



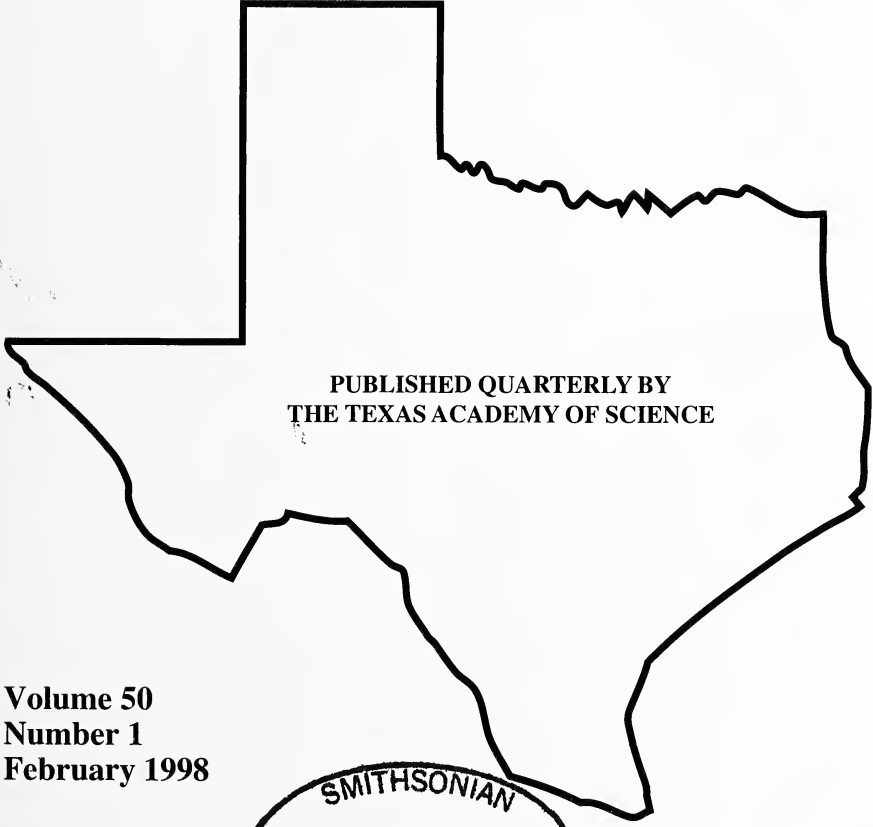






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## CLASSIFICATION OF PRONGHORN FAWNING HABITAT USING LANDSAT THEMATIC MAPPER DATA

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**Abstract.**—Management of wildlife resources requires accurate information about habitat characteristics. Six maps of pronghorn (*Antilocapra americana*) fawning habitat from the Trans-Pecos region of Texas were developed from Landsat 5 Thematic Mapper (TM) data. The goal was to classify fawning habitat and compare unsupervised and supervised habitat classification techniques. Unsupervised and supervised classification training sets were developed for each of two TM scenes taken during the 1990 and 1991 pronghorn breeding season. Data from 12 reference microhabitat variables were used to assess accuracy of the classifications. Additionally, principal components analysis was evaluated as a data reduction technique for the TM data sets. Unsupervised classification accuracy for 1990 and 1991 maps were 41 and 44%, respectively; supervised classification accuracy for each respective map was 29 and 39%. Accuracy of maps after principal components analysis was 31% for unsupervised and 69% for supervised classifications. Supervised training sets performed better than unsupervised training sets in identifying specific fawning habitat signatures. Of the training sets examined, the 1990 supervised set performed the best in identifying critical fawning habitat during drought conditions.

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Satellite acquired data sets are useful for mapping large areas of wildlife habitat (Tueller 1987). Landsat Multispectral Scanner (MSS) data have been used to classify elk (*Cervus elaphus*) habitat in the Blue Mountains of Oregon (Isaacson et al. 1982), American kestrel (*Falco sparverius*) nesting habitat in Oregon (Lyon 1983), and white-tailed deer (*Odocoileus virginianus*) habitat in the Saginaw River Basin of Michigan (Ormsby et al. 1985).

Habitat used by pronghorn (*Antilocapra americana*) during fawning is important to fawn survival (Autenrieth 1982). The ability to remotely identify and assess fawning habitat would be beneficial to pronghorn management programs. Since fawns often occur in specific vegetation types (Pyrah 1974; Autenrieth 1976; 1982; Barrett 1981), vegetation occurring in fawning habitat should produce different spectral signatures, which could be classified from Landsat Thematic Mapper (TM) data.

Although the Trans-Pecos region of Texas represents important habitat for pronghorn, little specific information is available on fawning habitat (Canon 1993). The present study was initiated to evaluate Landsat TM digital data in classifying microhabitat features of pronghorn fawning habitat in the Trans-Pecos region of Texas. Specifically, the objectives were to: (1) evaluate the effectiveness and accuracy of supervised and unsupervised training techniques in a classification scheme using Landsat TM data, and (2) determine if Landsat TM data can be used to classify vegetation used by pronghorn for fawning habitat.

#### STUDY SITE AND METHODS

The study was conducted in the Trans-Pecos region of Texas, on approximately 38,955 ha of the Double U Ranch in Hudspeth County, Texas. The Double U Ranch is part of the University of Texas Lands System. This area includes some of the best pronghorn habitat in the region (Huber 1992).

The climate is arid; annual precipitation averages 22.5 cm at Cornudas, Texas (NESDIS 1991), which is located 16 km east of the eastern boundary of the study area. Topography is characterized as undulating with broad flat draws (Buechner 1950). Four range sites found on the study area are loamy sites, gravelly sites, draws, and limestone hills and mountains.

Major vegetation types are creosote bush (*Larrea tridentata*) and tarbush (*Florensia cernua*) desert shrub, grama (*Bouteloua* sp.) grassland, and yucca (*Yucca* sp.) savannah (Correll & Johnston 1979). Dominant vegetation is blue grama (*Bouteloua gracilis*), burrograss (*Scheropogon brevifolius*) and tobosagrass (*Hilaria mutica*), with some areas having considerable densities of succulent and woody plants including yucca (*Yucca elata*), creosote bush, tarbush, lotebush (*Ziziphus obusifolia*), allthorn (*Koeberlina spinosa*), lechuguilla (*Agave lechuguilla*), and javelina bush (*Condalia ericoides*).

Landsat 5 TM scenes of the study area were taken on 19 June 1990 and 16 April 1991 to coincide with pronghorn parturition (April-June). Cloud cover on the study area prevented use of same day and month scenes for 1990 and 1991.

Landsat TM data for the 1990 scene were extracted from a magnetic tape in four 1024 by 1024 pixel segments six bands deep. Data were then partitioned (SUBSET; Earth Resources Data Analysis System [ERDAS] 1990) to complete a six band 1024 by 1024 pixel image of the

study area that represented 30 by 30 m ground resolution. Problems with downloading band 6, the thermal infrared band, prevented its use in the classification process. The 1991 data were extracted in two 1024 by 1024 pixel segments six bands deep.

Two clustering techniques (STATCL and ISODATA; ERDAS 1990) were used to develop unsupervised training sets for both images. Since it was unknown how spectrally distinct the fawning habitat was, training sets were developed with the maximum number of distinct signatures. A training set with 10 signatures was selected following evaluation of numerous training sets from both clustering techniques (Huber 1992). Ellipse plots (ELLIPSE; ERDAS 1990) were used to evaluate signature distinctness.

Seed clusters were used to build the supervised training sets for both images (SEED; ERDAS 1990). This method allowed the building of training sets using known pixel locations and previous knowledge of pertinent environmental variables. For the 1990 image, characteristics of 14 known fawn bed sites obtained from radio telemetry (Canon 1993) were used to build a signature for the fawning habitat. Signature 10 was the fawning habitat signature for the 1990 error classification matrix of the supervised training set, whereas signature 1 was the fawning habitat signature for the 1991 supervised training set.

The 1991 image was rectified to the 1990 image using nearest-neighbor resampling. Principal components analysis was then performed on the resulting 12 (6 band image for 1990 and 6 band image for 1991) band file (PRINCE; ERDAS 1990). This analysis indicated that a 3-band principal component image was appropriate to capture most (93%) of the variance in the data. The 3-band file was then used to develop unsupervised and supervised training sets. Each training set contained 10 signatures. Signature 1 represented fawning habitat for the principal components supervised training set.

Following development of the training sets, each Landsat data set was classified twice (once for each training set) using a maximum likelihood decision rule (MAXCLAS; ERDAS 1990). A first-pass, parallelepiped classification was used to shorten the time required to run the classification and to avoid potential normality problems of the data (Campbell 1987). These data sets were then stored as Geographic Information System (GIS) files.

The 1990 Landsat data was georectified to the Universal Transverse Mercator grid (GCP, COORDN, and LRECTIFY; ERDAS 1990). A GIS

Table 1. Classification error matrix for the 1990 unsupervised and supervised classifications.

		Supervised														
		Unsupervised					Reference clusters									
Training set signature	Reference clusters					Training set signature	User Acc.	Total	3	4	9	10	11	14	Total	User Acc.
	1	2	3	3	3											
1	16	1	19	36	44%	1	0	0	0	0	1	0	0	1	1	44%
2	12	14	56	82	17%	2	10	0	1	0	4	10	0	25	40%	
3	19	9	69	97	71%	3	7	1	1	0	4	4	0	17	6%	
4	1	6	9	16		4	2	0	1	0	7	7	0	17	6%	
5	0	1	0	1		5	4	0	0	0	0	0	0	4		
6	5	1	2	8		6	0	2	1	5	0	1	0	9		
9	0	2	1	3		8	0	0	0	0	7	1	1	10		
10	0	0	1	1		9	4	1	1	15	2	5	6	34	44%	
						10	4	2	0	1	14	14	3	38	37%	
						11	4	0	0	0	12	17	3	36	47%	
						12	0	0	0	2	0	1	0	3		
						14	5	0	3	3	16	8	2	37	5%	
Total	53	34	157	244		40	6	8	27	67	68	15	231			
Producer Acc.	30%	41%	44%			25%	17%	13%	56%	21%	25%	25%	13%			

Training set signature 10 in the supervised classification matrix is the fawning habitat signature.



data set was then developed for both of the 1990 classified GIS files. For accuracy assessment of locations, 512 stratified random samples were selected with replacement from each of the two 1990 GIS files. Each sampling location was a 3 by 3 pixel array, representing 90 by 90m. Because sampling was random, the 3 by 3 pixel arrays could include more than one signature type. Consequently, the 200 best locations (also stratified), based on purity of the signature class within the 3 by 3 array, were selected from each set of sampling locations for a total of 400 locations. The locations were then plotted on U.S. Geologic Survey 7.5 minute topographic maps.

All signatures were included in the error classification matrix to show misclassifications to the other signatures. Correctly classified pixels were presented on the diagonal, whereas misclassified pixels were on the off-diagonal. Statistics were computed for those signatures that had sufficient reference data.

Reference samples were collected in the summers of 1991 and 1992. Sampling locations were randomly selected. Twelve reference variables were measured at each location. Ten randomly located 0.1m<sup>2</sup> foliar cover plots (Hays et al. 1981) were sampled within a 30 m radius of the center of each sampling location. Percent foliar cover (grass, forb and total foliar cover) and rock cover were estimated in each plot. Also, 10 ten-point frames (Hays et al. 1981) were randomly located from which rock, bare ground, vegetation litter and basal cover (grass, forb and total) were recorded. Succulent and woody plant cover was estimated along four 30 by 2 m belt transects (one in each cardinal direction from the center of each sampling location) in which succulent and woody plant canopy and height were measured.

Data from 256 field sample locations were used to assess map accuracy. Variables were standardized prior to clustering. The EML clustering algorithm was used to group the data into meaningful clusters (SAS Institute Inc. 1989). Final clusters contained five variables; these were succulent and woody plant canopy, bare ground, vegetation litter, rock, and total basal cover. Descriptive statistics were calculated for each cluster and used in the accuracy assessment (CLASERR; ERDAS 1990).

Statistics reported for classification error matrices included overall accuracy (total pixels correctly classified/total pixels), user's accuracy (total pixels correctly classified in a signature/total signature locations referenced), producer's accuracy (total pixels correctly classified/total

Table 2. Mean values of the five habitat variables used to assess the accuracy of the 1990 and 1991 unsupervised and supervised classifications.

Variable	1990										1991														
	Unsupervised Cluster					Supervised Cluster					Unsupervised Cluster					Supervised Cluster									
	1	2	3	2	3	4	3	4	9	7	10	10	11	14	1	2	3	3	9	9	1	2	3	4	5
Brush canopy	1.8	3.7	1.4	1.5	2.3	3.3	3.7	3.7	1.5	1.0	2.2	2.2	3.7	1.3	1.8	2.2	1.8	2.2	2.2	1.5	1.5	1.9	1.0	2.2	3.7
Bare ground	30.4	17.3	53.3	33.4	12.6	28.3	17.3	46.4	59.9	45.7	45.7	30.4	52.9	30.4	45.7	46.4	30.4	45.7	46.4	30.4	59.9	45.7	45.7	45.7	17.3
Litter	53.6	21.7	31.9	53.8	75.7	39.5	21.7	38.5	27.4	18.6	21.7	33.1	53.7	18.6	38.5	54.0	27.4	18.6	38.5	54.0	27.4	18.6	21.7	18.6	21.7
Rock	5.3	55.1	8.0	2.7	1.0	18.4	55.1	7.1	5.2	28.1	5.1	6.2	5.3	28.1	7.1	5.2	5.2	28.1	7.1	5.2	28.1	5.2	5.2	28.1	5.2
Basal cover	9.9	5.8	7.6	10.0	10.7	9.7	5.8	7.7	7.5	7.5	7.5	7.6	9.9	7.5	7.7	10.0	7.5	7.5	7.7	10.0	7.5	7.5	7.5	7.5	5.8

Table 3. Classification error matrix for the 1991 unsupervised and supervised classifications.

Training set signature	Unsupervised										Supervised														
	Reference clusters					Training set signature					Reference clusters					Training set signature									
	1	2	3	9	4	1	2	3	9	4	1	2	3	9	4	1	2	3	9	4	1	2	3	9	4
1	8	22	4	4	38	1	46	19	41	7	46	19	41	7	114	1	50	26%	62%	230	1	13	46%	46%	40%
2	1	78	14	7	100	2	2	6	5	0	2	6	5	0	13	0	0	0	0	0	0	1	42%	42%	42%
3	2	32	17	1	52	3	8	15	18	0	8	15	18	0	24	3	8	16	0	0	8	24	13%	13%	70%
4	0	5	7	0	12	4	10	2	2	3	5	2	2	3	23	1	1	0	0	0	3	23	0	0	0
6	5	0	0	1	6	5	0	2	2	0	6	2	2	0	6	0	0	0	0	0	0	6	0	0	0
7	0	2	1	0	3	6	1	3	2	0	1	3	2	0	1	0	0	0	0	0	0	1	0	0	0
8	0	2	2	0	4	7	1	0	0	0	7	0	0	0	1	0	0	0	0	0	0	1	0	0	0
9	10	4	2	0	16	9	0	2	0	0	9	2	0	0	2	0	0	0	0	0	0	2	0	0	0
10	1	2	0	0	3	10	3	1	1	0	10	1	1	0	5	0	0	0	0	0	0	5	0	0	0
Total	28	147	47	13	235	65%	12%	26%	62%	230	65%	12%	26%	62%	230	65%	12%	26%	62%	230	65%	12%	26%	62%	230
Prod. Acc.	29%	53%	36%	0%																					

Training set signature 1 in the supervised classification matrix is the fawning habitat signature.

pixels classified to signature). A kappa-like statistic (Foody 1992) was calculated to exclude the possibility of chance agreement in overall accuracy (overall accuracy -  $1/n / 1-1/n$  where  $n$  = number of signatures of interest). Clusters were given the same number as the signature they represented in all error matrices.

A GIS file of known fawn locations was constructed (DIGSCRN and GRDPOL; ERDAS 1990), which determined the GIS file values for the classified images (INQUIRE; ERDAS 1990). This data set contained 480 fawn locations, of which 209 were bed-site locations.

## RESULTS

*Cluster analyses.*—The classification error matrix for the 1990 unsupervised classification included 10 training set signatures and 244 reference locations (Table 1). Only three signatures were referenced sufficiently for inclusion in the error matrix. These signatures received 87% of the pixels classified. Overall classification accuracy was 41%, but the kappa-like statistic was 12%. Clusters 1, 2 and 3 in the 1990 unsupervised classification were primarily draw sites, limestone hills and mountains, and loamy sites, respectively (Table 2).

The error matrix for the 1990 supervised classification included seven reference clusters for signatures 2, 3, 4, 9, 10, 11 and 14, and 231 reference locations (Table 1). These seven signatures received 80% of the pixels in the classification. Signature 10, the fawning habitat signature, had an user accuracy of 37%. Overall accuracy was 26%; the kappa-like statistic generated an accuracy estimate of 14%. Reference clusters 2 and 3 were equated to draw sites, clusters 4 and 14 gravelly sites, cluster 9 limestone hills and mountains, and clusters 10 and 11 loamy sites (Table 2).

The classification error matrix for the 1991 unsupervised classification included four clusters and 235 reference locations (Table 3). The overall accuracy of this map was 44%; user accuracy ranged from 78% for signature 2 to 0% for signature 9 (Table 3). The kappa-like statistic produced an accuracy value of 25%. Cluster 1 represented limestone hills and mountains, cluster 2 loamy sites, cluster 3 draws, and cluster 9 gravelly sites (Table 2). The signatures that were evaluated (1, 2, 3 and 9), received 86% of the pixels in the classification.

The error matrix of the 1991 supervised classification contained five clusters and 230 reference locations (Table 3). Signatures 1 through 5 were sufficiently ground-truthed for accuracy evaluations. Signature 1

was the fawning habitat signature and its accuracy was 40% (user accuracy). Signature 5 also performed well, with user accuracy of 70%. Overall accuracy was 39%; the kappa-like statistic was 23%. Clusters 1 and 3 were equivalent to loamy sites, cluster 2 draws, cluster 4 gravelly sites, and cluster 5 limestone hills and mountains (Table 2).

*Principal components analysis.*—Principal components analysis indicated that six bands (1-5 and 7) in the 1990 and 1991 classifications were sufficient in capturing the important characteristics of the data. No single band or subset of bands from either image predominated in principal component 1 (Huber 1992). This indicated that each band was explaining some unique variation.

The error matrix for unsupervised principal components classification included four clusters (1, 2, 3 and 10) and 242 reference locations. Signature 3 performed best (62% user accuracy), whereas signature 10 performed worst (3% user accuracy). The four signatures that were evaluated received 86% of the pixels. Overall accuracy was 31%, but the kappa-like statistic was 10%.

The error matrix for the supervised principal components classification was reduced to two components (clusters 1 and 2) because signature 1, the fawning habitat signature, received 67% of the pixels in the classification. The fawning habitat signature (signature 1) performed well, with an user accuracy of 72%. Overall accuracy for the classification was 69%; however, the kappa-like statistic generated an accuracy estimate to 37%.

*GIS analysis.*—About 87% of the pixels were classified to signatures 1, 2 and 3 (Table 4) in the 1990 unsupervised training set. About 95% of the fawn locations and bed sites were in signatures 1, 2 and 3. Similar results were also found in the 1991 unsupervised training set (Table 5) and the principal components unsupervised training set (Table 6).

Classification of the 1990 supervised training set resulted in 40% of the pixels being classed in signatures 3, 9 and 10 (Table 4). About 79% of the fawn bed sites and fawn locations occurred in signatures 3, 9 and 10. Signature 10 (fawning habitat signature) received only 18% of the pixels in the classification but accounted for 44% of the fawn locations and 43% of the bed site locations.

The supervised 1991 training set (Table 5) did not perform as well in identifying fawn locations or bed sites as the training set for the 1990 supervised classification. Signature 10 had 3.7% of all pixels, and accounted for only 2.5 and 2.4% of fawn locations and bed sites,

Table 4. Percentage of fawn locations ( $n = 480$ ) and fawn bed sites ( $n = 209$ ) in a signature in relation to the percentage of pixels that were classified to a signature for the 1990 unsupervised and supervised classifications.

Signature	Unsupervised			Supervised		
	Pixels %	Fawn locations %	Fawn bed sites %	Pixels %	Fawn locations %	Fawn bed sites %
1	20.9	16.7	17.8	0.7	1.0	0.5
2	29.8	32.3	32.2	8.8	1.5	1.5
3	36.0	45.8	45.1	12.7	19.6	19.2
4	6.1	4.6	4.8	6.9	3.8	1.9
5	0.4	0.0	0.0	4.8	5.4	7.3
6	3.5	0.2	0.0	6.1	2.1	1.4
7	0.2	0.0	0.0	1.0	0.2	0.5
8	0.7	0.4	0.1	5.2	3.1	3.8
9	1.8	0.0	0.0	10.1	16.3	16.9
10	0.6	0.0	0.0	17.5	44.0	43.1
11				10.8	0.3	1.0
12				1.2	0.2	0.5
13				0.8	0.0	0.0
14				13.4	2.5	2.4
Total	100.0	100.0	100.0	100.0	100.0	100.0

Table 5. Percentage of fawn locations ( $n = 480$ ) and fawn bed sites ( $n = 209$ ) in a signature in relation to the percentage of pixels that were classified to a signature for the 1991 unsupervised and supervised classifications.

Signature	Unsupervised			Supervised		
	Pixels %	Fawn locations %	Fawn bed sites %	Pixels %	Fawn locations %	Fawn bed sites %
1	14.3	13.1	14.5	44.8	48.9	52.7
2	37.4	38.2	40.8	7.2	9.2	9.7
3	24.0	28.6	27.4	14.7	15.6	12.1
4	4.8	7.7	6.8	9.0	7.5	7.8
5	0.6	0.0	0.0	13.0	9.6	11.0
6	3.4	2.3	1.9	4.4	4.4	2.9
7	0.7	0.8	0.0	1.2	0.4	0.0
8	2.5	1.0	0.0	1.1	1.9	1.4
9	9.9	6.0	5.3	0.9	0.0	0.0
10	2.4	2.3	3.3	3.7	2.5	2.4
Total	100.0	100.0	100.0	100.0	100.0	100.0

respectively (Table 5). Most pixels were assigned to signatures 1, 3 and 5, which together accounted for 72% of the pixels.

The fawning habitat signature (signature 1) in the principal components supervised training set (Table 6) performed in a similar manner to the 1990 supervised signatures 3, 9 and 10. The classification

Table 6. Percentage of fawn locations ( $n = 480$ ) and fawn bed sites ( $n = 209$ ) in a signature in relation to the percentage of pixels that were classified to a signature for the principal components unsupervised and supervised classifications.

Signature	Unsupervised			Supervised		
	Pixels %	Fawn locations %	Fawn bed sites %	Pixels %	Fawn locations %	Fawn bed sites %
1	20.4	19.4	17.8	67.3	84.2	83.7
2	16.3	19.6	18.8	2.7	1.0	1.0
3	36.4	46.2	45.6	1.6	0.0	0.0
4	0.1	0.2	0.0	6.5	2.9	3.8
5	6.5	1.7	1.4	6.4	3.8	1.9
6	0.8	0.4	0.0	4.0	1.2	1.9
7	0.6	0.0	0.0	1.8	1.5	1.4
8	6.2	2.9	4.8	4.2	1.0	1.4
9	0.1	0.0	0.0	0.3	0.6	1.1
10	12.6	9.6	11.6	5.2	3.8	3.8
Total	100.0	100.0	100.0	100.0	100.0	100.0

allocated 67% of the pixels to signature 1; whereas, about 84% of the fawn locations and fawn bed sites were found in signature 1 (Table 6).

## DISCUSSION

Ormsby et al. (1985) used Landsat TM data incorporated into a GIS to develop a habitat suitability map for white-tailed deer in Michigan, with classification accuracy ranging from 32 to 81% for different cover types. Huber & Casler (1990) reported preliminary results of elk habitat mapping in Colorado using Landsat TM and digital elevation model data, in which the best results produced were from six classifications yielding 63% and 57% accuracy levels. Results from this study indicated relatively low accuracy for the various methods used to assess pronghorn fawning habitat. This may be due to variability in habitats that are hard to measure remotely, the general characteristics of habitat for fawning, the arid nature and variability of the study area, or the assessment methods used.

Difficulties have been encountered in mapping arid communities (Ustin et al. 1986; Tueller 1987). Belward et al. (1988) noted that cover classes should be selected to have spectral homogeneity as well as ecological significance. Because the reflectance from a 30 by 30 m area can have so many constituents (rock, bare ground, shadows, rock type, species composition, elevation, aspect, slope and others), it is not unusual for confusion to exist. In this study, training set signatures,

particularly the unsupervised signatures, were spectrally homogeneous, but in an ecological sense they confuse types that are obviously different. An example would be loamy sites and draws that were confused in every classification. Although reference variables were selected that were used to measure microhabitat characteristics of fawn bed sites described by Canon (1993), those variables could not be used to adequately model or predict brightness levels in the six spectral bands of Landsat data.

The number of signatures per training set may have influenced the results. Possibly too many signatures per training set were developed, which reduced the ecological significance of the signatures by including mixed habitat characteristics. This was apparent in relation to the range of variation found in the reference data. Five or six signatures may have provided a more realistic assessment. However, it was also apparent that too few signatures were developed within the narrow Landsat TM data range surrounding the means in each band, which was evident in the percentage of pixels classified to signatures in the classifications. Each classification, except the 1990 supervised classification, had three signatures that received 70 to 86% of the pixels classified. A more realistic approach may have been to define four or five signatures around the band means with a broad signature in the bright range and a broad signature in the dark range.

