246	991.653	0.471	0.560	1.127	0.000	5.943	0.000
JB5	0.135	991.184	0.593	0.468	1.154	0.000	6.138	0.000
KC1	0.000	987.683	0.539	1.090	2.032	0.000	8.160	0.000
KC2	0.139	989.736	0.403	1.473	1.501	0.000	6.428	0.000
KC3	0.099	989.565	0.409	1.711	1.524	0.000	6.692	0.000
KC4	0.087	990.436	0.439	1.036	1.549	0.000	6.204	0.000
KC5	0.096	988.541	0.400	0.970	1.845	0.000	8.147	0.000

ethanol, at the beginning of all of the chromatograms was selected as a reference point. It was assigned a relative retention time (RRT) of ten minutes. A relative retention time was then calculated for the other seven peaks. The data were further treated by normalizing each peak area to the total area of the eight peaks chosen for analysis. This was done in order to eliminate any differences due to sample concentration or the size of sample injected. Table 1 contains the RRT and normalized peak area for the samples. The subtle differences in peak area are difficult to compare when one is comparing several chromatograms (Looney 1998); therefore the data were subjected to cluster analysis.

Hierarchical cluster analysis (HCA) is a method by which distances between points in N-dimensional space, where N is the number of variables measured in the samples, are calculated. A dendrogram, a tree-shaped map, is developed from these distance calculations by linking samples and clusters of samples as a function of distance. Initially, each sample is considered a cluster of one, and then the two most similar samples are linked together. Once this cluster is linked, it searches for a cluster of similar characteristics to link to. The distances

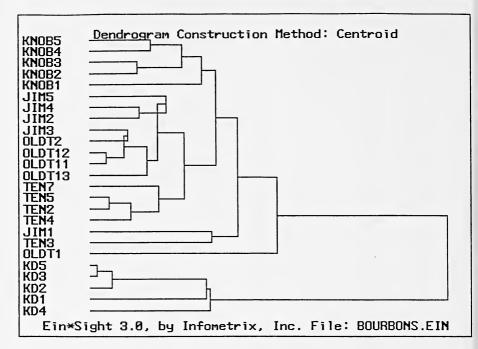


Figure 2. A hierarchical cluster analysis dendrogram produced by Pirouette 2.7 for the data set of the five brands of bourbon whiskey using eight chromatographic peaks. The preprocessing technique was autoscale and the clustering technique was centroid. Note the presence of two distinct clusters representing Knob Creek (KNOB) the top cluster and Kentucky Deluxe (KD) the bottom cluster. The other three brands cluster somewhat, but there is more overlap present.

between all existing clusters are computed, and the smallest distance is again searched and another cluster is created. Continuing this process links all of the samples at some level of similarity. A dendrogram is produced representing all of the links between the samples. The branches of the dendrogram have lengths that are proportional to the distances between the connected clusters.

The data shown in Table 1 were subjected to cluster analysis. Different combinations of preprocessing and linkage methods were used in order to find optimum values. Pirouette offers four types of preprocessing; mean centering, variance scaling, range scaling and auto scaling. The method of choice is usually determined by trial and error. The clustering techniques available in HCA are single link, complete link, centroid, incremental, median, group average and flexible. The preprocessing technique chosen for this data was autoscale. The clustering technique chosen was centroid. A dendrogram (Figure 2) was

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constructed using this data. The top cluster consists of all five Knob Creek (KNOB) samples, the most expensive brand analyzed. The bottom cluster consists of all five Kentucky Deluxe (KD) samples, the least expensive brand. The remaining three brands all clustered in the middle, but it is apparent that these also can be differentiated. Three possible outliers, one representing each brand exist, but no attempt was made to eliminate these from the data set.

### **CONCLUSIONS**

The detection of counterfeit whiskey poses a problem in forensic science. In some bars, the practice of placing an inexpensive brand of liquor into a bottle of a known brand of more expensive liquor generally goes undetected by the consumer. Unfortunately, the distiller has no way of proving this takes place except through a method that could distinguish the liquors. Successful prosecution of a violator requires that an adequate showing be made that the contents of the bottle are not of the brand of liquor represented by the bottle. This particular study used gas chromatography coupled with cluster analysis to analyze five different brands of bourbon to find a distinct difference among brands of bourbon.