Curran & Williamson (1985) found that ground truth data can be less accurate than remotely sensed data. Apparently, in this study, the reference data clusters were a source of error. Also, the variables that were measured probably did not model all of the information contained in the Landsat TM data. Differences in the scale of data collection may have influenced the results since comparisons were made between 30 m circular plots and 3 by 3 pixel arrays (90 by 90 m).

Unfortunately, a classification error matrix could not be constructed from GIS data. There was no way of determining when an area that was not habitat was classified into the habitat signature. If the locations that were referenced for the cluster analysis had been monitored for fawn presence or absence through the study period, then presence or absence at the locations could have been used to construct an error matrix (Congalton 1991).

Fawn locations and bed sites found in a signature in the unsupervised classifications were approximately proportional to the percentage of the pixels classified to a signature. The same was true for the 1991

supervised classification. The result of this proportional indifference was that as a larger percentage of the fawn locations or bed sites were mapped, the same percentage of the study area was also mapped. For instance, about 95% of the fawn locations and bed sites in the 1990 unsupervised classification were in signatures 1, 2 and 3. These signatures included about 86% of the pixels classified (or area mapped). If this represents a fawning habitat map that was 95% accurate, then a blank sheet of paper said to be the fawning habitat map would only be wrong 18%  $\{[1 - (0.86 \times 0.95)] \times 100\}$  of the time. Such a fawning habitat map would be neither practical nor useful.

Supervised training sets appeared to be more effective in classifying pixels to the fawning habitat signature. In the classification of the principal components supervised training set, the fawning habitat signature received about 67% of the pixels. About 84% of the fawn locations and bed sites were in the fawning habitat signature. However, this situation could have been the result of chance agreement. The fawning habitat signature for the 1990 supervised classification (signature 10) received about 18% of the pixels and 44% of the fawn locations and bed sites. It is unlikely that this is a result of chance agreement alone. From the substantial telemetry data obtained on fawn locations and their corresponding microhabitats (Canon 1993), the 1990 supervised training set was the most effective in mapping pronghorn fawning habitat.

Drought conditions in 1990 limited the area used by pronghorn fawns resulting in the fawning habitat signature receiving only about 18% of the pixels classified. As conditions continually improved through 1991, use of the habitat increased. This resulted in the fawning habitat signature for 1991 receiving about 45% of the pixels classified. The fawning habitat signature in the principal components training set was even higher at 67%. Thus, as the size of the habitat used for fawning increased, so did the area included in the classified signatures. These results suggest that the habitat used by pronghorn in 1990 could be viewed as critical fawning habitat due to the drought conditions that year. A critical fawning habitat map would be more practical to develop than attempting to develop fawning habitat signatures when fawn locations become proportionally indifferent as range conditions return to normal.

## CONCLUSIONS

Several conclusions can be drawn from the results of this study.



First, a supervised classification using known locations performed better than an unsupervised classification for identifying pronghorn fawning habitat. The GIS analysis indicated that the natural variability in the Landsat data, which the unsupervised clustering algorithm accents, may not effectively model the spectral response of pronghorn fawning habitat. Second, the microhabitat variables that were measured, though possibly important in pronghorn fawning habitat, were incomplete in evaluating spectral response and map accuracy. A multiple regression study to determine the most important variables related to spectral response would be useful to better identify pertinent habitat variables in pronghorn fawning habitat. Third, if the appropriate field reference parameters had been measured for use in evaluating the 1990 supervised classification, the results may have indicated a fairly useful fawning habitat map for drought years. Fawning habitat in years with normal or above normal precipitation appears to be so widespread as to make mapping impractical.

#### ACKNOWLEDGMENTS

We thank S. Hartman of the University of Texas Lands for access on the Double U Ranch. We thank S. Meek, who provided field assistance. We are especially indebted to Dr. Ernest B. Fish who provided much of the background support and expertise to see this study through to completion.

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## SALINITY RECOVERY IN TEXAS BAYS

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**Abstract.**—A long-term program of continuous measurement of salinities at various locations in Texas estuaries recorded instances of salinity drop and recovery during and after flood inflows. Rates of salinity increase following floods were calculated and compared. Average rates varied from 0.15 psu/day to 0.30 psu/day among bays. The rate of salinity recovery was used with other data to estimate the efficiency of mixing of tidal exchange between Matagorda Bay and the Gulf of Mexico. Results show that approximately 10% of the total tidal influx of new Gulf water is retained in the bay.

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Texas estuaries, especially those of the lower coast, demonstrate great variation in salinities, corresponding to times of drought and episodes of large flushing flood flows from tributaries. Freshening of the bays with flood-inflows, while beneficial in many respects, is a system disturbance with both natural and economic consequences. Low bay salinities raise concerns among commercial fishermen and others relative to the duration of water conditions which are potentially deleterious to their enterprise. Populations of mobile estuarine biota may migrate to seek preferred salinities. So the question arises as to how long will it take before the bay salinities return to "normal". Rates of salinity recovery are offered here as a guide to answering that question.

The process of estuary salinity recovery also provides insight into the hydraulic parameters which determine characteristics of each estuary. Specifically, analysis of salinity dynamics are a means of estimating parameters of the mixing of bay and Gulf waters. Both evaporation and bay-Gulf exchange operate to make the estuaries saltier, countering freshwater inflow. In estuaries where precipitation usually exceeds evaporation, Gulf exchange is the major means by which the characteristic estuarine salinities are maintained. The exchange of materials and water which occurs with tidal movement is important for other reasons as well, contributing to the flushing of pollutants, for example. The determination of volumes of tidal inflow (and outflow) captured by the bay (or Gulf on ebb tide) is important in calculations of estuary materials budgets, as has been recently pointed out by Nixon et al. (1994). Tidal exchange has also been shown to be important in nitrogen balances compiled for several Texas estuaries (Brock 1994; 1996). Actually,

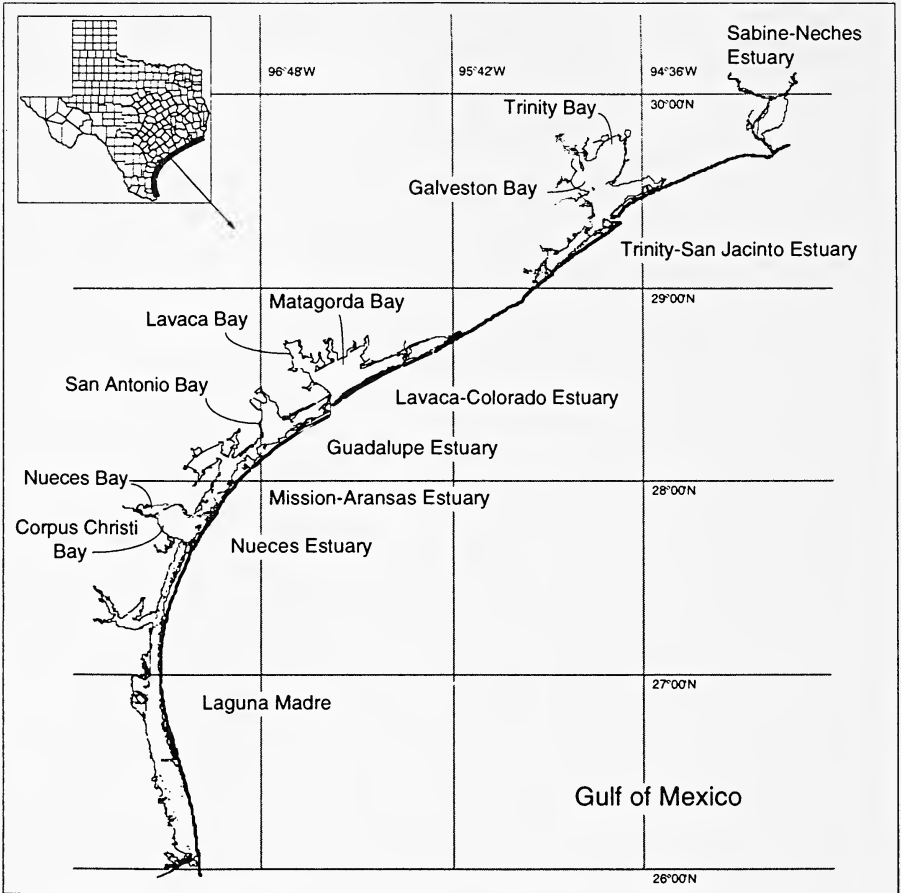


Figure 1. Estuaries of the Texas Coast and Bays discussed in salinity analyses.

reference here to "tidal" is a simplification. As used in this paper, tidal exchange includes not only astronomically driven movements, but also flows from longer cycle, meteorologically driven sea level changes and flows generated by winds.

## DATA AND METHODS

Texas bays are typically broad and relatively shallow, vertically well mixed, but with the potential for lateral as well as down-estuary salinity gradients. Salinity data in Texas bays are collected by several agencies to meet needs of fisheries regulation, inflow management, and protection of resources. For this study, salinity data are included from

the Texas Water Development Board (TWDB) Coastal Data System, TWDB Datasondes, Texas Natural Resource Conservation Commission Statewide Monitoring Network, Texas Parks & Wildlife Department's (TPWD) Coastal Fisheries Division, and Texas Department of Health Shellfish Sanitation Monitoring Program. Most monitoring data includes depth profiles, with samples at the surface, mid-depth, and near the bottom. Some monitoring programs report only salinities at depths of concern, such as near reef surface. Salinity data from all monitoring programs were compiled and summarized into daily averages, including all depths, for selected areas. That is, where stations from several monitoring activities occurred in close proximity, data from those sites were combined. In most cases salinities are not measured directly but calculated from conductivities measured by probe (Yellow Springs Instrument and Hydrolab) and temperature compensated. Conversion to practical-salinity-scale salinities were based on formulations in Lewis & Perkin (1981). Salinity units are "psu", equivalent for most purposes to "ppt". Sites monitored by the agencies are distributed around the bays and provide adequate characterization of estuary-wide conditions. However, the number of sites sampled has varied over the years. For the whole-estuary analysis presented below, salinity averages were computed by volume-weighting data from various sites, based on a rough partitioning of bay areas around each site and depth at the site. Estuaries discussed below are shown in Figure 1.

For the purposes of this study, Gulf salinities were represented by averages from data collected by the TPWD in conjunction with trawl sampling of fisheries along Gulf beaches (Dailey et al. 1991). These samples are usually taken within 24 km along shore of major passes.

Beginning in late 1986, TWDB installed instruments to continuously record changes in water quality at selected sites. These instruments, Hydrolab Datasondes, were programmed to record conductivities and salinities every 60, 90, or 120 minutes and were exchanged monthly with freshly calibrated units. Although there are gaps in the time-series of salinities from these instruments, resulting from instrument loss, failure, and temporary relocation, this data set provides a valuable record of salinity dynamics with which to work. Probe fouling did occur during the monthly tenure of instrument deployment. The degree of foulant effect was assessed in decalibration and comparison of final recorded measurements with independent field measurements taken at times of instrument exchange. The fouling effect on salinity measure-

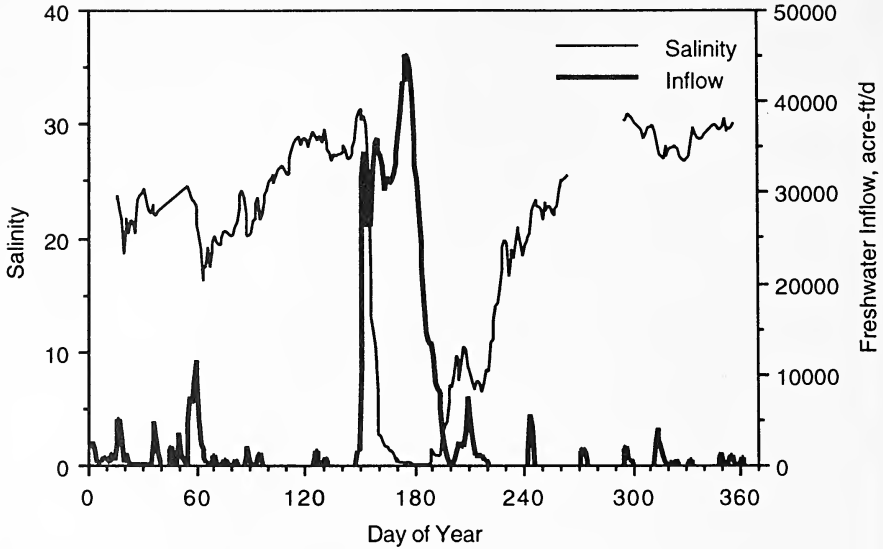


Figure 2. Nueces River inflow and Nueces Bay salinities, 1987.

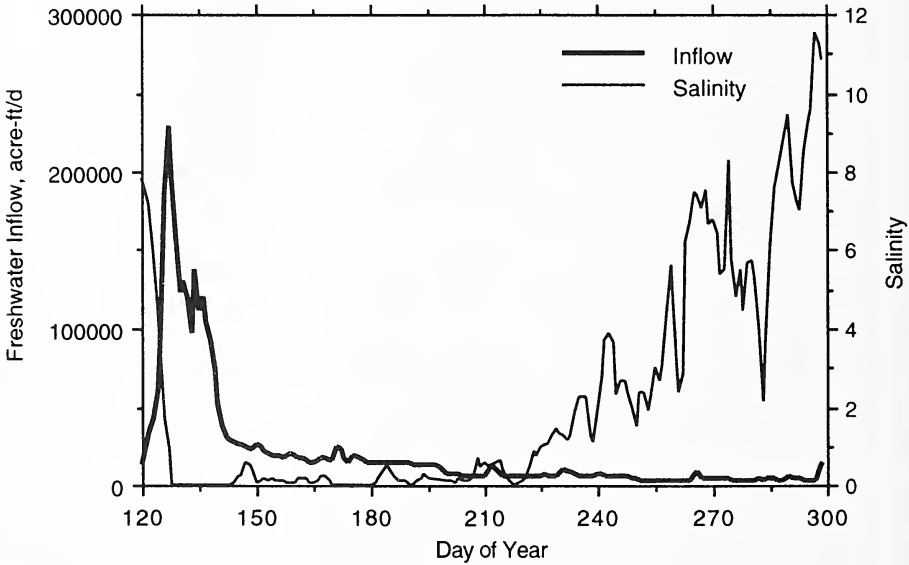


Figure 3. Flood flows and salinity changes in upper San Antonio Bay, June-November, 1987.

ment was typically small, and is not addressed here. In most of the data sets, an algorithm was used to correct for foulant effects when effects were greater than 1 psu, as determined by independent field checks.

Table 1. Locations of principal salinity measurement sites referred to in the text.

Bay	Description	Latitude	Longitude
Nueces	Mid bay at upstream powerline	27°50'56"N	97°25'26"W
San Antonio	Near Junction of Seadrift Channel and Victoria Barge Canal	28°22'53"N	96°44'33"W
Lavaca	At Lavaca Causeway, north of channel	28°39'12"N	96°35'44"W
Trinity	Open bay NW of Double Bayou Channel	29°39'40"N	94°44'45"W

Locations of principal salinity monitoring sites are given in Table 1. Salinity records from these instruments are available from the author or the TWDB web site through electronic media at:

<http://www.twdb.state.tx.us/www/twdb/planning/environmental/sondpage.html>

Hydrology data were compiled on gaged flows, ungaged rainfall runoff, diversions, wastewater return flows, direct precipitation, and evaporation by Solis (1994). "Freshwater inflows" in discussions below refers to the balance of the above sources and losses, that is, to net inflows. Therefore, the impact of evaporation on salinity change is taken into account.

Choice of sequences of salinity changes presented in this paper was somewhat subjective, but more importantly limited by data availability. Both inflow and salinity time-series data were examined to choose periods in which an estuary responded to a strong peak freshwater flow and then recovered during the subsequent period without major large inflows. For each recovery period, simple linear regressions on salinity versus days since start were run. The starting date was the date of minimum salinity or date of initiation of salinity increase, when from a continuous record. The slope of the salinity change time series line is used as the recovery rate.

## RESULTS AND DISCUSSION

*Flood effects on a bay.*—Figure 2 shows the time sequence of daily average salinities recorded at an upper-estuary location, mid Nueces Bay, in response to a major flood of the Nueces River. Figure 3 shows a similar sequence of salinities measured in upper San Antonio Bay near Seadrift, before, during, and after a flood on the Guadalupe River. In both cases, salinity drops are abrupt, salinities are depressed in the estuary for a time, and recovery is gradual. Figure 4 shows a sequence of salinity change for a flood and recovery sequence of inflows in

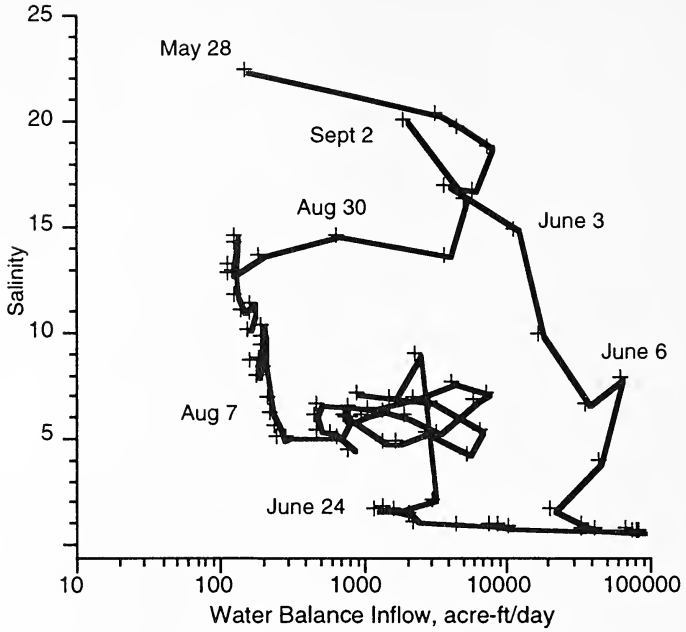


Figure 4. Time trace of Lavaca Bay salinity change with inflow, May 28, Sept. 2, 1987.

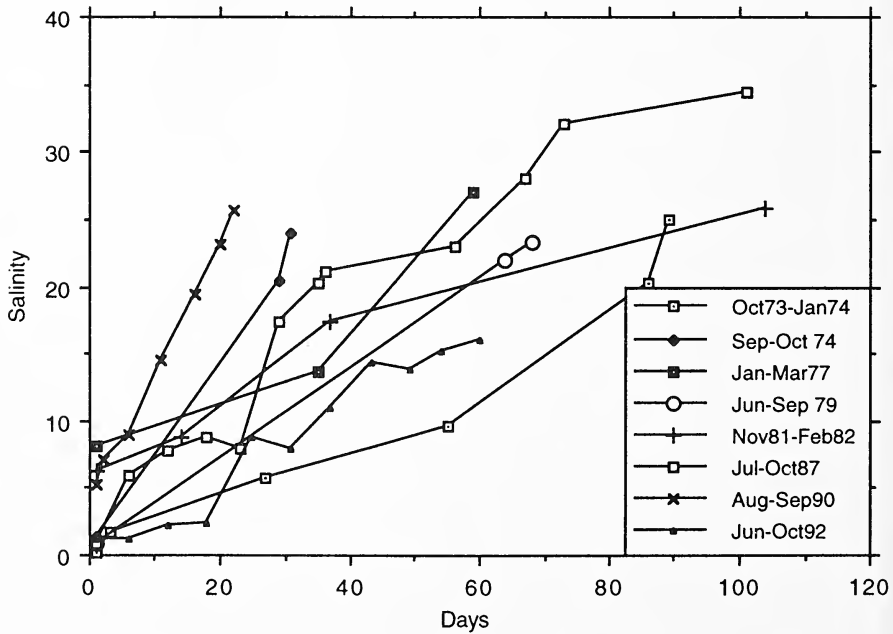


Figure 5. Salinity recovery series from mid Nueces Bay.



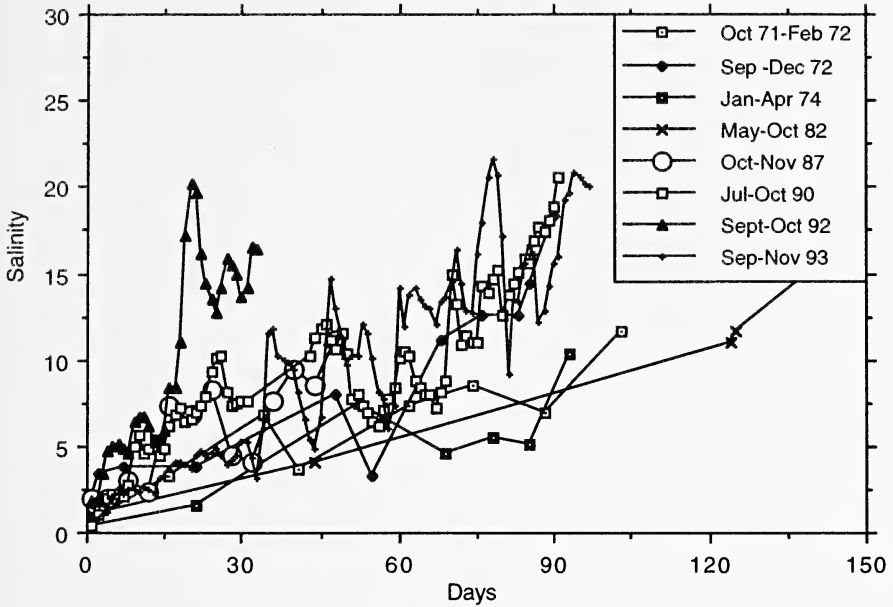


Figure 6. Salinity recovery series from the Seadrift area, San Antonio Bay.

Lavaca Bay. This plot illustrates the phenomenon of hysteresis. Thus, salinity recovery is not the mirror of salinity fall as a function of inflows. A given amount of inflow during a period when the bay was already fresh is associated with much lower salinities than it was when the bay was salty. This means that in a flashy estuary, a given inflow is not associated with one salinity, but can be associated with higher or lower salinities, depending on the recent inflow history.

*Salinity recovery rates.*—Time series of salinity recoveries are illustrated for Nueces Bay in Figure 5, for San Antonio Bay in Figure 6, for Lavaca Bay in Figure 7, and for Trinity Bay in Figure 8. Inspection of the figures suggests that some recoveries are rapid, others slow, and many rates are similar. There is a gross level of similarity of salinity recovery vectors, although the plots reveal variation among and within bays.

For a given bay, salinity recovery rates would be expected to be influenced by seasonal evaporation rate, inflows during the recovery period, and degree of tidal exchange with the Gulf. The Gulf effect depends on low-frequency components of tidal exchange (Ward 1997), barometric pressure gradients, wind sets, and variation in Gulf salinity,

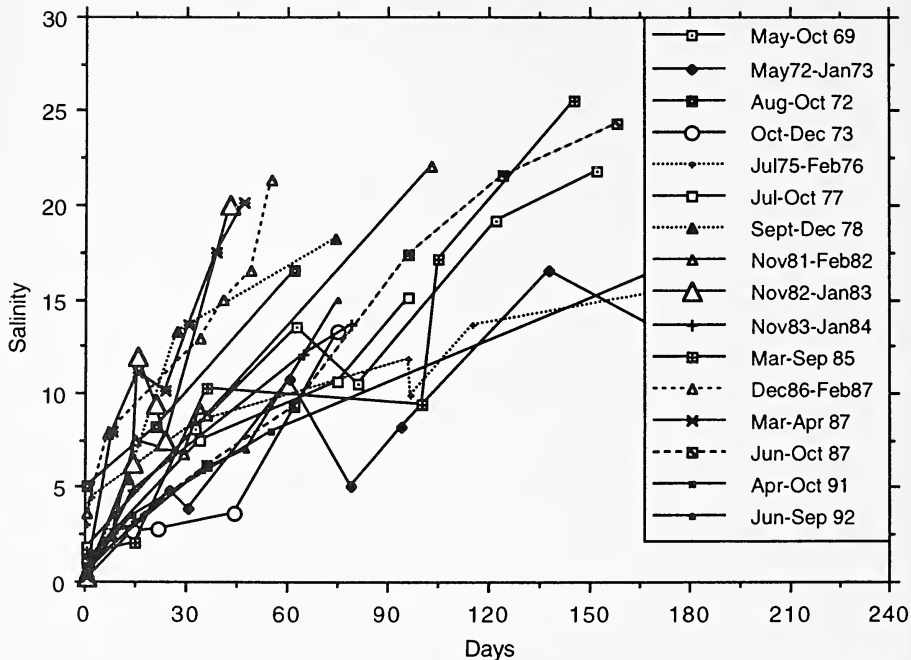


Figure 7. Salinity recovery series from the Lavaca Bay Causeway.

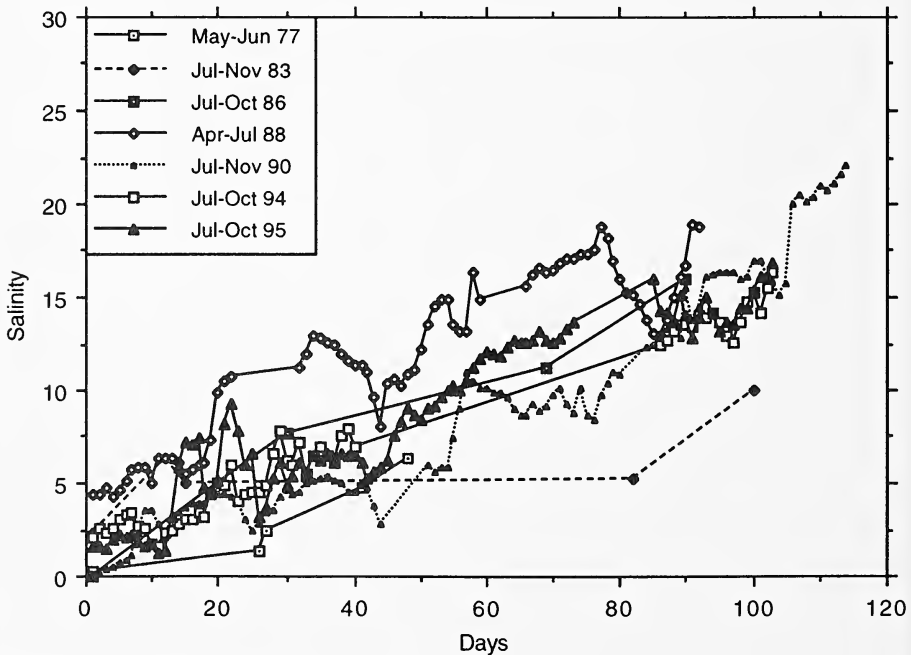


Figure 8. Salinity recovery series from mid Trinity Bay.

Table 2. Correlation of bay salinity recovery rate with initial salinity and intervening freshwater inflow.

	<u>Trinity Bay</u>	<u>Lavaca Bay*</u>	<u>San Antonio Bay</u>	<u>Nueces Bay</u>
Number of Periods	5	10	5	8
Initial Salinity	.153	-.394	.735	-.665
Inflow	-.889	.183	-.710	.081

\* Lavaca cases limited to those with initial Matagorda Bay salinities less than 20 psu.

which is important on the near-shore continental shelf. In general, the Gulf off the Texas Coast varies in level seasonally (Marmier 1954) with lows in January and July, and peaks in April and October. The total difference in mean sea levels is only around 1 foot, however a variation in a few inches spread over the area of the bay translates into a significant volume. The result of these long-cycling or non-cyclic influences is that at times the bay is either slowly draining or slowly filling. This change could be influential in rates of salinity recovery.

Intuitively, one would also expect that the greater the freshwater displacement of salt in the bay, the slower would be the rate of salinity recovery. Thus, very low initial salinities, reflecting major freshening, would signal a slower recovery rate. A substantial rate of freshwater inflow during recovery would also be expected to suppress recovery rates. Attempts to test the above as potential causes of variation in rates were hampered, particularly for data prior to 1986, by sparse data both for the bay and for the Gulf, and by inconsistent temporal data density. Correlations were calculated between recovery rates, inflow, and beginning salinity for the bays examined. Slopes of salinity recovery time series, from linear fit lines, represent recovery rates. Initial results (Table 2) were not as promising as would be anticipated. It is likely the sparse data available for some of the flood-and-recovery periods misrepresents the extent of bay changes. In addition, the process of selecting periods with obvious recovery trends may have excluded those cases which would demonstrate the importance of one or another process. In Figure 9 are plotted recovery rates and intervening inflow for each estuary. "Intervening inflow" is the freshwater flow during the recovery period, given in terms of bay volume for comparative purposes. Trinity Bay and San Antonio Bay are grouped together with relatively high inflows and low recovery rates. The Nueces data show a negative association between inflow and recovery rate even excluding one high value.

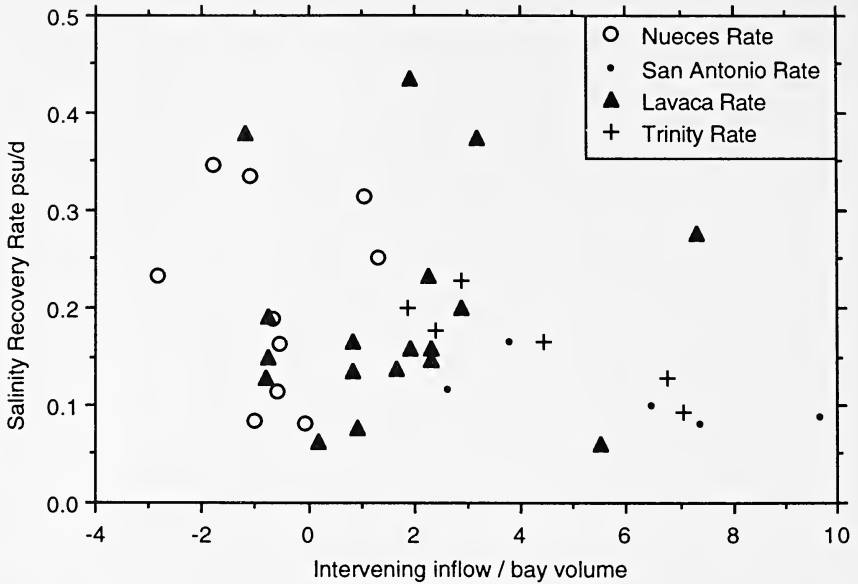


Figure 9. Salinity recovery rates versus intervening freshwater inflow, per bay volume.

Given the unknown influence of factors such as wind conditions on salinity recovery rates among the series plotted, rates of recovery in general are relatively close, so that the average or median rate is useful for rough planning purposes. Table 3 gives rate statistics for each bay investigated, again using slopes of lines fit to recovery series as recovery rates. Variation among bays is in line with the gradient from wet to dry along the coast. Nueces Bay recoveries can proceed almost 1 psu/day in the most favorable conditions, much faster than the fastest recovery in the Lavaca Bay, 0.4 psu/day. The difference between these two bays probably reflects the proportionately larger role of evaporation in Nueces Bay. Also, Nueces Bay has tidal exchange with the larger-volume Corpus Christi Bay, which is typically more salty than Matagorda Bay, the primary bay exchanging with Lavaca Bay. The basic similarity of the rates may reflect the features common to most Texas estuaries. They are separated from the Gulf by barrier islands except for relatively narrow passes, which are dredged to fairly similar cross sectional dimensions.

The emphasis in this analysis has been on portions of salinity records with clear trends of salinity change. During these periods, salinity increases often appear linear. However, recovery is not necessarily linear. Salinity recovery usually begins, at least in the upper estuary,

Table 3. Rates of salinity recovery, psu/day, at long-term sampling site in each of four Texas Estuaries, with descriptive statistics and quartile ranges.

	<u>Trinity Bay</u>	<u>Lavaca Bay</u>	<u>San Antonio Bay</u>	<u>Nueces Bay</u>
Number of Periods	8	18	8	14
Mean	.160	.193	.153	.302
S D	.043	.109	.086	.249
10%	.10	.067	.083	.083
25%	.129	.136	.095	.144
50%	.162	.157	.127	.242
75%	.188	.232	.178	.334
90%	.219	.379	.299	.748

with two or three days of sustained low salinities following cessation of flood inflows. One might expect that the duration of this period would depend on the degree to which the entire estuary was freshened, particularly the downstream section. Tests for this idea were impeded, since many floods occurred in the midst of generally wet periods, so that the freshening effect of one hydrograph was difficult to show. The magnitude of salinity changes with floods is also strongly dependent on the location within an estuary. Figure 10 illustrates various degrees of salinity response to 1987 inflow events at Matagorda Bay stations along a series from upper to lower bay.

*Salinity recovery and Gulf tidal exchange.*—The sequence of salinity change provides data for investigating the rate of tidal flushing of Texas bays and net transport of materials between bays and the Gulf. Gulf tidal flushing helps determine properties of an estuary, including nutrient retention and pollutant dilution. The net flow of river water out of the estuary effects material transport, but tidal exchange also effects transport and may be the principal agent of flushing in estuaries with a negative freshwater balance.

From calculation of the intertidal volume using tide records, or from results of simulation of bay hydraulics, one can calculate the gross volume of water which comes into a bay on flood tide. How much of this volume is effectively intermixed with bay water (and how much ebb tidal bay water effectively leaves the bay) is harder to determine. A measure of tidal entrainment, or mixing efficiency was sought, which could be applied to gross tidal volumes to provide volumes for material transport computations. Pritchard (1960) and others have made use of

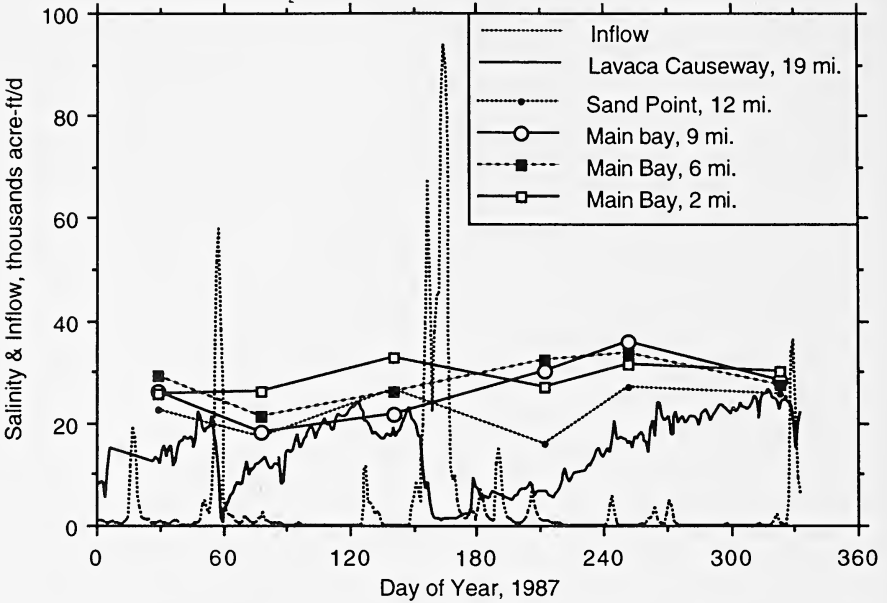


Figure 10. Salinity changes at Matagorda Bay sites with 1987 inflows. Measurement sites are identified by distance from the Matagorda Entrance Channel.

salt-balance relationships to determine the amount of seawater which must be mixed inward (entrained) to achieve observed bay salinities. Given an available gross tidal inflow and a calculated entrained volume, a mixing efficiency should be the ratio, entrained/total.

Solis & Powell (1997) compute "bulk mixing efficiencies" for Gulf of Mexico estuaries based on estuary average inflows, estuary volumes, inter-tidal volumes, and salinities of bay and Gulf. Bulk mixing efficiencies for Texas major estuaries were all below 0.3, except for Sabine Lake. For estimates of mixing efficiency which may be more applicable to shorter time scales, estimates based on system dynamics rather than equilibrium conditions may be useful. The following presents a calculation of mixing efficiency from salinity recovery data.

Using salinity data from monitoring stations distributed around the Lavaca-Colorado Estuary (Matagorda Bay system) from the TWDB Coastal Data System, the volume-weighted average salinities were calculated for several periods identified as flood-recovery periods. Unfortunately, samples were collected quarterly or bimonthly, and there were incomplete whole-estuary sets for many of the periods

Table 4. Data for application of simple salinity models to Matagorda Bay.

General Data					
Volume of Estuary					
Average net freshwater inflow					
Median net Freshwater Inflow					
Average Bay Salinity					
Average Gulf Salinity					
Gross Tidal Volume					
Period	Freshwater Inflow*	Initial Bay Salinity**	Average Bay Salinity	Ending Bay Salinity	Gulf Salinity
May-Aug 1974	2.24	17.7	22	27.2	31
Apr-Sep 1985	6.19	16.4	26	32.0	32
Mar-Apr 1987	5.76	18.4	22	26.8	30
Jun-Sep 1987	18.77	9.7	16	26.2	32

\* Inflows are  $10^6$  m<sup>3</sup>/day. Actual daily values were used in the iterative model.

\*\* Salinities are volume-weighted system averages.

identified as Lavaca Bay recoveries. Thus, data were limited for this analysis. Data for the system and applications of relationships discussed below are give in Table 4. Estuary salinities listed in the table are volume-weighted averages.

Pritchard (1960: equation 4) incorporates rate of salinity change in a salt-balance relationship to compute tidal exchange rate:

$$DS = Q_i/V \cdot (S_g - S_b) - (R/V) \cdot S_b$$

where  $Q_i$  = new Gulf water, not previously moving out of bay on ebb tide

$V$  = volume of bay

$S_g$  = salinity of Gulf

$S_b$  = salinity of outflowing bay water (here the bay weighted average salinity)

$R$  = volume of freshwater inflow (including precipitation and evaporation)

$DS$  = rate of salinity change.

If one substitutes  $Q_i = E \cdot Q_g$ , where  $E$  is the mixing efficiency and  $Q_g$

Table 5. Terms and equation for a salt-balance in a simple well-mixed estuary.

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Vb	=	volume of bay
Qt	=	total volume of Gulf flood tide per day
E	=	entrainment rate
Qi	=	volume of Gulf water entrained inward per day
Qo	=	volume of bay water entrained outward each day
Sg	=	salinity of Gulf water
Sb	=	beginning salinity of bay
Se	=	ending salinity
$\Delta S_b$	=	$S_e - S_b$
Sbnew	=	salinity at end of day time step
R	=	volume of river inflow per day
$Q_i = Q_t \cdot E$		
$Q_o = Q_t \cdot E$		
$S_b \text{ new} = [(V_b \cdot S_b) + (Q_i \cdot S_g) - (Q_o \cdot S_b) - (R \cdot S_b)] / V_b$		

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is total tidal inflow, the above relationship can be used with salinity recovery rates to estimate mixing efficiency. Applying this relationship to the periods presented in Table 4 produced  $Q_i$  values ranging from  $36 - 63 \cdot 10^6 \text{ m}^3$ , and mixing efficiencies from 0.11-0.20, near Solis & Powell's (1997) value of 0.10 for this estuary. However, both approaches (and that discussed below) treat the bay-system as one well-mixed reactor, so corroborating results do not necessarily imply reality. It is possible that mixing efficiencies are really related to conditions which influence salinity stratification in the entrance channels, to the contrast between bay and Gulf salinity, or to other conditions near passes unrelated to average bay salinities or river inflows.

The problem of calculating a mixing efficiency can also be approached with an iterative application of a salt-balance model, with comparison of results to observed salinity change. Table 5 gives the terms and equation of a simple salt balance which can be applied iteratively to an inflow series. The equation states that salinity on day  $i$  is equal to the mass of salt divided by the volume of the bay, where the mass of salt equals what was in the bay on day  $i-1$ , plus what the Gulf delivered, subtracting riverine flushing of bay salt and tidal flushing of bay salt. The entrained volume in the equation is the portion of total flood-tidal inflow volume which is new Gulf water captured by the bay, that is, mixed into the bay waters. The amount of salt or other material contributed to the bay from the Gulf per tidal cycle can be thought of as the amount contained in the parcel of entrained water.



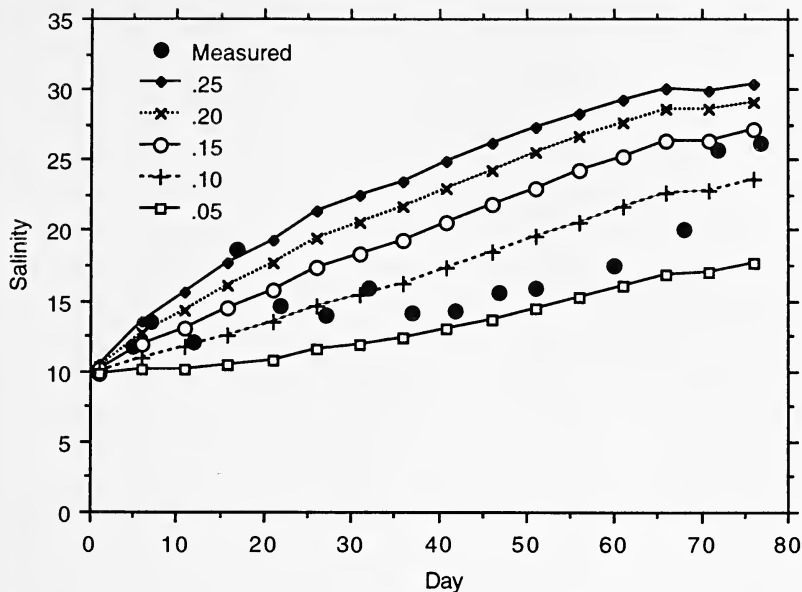


Figure 11. Matagorda Bay average salinities, June-Sept., 1987: observed salinities and salinities simulated using a range of mixing rates.

Salinity recovery in the estuary was simulated by applying the above salt-balance equation with a daily iteration, using data for each period in Table 4. River inflow data available included inflow from ungaged areas, diversion, return flows, precipitation, and evaporation (Solis 1994). Gulf salinities were taken from monthly average bottom water salinities collected during fisheries trawl sampling in shelf waters near the pass (Dailey et al. 1991). Gross tidal volumes, as 24-hr summed flows coming in and going out of the passes, were available from runs of the finite element model, TxBlend (Matsumoto 1993), generated for analyses of Matagorda Bay by the Lower Colorado River Authority (Martin et al. 1997). A daily average tide volume was computed from annual data, so the volume reflects a combination of tidal components. Flood and ebb tides can be assumed symmetrical with respect to volume, within the resolution of the particular model application. Salinity recovery iterations were run with several candidate entrainment rate values. The results, including observed salinities, are shown in Figure 11 for the June-September, 1987 period. The best entrainment rate is that which increases the bay salinity in the same number of days as was observed in the field with an appropriate slope. This model is too simple for the form of recovery to match well, and may not be useful

for conditions in which bay salinities approach sea-water (because the mixing rates become unrealistically large). Best values for mixing rate were 0.12, 0.40, 0.30, and 0.13, in year-sequence of cases. The 0.40 value is suspect because bay salinities approached Gulf levels.

From these exercises it is clear that the average mixing rate between bay waters and the flood tidal plume is rather low. From the above work with Matagorda Bay data and preliminary analyses on other bays such as Galveston Bay (Brock 1996), the portion of total flood tide volume which is new Gulf water that remains in the bay, replacing an equivalent volume of bay water, is on the order of 10%. However, for some Texas bays, this percentage results in a volume greater than the average river inflow rate, and thus represents an important flow.

Regarding these results, it should be mentioned that for most of the period considered here, much of the Colorado River flow by-passed the Lavaca-Colorado Estuary. That situation changed in 1992, with the completion of a diversion project which rerouted all river flows into the eastern arm of Matagorda Bay. Therefore, some of the rates of salinity change given here for Matagorda Bay may no longer apply.

Although it is common for finite element computer simulation models to represent movement of materials in an estuary using parameters of advection and diffusion, a mixing efficiency parameter is useful in some one-dimensional models. Pritchard (1960) used an unnamed constant to include in formulating the proportion of tidal flood flow effective in the tidal renewal of Chincoteague Bay. Dyer & Taylor (1973) introduce a mixing parameter "*a*", which they suggest might be 0.5 - 0.8, for the exchange between segments of an estuary box model. Smith (1985) estimated a mixing rate for tidal flows through the Corpus Christi Bay entrance channel for use in a model of bay-shelf exchange.

The analyses developed above were possible because of the existence of several long-term monitoring programs. Together, they provide a spatial distribution of salinity data across the estuaries to at least minimally characterize estuary response to inflows and other forcing factors. New recording water quality meters have made it possible to obtain excellent time-series data. However, these instruments have been set out at only a few locations within selected bays. Hopefully, continued investment in sites where salinity and other parameters are

measured with high frequency will provide data to improve our understanding of estuary salinity dynamics.

#### ACKNOWLEDGMENTS

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## POTENTIAL FOR SEAGRASS RESTORATION IN GALVESTON BAY, TEXAS

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**Abstract.**—Seagrass meadows were lost from West Bay, part of the Galveston Bay system, between 1956 and 1982 due apparently to acute and chronic effects of waterfront development and water quality degradation. Various transplanting methods and materials were used to determine whether *Halodule wrightii* and *Ruppia maritima* could be restored to the area since environmental conditions have improved. Plugs of *Halodule* in peat pots survived better than did bare root transplants. Faunal exclusion cages aided survival and growth of those transplants, but only when mesh size was small enough ( $< 3$  cm) to block small fishes and decapods typical of West Bay that were observed to disturb transplants. Cages need to protect transplants 60-90 days to be effective. Enclosing propagules in cheesecloth bags may be more useful and less time-consuming than using plugs, more so for faster-growing *Ruppia* than for slower-growing *Halodule*. There were no indications that planting on 0.5 m vs. 1.0 m centers or in square vs. rectangular beds produced better survival. *Halodule* transplanted in peat pots on 0.5 m centers was able to survive over two years after removal of small mesh cages. Transplanting *Halodule* was effective only during April-August, but *Ruppia* grew quickly and produced seeds within 47 days after planting in September. Mixed transplants of *Ruppia* and *Halodule* could be advantageous, as the annual *Ruppia* is able to spread rapidly and stabilize a transplant site whereas the perennial *Halodule* spreads more slowly but becomes the dominant species over time. In the future, seagrasses can be transplanted successfully into western Galveston Bay if certain precautions are taken to insure survival and growth.

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Seagrasses provide food and shelter for juveniles and adults of many commercially and recreationally important species of fish and shellfish and their forage organisms (Fonseca 1989; Zieman & Zieman 1989). As a result, they often support faunal densities much greater than those found in adjacent sand or mud habitats. Seagrass meadows are a sensitive habitat and are adversely affected by such factors as industrial and agricultural run-off, eutrophication, dredging, increased turbidity, bioturbation, storm-associated scour, and subsidence (Zieman & Zieman 1989; Fonseca 1992). Such factors have caused a decline in acreage of this valuable habitat along the margins of the northern Gulf of Mexico by 30-80% over the past four decades (Duke & Kruczynski 1992).

Seagrass acreage in the western arm of Galveston Bay, Texas,

declined from 458 ha in 1956 to 0 by 1987 (Pulich & White 1991). Most of these seagrass meadows (primarily shoalgrass *Halodule wrightii*) grew along the barrier island edges of western West Bay. The only seagrass beds remaining in this estuary grow in Christmas Bay, a semi-isolated embayment southwest of West Bay, although acreage has declined there as well; 502 ha in 1971-72 versus 113 ha in 1987 (Adair et al. 1994). Seagrass loss was attributed primarily to waterfront dredging, spoil placement on seagrass beds, and subsequent increases in turbidity, sedimentation and erosion in West Bay (Pulich & White 1991). Secondary causes included overwash from Hurricane Carla in 1961 and increased turbidity and algal blooms after the 1950's drought ended. Point source pollution was suggested as an alternative stressor (Adair et al. 1994), but a previous assessment of water and sediment quality has indicated few pollutant impacts in West Bay (Ward & Armstrong 1992).

Seagrass restoration now seems possible in areas that once supported lush beds. Water clarity has improved since the 1960s (Ward & Armstrong 1992). Turbidity and salinity in West Bay are now similar to those in Christmas Bay (Pulich & White 1991). Waterfront dredging has declined since the period of maximum seagrass loss (prior to 1979), and upland placement of maintenance dredge materials from canal housing developments is now required (J. Boslet, U. S. Army Corps of Engineers, Galveston District, pers. comm.). Natural recolonization by *Halodule* may now be prevented by lack of a nearby propagule or seed source (these are unlikely to escape through the narrow passes from Christmas Bay except during extreme tides). Successful restoration of seagrass beds should increase habitat for forage organisms and fisheries species such as penaeid shrimps *Penaeus* spp. and spotted seatrout *Cynoscion nebulosus* (cf. Zieman & Zieman 1989).

The goal of this project was to determine whether restoration of viable *Halodule* habitat to its former range in West Bay is possible. The objective was to assess factors associated with transplant methodology that could affect survival and growth of transplanted seagrass. Field and laboratory experiments and qualitative observations were conducted to determine whether *Halodule* and the salt-tolerant, freshwater wigeon-grass *Ruppia maritima* (spelling of common name follows Kantrud 1991) would survive and grow using various construction materials and transplant methods suggested by Fonseca (1994).

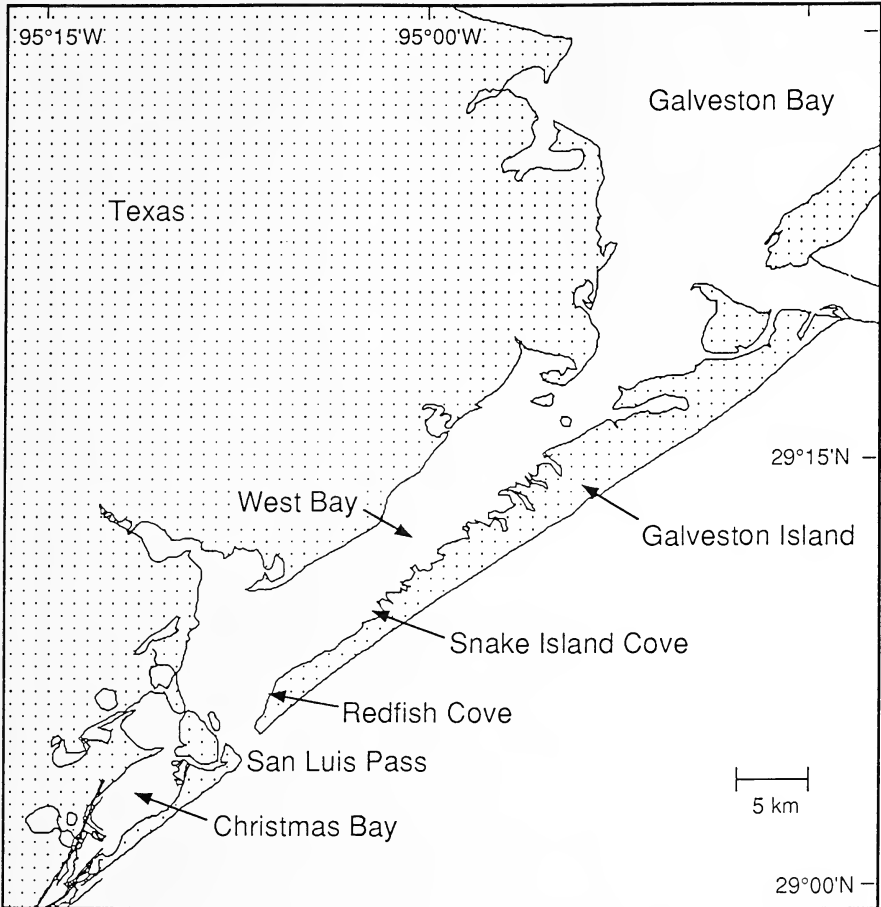


Figure 1. Experimental and natural seagrass sites in West Bay, part of the Galveston Bay estuary.