Although this study proved that there is a major distinction between samples based on cost, there are still many questions facing the forensic scientist in the study of liquor counterfeiting. In order for the scientist to obtain a sample of the whiskey, he must go into the field and obtain a sample himself to make sure that the sample is valid. A database must be compiled of all of the different brands of liquor and their chromatograms in order to compare the suspect sample.

This method appears to have value as a chromatographic technique for the differentiation of bourbon whiskies, further study is warranted. Other samples of bourbons as well as other types of aged whiskies must be analyzed to ensure that differentiation can be made. A variable that might affect the analysis, but difficult to determine, is the length of time the bottles have been opened. Another variable might be the same brand of bourbon coming from different lots.

### ACKNOWLEDGMENTS

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MML at: mlooney@schreiner.edu

### GENERAL NOTES

### NOTEWORTHY RECORD OF THE SEMINOLE BAT, LASIURUS SEMINOLUS (CHIROPTERA: VESPERTILIONIDAE), IN VAL VERDE COUNTY, TEXAS

Joel G. Brant\* and Robert C. Dowler
Department of Biology, Angelo State University
San Angelo, Texas 76909
\*Current Address:
Department of Biological Science

Texas Tech University Lubbock, Texas 79409

The Seminole bat, Lasiurus seminolus, occurs in the eastern third of Texas and is usually associated with forested areas (Schmidly 1991). It is considered the only non-migratory species of Lasiurus and often utilizes pine forests as roosts (Menzel et al. 1999). The western limits of the Seminole bat's distribution in Texas are thought to be the eastern portion of the state with records for Austin, Burleson, Wharton (Davis & Schmidly 1994) and Fayette counties (Yancey & Jones 1996). Fieldwork in Val Verde County has resulted in documentation of the Seminole bat at a site beyond the currently reported range of the species.

On 10 September 1999, a single female *L. seminolus* was collected from Val Verde County, Devils River State Natural Area, Dolan Springs on Dolan Creek (29° 53.8' N, 100° 59.1' W). This site was sampled for 72 net hours from March to September. The bat was collected in a mist net over the creek a few meters downstream from the springs near a large grove of Plateau live oak (*Quercus fusiformis*) with growths of ball moss (*Tillandsia recurvata*) on their branches. The specimen was deposited in the Angelo State Natural History Collection (ASNHC 10650). Measurements (in mm) for the specimen were: total length, 106; length of tail, 47; length of hind foot, 8; length of ear, 15; length of forearm, 44; length of tragus, 6. The specimen had a mass of 10.5 grams. Other bats collected at this locality were *Tadarida brasiliensis*, *Lasiurus cinereus*, *Myotis velifer*, *Antrozous pallidus* and *Pipistrellus hesperus*.

This record is the farthest west reported for *L. seminolus*. The collecting locality in Val Verde County is approximately 250 miles (425

km) west of the Burleson County record reported by Lee (1987). An additional specimen (TTU 76878) that extends the range to Travis County was located in the Collection of Mammals, Texas Tech University, which was secured through the rabies surveillance program of the Texas Department of Health (M. A. Revelez & C. Jones, pers. comm.).

In Texas, extralimital records for *L. seminolus* have been reported in McLennan County (Wilkins 1987) and Cameron County (Hall 1981). Wilkins (1987) suggested that the extralimital records of the Seminole bat are a result of storms blowing the bats off course. Chapman & Chapman (1999) stated that young Seminole bats tend to wander extensively after they have been weaned and suggested that this, in conjunction with storms, account for the extralimital records in the autumn.

Another possible explanation for this record is that the Rio Grande Valley acts as a corridor for this species to move into west Texas. Baker (1956) suggested this possibility in reference to the dispersal of the eastern pipistrelle (*Pipistrellus subflavus*) into Coahuila, Mexico. The Seminole bat and the eastern pipistrelle have both been recorded seasonally using Spanish moss, *Tillandsia usneoides*, as roost sites (Menzel et al. 1999). Both *Tillandsia usneoides* and *T. recurvata* occur in southern Texas (Correll & Johnston 1979). The Seminole bat could utilize the *T. recurvata* that occur along the river as roost sites and move up the Rio Grande. The Devils River is just a half-mile to the west of the collection site and empties into the Rio Grande 40 miles (68 km) from this site.