## METHODS AND MATERIALS

Potential restoration sites were chosen from historical photographs and actual 1993 conditions. Aerial photographs from the Texas Natural Resources Information System archives and from the Texas Parks and Wildlife Department, Resource Protection Division, for the years 1930, 1956, 1965, 1975, 1982, 1987, and 1989 were examined. In November 1930 and in August 1956, seagrasses grew along the southeastern bay shore in a band of varying width (100-400 m at widest points) and density from San Luis Pass on the western tip of Galveston Island eastward approximately 12 km to Snake Island Cove (Fig. 1). Seagrasses became patchy and extended perhaps another 5 km eastward of Snake Island

Cove. By October 1965, seagrass beds had thinned out in general and had disappeared from areas adjacent to canal housing developments on western Galveston Island. By October 1975, seagrasses had become restricted to narrow beds on the western tip of the island. No seagrasses were detected along Galveston Island in 1982, 1987, or 1989 photographs, although seagrasses remained visible in Christmas Bay (Fig. 1). This historical distribution led to selection of two experimental sites: Redfish Cove (29° 09' N, 95° 02' W) because it was the last area to harbor seagrasses, and Snake Island Cove (29° 05' N, 95° 07' W) because it is an embayment protected by an offshore bar (Fig. 1).

Sediment samples (top 5 cm) were collected at these two sites and near both ends of the southeastern Christmas Bay seagrass bed in February 1993 to compare sediment organic content (Dean 1974) and particle size (Folk 1974). Turbidity samples were collected in February and March 1993 and analyzed with an HF Scientific DRT 100B Turbidimeter. There were no appreciable differences in sediments among the sites (organic content: 0.69-0.92%; sand: 81-89%; silt: 3-6%; clay: 8-13%). Turbidity was higher in Christmas Bay (26-62 NTU) than at either experimental site (7-18 NTU).

*Halodule* used in transplant experiments was collected from Christmas Bay. *Ruppia* was obtained from Gangs Bayou, 15 km east of Snake Island Cove, although it was occasionally mixed with *Halodule* in Christmas Bay samples.

*Experiment 1: Bed configuration.*—Experimental seagrass beds were constructed at Redfish Cove and Snake Island Cove to assess effects of bed shape and density of transplants on survival and growth. Bed shape was either 3 m by 3 m or 2 m by 10 m, and transplant density was either 0.5 m or 1.0 m centers. One bed of each shape and density was constructed at each site. Each bed was surrounded with 1.2 m high, 5 cm mesh, galvanized chicken wire fencing to deflect large fishes and decapods that could disturb transplants (Fonseca 1994). Experimental beds were located at depths similar to those in Christmas Bay (approximately 1 m during spring high tides).

Transplanting units (TPUs) of *Halodule* were collected on 19-20 April 1993 after the seagrass leaves had developed sufficiently, following the methods of Fonseca (1994). A 7.5 cm diameter circular sod plunger was used to extract TPUs from the donor bed. Each TPU was then



inserted directly into a 7.5 cm diameter peat pot. TPUs were transported in a seawater-filled holding tank and covered with wet burlap to prevent desiccation. Once at the transplant site, the sod plugger was used to create holes into which TPUs were placed, after ripping the sides of the peat pots to allow rhizome spread. Periodic visits were made to both sites to insure that fencing was intact around each transplant bed. All TPUs were examined by snorkeling over the beds on 25 June 1993 (66 days post-transplant) and on 23 and 26 July 1993 (94-97 days post-transplant). On 7 July 1993, all chicken wire fence was removed (due to rusting) and replaced with plastic safety fence (3.8 cm mesh), but no observations were made of the plants. Percent survival was assessed with one-way analysis of variance (*ANOVA*) of the four combinations of planting density and bed shape.

*Experiment 2: Faunal exclusion cages and transplant methods.*—Bioturbation and herbivory can contribute to transplant failure as much as the choice of transplant method (Fonseca 1994). A second restoration experiment, testing faunal exclusion cages of four mesh sizes and two transplant methods, was conducted at Redfish Cove during 6 August - 18 November 1993. Submerged exclusion cages (1.5 m by 1.5 m by 0.25 m high) were designed with skirts to keep fishes and invertebrates from entering beneath fencing at the sediment surface and with lids to enable access during the experiment. Cage walls and lids were constructed of four different materials with varying mesh sizes: galvanized hardware cloth (1.3 cm), vinyl-covered hardware cloth (2.5 cm), plastic safety fence (3.8 cm), and galvanized chicken wire (5.0 cm). Exclusion cages were placed in two rows of 12 cages each, parallel to and approximately 30 m from the shoreline in < 1 m depths, adjacent to the Experiment 1 site. Six cages of each mesh size were constructed, with three cages of each mesh size allotted to test one of two transplanting methods.

Two methods of transplanting were tested, bare roots and peat pots (reviewed by Fonseca 1994). A 7.5 cm diameter sod plugger was used to extract all *Halodule*, ensuring that TPU biomasses were approximately equal. To create the bare root TPUs, *Halodule* was washed of surrounding sediments and attached to U-shaped staples (fashioned from 20 cm lengths of 16 gauge aluminum wire) with paper-covered twist ties. A dive knife was used to dig holes into which bare root TPUs were placed, with the staple bridge just under the sediment surface. Peat pot TPUs were transplanted as described previously. Each cage

received nine TPUs planted on 0.5 m centers.

Lids were attached to the cages after transplanting. Cages and TPUs were monitored at 13, 21, 35, 48, 63 and 104 days post-transplanting (ending 18 November 1993). All TPUs were accounted for by removing lids, manually locating each TPU, and categorizing each by *Halodule* appearance as healthy (lush, long shoots), unhealthy (sparse, short shoots), or absent. These subjective observations were assigned numerical values of 2, 1, and 0, respectively, and mean values for each cage were derived for each sampling date. *Halodule* health on each date was tested with two-way analysis of variance (ANOVA) of the cage means for effects of planting method and cage mesh size. Comparison of treatment means for significant main effects employed Ryan's Q test (Day & Quinn 1989).

Qualitative observations of the potential effects of bioturbators or herbivores were collected during the same time period from transplants installed in an outdoor fiberglass tank receiving flowing seawater at the National Marine Fisheries Service Galveston Laboratory. Sediment from Redfish Cove was placed in the tank to a depth of 10 cm. The circular tank (1.8 m diameter) was partitioned into four equal sections with 2.5 cm mesh vinyl-coated hardware cloth. Water depth was maintained at 23-27 cm and flow was set for one turnover in tank volume per day. Fifteen peat pot TPUs of mixed *Halodule* and *Ruppia* were placed in each section on 20 September 1993. Eight additional TPUs were sieved to remove sediments and refrigerated to measure pre-transplant characteristics. Each section of the tank received one of three species of organisms with potential for site disturbance that were commonly seen at the transplant sites: thinstripe hermit crab *Clibanarius vittatus*, blue crab *Callinectes sapidus*, and pinfish *Lagodon rhomboides*. Crabs have been cited as bioturbators by Fonseca (1994), and both *Callinectes* and *Lagodon* consume plant material (Stoner 1980; Laughlin 1982). Densities were 12 *Clibanarius* (2-5 cm shell diameter), four *Callinectes* (5-10 cm carapace width), or four *Lagodon* (5-8 cm total length) per section. The fourth section was kept free of animals. *Clibanarius* and *Lagodon* were introduced on 22 September, and *Callinectes* was introduced on 30 September. Caged animals were fed frozen shrimp on Monday, Wednesday, and Friday of each week. Periodic observations were made of organism behavior.

The tank was drained on 22 October 1993. TPUs were dug up, peat

pots were removed, and seagrasses were sieved to remove sediments. Each TPU, including the pre-transplant TPUs, was separated into aboveground and belowground biomass. Leaves were counted and length measurements of 10 randomly selected leaves per TPU were recorded. All samples were soaked in 10% phosphoric acid to remove calcification, rinsed, and dried at 85-100°C for 24 hr. Seagrass leaf count, leaf length, and aboveground and belowground biomasses were noted.

*Experiment 3: Halodule versus Ruppia, peat pots versus cheesecloth bags.*—Survival and growth of *Halodule* and *Ruppia* were compared using peat pot and cheesecloth bag TPUs in an outdoor fiberglass tank receiving flowing seawater. Durako et al. (1993) suggested the cheesecloth bag method as an alternative to diver-oriented transplant methods because it may be less time-consuming. *Ruppia* was used to assess whether mixed transplants would affect overall success of transplant beds. The rectangular tank (6.1 m by 1.8 m by 0.8 m high) received water and sediment as described previously. A 7.5 cm diameter sod plugger was used to collect TPUs in order to keep all biomasses similar. Cheesecloth bags were 1-ply, 15 cm by 15 cm, and sewn on three sides with cotton thread. Quarry rocks were sewn into the corners to weight the bags down. After plant material was inserted into the bag, the open side was sewn shut and the bags were placed in buckets of seawater. Peat pot TPUs were prepared as described previously. Sixty TPUs (15 of each type) were placed in the tank on 0.25 m centers in 15 parallel rows of four TPUs each. One TPU of each type was set randomly in each row. A sod plugger was used in planting peat pots, whereas cheesecloth bags were placed directly on the sediment surface.

TPUs were planted on 20 September 1993, and growth of rhizomes from TPUs was measured after 25, 33, and 47 days. Coverage was estimated by measuring seagrass spread in four directions. The longest rhizome from each TPU was measured first, then three other measurements were taken at 90°, 180° and 270° from the first measurement. Actual rhizome length measurements (mm) were recorded for each TPU after 25 and 33 days and were used to determine growth rates for the four types of TPUs. After 47 days, TPUs had begun to coalesce and it was no longer possible to determine the source TPU for many rhizomes. The shortest distance between edges of two TPUs was 235 mm; therefore, 112.5 mm was established as the maximum rhizome length before potentially crossing another rhizome.

Table 1. Analysis of variance (*ANOVA*) comparisons of *Halodule wrightii* health relative to planting method and mesh size of faunal exclusion cage at six intervals after transplanting on 6 August 1993. Mean health of nine transplant units (TPU) per cage was used as the observation.  $N = 3$  per combination of method and cage mesh. *ANOVA*  $df = 7$  (Model), 1 (Method), 3 (Cage mesh), and 16 (Error).

Source	MS	<i>F</i>	<i>P</i>	MS	<i>F</i>	<i>P</i>
	Day 13			Day 21		
Model	0.263	3.22	0.025	0.628	6.72	0.008
Method (M)	0.700	8.58	0.010	2.600	27.86	0.001
Cage mesh (C)	0.355	4.35	0.020	0.498	5.34	0.010
M x C	0.026	0.32	0.812	0.099	1.06	0.392
Error	0.082			0.093		
	Day 35			Day 48		
Model	0.975	10.73	0.001	0.972	12.68	0.001
Method (M)	3.227	35.52	0.001	3.300	43.05	0.001
Cage mesh (C)	1.007	11.09	0.001	0.899	11.73	0.001
M x C	0.192	2.12	0.138	0.268	3.50	0.040
Error	0.091			0.077		
	Day 63			Day 104		
Model	1.070	10.19	0.001	0.614	3.82	0.013
Method (M)	3.450	32.86	0.001	1.550	9.64	0.007
Cage mesh (C)	0.865	8.24	0.002	0.583	3.62	0.036
M x C	0.482	4.59	0.017	0.333	2.07	0.145
Error	0.105			0.161		

Length of each of the four measured rhizomes was used as an indicator of coverage in each quadrant around a TPU. The four measurements were added to estimate coverage for the whole TPU area, with a total of 450 mm equal to 100% coverage. Mean percent coverage was calculated for each type of TPU and each observation date, and these means were used to estimate coverage rates for each type of TPU. Differences in rhizome length and percent coverage (arcsine transformed) were tested with one-way *ANOVA*. Comparison of treatment means employed either the GT2 test for unbalanced designs (rhizome length; Day & Quinn 1989) or Ryan's Q test for balanced designs (coverage).

## RESULTS

*Experiment 1: Bed configuration.*—Survival of peat pot TPUs planted on 20 April 1993 was 65% in Redfish Cove and 67% in Snake Island

Cove 66 days after transplanting. There were no significant differences in mean *Halodule* survival among the four combinations of bed shape and planting density, but replication of each bed configuration was minimal ( $n = 2$ ) and thus provided low power.

Redfish Cove was revisited on 23 July 1993 (94 days after transplanting), and there was almost no live *Halodule* present. Only two of the original 142 TPUs contained any *Halodule*, and these contained only a few short shoots. Four bare TPUs were removed and none contained any live roots or rhizomes. Snake Island Cove was revisited three days later, and none of the 142 TPUs had survived. Two bare TPUs were examined and contained some root material, but three additional TPUs contained no live roots or rhizomes. Neither of these beds exhibited any growth in 1994.

*Experiment 2: Faunal exclusion cages and transplant methods.*—Failure of the Experiment 1 transplants may have been due to transplant method or to the presence of bioturbators that gained access through screens of relatively large mesh (safety fence and chicken wire). Analyses of *Halodule* health and survival in relation to peat pot versus bare root transplant methods and to faunal exclusion cages of four mesh sizes were made on six observation dates over 104 days (6 August - 18 November 1993). Health of *Halodule* TPUs was significantly affected by both planting method and cage mesh size on all observation dates (Table 1). Mean *Halodule* health declined over time for both peat pot and bare root TPUs, in part due to inclusion of typical fall senescence into the test period. However, health of peat pot TPUs was always significantly better than that of bare root TPUs (Fig. 2), and health in safety mesh cages (3.8 cm) was always significantly lower than in other mesh sizes (Fig. 3). There were no significant differences in *Halodule* health among other mesh sizes except on the last observation date. After 104 days, the relatively healthy TPUs in chicken wire cages (5.0 cm mesh) were found to be in poor condition (Fig. 3). On the last observation date, only peat pot TPUs protected by 1.3 cm and 2.5 cm cages appeared healthy enough to overwinter. Three peat pot TPU beds that had survived unprotected for two years were found in December 1995, but cage mesh size could not be determined.

Inclusion of common estuarine fauna in an outdoor tank with transplanted *Halodule* resulted in alterations to some floral characteristics. After approximately 30 days in the tank sector free of organisms, leaves

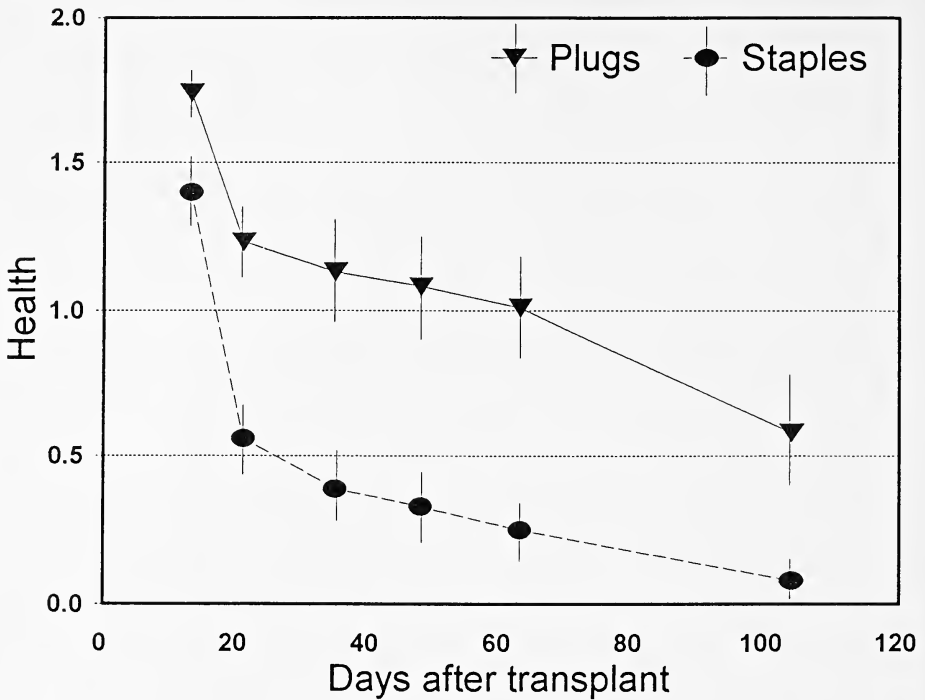


Figure 2. Mean health of *Halodule wrightii* after transplanting by plugs in peat pots or by bare root staples. Maximum health value = 2. Mean health of 9 transplant units per cage was used as the observation. N = 12 cages per method. Vertical bar =  $\pm$  standard error.

were longer (mean 108 mm) than in sectors containing *Lagodon* (61 mm), *Callinectes* (80 mm), or *Clibanarius* (84 mm). No trend was observed for total number of leaves per TPU among tank sectors. *Halodule* biomass (aboveground plus belowground) appeared to be reduced in *Clibanarius* and *Lagodon* sectors (218 and 219 mg dry weight per TPU, respectively) when compared to *Callinectes* and animal-free sectors (339 and 375 mg dry weight per TPU, respectively). Rhizome growth out of the TPUs was not observed during the test period (20 September - 22 October).

*Experiment 3: Halodule versus Ruppia, peat pots versus cheesecloth bags.*—Growth rates for *Halodule* and *Ruppia* in peat pot and cheesecloth TPUs were determined by plotting rhizome length against the number of days elapsed since transplanting (Fig. 4). Mean growth rate of *Ruppia* in peat pot TPUs was 5.5 mm/day over 33 days, with a possible increase in growth rate between day 25 and day 33. All 15 *Ruppia* in peat pot TPUs expanded. Mean growth rate of *Ruppia* in

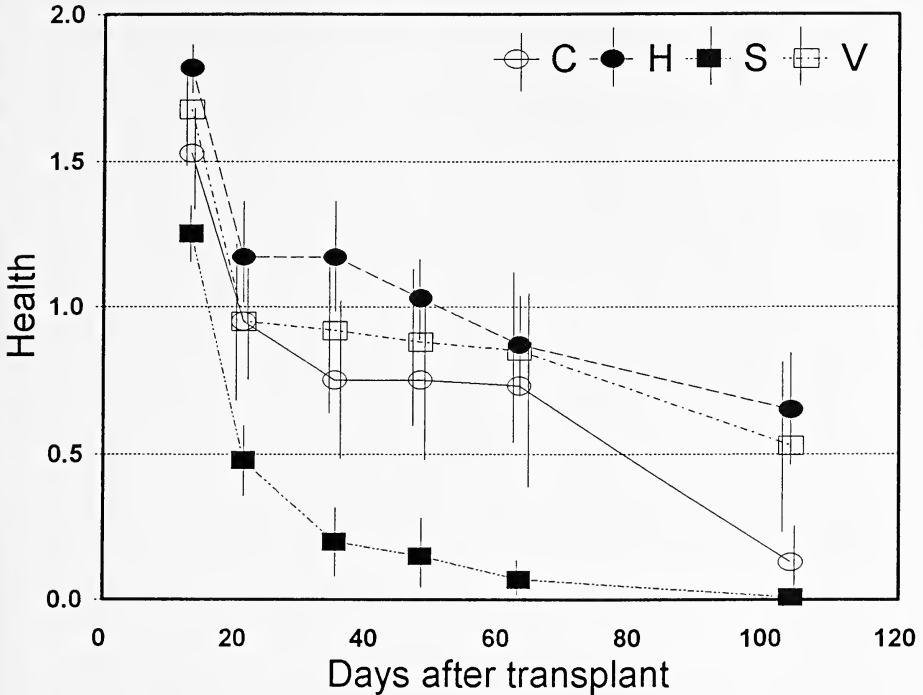


Figure 3. Mean health of *Halodule wrightii* after transplanting into cages of hardware cloth (H; 1.3 cm mesh), vinyl-coated wire (V; 2.8 cm), plastic safety fence (S; 3.8 cm), or chicken wire (C; 5.0 cm). Maximum health value = 2. Mean health of 9 transplant units per cage was used as the observation.  $N = 6$  cages per mesh size. Vertical bar =  $\pm$  standard error.

cheesecloth TPUs was 3.1 mm/day over 33 days. Ten of the 15 *Ruppia* in cheesecloth TPUs expanded. Mean growth rate of *Halodule* in peat pot TPUs was 1.8 mm/day over the 33-day period, but only six of 15 TPUs showed any expansion. Only one of 15 *Halodule* in cheesecloth TPUs (not figured) grew outside the confines of its container and its mean growth rate was 0.9 mm/day, mostly between days 25 and 33. Excluding the *Halodule* in cheesecloth data, mean rhizome lengths were not significantly different among the three combinations after 25 days ( $ANOVA F = 1.33, P = 0.274, df = 2,47$ ), but mean length of *Ruppia* rhizomes from peat pots was significantly greater after 33 days ( $ANOVA F = 7.70, P = 0.001, df = 2,65, GT2$  test).

*Ruppia* in peat pot TPUs exhibited the greatest total coverage (83%) over the 47 day study period, while *Ruppia* in cheesecloth TPUs covered much less area (32%; Figure 5). *Halodule* in peat pot TPUs covered only 5% of the area, and *Halodule* in cheesecloth TPUs (not figured)

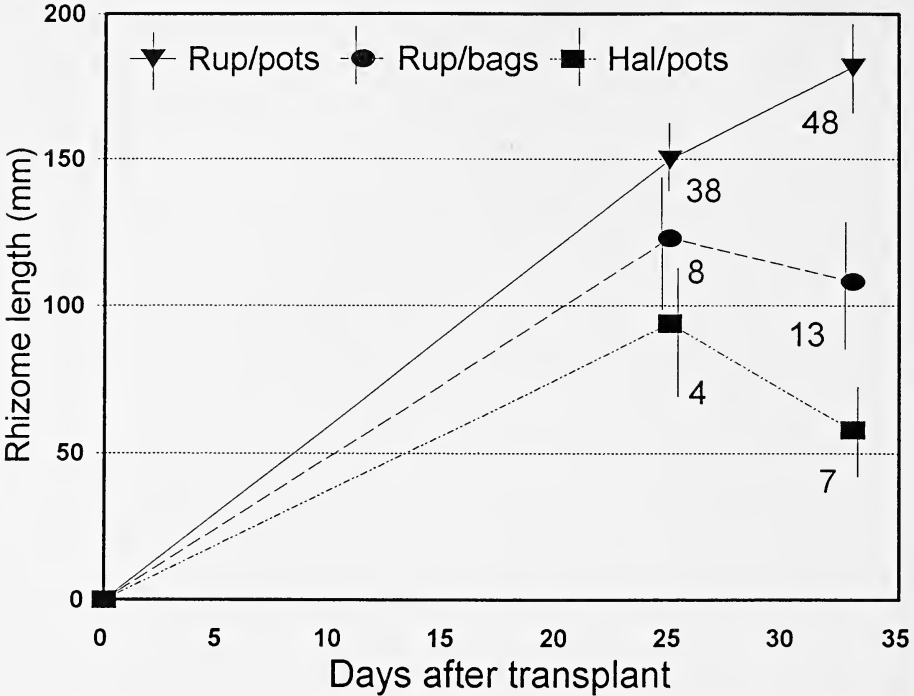


Figure 4. Mean lengths of rhizomes growing from *Ruppia maritima* (Rup) and *Halodule wrightii* (Hal) transplanted in peat pots or cheesecloth bags. *Halodule* in bags not figured. Vertical bar =  $\pm$  standard error. N = number of rhizomes measured.

exhibited <1% coverage. Many *Ruppia* TPUs had flowered and developed seeds by the end of the experiment (5 November). Mean coverage by *Ruppia* in peat pot TPUs was significantly greater than by other treatments after 25, 33, and 47 days (*ANOVA*  $F = 37.35, 54.92,$  and  $36.32,$  respectively,  $P < 0.001,$   $df = 3, 56,$  Ryan's Q test). By day 47, mean coverage by *Ruppia* in cheesecloth TPUs was also significantly higher than by either *Halodule* treatment.

#### DISCUSSION

Field and laboratory experiments have indicated that submerged aquatic vegetation (*Halodule wrightii* and *Ruppia maritima*) can be successfully transplanted into western Galveston Bay, if certain precautions are taken to insure survival and growth. Factors considered in these experiments were (1) transplant method, (2) type and mesh size of protective fencing, (3) time of the year, and (4) bed configuration.

Transplanting *Halodule* and *Ruppia* using peat pots was more success-



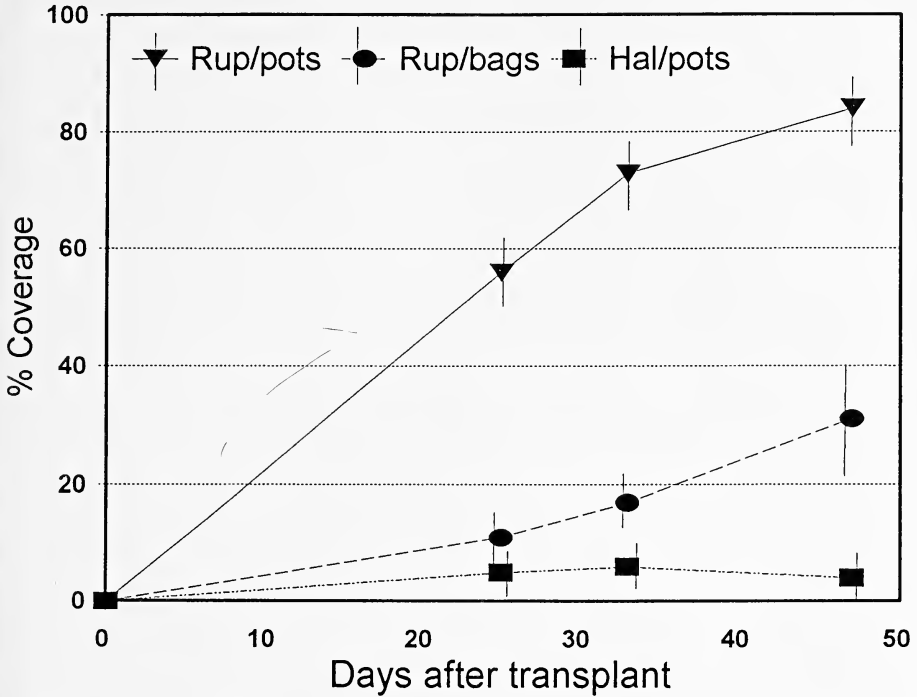


Figure 5. Mean coverage of *Ruppia maritima* (Rup) and *Halodule wrightii* (Hal) transplanted in peat pots or cheesecloth bags. *Halodule* in bags not figured. Vertical bar =  $\pm$  standard error. N = 15 measurements per type per day.

ful than with bare roots or cheesecloth bags. Although the use of a sod plugger may be stressful to plants, transporting sediment with each TPU likely causes less stress or damage to intact plant biomass than removal of sediments for bare root or cheesecloth methods. The bare root method is not recommended for Galveston Bay since survival was extremely low, even though bare roots have been successful elsewhere (Fonseca 1994; Fonseca et al. 1994). However, trials with the cheesecloth bag method early in the growing season still need to be conducted, since this method is less time consuming at the transplant site. Cheesecloth bags can be distributed by dropping them from a boat or while walking and should remain in place if currents are weak and bioturbators, including wading birds, are excluded (Durako et al. 1993).

Materials used for protective fencing are also important. Health of TPUs enclosed in 1.3 cm and 2.5 cm mesh was nearly always better than TPUs enclosed in 3.8 cm and 5.0 cm mesh. Smaller mesh will exclude a wider size range of herbivores or bioturbators, and cages need

only remain in place until transplants are established (60-90 days in this study; 90 days recommended by Fonseca et al. 1994). *Halodule* transplants accessible to typical West Bay macrofauna such as *Clibanarius*, *Callinectes* or *Lagodon* appear to exhibit shorter leaves or lower biomass or both. All three species were observed to sift through the sediment, presumably in search of benthic or epibenthic prey, and thus may have impacted root functions. In addition, *Lagodon* are known to bite tips off seagrass leaves (Stoner 1985). Experimental verification of the validity of these observations, as well as elucidation of mechanisms, still need to be conducted. Smaller mesh sizes probably excluded most bioturbators and may have provided more protection from currents, resulting in better survival of transplants. Mesh material seemed to make no difference in seagrass survival, although plastic mesh or vinyl-coated wire mesh will last longer than uncoated, galvanized wire meshes. Chicken wire (5 cm mesh) is not recommended: it may be useful in excluding rays (Fonseca et al. 1994), but it would be ineffective in excluding many small fishes and decapods common to Galveston Bay and it rusts quickly.

*Ruppia* transplants grew faster than *Halodule* transplants, at least in the fall. *Ruppia* is found in the relatively high salinity waters of Christmas Bay and in tidal ponds along West Bay, but it is more abundant in fresh and brackish water near river mouths (Adair et al. 1994). In addition, *Ruppia* is an annual and grows back from seeds in the Galveston Bay area, as it does in southern Texas (Dunton 1990). *Ruppia* is a potential tool for brackish and freshwater restorations in Galveston Bay and for mixed plantings with *Halodule*, where *Ruppia* could provide initial stability to a transplanted area due to its faster growth (Dunton 1990). *Halodule* is the plant of choice for higher salinity restorations in West Bay, since it dominates the Christmas Bay seagrass meadows (Adair et al. 1994). *Halodule* transplanted in April and August exhibited survival and growth, while September transplants showed little growth. Thus, *Halodule* should be planted as early in the growing season as possible to allow maximum spread and overwintering potential.

Transplant bed configuration was not clarified. Neither compact beds of dense plantings (0.5 m centers) nor large beds of sparse plantings (1 m centers) could be recommended, nor is it known whether beds should have more or less edge (e.g., a rectangular bed has more edge than a square bed of the same area). In West Bay, dense plantings are

expected to have a better chance of coalescing over the relatively short growing season (April-September) and of resisting effects of currents and bioturbation than sparse plantings.

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## REPRODUCTION IN THE MEXICAN VINE SNAKE *OXYBELIS AENEUS* (SERPENTES: COLUBRIDAE)

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**Abstract.**—Reproductive tissue was examined in 40 museum specimens of *Oxybelis aeneus* from Arizona and the Mexican states of Sonora, Sinaloa, Nayarit, Jalisco, Colima, Oaxaca and Chiapas. The results of this examination support the premise that this species of vine snake exhibits a reproductive cycle with sperm formation occurring in the spring and that clutch sizes for this species may be as large as eight.

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The Mexican vine snake, *Oxybelis aeneus* occurs from extreme southern Arizona to southeastern Brazil (Stebbins 1985). There are only anecdotal reports on its reproduction including clutch sizes (Mole 1924; Campbell 1934; Beebe 1946; Cochran 1946; Stebbins 1954; Rand 1957; Sexton et al. 1964; Sexton & Heatwole, 1965; Hardy & McDiarmid 1969; Emsley 1977; Lowe et al. 1986; Rossi & Rossi 1995), size at maturity (Keiser 1967) and seasonal ovarian cycle (Neill 1962). The biology of *O. aeneus* is summarized by Keiser (1967; 1982). The purpose of this investigation is to provide information on the seasonal ovarian and testis cycles of *O. aeneus* and to report additional clutch sizes.

### METHODS AND MATERIALS

A sample of 40 specimens of *Oxybelis aeneus* (22 females, Mean Snout-Vent Length, SVL = 788.9 mm  $\pm$  18.7 SE, range 613-922 mm; 18 males, Mean SVL = 733.6 mm  $\pm$  15.7 SE, range 605-830 mm) from Arizona and México was examined from the herpetology collections of the Natural History Museum of Los Angeles County and the University of Arizona, Tucson. Counts were made of oviductal eggs or enlarged follicles (> 6 mm diameter). The left testis, epididymis, vas deferens and part of the kidney were removed from males; the left ovary was removed from females for histological examination. Tissues were embedded in paraffin and cut into sections at 5  $\mu$ m. Slides 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. Epididymides

Table 1. Monthly distribution of conditions in seasonal testicular cycle of *Oxybelis aeneus* from Arizona and México. Values shown are the numbers of males exhibiting each of the three conditions.

Month	N	Regressed	Recrudescence	Spermiogenesis
February	1	0	0	1
April	2	0	0	2
June	3	0	0	3
July	6	5	0	1
August	4	4	0	0
October	1	0	1	0
November	1	0	1	0

and vasa deferentia were examined for sperm. Slides of kidney sexual segments were examined for secretory activity. Histology slides are deposited in the herpetology collection of the Natural History Museum of Los Angeles County.

*Material examined.*—The following specimens of *Oxybelis aeneus* were examined from the herpetology collections of the Natural History Museum of Los Angeles County (LACM) and The University of Arizona (UAZ).

ARIZONA: SANTA CRUZ COUNTY, three specimens (UAZ 16787, 39545, 47314).

MÉXICO: SONORA, 16 specimens (LACM 103644; UAZ 16794, 16796, 16800, 25796, 26972-26973, 42849, 103643, 103645-103646, 25795, 28279, 45899, 45989, 46662); SINALOA, 14 specimens (LACM 7012-7020, 7235-7236, 103642, 126259, UAZ 37700; NAYARIT, one specimen (LACM 25249); JALISCO, one specimen (LACM 136959); COLIMA, two specimens (LACM 37326; UAZ 27000); OAXACA, two specimens (UAZ 16789, 27050); CHIAPAS, one specimen (UAZ 25814).

## RESULTS AND DISCUSSION

Data on the male specimens of *O. aeneus* seasonal testicular cycle are presented in Table 1. Testicular histology was similar to that reported by Goldberg & Parker (1975) for two colubrid snakes, *Masticophis taeniatus* and *Pituophis melanoleucus*. In the regressed testes, seminiferous tubules contained spermatogonia and Sertoli cells. In

Table 2. Monthly distribution of conditions in seasonal ovarian cycle of *Oxybelis aeneus* from Arizona and México\*. Values shown are the numbers of females exhibiting each of the four conditions.

Month	N	Inactive	Yolk deposition	Enlarged follicles	Oviductal eggs
January	2	2	0	0	0
March	1	1	0	0	0
April	1	1	0	0	0
May	2	2	0	0	0
June	1	0	1	0	0
July	6	3	0	2	1
August	5	2	1	2	0
September	3	3	0	0	0

\* One additional female collected from March to May contained oviductal eggs (the exact month of collection was not given).

recrudescence, there was renewal of spermatogenic cells characterized by spermatogonial divisions; primary and secondary spermatocytes and spermatids may have been present. In spermiogenesis, metamorphosing spermatids and mature sperm were present. Males undergoing spermiogenesis were found in February, April, June and July; regressed males were found in July and August. Males with testes in recrudescence were found in October and November. This suggests a prenuptial type of reproductive cycle (*sensu* Saint Girons 1982) in which spermatogenesis occurs in spring. Epididymides and vasa deferentia of spermiogenic males contained sperm. Sperm was also present in the vasa deferentia of one July male and two August males with regressed testes as well as one October male with recrudescing testes. This may suggest males are capable of inseminating females outside the period when testes are undergoing spermiogenesis. The smallest spermiogenic male measured 605 mm SVL. The sexual segment of the kidney was enlarged and contained densely staining secretory granules in spermiogenic males. Mating coincides with hypertrophy of the kidney sexual segment (Saint Girons 1982).

Data on the *O. aeneus* seasonal ovarian cycle are presented in Table 2. The smallest reproductively active female (enlarged follicles) measured 773 mm SVL. Keiser (1967) reported fully formed eggs in *O. aeneus* measuring 590 mm SVL. To avoid the possibility of using immature snakes, only female *O. aeneus* of this size or larger were included in this analysis of the female reproductive cycle (N = 22).

Table 3. Published clutch sizes for *Oxybelis aeneus*.

Date	Locality	Clutch Size	Author
17 March	Venezuela	3	Beebe 1946
24 July	Arizona	4	Campbell 1934
18 July	Panamá	4	Cochran 1946
June	Trinidad and Tobago	ca 6	Emsley 1977
16 August	Sinaloa, México	4, 6	Hardy & McDiarmid 1969
not given	Arizona	3-5	Lowe et al. 1986
June	Trinidad	6	Mole 1924
21 March or 21 May	El Salvador	5	Rand 1957
31 August	captivity	3	Rossi & Rossi 1995
1 July	Panamá	4	Sexton & Heatwole 1965
not given	Arizona	4	Stebbins 1954

The time when reproductive activity begins in females is not clear as the earliest female with oviductal eggs had a collection date of March to May (the exact month of collection was not given); other reproductively active females were June to August. Previous reports have indicated *O. aeneus* eggs are produced spring to summer (Mole 1924; Campbell 1934; Beebe 1946; Cochran 1946; Rand 1950; Sexton & Heatwole 1965; Hardy & McDiarmid 1969; Emsley 1977; Lowe et al. 1986). The following six clutch sizes are herein recorded: March-May (the exact month of collection was not given) Oaxaca, SVL = 790 mm, 3 oviductal eggs; 3 July, Sinaloa, SVL = 883 mm, 8 enlarged follicles; 11 July, Sinaloa, SVL = 829 mm, 6 enlarged follicles; 24 July, Colima, SVL = 890 mm, 7 oviductal eggs; 8 August, Sinaloa, SVL = 777 mm SVL, 6 enlarged follicles; 16 August, Sinaloa, SVL = 773 mm SVL, 4 enlarged follicles. The presence of a total of four females from March, April and May with inactive ovaries during the reproductive period (Table 2) may suggest the possibility that not all mature females breed each year. The three July and two August females with inactive ovaries may have already deposited eggs. Neill (1962) reported an adult female *O. aeneus* collected in April from British Honduras did not contain eggs. Obviously, more females will need to be examined before this is known. Published clutch sizes for *O. aeneus* are summarized in Table 3 and indicate a range of 3-6. The two females containing seven oviductal eggs and eight enlarged follicles in the present study represent the largest clutch sizes recorded for *O. aeneus*. In Arizona hatching occurs in September (Lowe et al. 1986).



## CONCLUSIONS

In conclusion, this study indicates that *Oxybelis aeneus* has a reproductive cycle with sperm formation occurring in spring and that clutch sizes for this species may be as large as eight (the previous maximum clutch size for *O. aeneus* was six). Due to the large geographic range of *O. aeneus* (southern Arizona to Brazil), additional studies from other parts of its range will be needed before the reproductive biology of this species is fully known.

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## DATA RELATABILITY DEPENDENCIES AND THE DECOMPOSITION OF DATABASE RELATIONS

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**Abstract.**—The decomposition of database relations provides a reasonable and powerful approach for designing a database schema which minimizes the presence of anomalies. Traditionally, functional dependencies and multivalued dependencies have been the basis for the axiomatic foundation underlying the development of the related decomposition algorithms. One-to-one and one-to-many relationships between attributes are captured by functional and multivalued functional dependencies respectively. When functional or multivalued functional dependencies are the basis for a decomposition, then the database designer must employ multiple relational schemes to represent many-to-many relationships between attributes. These many-to-many relationships between attributes are commonly required by integrity constraints in the related database schema. To reveal the latter integrity constraints, then the related relational schemes must be joined, an expensive and time consuming operation. Many-to-many relationships between attributes are the basis for the operational definition of a data relatability dependency. A decomposition based upon a combination of functional and data relatability dependencies exhibits better control for update, insertion and deletion anomalies. Data relatability dependencies ensure a decomposition where the totality of the functional dependencies in the associated universal relation are completely defined. This paper presents an axiomatization for data relatability dependencies. The presence of a nontrivial data relatability dependency in a relation is a necessary and sufficient condition for the relation to be decomposable.

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Recent research in relational database design has focused on the elimination of various types of anomalies. References (Codd 1971; 1972) raised the issue of update anomalies and initiated the search for normal forms that prevent them by introducing second and third normal forms. Third normal form eliminates insertion, deletion and update anomalies due to the elimination of the related transitive dependencies (Codd 1972). Boyce-Codd normal form (BCNF) was introduced by Codd (1974) to provide a normal form simpler than 3NF. Fourth normal form was introduced in Fagin (1977). Even richer normal forms include project-join normal form (PJ/NF) (Fagin 1979) and domain-key normal form (Fagin 1981). Domain-key normal form eliminates insertion and deletion anomalies (Fagin 1981). Elimination of intersection anomalies in a relational database design has been extensively studied (Beeri & Kifer 1983;1986;1987). Another approach to relational database design is to employ schemes that have no update anomalies due to functional dependencies (Vossen 1988; Chan 1989; Vincent & Srinivasan 1994a). These update anomalies, referred to as replacement

anomalies can be detected and eliminated from the schema design. Insertion anomalies can be defined in terms of multivalued functional dependencies and are present if the relation is not in fourth normal form (Vincent & Srinivasan 1995). Key-based update anomalies can be eliminated if the relation is in fourth normal form (Vincent & Srinivasan 1994b).

When a schema is created using traditional functional dependencies, FDs, and multivalued dependencies, MVDs, the many-to-many relationships may not be represented. A relational scheme containing two MVDs having the same determinant group can be decomposed into two relational schemes (Dutka & Hanson 1989). One scheme in the decomposition contains the determinant group and a set of attributes that are multivalued determined by the determinant group. The second relational scheme in the decomposition has the determinant group and the other set of attributes that it determines. The resulting decomposition has no way of enforcing certain integrity constraints that represent policy matters if many-to-many relationships exist between the determinant group and the two groups of determined attributes. The lack of representation of a many-to-many relationship causes insertion, deletion, and update anomalies. This is because the relationship is expressed as an interrelational constraint, or not within the same relation (Date 1990). The only way to make the interrelational constraint intrarelational, or within the same relation, is to join the relations. Joining the relations is a time-consuming way to prevent these problems.

In addition to introducing second and third normal form, Codd (1972) initiated the search for normalization algorithms by proposing the first decomposition algorithms. This initiated other research on decomposition (Delobel & Casey 1972; Rissanen & Delobel 1975; Paredaens & Janssens 1981) and synthesis (Bernstein et al. 1975; Bernstein 1976; Biskup et al. 1979). The fact that these two criteria are not equivalent was stressed in Rissanen (1977) where it is proposed that both be attempted. Algorithms for synthesis into 3NF include Bernstein (1976) and Biskup et al. (1979), for decomposition into BCNF include Tsou & Fischer (1982), and for decomposition into 4NF include Fagin (1977). The more formal study of decompositions and their properties was initiated in Rissanen (1977), which considered decompositions into two-element sets and proposed the notion of independent components; and Arora & Carlson (1978), which studied decompositions with lossless joins and dependency preservation. This was extended independently to arbitrary decompositions over FDs by Beeri & Rissanin (1980) and

Maier et al. (1980). Lossless join was further investigated in Vardi (1982), in which an example of a schema where the dependencies were expressed in first-order logic and a decomposition was constructed which was one-to-one but did not have the lossless join dependency due to the presence of embedded dependencies. A very different form of decomposition, called *horizontal decomposition*, is introduced in DeBra & Paredaens (1984). This involves splitting a relation into pieces, each of which satisfies a given set of FDs. If a relation schema is in Boyce-Codd normal form and *some* key is simple, consists of a single attribute, then the relation is in fourth normal form, but not necessarily projection-join form. The latter results give the database designer simple sufficient conditions, with respect to FDs alone, that guarantee that the decomposition is in higher normal forms (Date & Fagin 1992).

Surveys of database systems often include sections on schema design and related ideas. For instance, an overview of some types of anomalies and normal forms is given in Vossen (1991). Different types of dependencies, such as functional, multivalued, and inclusion dependencies are discussed in Atzeni & De Antonellis (1993).

The issue of finding the most desirable way of mapping one-to-one predicates to database schemata is addressed in Ritson & Halpin (1993). In addressing this issue, certain goals for favorable database schemata are stated.

One such characteristic is that of a reduced number of relations. A smaller number of relations in a database schema has many benefits. These advantages include fewer update anomalies and fewer interrelational constraints. Another advantage is that queries do not require as many joins as in schemata with larger numbers of relations (Ritson & Halpin 1993).

Another goal for desirable database schemata is to make as many of the constraints readily enforceable. A constraint that resides in a single table, an intrarelational constraint, is enforced simply. Some ways of upholding the conditions of such a constraint include nonnull and unique constraints. Having mostly constraints that are easy to enforce gives the database designer control over update anomalies caused by the presence of more difficult to enforce constraints (Ritson & Halpin 1993).

The goals of desirable database schemata are realized through the use of data reliability dependencies in schema design. The DRDs allow the



**Axioms and Theorems:**

Reflexivity:	If $Y \subseteq X$ then $X \rightarrow Y$ .
Augmentation:	If $Z \subseteq W$ and $X \rightarrow Y$ then $XW \rightarrow YZ$ .
Transitivity:	If $X \rightarrow Y$ and $Y \rightarrow Z$ then $X \rightarrow Z$ .
Complementation:	If $X \rightarrow Y$ , then $X \rightarrow \neg Y$ .
MVD Augmentation:	If $X \rightarrow Y$ and $W \supseteq Z$ , then $WX \rightarrow YZ$ .
MVD Transitivity:	If $X \rightarrow Y$ and $Y \rightarrow Z$ , then $X \rightarrow (Z-Y)$ .
Replication:	If $X \rightarrow Y$ , then $X \rightarrow Y$ .
Coalescence:	If $X \rightarrow Y$ and there exists $W$ with the properties that (a) $W \cap Y$ is empty, (b) $W \rightarrow Z$ , and (c) $Y \supseteq Z$ , then $X \rightarrow Z$ .
Pseudotransitivity:	If $X \rightarrow Y$ and $YW \rightarrow Z$ then $XW \rightarrow Z$ .
MVD Pseudotransitivity:	If $X \rightarrow Y$ and $WY \rightarrow Z$ , then $WX \rightarrow (Z-WY)$ .
MVD Mixed Pseudotransitivity:	If $X \rightarrow Y$ and $XY \rightarrow Z$ , then $X \rightarrow (Z-Y)$ .
Union:	If $X \rightarrow Y$ and $X \rightarrow Z$ then $X \rightarrow YZ$ .
MVD Union:	If $X \rightarrow Y$ and $Y \rightarrow Z$ , then $X \rightarrow YZ$ .
Decomposition:	If $X \rightarrow YZ$ then $X \rightarrow Y$ and $X \rightarrow Z$ .
MVD Decomposition:	If $X \rightarrow Y$ and $X \rightarrow Z$ , then $X \rightarrow (Y \cap Z)$ , $X \rightarrow (Y-Z)$ , and $X \rightarrow (Z-Y)$ .

**DATA RELATABILITY DEPENDENCY AXIOMATICS**

Likewise, a Borel algebra for data relatability dependencies can be developed. A discussion of how values map to other values is necessary in order to define a data relatability dependency. First consider two disjoint attributes  $A$  and  $B$  from a relation  $R$ . Considering a tuple  $r \in R$ ,  $r[A]$  is the concatenated set of value assignments of  $A$  for  $r$ .  $M_B(r[A])$  is the *mapping from attribute set  $A$  to attribute set  $B$* . It is the set of  $B$  values associated with  $r[A]$  and is described by the set shown below.

$$M_B(r[A]) = \{r'[B] \mid r' \in R \text{ and } r'[A] = r[A]\}.$$

Note that  $M_B$ , the set of  $B$  values associated with  $r[A]$ , is a mapping from  $\Pi_A(R)$  into the family of subsets of  $\Pi_B(R)$ . Let  $C$  be the set of all attributes in  $R$ . When  $A \cup B = C$  and  $A \cap B = \emptyset$ ,  $M_B(r[A])$  is the image set of  $r[A]$  under  $R$ . A formal definition for a data relatability dependency is stated below.

**Definition of data relatability dependency:** The attribute set  $A$  is said to be data relatability dependent, in relation  $R$  defined over the attribute set  $C$ , on the attribute set  $B$  when, letting  $D=C-B-A$ , for any pair of tuples  $r_1, r_2 \in \Pi_{D \cup B}(R)$ ,

$$(r_1[B]=r_2[B]) \rightarrow (M_A(r_1)=M_A(r_2)).$$

Thus, the mapping  $M_A$  is solely a function of the  $B$  attributes and not of the  $D$  attributes.

It is important to notice that a data relatability dependency is related to a functional dependency. If  $\|M_A\| = 1$  (the cardinality of the mapping is 1) then the definition of data relatability dependency is equivalent to the definition of a functional dependency.

As with functional and multivalued dependencies, data relatability dependencies may be trivial. A trivial data relatability dependency occurs when  $D=\emptyset$  ( $A \cup B=C$ ) or when  $A \subseteq B$ . If  $r_1[B]=r_2[B]$ , then  $M_A(r_1)=M_A(r_2)$  is always true.

The following proposition enables the proofs for the majority of the Borel Algebraic laws for DRDs.

**Proposition A:**  $\Gamma \rightarrow \Delta$  in  $R(\Omega)$  if and only if  $(x_1, x_2, \dots, x_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in R$  and  $(y_1, y_2, \dots, y_p, x_{p+1}, \dots, x_r) \in \Pi_{\Lambda \cup \Gamma}(R)$  implies that  $(y_1, y_2, \dots, y_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in R$ .

For the following theorems, let  $\Gamma, \Delta, \Lambda$ , and  $\Psi$  be nonempty subsets of  $\Omega$ . Then the DRDs of a relation  $R(\Omega)$  have the following properties:

Reflexivity:	$\Delta \rightarrow \Delta$ .
Augmentation:	If $\Gamma \rightarrow \Delta$ and $\Gamma \subseteq \Psi$ , then $\Psi \rightarrow \Delta$ .
Projectability:	If $\Gamma \rightarrow \Delta$ in $R(\Omega)$ then $\Gamma \rightarrow \Delta'$ , in any $\Pi_R(\Omega')$ where $\Gamma \subseteq \Omega' \subseteq \Omega$ and $\Delta' = \Omega' \cap \Delta \neq \emptyset$ .
Additivity:	If $\Gamma \rightarrow \Delta$ and $\Gamma \rightarrow \Psi$ then $\Gamma \rightarrow (\Delta \cup \Psi)$ .
Transitivity:	If $\Gamma \rightarrow \Delta$ and $\Delta \rightarrow \Psi$ then $\Gamma \rightarrow \Psi$ .
Pseudotransitivity:	If $\Gamma \rightarrow \Delta$ and $(\Lambda \cup \Delta) \rightarrow \Psi$ then $(\Gamma \cup \Lambda) \rightarrow \Psi$ .

The proofs of the previously stated formal properties of DRDs are given in the subsections that follow.

### Proof of Proposition A:

$\Gamma \rightarrow \Delta$  in  $R(\Omega)$  if and only if  $(x_1, x_2, \dots, x_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in R$  and  $(y_1, y_2, \dots, y_p, x_{p+1}, \dots, x_r) \in \Pi_{\Lambda \cup \Gamma}(R)$  implies that  $(y_1, y_2, \dots, y_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in R$ .