Scheel et al. (1996) predicted that tree-roosting bats would utilize new vegetation types in response to global warming, based on an existing vegetation model and general circulation models. These climate changes would allow tree-roosting bats to expand their ranges west and cause an increase in species richness of these bats in southwestern Texas. This record, along with the occurrence of another eastern tree-roosting species, *Nycticeius humeralis*, in the same area (Dowler et al. 1999) are consistent with that prediction.

### **ACKNOWLEDGMENTS**

This research was funded in part through a contract with the Texas Parks and Wildlife Department to survey terrestrial vertebrates of Devils River State Natural Area. We wish to thank Bill Armstrong and Jim Finegan from Devils River State Natural Area for their help and support in field studies. Joshua B. Coffey, Phillip Balfanz and Marisol Salazar assisted in collecting and Brandy J. Martin and Richard A. Humbertson helped in cataloging the specimen. Special thanks go to Ned E. Strenth of Angelo State University and David J. Schmidly of Texas Tech University for help with the literature. Thanks also are due to Terry C. Maxwell of Angelo State University, Clyde Jones of Texas Tech University, and one other anonymous reviewer for comments on the manuscript.

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RCD at: robert.dowler@angelo.edu

### PRELIMINARY OBSERVATIONS ON BREEDING BY THE LLANO POCKET GOPHER, GEOMYS TEXENSIS

### Richard M. Pitts, Jerry R. Choate and Chad K. Mansfield\*

Sternberg Museum of Natural History Fort Hays State University Hays, Kansas 67601 \*Jesuit College Preparatory High School 1234 Inwood Road, Dallas, Texas 75026

The Llano pocket gopher, Geomys texensis, was only recently recognized as a species distinct from the plains pocket gopher, Geomys bursarius, by Block & Zimmerman (1991). Populations now assigned to G. texensis formerly were referred to the subspecies G. bursarius texensis and G. bursarius llanensis. Because the biology of those populations had not been studied extensively, little is known about the biology of G. texensis (cf. Goetze 1998).

The purpose of this note is to present preliminary observations regarding the time of breeding in this species. Voucher specimens are deposited in the Sternberg Museum of Natural History (MHP) at Fort Hays State University, Hays, Kansas, and the Texas Cooperative Wildlife Collections (TCWC), Texas A&M University, College Station, Texas.

On 25 November 1986, a Llano pocket gopher (TCWC 53480) containing three fetuses with crown-rump lengths of about 18 mm was captured 16 mi S of Sabinal, Uvalde County, Texas. On 30 December 1997, a female (MHP 33797) containing two fetuses with crown-rump lengths of about 25 mm was trapped 4.2 mi NE of Bend, Lampasas County, Texas. Finally, on 6 January 1984, a lactating female (TCWC 54100) was trapped 6.2 mi W of Hondo, Medina County, Texas. These records demonstrate that the Llano pocket gopher breeds at least in November and December. Additionally, Davis & Schmidly (1994) presented evidence that this gopher breeds in March.

The period of reproduction in Texas in other species of *Geomys* has been described as follows: *G. attwateri*, October through June; *G. breviceps*, December through August; *G. bursarius*, January through November (essentially year round); *G. knoxjonesi*, presumably similar

to G. bursarius; G. personatus, essentially year round (Pitts et al. 1992; Davis & Schmidly 1994; Goetze 1998). At this time, it is impossible to tell whether G. texensis breeds primarily in winter (like G. attwateri), primarily in summer (like G. breviceps), or essentially year round (like G. personatus, G. bursarius and possibly G. knoxjonesi). It is noteworthy that all these species of Geomys are closely related and that time of reproduction conceivably represents a pre-zygotic form of reproductive isolation of populations where their geographic distributions abut.

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JRC at: jchoate@fhsu.edu

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