**Proof:**

Case 1:

Consider a pair of tuples:

$$(x_1, x_2, \dots, x_p, x_{p+1}, \dots, x_r) \in \Pi_{\Delta \cup \Gamma}(\mathbf{R}) \text{ and } (y_1, y_2, \dots, y_p, y_{p+1}, \dots, y_r) \in \Pi_{\Delta \cup \Gamma}(\mathbf{R})$$

Assume that  $(x_{r+1}, \dots, x_n) \in M_{\Delta}(x_1, x_2, \dots, x_p, x_{p+1}, \dots, x_r)$ . Then  $(x_1, x_2, \dots, x_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in \mathbf{R}$ . Then it follows that  $(y_1, y_2, \dots, y_p, y_{p+1}, \dots, y_r, x_{r+1}, \dots, x_n) \in \mathbf{R}$

Thus,  $M_{\Delta}(x_1, \dots, x_p, x_{p+1}, \dots, x_n) \subseteq M_{\Delta}(y_1, \dots, y_p, x_{p+1}, \dots, x_n)$ . Then  $\Gamma \rightarrow \Delta$ .

This completes the proof for the sufficiency case.

Case 2:

{Assume  $\Gamma \rightarrow \Delta$ } and  $\{(x_1, x_2, \dots, x_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in \mathbf{R}$  and  $(y_1, y_2, \dots, y_p, x_{p+1}, \dots, x_r) \in \Pi_{\Delta \cup \Gamma}(\mathbf{R})\}$ . Then  $(x_{r+1}, \dots, x_n) \in M_{\Delta}(x_1, \dots, x_p, x_{p+1}, \dots, x_r)$ .

But  $\Gamma \rightarrow \Delta$  implies  $M_{\Delta}(x_1, \dots, x_p, x_{p+1}, \dots, x_r) = M_{\Delta}(y_1, \dots, y_p, x_{p+1}, \dots, x_r)$ .

Therefore,  $(x_{r+1}, \dots, x_n) \in M_{\Delta}(y_1, \dots, y_p, x_{p+1}, \dots, x_r)$ . Thus,  $(y_1, \dots, y_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in \mathbf{R}$ .

Therefore, the proof for the necessity part is complete.

Proofs of Reflexivity and Augmentation:

Reflexivity:  $\Delta \rightarrow \Delta$ .Augmentation: If  $\Gamma \rightarrow \Delta$  and  $\Gamma \subseteq \Psi$ , then  $\Psi \rightarrow \Delta$ .

**Proof:** The proofs for reflexivity and augmentation follow directly from the definition for DRD.

Proof of Projectability:

If  $\Gamma \rightarrow \Delta$  in  $\mathbf{R}(\Omega)$  then  $\Gamma \rightarrow \Delta'$  in any  $\Pi_{\mathbf{R}}(\Omega')$  where  $\Gamma \subseteq \Omega' \subseteq \Omega$  and  $\Delta' = \Omega' \cap \Delta \neq \emptyset$ .

**Proof:** Assume that  $\Gamma \cap \Delta = \emptyset$ , and that  $\Lambda = \Omega - \Gamma - \Delta$ . Without loss of generality, one can then assume that these sets are defined by  $\Omega = \{A_1, \dots, A_p, A_{p+1}, \dots, A_r, A_{r+1}, \dots, A_n\}$ , where  $\Lambda = \{A_1, \dots, A_p\}$ ,  $\Gamma = \{A_{p+1}, \dots, A_r\}$ ,  $\Delta = \{A_{r+1}, \dots, A_n\}$

$$(x_1, \dots, x_p, x_{p+1}, \dots, x_r) \in \Pi_{\Delta \cup \Gamma}(\mathbf{R}),$$

$$(y_1, \dots, y_p, x_{p+1}, \dots, x_r) \in \Pi_{\Delta \cup \Gamma}(\mathbf{R}),$$

and  $(x_{r+1}, \dots, x_n) \in M_{\Delta}(x_1, \dots, x_p, x_{p+1}, \dots, x_r)$  implies  $(x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in \Pi_{\Gamma \cup \Delta}(\mathbf{R})$ .

Let  $\Omega'$  contain all the attributes in  $\Omega$  but the last one:

$\Omega' = \{A_1, \dots, A_p, A_{p+1}, \dots, A_r, A_{r+1}, \dots, A_{n-1}\}$ , where  $\Delta = \{A_1, \dots, A_p\}$ ,  $\Gamma = \{A_{p+1}, \dots, A_r\}$ ,  $\Delta' = \{A_{r+1}, \dots, A_{n-1}\}$ . Thus,  $\Delta' = \{A_{r+1}, \dots, A_{n-1}\}$ , where  $r \leq n-1$ .

Then if  $(x_1, \dots, x_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_{n-1}) \in \Pi_{\Omega'}(\mathbf{R})$

and  $(y_1, \dots, y_p, x_{p+1}, \dots, x_r) \in \Pi_{\Lambda \cup \Gamma}(\mathbf{R})$ , one can find an  $x_n$  such that:

$(x_1, \dots, x_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_{n-1}, x_n) \in \mathbf{R}(\Omega)$ .

But since  $\Gamma \twoheadrightarrow \Delta$  one has:  $(y_1, \dots, y_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_{n-1}, x_n) \in \Pi_{\Omega}(\mathbf{R})$ .

Thus,  $(y_1, \dots, y_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_{n-1}) \in \Pi_{\Omega}(\mathbf{R})$ .

This proves the invariance of DRDs over projection operations where one domain is eliminated from a leaf subset. The general case is then derived by induction on the number of domains eliminated from  $\Omega$  during the projection operation.

This completes the proof for Projectability.

Counterexample to Reverse Projectability for DRDs: The projectability property that applies to DRDs is analogous to the projectability property for FDs. The projectability property for DRDs states that if  $\Gamma \twoheadrightarrow \Delta$  in  $\mathbf{R}(\Omega)$  then  $\Gamma \twoheadrightarrow \Delta'$  in any  $\Pi_{\mathbf{R}}(\Omega')$  where  $\Gamma \subseteq \Omega' \subseteq \Omega$  and  $\Delta' = \Omega' \cap \Delta \neq \emptyset$ . Similarly, the projectability property for FDs states that if  $\Gamma \rightarrow \Delta$  in  $\mathbf{R}(\Omega)$  then  $\Gamma \rightarrow \Delta'$  in any  $\Pi_{\mathbf{R}}(\mathbf{R})$  where  $\Gamma \subseteq \Omega' \subseteq \Omega$  and  $\Delta' = \Omega' \cap \Delta \neq \emptyset$ . The reverse projectability property for FDs states that if  $\Gamma \rightarrow \Delta$  in a projection of  $\mathbf{R}$ , then  $\Gamma \rightarrow \Delta$  in  $\mathbf{R}$ . However, the reverse projectability property that applies to FDs does not apply to DRDs. In the following example, the relation  $\mathbf{R}$  has attributes  $A_1, A_2, A_3, A_4$ , and  $A_5$  and is shown below. There are two tuples in this relation.

Sample Relation  $\mathbf{R}(A_1, A_2, A_3, A_4, A_5)$ .

$A_1$	$A_2$	$A_3$	$A_4$	$A_5$
$x_1$	$x_2$	$x_3$	$x_4$	$x_5$
$x_1$	$x_2$	$y_3$	$x_4$	$y_5$

Let  $\Omega = \{A_1, A_2, A_3, A_4, A_5\}$ ,  $\Omega' = \{A_1, A_2, A_3, A_4\}$ ,  $\Gamma = \{A_1\}$ ,  $\Delta = \{A_2, A_3\}$ .

Let  $\Lambda = \Omega' - \Gamma - \Delta = \{A_4\}$ . Assume  $\Gamma \twoheadrightarrow \Delta$  holds in  $\mathbf{R}(\Omega')$ . Then, for any pair of tuples  $r_1, r_2 \in \Pi_{\Lambda \cup \Gamma}(\mathbf{R})$ ,  $r_1(\Gamma) = r_2(\Gamma) \rightarrow M_{\Delta}(r_1) = M_{\Delta}(r_2)$  by definition of data reliability dependency.

For example,  $r_1 = (x_1, x_4)$ ;  $r_2 = (x_1, x_4)$ . Note:  $r_1[\Gamma] = r_2[\Gamma]$ .  $M_{\Delta}(r_1) = M_{\Delta}(x_1, x_4)$  and

$M_{\Delta}(x_1, x_4) = \{(x_2, x_3), (x_2, y_3)\}$  and  $M_{\Delta}(r_2) = M_{\Delta}(x_1, x_4) = \{(x_2, x_3), (x_2, y_3)\} = M_{\Delta}(r_1)$ .

The mappings of  $r_1$  and  $r_2$  onto  $\Delta$  are equal because the DRD  $\Gamma \twoheadrightarrow \Delta$  holds in  $R(\Omega')$ .

The following example will demonstrate that  $\Gamma \twoheadrightarrow \Delta$  does not hold in  $R(\Omega)$ , even though  $\Gamma \twoheadrightarrow \Delta$  holds in  $R(\Omega')$ : Let  $\Lambda = \Omega - \Gamma - \Delta = \{A_4, A_5\}$ .

Suppose  $\Gamma \twoheadrightarrow \Delta$  in  $R(\Omega)$ . Then, for any pair of tuples  $r_1, r_2 \in \Pi_{\mathbb{R}}(\Lambda \cup \Gamma)$ ,  $r_1(\Gamma) = r_2(\Gamma) \rightarrow M_{\Delta}(r_1) = M_{\Delta}(r_2)$ .

For example,  $r_1 = (x_1, x_4, x_5)$ ;  $r_2 = (x_1, x_4, y_5)$ . Note:  $r_1[\Gamma] = r_2[\Gamma]$ .  $M_{\Delta}(r_1) = M_{\Delta}(x_1, x_4, x_5) = \{(x_2, x_3)\}$  and  $M_{\Delta}(r_2) = M_{\Delta}(x_1, x_4, y_5) = \{(x_2, y_3)\} \neq M_{\Delta}(r_1)$ .

Note that a contradiction to the supposition has been reached. The mappings of  $r_1$  and  $r_2$  onto  $\Delta$  are not equal. The mappings would be equal if the DRD  $\Gamma \twoheadrightarrow \Delta$  held in  $R(\Omega)$ . Thus, the DRD  $\Gamma \twoheadrightarrow \Delta$  does not hold in  $R(\Omega)$ , even though  $\Gamma \twoheadrightarrow \Delta$  holds in  $R(\Omega')$ . The result of this counterexample is that the presence of a DRD in the projection of a relation does not imply that the DRD is present in the original relation. Therefore, the reverse projectability property does not apply to DRDs.

Proof of Additivity: If  $\Gamma \twoheadrightarrow \Delta$  and  $\Gamma \twoheadrightarrow \Psi$  then  $\Gamma \twoheadrightarrow (\Delta \cup \Psi)$ .

Assume that  $\Gamma \cap \Delta = \Psi$  and let  $\Lambda = \Omega - \Gamma - \Delta - \Psi$ . If  $\Psi$  and  $\Lambda$  are empty then the theorem is trivial. Otherwise, order the variations as:  $\Lambda = \{A_1, \dots, A_r\}$  where  $0 < r$ ,  $\Gamma = \{A_{r+1}, \dots, A_p\}$  where  $r < p$ ,  $\Delta = \{A_{p+1}, \dots, A_m\}$  where  $p < m < n$ , and  $\Psi = \{A_{q+1}, \dots, A_n\}$  where  $p < q \leq m$ , all where  $\Omega = \{A_1, \dots, A_r, A_{r+1}, \dots, A_p, A_{p+1}, \dots, A_{q+1}, \dots, A_m, \dots, A_n\}$ . Then  $\Delta$  and  $\Psi$  can overlap, although neither  $\Delta$  can contain  $\Psi$ , nor vice versa. (Otherwise, the theorem is trivial.) One needs to prove that assuming  $\Gamma \twoheadrightarrow \Delta$  and  $\Gamma \twoheadrightarrow \Psi$ , then  $(x_1, \dots, x_r, x_{r+1}, \dots, x_p, x_{p+1}, \dots, x_n) \in \mathbb{R}$  and  $(y_1, \dots, y_r, x_{r+1}, \dots, x_p) \in \Pi_{\Lambda \cup \Gamma}(\mathbb{R})$  and  $(y_1, \dots, y_r, x_{r+1}, \dots, x_p, x_{p+1}, \dots, x_n) \in \mathbb{R}$ . From the projectability property of DRDs, one has  $\Gamma \twoheadrightarrow (\Delta - \Psi)$  in  $\Pi_{\Omega - \Psi}(\mathbb{R})$ .

Then if  $(x_1, \dots, x_r, x_{r+1}, \dots, x_p, x_{p+1}, \dots, x_n) \in \mathbb{R}$  and  $(y_1, \dots, y_r, x_{r+1}, \dots, x_p) \in \Pi_{\Lambda \cup \Gamma}(\mathbb{R})$  are true, then  $(y_1, \dots, y_r, x_{r+1}, \dots, x_p, x_{p+1}, \dots, x_{q-1}) \in \Pi_{\Omega - \Psi}(\mathbb{R})$ .

Since  $\Gamma \twoheadrightarrow \Psi$  in  $\mathbb{R}$  then  $(x_1, \dots, x_r, x_{r+1}, \dots, x_p, x_{p+1}, \dots, x_n) \in \mathbb{R}$  and  $(y_1, \dots, y_r, x_{r+1}, \dots, x_p, x_{p+1}, \dots, x_{q-1}) \in \Pi_{\Omega - \Psi}(\mathbb{R})$  then  $(y_1, \dots, y_r, x_{r+1}, \dots, x_p, x_{p+1}, \dots, x_n) \in \mathbb{R}$ .

The general case in which  $\Gamma$  and  $\Delta$ , and/or  $\Gamma$  and  $\Psi$  are not disjoint follows immediately. In fact:  $\Gamma \twoheadrightarrow \Delta$  implies  $\Gamma \twoheadrightarrow (\Delta - \Gamma)$ , and  $\Gamma \twoheadrightarrow \Psi$  implies  $\Gamma \twoheadrightarrow (\Psi - \Gamma)$ . Then from the proof just completed one has:  $\Gamma \twoheadrightarrow (\Delta - \Gamma) \cup (\Psi - \Gamma)$ . Therefore,  $\Gamma \twoheadrightarrow (\Delta \cup \Psi) - \Gamma$ , which implies  $\Gamma \twoheadrightarrow (\Delta \cup \Psi)$ .

The proof to the additivity property is complete.

Counterexample to Distributivity for DRDs: The additivity property for DRDs is similar to the additivity property of FDs. The additivity property for DRDs states that if  $\Gamma \twoheadrightarrow \Delta$  and  $\Gamma \twoheadrightarrow \Psi$  then  $\Gamma \twoheadrightarrow (\Delta \cup \Psi)$ . Likewise, the additivity property for FDs states that if  $\Gamma \rightarrow \Delta$  and  $\Gamma \rightarrow \Psi$ , then  $\Gamma \rightarrow (\Delta \cup \Psi)$ . The distributive property for FDs states that if  $\Gamma \rightarrow (\Delta \cup \Psi)$  then  $\Gamma \rightarrow \Delta$  and  $\Gamma \rightarrow \Psi$ . However, the distributive property that applies to FDs does not apply to DRDs. On page 64, the sample relation R, has attributes  $A_1, A_2, A_3, A_4$ , and  $A_5$ . There are two tuples in this relation. Let  $\Omega = \{A_1, A_2, A_3, A_4, A_5\}$ ,  $\Gamma = \{A_4\}$ ,  $\Delta = \{A_2, A_3\}$ ,  $\Psi = \{A_5\}$ . Let  $\Theta = \Omega - \Gamma - (\Delta \cup \Psi) = \{A_1\}$ . Assume  $\Gamma \twoheadrightarrow (\Delta \cup \Psi)$  holds in  $R(\Omega)$ . Then, for any pair of tuples  $r_1, r_2 \in \Pi_{\Theta \cup \Gamma}(R)$ ,  $r_1(\Gamma) = r_2(\Gamma) \rightarrow M_{\Delta \cup \Psi}(r_1) = M_{\Delta \cup \Psi}(r_2)$  by definition of data relatability dependency. For example,  $r_1 = (x_1, x_4)$ ;  $r_2 = (x_1, x_4)$ . Note:  $r_1[\Gamma] = r_2[\Gamma]$ .  $M_{\Delta \cup \Psi}(r_1) = M_{\Delta \cup \Psi}(x_1, x_4)$  and  $M_{\Delta \cup \Psi}(x_1, x_4) = \{(x_2, x_3, x_5), (x_2, y_3, y_5)\}$  and  $M_{\Delta \cup \Psi}(r_2) = M_{\Delta \cup \Psi}(x_1, x_4)$  where  $M_{\Delta \cup \Psi}(x_1, x_4) = \{(x_2, x_3, x_5), (x_2, y_3, y_5)\} = M_{\Delta \cup \Psi}(r_1)$ . The mappings of  $r_1$  and  $r_2$  onto  $(\Delta \cup \Psi)$  are equal because the DRD  $\Gamma \twoheadrightarrow (\Delta \cup \Psi)$  holds in R.

The following example will demonstrate that  $\Gamma \twoheadrightarrow \Delta$  does not hold, even though  $\Gamma \twoheadrightarrow (\Delta \cup \Psi)$  holds: Let  $\Theta = \Omega - \Gamma - \Delta = \{A_1, A_5\}$ .

Suppose  $\Gamma \twoheadrightarrow \Delta$ . Then, for any pair of tuples  $r_1, r_2 \in \Pi_{\Theta \cup \Gamma}(R)$ ,  $r_1(\Gamma) = r_2(\Gamma) \rightarrow M_{\Delta}(r_1) = M_{\Delta}(r_2)$  by definition of data relatability dependency. For example,  $r_1 = (x_1, x_4, x_5)$ ;  $r_2 = (x_1, x_4, y_5)$ . Note:  $r_1[\Gamma] = r_2[\Gamma]$ .  $M_{\Delta}(r_1) = M_{\Delta}(x_1, x_4, x_5) = \{(x_2, x_3)\}$  and  $M_{\Delta}(r_2) = M_{\Delta}(x_1, x_4, y_5) = \{(x_2, y_3)\} \neq M_{\Delta}(r_1)$ .

Note that a contradiction to the supposition has been reached. The mappings of  $r_1$  and  $r_2$  onto  $\Delta$  are not equal. The mappings would be equal if the DRD  $\Gamma \twoheadrightarrow \Delta$  held. Thus, the DRD  $\Gamma \twoheadrightarrow \Delta$  does not hold, even though  $\Gamma \twoheadrightarrow (\Delta \cup \Psi)$  holds.

The outcome of this counterexample is that the presence of the DRD  $\Gamma \twoheadrightarrow (\Delta \cup \Psi)$  does not imply the presence of the DRDs  $\Gamma \twoheadrightarrow \Delta$  and  $\Gamma \twoheadrightarrow \Psi$ . Therefore, the distributive property does not apply to DRDs.

Proofs of Pseudotransitivity and Transitivity:

Pseudotransitivity: If  $\Gamma \twoheadrightarrow \Delta$  and  $(\Delta \cup \Gamma) \twoheadrightarrow \Psi$  then  $(\Gamma \cup \Delta) \twoheadrightarrow \Psi$ .

Transitivity: If  $\Gamma \twoheadrightarrow \Delta$  and  $\Delta \twoheadrightarrow \Psi$  then  $\Gamma \twoheadrightarrow \Psi$ .

**Proof:** Pseudotransitivity can now be proven. Note that transitivity follows pseudotransitivity as a special subcase.

Let  $\Psi' = \Psi - (\Gamma \cup \Lambda \cup \Delta)$ ,  $\Delta' = \Delta - (\Gamma \cup \Lambda)$ ,  $\Theta = \Omega - (\Psi' \cup \Gamma \cup \Lambda \cup \Delta')$ . If  $\Psi'$  and  $\Delta$  are empty, the theorem is trivial. The sets  $\Psi'$ ,  $\Delta'$ , and  $(\Gamma \cup \Lambda)$  are pairwise disjoint. Assume without loss of generality that the attributes are ordered as follows:  $\Psi' = \{A_1, \dots, A_p\}$  where  $0 < p$ ,  $\Lambda \cup \Gamma = \{A_{p+1}, \dots, A_q\}$  where  $p < q$ ,  $\Delta' = \{A_{q+1}, \dots, A_r\}$  where  $q < r$ ,  $\Theta = \{A_{r+1}, \dots, A_n\}$  where  $r \leq n$ , and  $\Omega = \{A_1, \dots, A_p, A_{p+1}, \dots, A_q, A_{q+1}, \dots, A_r, A_{r+1}, \dots, A_n\}$ .

**Claim:**  $(\Gamma \cup \Lambda) \twoheadrightarrow (\Delta' \cup \Theta)$ .

**Proof to claim:** Assume:  $(x_1, \dots, x_p, x_{p+1}, \dots, x_q, x_{q+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in R$  and  $(y_1, \dots, y_p, x_{p+1}, \dots, x_q) \in \Pi_{\Psi' \cup \Gamma \cup \Lambda}(R)$ . Then by the augmentation property one has  $(\Gamma \cup \Lambda) \twoheadrightarrow \Delta'$ . Then according to projectability,  $(\Gamma \cup \Lambda) \twoheadrightarrow \Delta'$  in  $\Pi_{\Omega - \Theta}(R)$ . But according to Proposition A for DRDs which states that  $\Gamma \twoheadrightarrow \Delta$  in  $R(\Omega)$  if and only if  $(x_1, x_2, \dots, x_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in R$  and  $(y_1, y_2, \dots, y_p, x_{p+1}, \dots, x_r) \in \Pi_{\Lambda \cup \Gamma}(R)$  implies that  $(y_1, y_2, \dots, y_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in R$ , then  $(x_1, \dots, x_p, x_{p+1}, \dots, x_q, x_{q+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in R$  and  $(y_1, \dots, y_p, x_{p+1}, \dots, x_q) \in \Pi_R(\Psi' \cup \Gamma \cup \Lambda)$  implies that  $(y_1, \dots, y_p, x_{p+1}, \dots, x_q, x_{q+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in R$ . This proves that  $(\Gamma \cup \Lambda) \twoheadrightarrow (\Delta' \cup \Theta)$  in  $R$ . Thus,  $(\Gamma \cup \Lambda) \twoheadrightarrow \Psi'$  in  $R$ . But  $(\Gamma \cup \Lambda) \twoheadrightarrow (\Gamma \cup \Lambda \cup \Delta)$ , and then by applying the additivity property, one obtains  $(\Gamma \cup \Lambda) \twoheadrightarrow \Psi$ .

Thus the proof is complete.

Note that when  $\Gamma = \Lambda$ , one has  $(\Gamma \cup \Delta) \twoheadrightarrow \Psi$  and  $\Gamma \twoheadrightarrow \Delta$ . Then the pseudotransitivity property implies that  $\Gamma \twoheadrightarrow \Psi$ . This is very similar to the case for FDs:  $(\Gamma \cup \Delta) \twoheadrightarrow \Psi$  and  $\Gamma \twoheadrightarrow \Delta$  then  $\Gamma \twoheadrightarrow \Psi$ .

FORMAL PROPERTIES OF FDS AND DRDS

The following presents a comparison of the formal properties of FDs, MVDs and those of DRDs.

Property	FD	DRD	MVD
Reflexivity	yes	yes	no
Augmentation	yes	yes	yes
Additivity	yes	yes	yes
Distributivity	yes	no	yes
Projectability	yes	yes	yes
Reverse Projectability	yes	no	no
Transitivity	yes	yes	yes
Pseudotransitivity	yes	yes	yes

## DECOMPOSITION THEOREM

The combination of the algebraic laws for FDs and DRDs allows for a relational scheme to be decomposed. The following theorem relates DRDs to the decomposition for a relational scheme.

**Theorem:**

A nontrivial DRD in a relation is both a sufficient and a necessary condition for its decomposability.

**Proof:**

**Proposition B:** Given  $\{\Lambda, \Gamma, \Delta\}$  a three element partition of  $\Omega$ ,  $\Gamma \twoheadrightarrow \Delta$  in  $R(\Omega)$  if and only if  $R(\Omega) = \Pi_{\Lambda \cup \Gamma}(R) \mid x \mid \Pi_{\Gamma \cup \Delta}(R)$ . Note that  $\mid x \mid$  symbolizes a natural join operation.

Proof: The order of the attributes is immaterial; therefore, one can arrange them to simplify our notation. The  $\Lambda$  attributes come first, then the  $\Gamma$  attributes, and finally the  $\Delta$  attributes.  $\Lambda = \{A_1, A_2, \dots, A_p\}$  where  $0 < p$ ,  $\Gamma = \{A_{p+1}, \dots, A_r\}$  where  $p < r$ ,  $\Delta = \{A_{r+1}, \dots, A_n\}$  where  $r < n$ ,  $\Omega = \{A_1, \dots, A_p, A_{p+1}, \dots, A_r, A_{r+1}, \dots, A_n\}$ .

Case 1: Assume that  $R(\Omega) = \Pi_{\Lambda \cup \Gamma}(R) \mid x \mid \Pi_{\Gamma \cup \Delta}(R)$ .

- Let  $(x_1, \dots, x_p, x_{p+1}, \dots, x_r) \in \Pi_{\Lambda \cup \Gamma}(R)$  and  $(y_1, \dots, y_p, x_{p+1}, \dots, x_r) \in \Pi_{\Lambda \cup \Gamma}(R)$ .
- If  $(x_{r+1}, \dots, x_n) \in M_{\Delta}(x_1, \dots, x_p, x_{p+1}, \dots, x_r)$ , then (i)  $(x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in \Pi_{\Gamma \cup \Delta}(R)$ .
- According to the definition of a natural join, one then has (due to (i)):

$$(y_1, \dots, y_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in \Pi_{\Lambda \cup \Gamma}(R) \mid x \mid \Pi_{\Gamma \cup \Delta}(R)$$

$$\text{or } (y_1, \dots, y_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in R(\Omega).$$

$$\text{Therefore, } (x_{r+1}, \dots, x_n) \in M_{\Delta}(y_1, \dots, y_p, x_{p+1}, \dots, x_r).$$

Therefore,  $M_{\Delta}(x_1, \dots, x_p, x_{p+1}, \dots, x_r) \subseteq M_{\Delta}(y_1, \dots, y_p, x_{p+1}, \dots, x_r)$  and vice versa by symmetry.

Therefore,  $R(\Omega) = \Pi_{\Lambda \cup \Gamma}(R) \mid x \mid \Pi_{\Gamma \cup \Delta}(R)$  implies  $\Gamma \twoheadrightarrow \Delta$ .

Case 2: Assume  $\Gamma \twoheadrightarrow \Delta$ . Note that  $R(\Omega) \subseteq \Pi_{\Lambda \cup \Gamma}(R) \mid x \mid \Pi_{\Gamma \cup \Delta}(R)$  is always true.

$$\text{Let } (x_1, \dots, x_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in \Pi_{\Lambda \cup \Gamma}(R) \mid x \mid \Pi_{\Gamma \cup \Delta}(R)$$

$$\text{Then } (x_1, \dots, x_p, x_{p+1}, \dots, x_r) \in \Pi_{\Lambda \cup \Gamma}(R)$$

and there exists a tuple  $(y_1, \dots, y_p)$  such that:

$$(y_1, \dots, y_p, x_{p+1}, \dots, x_r, x_{r+1}, \dots, x_n) \in R.$$

Thus,  $(x_{r+1}, \dots, x_n) \in M_{\Delta}(y_1, \dots, y_p, x_{p+1}, \dots, x_r)$ ; but since  $\Gamma \twoheadrightarrow \Delta$ ,  $(x_{r+1}, \dots, x_n) \in M_{\Delta}(x_1, \dots, x_p, x_{p+1}, \dots, x_r)$ .  
 Thus  $\Gamma \twoheadrightarrow \Delta$  implies  $R(\Omega) = \prod_{\Lambda \cup \Gamma} (R) \mid x \mid \prod_{\Gamma \cup \Delta} (R)$ .

This completes the proof of Proposition B.

By observing that  $\Lambda$  and  $\Delta$  play a symmetric role in  $R(\Omega) = \prod_{\Lambda \cup \Gamma} (R) \mid x \mid \prod_{\Gamma \cup \Delta} (R)$ , one obtains the following corollary:

**Proposition C:** In a relation  $R(\Omega)$  the nontrivial DRD “ $\Gamma \twoheadrightarrow \Delta$ ” implies the nontrivial DRD “ $\Gamma \twoheadrightarrow \Lambda$ ” where  $\Lambda = \Omega - \Delta - \Gamma$ .

The attribute set  $\Gamma$  will be called the *kernel* subset while the combinations  $\Lambda$  and  $\Delta$  will be called the *leaf* subsets. Generally speaking, therefore, when designers recognize the pattern of a nontrivial DRD, they know that the relation is affected by anomalies. Then they may solve the problem by decomposing the relation. In many cases, multiple decompositions are possible as a network of FDs and DRDs characterizes the internal structure of the relation. Three definitions are in order:

**Definition of Decomposability:**  $R(x_1, x_2, \dots, x_n)$  is decomposable when there exists a family of pairwise distinct nonempty proper subsets,  $F$ , of  $\{x_1, x_2, \dots, x_n\}$  where  $F = \{y_1, y_2, \dots, y_m\}$  with  $m \geq 1$  such that  $R(x_1, x_2, \dots, x_n) = \prod_{y_1} (R) \mid x \mid \prod_{y_2} (R) \mid x \mid \dots \mid x \mid \prod_{y_m} (R)$  or  $R(x_1, x_2, \dots, x_n)$  is obtainable as the natural join of two or more proper subprojections of  $R$ .

Note that  $\{x_1, x_2, \dots, x_n\} = \bigcup_{i=1}^m y_i$ .

**Definition of atomic relation:** A relation is said to be atomic if it is neither separable nor decomposable.

**Definition of Separability:**  $R(x_1, x_2, \dots, x_n)$  is separable if and only if there is a partition of  $\{x_1, x_2, \dots, x_n\}$   $P = \{y_1, y_2, \dots, y_m\}$  with  $m \geq 2$  where  $R(x_1, x_2, \dots, x_n) = \prod_{y_1} (R) \times \prod_{y_2} (R) \times \dots \times \prod_{y_m} (R)$ .

An atomic decomposition can be made that is based upon DRDs. The decomposition refers to the final schema that consists of relational schemes that are projections of the original scheme. The decomposition is considered atomic because all of the relations are atomic. Each relation is neither decomposable nor separable. Some of the relations are created in the schema design process because certain DRDs are present. The resulting schema is minimal. A decomposition is defined to be *minimal* when no redundancy enters into the design process.

An algorithm to determine whether or not a DRD is reducible is needed in order to detect partial or reducible DRDs. It is stated below.

**Partial DRD detection algorithm:** Let  $\{D_i: T_i \twoheadrightarrow \Delta_i \mid i \in I\}$ .  $I$  is the set of indices into the set of dependencies  $D$ . In order to tell if a dependency  $D_p$  is reducible, two steps are performed.

Consider the dependency  $D_p: \Gamma_p \twoheadrightarrow \Delta_p$ ,  $p \in I$ . The first step is to construct the subset  $I_p \subseteq I$ , where  $I_p = \{i \in I \mid \Gamma_i \subset \Gamma_p \text{ and } \Delta_i \subseteq \Delta_p\}$ . This subset is described as the set  $I_p$  of indices of dependencies which is the set of all  $i$ 's in  $I$  such that a proper subset of the determinant  $\Gamma_p$  determines a subset of the determined attributes  $\Delta_p$ .

The second step is where a DRD is determined to be reducible or irreducible.  $D_p: \Gamma_p \twoheadrightarrow \Delta_p$  is irreducible if and only if  $\Delta_p \neq \cup_{i \in I_p} \Delta_i$ . The dependency  $D_p$  is said to be irreducible if and only if the determined attribute set is not the same as the union of the attribute sets indexed by all the  $i$ 's in  $I_p$ . In other words, the dependency  $D_p: \Gamma_p \twoheadrightarrow \Delta_p$  is irreducible if and only if the attributes  $\Delta_p$  are not equal to the set of all attributes determined by any proper subset of  $\Gamma_p$ .

The concept of the minimal DRD pair is essential for making a schema design algorithm that is based on DRDs. Consider the pair of DRDs  $\Gamma \twoheadrightarrow \Delta$  and  $\Gamma \twoheadrightarrow \Lambda$ , where  $\Lambda = R - \Delta - \Gamma$ . The DRDs constitute a *minimal DRD pair* because  $\Gamma \twoheadrightarrow \Delta$  implies that  $\Gamma \twoheadrightarrow \Lambda$ .

In order to discuss a way of designing a schema that is based on DRDs, an algorithm for splitting relations according to DRDs is required. An algorithm is presented below.



**DRD-Based Decomposition Algorithm:** Given a set of nonseparable relations  $\Sigma = \{R_1, R_2, \dots, R_n\}$ , where  $R_1$  is over attribute set  $\Omega_1$ ,  $R_2$  over  $\Omega_2$ , ...,  $R_n$  over  $\Omega_n$ . Couple the relations with the set of DRDs that hold on them. Next, assume that relation  $R_i$  has a three element partition  $\{\Delta_i, \Gamma_i, \Delta_i\}$  over  $\Omega_i$ , where  $\Gamma_i \twoheadrightarrow \Delta_i$  and  $\Gamma_i \twoheadrightarrow \Delta_i$ . Thus, a minimal DRD pair exists. As a step in decomposing the scheme, replace  $R_i$  in  $\Sigma$  with the pair  $\Pi_{\Delta_i \cup \Gamma_i}(R_i)$  and  $\Pi_{\Gamma_i \cup \Delta_i}(R_i)$ .

A couple of definitions regarding minimal decompositions are necessary. Minimal decomposition sequence and minimal decomposition are defined below.

**Definition of a minimal decomposition sequence:** A *minimal decomposition sequence* is a sequence of steps in a minimal decomposition.

**Definition of minimal decomposition:** A *minimal decomposition* of  $\Sigma$  is a set of relations that are created by a succession of minimal decomposition steps.

A way of approaching the decomposition task is to use a tree construction approach. With a tree construction approach, the decomposition is created using dependencies to create a directed, unordered tree. Each dependency forms a relational scheme that will be in the final schema. Each branching point or nonterminal node represents a step in the decomposition sequence where a new relational scheme is created. A relational scheme at a terminal or leaf node is based on the dependency named at the nonterminal node just above it. The process is illustrated in Figure 1.

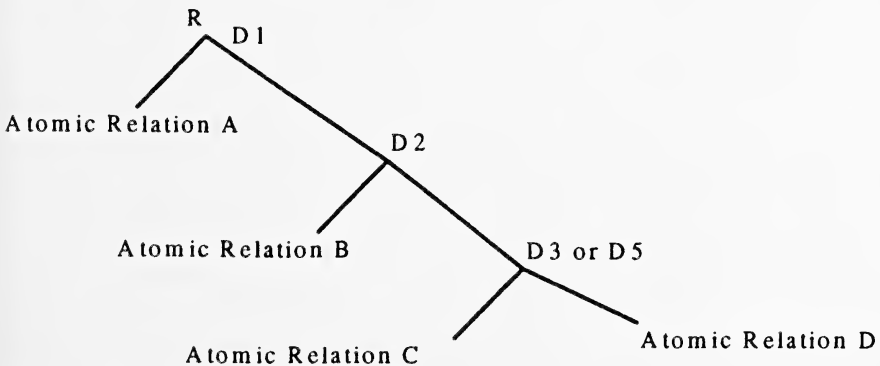


Figure 1. Tree Decomposition Approach.

Each node in the tree is a step in the minimal decomposition sequence. Each terminal node is a relational scheme that will be in the final schema. Therefore, the set of terminal nodes becomes the atomic decomposition of the original relational scheme. The tree construction approach uses the dependencies to determine the final schema. Some of the internal branching nodes will be DRDs and others will be FDs. The end result is a minimal decomposition of the original relation.

This decomposition has fewer anomalies than a decomposition that is not minimal. Since each atomic relation is formed based upon a DRD or a FD the decomposition created insures that all the DRDs and FDs are preserved and complete data relatability is guaranteed. Anything that applies to the decomposition of one relational scheme applies to the decomposition of multiple relational schemes. In fact, the decomposition of a set of relations is done by creating a forest of trees such as the one above.

This concludes the proof of the decomposability theorem.

### CONCLUSIONS

This study describes the data relatability dependency and its properties. Its ability to represent many-to-many relationships exceeds those of functional dependencies and multivalued dependencies. A necessary and sufficient condition for a relation to be decomposable is for the relation to possess a data relatability dependency. This is a result established by (Fagin 1977) that an MVD's presence is a necessary and sufficient condition for a relation scheme to have a lossless join decomposition.

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## CONIFER cDNA SEQUENCES ARE HIGHLY CONSERVED AMONG EUKARYOTIC GENOMES

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**Abstract.**—Computer search algorithms for *Pinus taeda* cDNA sequences revealed that 20 of 41 *Pinus taeda* cDNA sequences had conserved homology to angiosperms, animals and/or other conifers. Ten of the 20 homologous sequences coded for highly conserved amino acid products among conifers, flowering plants and animals. Among the 20 homologous sequences, there were 12 highly conserved query sequences. These sequences coded for DNA replication, RNA-binding protein, glycolysis and photosynthesis. A total of 34 plant species were identified with homologies to *P. taeda* cDNA sequences. Of these families, one family of conifers and one family of monocots were represented in the matches to *P. taeda*. The remaining 16 families were dicots. Using nucleotide sequences to infer evolutionary relationships among plants will improve as more plant genomic sequences drawn from a wider taxonomic spectrum are added to public databases.

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*Pinus taeda* L., commonly known as loblolly pine, is indigenous to Texas where it is considered a major agricultural commodity. In the past decade, *P. taeda* has also emerged as a model gymnosperm for genome analysis. Today, a comparative analysis of gymnosperm, angiosperm and other eukaryotic cDNA sequences is possible; such an analysis permits inference of the ancient eukaryotic genome in land plants.

Fossil evidence suggests the first conifers originated during the Carboniferous period (Table 1), about 325 million years ago (Stewart & Rothwell 1993). During this time the continents were in one large land mass and most of the land was covered in ice sheets. The conifers were mostly found in the middle latitudes of the northern hemisphere (Conkle 1992).

During the Cretaceous period, approximately 141 million years ago, a protogymnosperm ancestor gave rise to primitive flowering plants in the lower latitudes (Stewart & Rothwell 1993). During this period, the primitive angiosperms diverged into dicotyledonous (Magnoliopsida) and monocotyledonous (Liliopsida) plants. The land mass separated and the North American and Eurasian masses now had warm seas between them. The ice sheets had retreated and conifers had spread east and west on the North American and Eurasian land masses (Conkle 1992). This

Table 1. Paleobotanical time scale is based on the fossil record and compiled from Stewart & Rothwell (1993), Gifford & Foster (1989) and Millar (1991). Molecular clock data do not correspond to the same timeframe but the progression of events parallel the fossil record (Savard et al. 1994).

Era	Period	Epoch	Major Events	Onset in Millions of Years	Duration in Millions of Years	
Cenozoic	Quaternary	Recent			5,000 years	
		Pleistocene	Retreat of glaciers and appearance of modern man	2.5	2.5	
	Tertiary	Pliocene			7	4.5
		Miocene	Establishment of present-day forests	26	19	
		Oligocene	Widespread occurrence of now relic taxa to higher latitudes	38	12	
		Eocene	Diversification of Pinaceae	54	16	
		Paleocene		65	11	
Mesozoic	Cretaceous		Angiosperms rise to dominance  First recognition of angiosperms in Lower Cretaceous	141	76	
	Jurassic		Conifers worldwide in distribution; plant communities composed of ginkgos, ferns and cycads.	195	54	
	Triassic		Diversification of conifers and ferns	225	30	
Paleozoic	Permian		Rise of Coniferales	280	55	
	Carboniferous (Pennsylvanian)		Expansion of ferns, lycopods and calamites	325	45	
	Carboniferous (Mississippian)			345	20	
	Devonian		Major vascular plant groups (including protogymnosperms) except angiosperms present	395	50	
	Silurian		Simple vascular plants appear	435	40	
	Ordovician		First vertebrates	500		
	Cambrian		Algae	590		
Precambrian			Unicellular eukaryotic algae at end of era	4600	65	

period has been thoroughly studied since it was the last period with evidence of dinosaurs and the first with modern insects and pouched mammals (Stewart & Rothwell 1993). Starting in the Jurassic period, a period of weather and climate changes, the conifers became abundant in the northern latitudes and extensive pine forests dominated the land. After several more glacial cycles, the conifers retreated and the angiosperms became dominant in the middle and tropical latitudes (Millar 1991; Conkle 1992). The timeline of events varies between the fossil record and molecular clock reports (Savard et al. 1994; Crane et al. 1995) but the sequence of events is similar for either method.

Based on the fossil record, it is hypothesized that there is genic similarity between gymnosperm and angiosperm coding sequences. The genic similarity hypothesis is supported by common evolutionary descent and this hypothesis can be tested by comparing coding sequences. Conserved chromosomal regions among angiosperms have been detected through hybridization with heterologous cDNAs (e.g. Paterson et al. 1996). Ancient conserved regions in animals and yeast have been detected by computer search algorithms which match cDNA sequences and amino acid products (Green et al. 1993). The comparative analysis of this study is based on the latter method. The purpose of this analysis is to further test the extent of conserved DNA sequences, amino acid products and cellular function between gymnosperms and other eukaryotes.

#### METHODS AND MATERIALS

*Source of Pinus taeda cDNA sequences.*—A subset of the *Pinus taeda* complementary DNA (cDNA) sequences reported in Devey et al. (1994) and Groover et al. (1994) have been sequenced at the Institute of Forest Genetics. The cDNA library was prepared from total RNA isolation from 12-day old loblolly pine seedlings.

*Sequencing.*—The following cDNAs were sequenced at the Institute of Forest Genetics and reported via the World Wide Web at:

<http://s27w007.pswf.gov/> and <http://www.cbc.med.umn.edu>

The cDNAs from Devey et al. (1994) were PtIFG605, 669, 739, 1454, 1626, 1849, 1917, 1934, 2022, 2025, 2220, 2295, 2323, 2357, 2540, 2553, 2568, 2610, 2703. The cDNAs from Groover et al. (1994) were PtIFG66, 138, 459, 503, 701, 726, 975, 1635, 1636, 1672, 1902, 1916, 1919, 1955, 2006, 2104, 2166, 2253, 2707, 2781, 2889, 2931.

*Search algorithms and threshold values.*—The *P. taeda* cDNA (nucleotide) sequences were compared by searching for (1) homology to amino acid coding regions in other eukaryotes and (2) similarity to nucleotide sequences. Matching the query sequence to the amino acid products is superior when sequences are diverged due to degeneracy in the genetic code and to protein structure constraints (Gish & States 1993). Matching query nucleotides to other nucleotides in the databases is useful in determining genetic divergence between close relatives.

The BLAST (Basic Local Alignment Search Tool) algorithm measures local sequence similarity based on a matrix of similarity scores for all possible pairs (Karin & Altschul 1990; Altschul et al. 1990). BLAST identifies ungapped, aligned pairs of sequence segments which are “high-scoring segment pairs” (HSP). BLASTX, based on this algorithm, was used to find statistically significant HSP’s between a translated *P. taeda* nucleotide query sequence and target protein sequences in GenBank, CDS, PDB, SwissProt and PIR protein databases. Each *P. taeda* nucleotide query sequence is translated in all reading frames then compared as full-length translation products against a comprehensive protein database.

BLASTN was used to search for significant HSPs between each *P. taeda* query nucleotide sequence and nucleotide sequences in the GenBank, EMBL, DDBJ and PDB nucleotide databases. The BLASTN parameters are not designed to optimize for distantly-related coding sequences so detection of similarity has lower resolution relative to BLASTX. BLAST runs were made via network access to the National Center for Biotechnology Institute’s BLAST software at:

<http://www.ncbi.nlm.nih.gov/BLAST/>

Upward bias due to low-complexity regions gave spuriously high scores thus low-complexity and repetitive sequences were eliminated from the *P. taeda* query sequences by SEG and DUST filters for BLASTX and BLASTN respectively (Altschul et al. 1994; Wootton & Federhen 1996). The probability of a random match was estimated using the Poisson probability, denoted as P(N). A probability of .05 was the cutoff value for the most significant cluster of segments. This cutoff reflected the number of segment pairs expected by chance to have a score of at least x where x is dependent upon the length of the query nucleotide sequence. The value has a Poisson distribution with parameters  $e^{-\lambda(x-u)}$  adjusted downward to eliminate bias due to multiple comparison tests (Altschul et al. 1994).



BLAST results were summarized in two ways. First, the number of sequences with high similarity to the *P. taeda* query sequence were tallied independently for BLASTX and for BLASTN. Second, BLASTX and BLASTN results were jointly classified into five categories based on whether the cDNA sequence showed strong nucleotide similarity: 1 = another conifer sequence with high nucleotide homology, 2 = similarity to amino acid sequence of another conifer species, 3 = similarity to amino acid sequence of an angiosperm plant species, 4 = no similarity to plant amino acid sequences but similarity to animal amino acid sequences, 5 = no match to eukaryotic nucleotide or amino acid sequences in public databases. This numbering system reflects approximate degrees of genic conservation. The list of putative protein products shown for the *P. taeda* sequences reflects all matches with HSP values corresponding to P(N) values  $\geq .05$ . The range of these HSP values, the lowest P(N) value and the number of species with similar amino acid products are also shown. Patented, anonymous sequences were excluded. For the survey of representative taxonomic groups, the BLASTX data were compiled from the first five plant species significantly similar to conifer nucleotide sequences.

## RESULTS

Twenty of the 41 *P. taeda* query sequences showed similarity to plant sequences or to plants and animal sequences (Table 2). Seven of the 20 had similarities to fungal, dicot and monocot sequences (Table 3). Ten of the 20 had significant HSP values to angiosperm plants and animal sequence. Nine cDNAs showed similarity to other conifer sequences, reflecting a sparse public sequencing effort for gymnosperms in general. The remaining 19 query sequences had no eukaryotic DNA homology or protein similarity to public databases (17/19) or showed similarity to animal DNA sequences only (2/19).

Twelve of the twenty cDNA sequences are highly represented in the DNA sequence database (35 to > 250 matches). This group includes genes coding for cell replication, RNA-binding proteins, lipid transfer proteins and photosynthesis (Tables 2-3). For example, genomic cDNA probe PtIFG1934 is a chlorophyll a/b binding protein. The nucleotide sequence for this *cab* sequence corresponds to a protein product highly conserved among algae, bryophytes, ferns, gymnosperms, angiosperm dicots and monocots.

In general, the frequency of plant species represented by the sequences shows similarities to dicot sequences to be more prevalent

Table 2. *Pinus taeda* cDNA sequences and their putative protein products. High-Scoring Pairs (HSPs) for BLASTX with P(N)  $\geq$  .05 are included.

cDNA	Length (bp)	Putative product	Total matches	HSP* Range	Range in P(N)
PtIFG0066	197	$\beta$ -glucosidase	2	46 46	.049 .049
PtIFG0138	141	Elongation factor TU, chloroplast precursor	111	96 68	$7.2 \times 10^{-7}$ .042
PtIFG0459	153	Polyubiquitin	>250	202 59	$5.4 \times 10^{-30}$ .049
PtIFG0605	128	Peroxidase	3	83 76	$2.7 \times 10^{-4}$ $2.5 \times 10^{-3}$
PtIFG0669	185	Photosystem II 22 kD protein precursor	5	143 33	$9.8 \times 10^{-13}$ .035
PtIFG0975	483	B2 protein	2	120 47	$6.7 \times 10^{-6}$ .024
PtIFG1626	156	Metallothionein-like protein	6	79 65	$1.1 \times 10^{-11}$ .035
PtIFG1635	492	Ribulose biphosphate carboxylase small chain precursor	172	335 80	$2.7 \times 10^{-41}$ .039
PtIFG1849	148	Peroxisomal thiolase	2	196 178	$5.8 \times 10^{-7}$ $2.3 \times 10^{-5}$
PtIFG1919	251	Ubiquitin	>250	365 39	$1.9 \times 10^{-51}$ .049
PtIFG1934	477	Light-harvesting chlorophyll A-B binding protein	142	316 70	$2.4 \times 10^{-74}$ .030
PtIFG1955	230	Metallothionein-like protein	17	63 38	$1.2 \times 10^{-9}$ .043
PtIFG2006	159	Light-harvesting chlorophyll a/b binding protein	194	205 40	$3.1 \times 10^{-28}$ .040
PtIFG2022	974	Glutamine synthetase	130	829 75	$4.5 \times 10^{-20}$ .031
PtIFG2220	367	RNA-binding protein	141	171 74	$2.4 \times 10^{-30}$ .0049
PtIFG2295	210	Proliferating cell nuclear antigen	35	332 74	$1.8 \times 10^{-40}$ .030
PtIFG2357	172	Glutamine synthetase	71	107 70	$8.9 \times 10^{-11}$ .019
PtIFG2540	410	Nonspecific lipid transfer protein	88	598 72	$1.4 \times 10^{-77}$ .039
PtIFG2610	201	4-coumarate: CoA ligase	13	51 38	.0011 .045
PtIFG2889	273	Ubiquitin-conjugating enzyme E2-16 kD	66	199 77	$4.6 \times 10^{-27}$ .043

than to gymnosperm and monocot sequences. For the top five species for each of the 20 *P. taeda* query cDNA sequences, there were matches to 34 species, of which 22 were dicotyledonous angiosperms. *Arabidop-*

Table 3. Twenty *P. taeda* cDNA query sequences had matching amino acid sequences among angiosperm plants and among some or all of the following taxonomic groups: animals (AN), fungi (FU), algae (AL), bryophytes (BR), ferns (FE), conifers and other gymnosperms (CO), dicots (DI) and monocots (MO). The symbol (■) refers to a match.

Probe	Putative product	AN	FU	AL	BR	FE	CO	DI	MO
PtIFG2220	RNA-binding protein	■	■	0	0	0	0	■	■
PtIFG2889	Ubiquitin-conjugating enzyme E2-16 kD	■	■	0	0	0	0	■	■
PtIFG1919	Ubiquitin	■	■	0	0	0	■	■	■
PtIFG0459	Polyubiquitin	■	■	0	0	0	■	■	■
PtIFG2295	Proliferating cell nuclear antigen	■	■	0	0	0	0	■	■
PtIFG2022	Glutamine synthetase	■	■	0	0	0	■	■	■
PtIFG2357	Glutamine synthetase	0	0	0	0	0	■	■	■
PtIFG2540	Nonspecific lipid transfer protein	0	0	0	0	0	0	■	■
PtIFG0975	B2 protein	■	0	0	0	0	0	■	0
PtIFG0605	Peroxidase	0	0	0	0	0	0	■	0
PtIFG1849	Peroxisomal thiolase	0	0	0	0	0	0	■	0
PtIFG0066	$\beta$ -glucosidase	0	0	0	0	0	0	0	■
PtIFG0669	Photosystem II 22 kD protein precursor	0	■	0	0	0	0	■	■
PtIFG2006	Chlorophyll a/b binding protein 3A precursor	0	0	0	■	0	■	a/b	■
PtIFG1934	Chlorophyll A-B binding protein	0	0	■	■	■	■	■	■
PtIFG0138	Elongation factor TU, chloroplast precursor	■	■	■	0	0	0	■	0
PtIFG1635	Ribulose biphosphate carboxylase small chain precursor	0	0	0	0	0	■	■	■
PtIFG1626	Metallothionein-like protein	0	0	0	0	0	■	■	■
PtIFG1955	Metallothionein-like protein	■	0	0	0	0	■	■	0
PtIFG2610	4-coumarate: CoA ligase	0	0	0	0	0	0	■	■

*sis thaliana* had the highest frequency of matches relative to dicots with 11 sequence matches; *Nicotiana sylvestris* had the second highest number with six sequence matches. The highest number of matches to any one gymnosperm species was six matches to *Pinus sylvestris*. The highest number of matches in monocots were from *Orzya sativa* (5) and *Zea mays* (5). The species frequency was plotted again using families and eighteen families were represented. There were 16 dicot families and one family of monocots and one family of gymnosperms. The only gymnosperm family was the Pinaceae (five species) and the only monocot family was the Poaceae (five species). The Pinaceae family

had seven matches and Poaceae family had five. The Solanaceae was the highest dicot family with four matches.

### DISCUSSION

These results reveal considerable eukaryotic sequence homology and similarity among eukaryotic genomes. Twenty of the 41 query sequences had conserved homologies, suggesting that all eukaryote genomes share a class of conserved coding sequences (Tables 2-4). The query sequences code for DNA replication, transcription, translation and protein degradation, glycolysis and photosynthesis. For example, cDNA PtIFG2220 codes an RNA-binding protein and all eukaryotes require RNA-binding proteins for transcription. The cDNA probe PtIFG2022 codes for glutamine synthetase, a coding sequence for an isoenzyme highly conserved among many eukaryotic species. Other conserved sequences (PtIFG0669, 1934, 2006, 1635) perform metabolic functions related to the basic biochemistry reactions required for energy production.

The main differences in conserved sequences common to both plants and animals (Tables 3) are found in photosynthetic genes. Since plants use photosynthesis and animals consume other organisms to product sugar for the start of the energy cycle, it is expected that some putative products will not be similar between plants and animals. Proteins such as light-harvesting chlorophyll a/b should only be found in plants. Photosynthesis is the primary functional difference represented in the sequence similarity data between plants and animals (Table 3).

Viewing the BLAST results using taxonomic classifications gives a skewed view of genetic relationships among plants. These figures would lead one to think that there was little relationship between the families and species of monocots, dicots, and gymnosperms. As a case in point, the gymnosperms and monocots had very few matches and all were in a single family. The taxonomic bias is apparent; some of the largest dicot families, the Asteraceae and Rosaceae, had only one species that matched. Many smaller families that had many more matches than these larger families.

Public DNA sequence databases reflect a strong bias for the commodity value of representative plant and animal taxa. This is apparent with gymnosperms and monocots which are represented by a single family each. For example, the Pinaceae is the most important gymnosperm family for timber and paper products. Even though the

pine genome is very large and only sections of conserved DNA has been sequenced, this family is over-represented among conifers because conifers have high consumer value. The pattern also holds true with the Poaceae family. This monocot family includes many cereal crop species and these cereal species are over-represented in the DNA sequence databases because considerable amounts of research funding has gone into the rice, maize, and barley genome projects. The most prevalent dicot family was the Solanaceae. This family includes *Nicotiana sylvestris*, which is the tobacco plant. *Nicotiana sylvestris* sequences are over-represented in the DNA sequence database because tobacco represents an unparalleled international consumer demand relative to all crop species and because transgenic tobacco plants have become a model gene expression system. At the other extreme, low-frequency matches, such as *Catharanthus roseus* or Madagascar periwinkle, have very recent pharmaceutical value as a potential cancer drug and thus there are fewer sequences in the database for *C. roseus*. The strong bias for commodity value will eventually subside and the databases will begin to show more complete evolutionary relationships if funding for genome sequencing is allocated on the basis of taxonomic representation. Results reported this study under-represent the portion of the conserved eukaryotic genome due to commodity bias in the DNA sequence databases.

There is one notable exception: *Arabidopsis thaliana* (Brassicaceae). This small flowering plant has no consumer value but it has the smallest genome of all plants (Goodman et al. 1995). The *A. thaliana* genome is estimated to be from 50 Mb to 150 Mb in size. Since it is so small and contains a relatively low content of interspersed repetitive DNA, it is a major plant genome model worldwide. Its sequences are over-represented in the plant genome database because the entire genome is being sequenced (Goodman 1995).

#### CONCLUSION

A high proportion of *Pinus taeda* cDNA sequences code for conserved amino acid products in both angiosperm plants and conifers. These results extend beyond previous reports of conserved eukaryotic sequences (i.e. Green et al. 1993; Paterson et al. 1996). Many *P. taeda* sequences are common to plants and animals, including mammals. These conserved cDNA have the highest potential for studying syntenic relationships and phylogenetic differences between angiosperm and gymnosperm plants.

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## GENERAL NOTES

NATURAL OCCURRENCE OF *MACRACANTHORHYNCHUS INGENS*  
(ACANTHOCEPHALA: OLIGACANTHORHYNCHIDAE)  
IN A NORTHERN BLACK RACER,  
*COLUBER CONSTRICTOR CONSTRICTOR*  
(SERPENTES: COLUBRIDAE)

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The documentation of oligacanthorhynchid cystacanths in the suborder Serpentes is very limited. Identified oligacanthorhynchid species in reptile paratenic hosts from the United States have only been recorded by Elkins & Nickol (1983) from southern Louisiana and Bolette (1997) from Nolan County, Texas. This note provides additional paratenic host information on an oligacanthorhynchid acanthocephalan endemic to the geographical area.

Three northern black racers, *Coluber constrictor constrictor* were collected recently killed on a roadway in Chester County (39°96'N, 075°75'W), Pennsylvania, during July and October of 1995. The snakes were maintained frozen at -20°C and examined for parasites between 14 August 1995 and 11 December 1996. Helminths collected consisted of one single male oligacanthorhynchid cystacanth occurring within the mesentery adjacent to the proximal small intestine in an adult non-gravid female *C. constrictor constrictor*.

The acanthocephalan was collected on 11 December 1996, preserved in AFA, stained in borax-carmin, dehydrated, cleared in xylene, and mounted in Canada balsam. Morphological measurements and characteristics of the mounted everted specimen are identical to the everted cystacanths of *Macracanthorhynchus ingens* (Linstow 1879) Meyer 1933, described by Elkins & Nickol (1983) and consistent with the cystacanth description by Moore (1946). The everted specimen measures 4.41 mm long by 1.0 mm wide at its greatest width. The proboscis is sub-globular, measures 379 µm long by 520 µm wide, and

possesses six circles of six hooks each. Hooks in the anterior most two circles are non-barbed; all remaining circles of hooks possess chisel-shaped barbs at their tips. Hook measurements in an anterior to posterior sequence are as follows: first circle, 176.7  $\mu\text{m}$  long; second circle, 156.5-166.6  $\mu\text{m}$  long; third circle, 116.1-121.2  $\mu\text{m}$  long; fourth circle, 101.0-106.0  $\mu\text{m}$  long; fifth circle, 75.7-80.8  $\mu\text{m}$  long; and sixth circle, 70.7-80.8  $\mu\text{m}$  long. Testes measure 0.28-0.30 mm long by 0.08-0.09 mm wide. The specimen was compared to voucher specimens of *M. ingens* from the United States National Parasite Collection, USDA, Beltsville, Maryland, (USNPC Nos. 60191, 80828, & 81431), known *M. ingens* cystacanths that were reared from *M. ingens* eggs (Elkins & Nickol 1983) and to other forms of oligacanthorhynchid cystacanths maintained by the author. Based on these comparisons and considering the northeastern geographical area in which it had been recovered, the specimen is charily assigned to this species. The recovery of *M. ingens* in the naturally infected *C. constrictor constrictor* from Chester County, represents the first finding of this oligacanthorhynchid in a paratenic host from Pennsylvania, and is reported in the northern black racer for the first time. Additionally, this is only the second time *M. ingens* is reported from this state; being previously collected and reported by Chandler & Melvin (1951) as adults and immature worms (in assumed unnatural hosts).

*Macracanthorhynchus ingens* in reptile paratenic hosts has only been reported from southern Louisiana. Elkins & Nickol (1983) described natural infections in the following reptile hosts: *Agkistrodon piscivorus*, *Coluber constrictor*, (subspecies was not specified, *C. constrictor constrictor* is extralimital of Louisiana) *Lampropeltis getulus*, *Nerodia cyclopion* and *N. fasciata*. Additionally, laboratory reared *Thamnophis sirtalis* and *N. cyclopion*; and wildcaught *Elaphe obsoleta* and yellow-bellied racers, *C. constrictor flaviventris* (listed only as *C. constrictor* by Elkins & Nickol (1983)) were experimentally infected with *M. ingens*. The authors show that snakes, including *C. constrictor* can serve as suitable paratenic hosts for *M. ingens* and play a successful role in addition to arthropod intermediate hosts in the transfer of this parasite to a definitive host.

The raccoon, *Procyon lotor* (Procyonidae), has been reported as the normal definite host for *M. ingens* from numerous regions throughout the United States. Reports are abundant, listing occurrences in the



midwest (Boddicker & Progulske 1968; Barnstable & Dyer 1974; Bafundo et al. 1980; Smith et al. 1985; Snyder & Fitzgerald 1985; Cole & Shoop 1987; Robel et al. 1989; Richardson et al. 1992) and the southeastern United States (Holloway 1957; Babero & Shepperson 1958; Jordon & Hayes 1959; Harkema & Miller 1962; 1964; Schaffer et al. 1981; Elkins & Nickol 1983; Price & Harman 1983).

Occurrences of *M. ingens* in the raccoon are also reported from Texas (Chandler 1942), Pennsylvania (Chandler & Melvin 1951), Connecticut (Penner 1954), Maryland (Herman 1955), and New York (Ermer & Fodge 1986). Additionally, the black bear, *Ursus americanus* (Ursidae), has been reported to host this species in the southeastern United States (Crum et al. 1978) and Florida (Conti et al. 1983); as well as the ringtail, *Bassariscus astutus* (Procyonidae) in Texas (Pence & Willis 1978). Since *P. lotor* and *U. americanus* are omnivorous and commonly occur in Pennsylvania, it is probable that the northern black racer, *C. constrictor constrictor* serves as an epizootiological reservoir for *M. ingens* in this northern geographical area.

The host voucher specimen is deposited in the Carnegie Museum of Natural History, Section of Amphibians and Reptiles, Pittsburgh, Pennsylvania, USA, (CM No. 146,039). The parasite voucher specimen is deposited in the United States National Parasite Collection, United States Department of Agriculture, Beltsville, Maryland, USA, (USNPC No. 86962).

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\* \* \* \* \*

## A SUSTAINING POPULATION OF THE FLORIDA RED-BELLIED TURTLE, *PSEUDEMYNS NELSONI* (REPTILIA: EMYDIDAE), IN SPRING LAKE, HAYS COUNTY, TEXAS

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The headwaters of the San Marcos River are produced from a series of ca 200 springs that issue from the Edwards Aquifer along the Balcones Escarpment. These springs were dammed in 1849 to produce Spring Lake, which has 18 surface ha. A slough enters Spring Lake from the east near its center point. For many years Spring Lake and the surrounding area (37 ha combined) were maintained as a theme park, Aquarena Springs.

Southwest Texas State University purchased Aquarena Springs in 1994, reduced theme park activity, and made the area available for education and research. An extensive mark-recapture program on the aquatic turtles that inhabit Spring Lake and the slough was begun in 1996.

On 2 July 1996, a large adult female *Pseudemys nelsoni* [carapace length (CL), 286 mm; plastron length (PL), 267 mm; mass (MA), 3900 g] was collected with a dip-net from the slough (Fig. 1). This turtle was



Figure 1. Photograph of adult female *Pseudemys nelsoni* collected at Spring Lake, Hays County, Texas, in 1996 (photograph by John N. Senter).

x-rayed and found to have nine shelled eggs. Oxytocin injections, administered as per Ewert & Legler (1978), resulted in the passage of seven eggs, all of which were placed in moist vermiculite and maintained at 27 C<sup>0</sup>. These eggs began hatching on 1 September 1996 and all were indistinguishable from hatchling *P. nelsoni*; thus, the presence of one or more male *P. nelsoni* was assumed at the site. This female was released back into the slough. A large individual, presumed to be this turtle, occasionally was observed basking on logs over the course of the next few months.

On 3 May 1997, a hatchling *P. nelsoni* was dipped from the slough; four additional individuals were recovered within the next 30 days. All hatchlings had been in the water sufficient time to have accumulated well developed algal mats (*Cladophora* sp.) but all exhibited minimal growth

lines between epidermal lamellae. Undoubtedly these hatchlings were the result of the 1996 nesting season, and had over-wintered in the ground.

On 30 June 1997, a second large female *P. nelsoni* (CL, 305 mm; PL, 278 mm; MA, 4450 g) was found excavating a nest hole approximately 58 meters from the slough. After laying, 18 eggs were collected and placed in moist vermiculite to incubate. A third adult female *P. nelsoni* (CL, 286 mm; PL, 263 mm; MA, 4300 g) was taken in a basking trap on 20 October 1997.

Descriptive statistics for egg parameters of the two clutches (N = 25) from the study site, including mean  $\pm$  SD (minimum - maximum) follow: length, 37.2 mm  $\pm$  1.04 (35.2 - 38.8); width, 24.0 mm  $\pm$  0.45 (23.2 - 25.1); mass, 12.5 g  $\pm$  0.76 (11.1 - 14.1).

After an incubation period of 55 - 57 days (30 June to 24 - 26 August) all 18 eggs hatched. Descriptive statistics, mean  $\pm$  SD (minimum - maximum), for these hatchlings were as follows: CL, 33.2 mm  $\pm$  0.75 (31.9 - 34.3); CW, 33.7 mm  $\pm$  0.77 (32.1 - 35.0); PL, 32.0 mm  $\pm$  0.91 (30.5 - 33.5); mass, 10.1 g  $\pm$  0.48 (9.1 - 11.2).

*Pseudemys nelsoni* is a large, aquatic, emydine turtle inhabiting rivers, streams and ponds in Florida and extreme Southeastern Georgia (Ernst, et al. 1994). Although there are two allopatric populations, no subspecies are recognized. Other members of the proposed subgenus *Ptechemys* by Ward (1984) are all allopatric (*P. rubriventris*, *P. alabamensis*, *P. texana*). It is possible that the turtles assigned to *Pseudemys nelsoni* at Spring Lake might represent an undescribed species and genetic studies are planned. There appears to be no external morphological character(s) that distinguish individuals from Spring Lake from those found in Florida.

The origin of the *P. nelsoni* in Spring Lake is not known. Exotic fish and plants have been introduced into the lake for many years. *Pseudemys nelsoni* was sold routinely in pet stores and frequently is seen for sale at reptile expositions. Perhaps the large adults collected during this study represent animals that were released as juveniles several years ago. It appears unlikely that the large adults were released recently, but that possibility cannot be discounted. No males have been collected to

date from Spring Lake, and the possibility of sperm storage cannot be completely dismissed.

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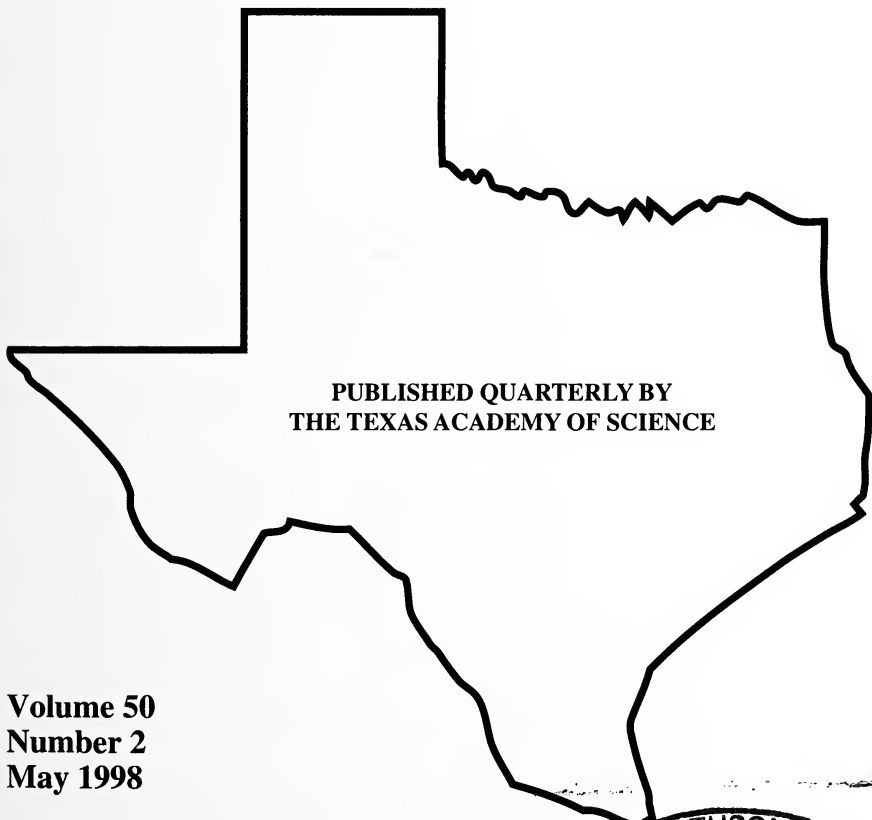
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NEOTYPE AND TYPE LOCALITY OF  
THE WESTERN HOGNOSE SNAKE, *HETERODON NASICUS*  
(SERPENTES: COLUBRIDAE)

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**Abstract.**—The original description of *Heterodon nasicus* Baird & Girard was based on one specimen (USNM 1272), which has since been lost. The holotype was credited to General Sylvester Churchill, who traveled from San Antonio to Presidio del Rio Grande (Mexican outpost downstream from Eagle Pass) and crossed the Rio Grande into Mexico. The type locality is here restricted to the Rio Grande, approximately 19 air miles (30.6 km) downstream from Eagle Pass, Maverick County, Texas. USNM 1249 from Eagle Pass is designated neotype of *Heterodon nasicus*. Other USNM reptilian species obtained under the same circumstances of collection are also listed.

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Eckerman (1996) in a detailed study of geographic variation of *Heterodon nasicus* did not resolve the confusion regarding the type and type locality of *H. nasicus* (see remarks in Degenhardt et al. 1996:275).

HOLOTYPE OF *HETERODON NASICUS*

In the brief, three-line original description of *Heterodon nasicus*, Baird & Girard (1852a:70) noted "Collected in Texas by Gen. Churchill"; the specific epithet was spelled "*nasicum*." Later, Baird & Girard (1852b:352-353) described the holotype in more elaborate fashion, noting "*Heterodon* [lapsus] *nasicus* [emendation]," and as "collected in Texas by General Churchill; a specimen is preserved at the Smithsonian Institution [= National Museum of Natural History, USNM]." Banta (1971) discussed these two papers by Baird & Girard and determined that the journal article (1852a) was published prior (April-June) to the report (July-August) included as part of Howard Stansbury's *Exploration of the Valley of the Great Salt Lake* (see Baird & Girard 1852b). Somewhat confusing is the inclusion of species' descriptions in the 1852b report that were based on material (e.g., Churchill's) not obtained during the course of Stansbury's sojourn.

The two descriptions of *Heterodon nasicus* (Baird & Girard 1852a; 1852b) are based on one and the same specimen credited to Churchill; they mentioned that (1852a:68) a "full description...will shortly appear

in Capt. Stansbury's Report..." and (1852b:353) "The only specimen of *H. nasicus* which we have seen is not a foot long." Later, Baird & Girard (1853:63) recorded data for the holotype as "Rio Grande. 138+1. 45. 23. 7 3/4. 1 1/4. Gen. S. Churchill." The authors noted Churchill's material from "Valley of the Rio Grande" (p. vi) and that the "five numbers" (p. viii) referred, respectively, to ventrals, subcaudals, dorsal scale rows, total length, and tail length in inches. Cope (1900:777) repeated the measurements and ventral and subcaudal counts of the holotype from "Rio Grande River."

Eckerman (1996:100) noted that data in the USNM catalog entry for the holotype of *H. nasicus* was entered in 1858 (16 February) (formal cataloging of reptiles not initiated until 1856) with the museum number as 1272, that it was designated as type and credited to "Genl. Churchill" and "Texas," and that a penciled notation of "Rio Grande" was written above the original locality. Degenhardt et al. (1996:273, 275) also noted the missing holotype as USNM 1272. Smith & Taylor (1945:72) noted the type as apparently lost, the locality as Texas. Cochran's (1961) list of USNM types did not include any entry for *H. nasicus nasicus*.

Other USNM specimens have been designated as the type of *Heterodon nasicus*. In Yarrow's (1883) listing of USNM specimens none of *H. nasicus* is credited to Churchill, but two specimens were indicated as "types": USNM 1285, a specimen obtained by Marcy & McClellan's (p. 140) *Exploration of the Red River of Louisiana, in the year 1852* (see Baird & Girard 1854), and USNM 4863 credited to a "Howard" from "Sante Fé, N. Mex." (p. 141). Likewise, Cope (1900:777) did not list any USNM specimens of *H. nasicus* sourced from Churchill, but he did include entries for USNM 4863 and 1285; only USNM 4863 was indicated as "type." These designations of USNM 1285 and 4863 as types of *H. nasicus* are erroneous.

Baird & Girard (1853:63) recorded data for all the specimens of *H. nasicus* available to them, and in an introductory comment (1853:viii) noted that "The specimen whose measurements are first given [= the Rio Grande, Churchill, holotype, see above], unless stated to the contrary [as noted below], has served as the type of the description." However, Gotte (pers. comm.) noted that the above-quoted comment seems to apply only to taxa described as new in the catalog. The specimen of *H. nasicus* described in Baird & Girard's 1853 catalog is not the holotype. Their description of *H. nasicus* (1853) is based on the Marcy & McClellan Red River specimen (later cataloged as USNM

1285), which these authors also described in Appendix F.—Reptiles (1854:193-195 [an earlier edition printed in 1853]) that accompanied the report by Marcy & McClellan. A side-by-side comparison of these two Baird & Girard descriptions is almost identical. In the 1854 account they cited the 1853 account (1854:193) and noted (p. 195) that "The specimen figured...Plate IV, is...upon which the foregoing description has been based." Although the basic loreal configuration (two loreals separating nasal and oculars) is the same in the holotype of *H. nasicus*, the upper is slightly larger than the lower loreal (ocular scales indistinct) in the snake illustrated in Plate IV, and differs from the "large,..lower loreal [sic] plate,...[and]...a second loreal [sic] above the first, much smaller than any of those of the orbital circle" described for the holotype (Baird & Girard 1852b:353). Also the pattern of few black markings on the posterior part of the belly and underside of the tail of the snake depicted on Plate IV differs from the "lower part of abdomen and tail is almost entirely black" as described for the holotype (Baird & Girard 1852b:353). Thus, the descriptive comments of *H. nasicus* in both of these accounts (Baird & Girard 1853; 1854) are regarded as based on USNM 1285 illustrated in Plate IV (1854), not the holotype.

#### TYPE LOCALITY OF *HETERODON NASICUS*

As noted in the foregoing discussion, the holotype of *Heterodon nasicus* credited to Churchill has been associated with the indefinite localities of Texas, Rio Grande, and Valley of the Rio Grande.

Schmidt (1953:179) noted the type locality of *Heterodon nasicus* as "Texas; restr. to Amarillo by R. A. Edgren (p.c.)." Edgren (1952b) did not mention a type specimen or type locality for *H. nasicus* in his abbreviated synopsis of the species of *Heterodon*, but in his detailed, unpublished account (1952a:203) he wrote "The type, according to Baird and Girard [he cited 1852b, 1853, and 1854], was collected by General Churchill in Texas; it is now apparently lost. According to the description it was fairly typical of this subspecies on the basis of dorsal blotch counts, and thus probably came from some area in northwest Texas. It seems logical then to restrict the type locality to the vicinity of Amarillo, Potter County, Texas, both on the basis of what is known of the type and the possibility that the city was visited by General Churchill." This restricted type locality (Amarillo, Texas) was reiterated by Platt (1969: 284, legend of Fig. 10 [map]). Edgren's restricted type locality is invalid, since Churchill's Texas excursion was nowhere near the Texas panhandle.

Sylvester Churchill (1783-1862) was a professional military man (ranks of Brigadier General, Colonel, Inspector General, U.S. Army); his only daughter (three younger sons), Mary Helen Churchill, married Spencer F. Baird in August 1846 (Rivinus & Youssef 1992:38). Churchill's Texas travels were a consequence of the war with Mexico. He accompanied General John Ellis Wool to Mexico via the "Wool Road" (Geiser 1948:272) from San Antonio to Presidio del Rio Grande in late September and early October, 1846. Goetzmann (1965:149) noted that Captain George W. Hughes commanded a detachment of Topographical Engineers that also traveled with Wool's Army into Mexico via Presidio del Rio Grande, Santa Rosa and Monclova to Parras.

Pool (1975:81, map) showed the "Wool Road" extending from San Antonio to Eagle Pass, but this is not precise as he noted (p. 78, 96) the Presidio Crossing as downstream a short distance from Eagle Pass. Pool (1975:97, map) also depicted two more or less straight routes (Upper and Lower Presidio Roads) from San Antonio to Presidio del Rio Grande. These two Presidio roads were also detailed on an 1876 Texas map (Rand McNally 1956) that depicts both roads merging about four miles before the river crossing. Only the Lower Presidio Road terminates in San Antonio; it is regarded as the 1846 "Wool Road." This Lower Road was generally south from San Antonio into Atascosa County (just west of Pleasanton) angling southwest through southeastern Frio County, northwestern La Salle County, and west across Dimmit County. The Upper Presidio Road crossed northwestern Dimmit County, southeastern Zavalla County, northwestern Frio County to Castorville then proceeded north (by-passing San Antonio) to Bandera and Kerrville; this Upper Road, with an intersecting road northwestward to Eagle Pass (now seemingly Farm or Ranch Road 1021) near the juncture with the Lower Road (vicinity present-day El Indio), probably was an established route after the Lower "Wool" Road. Presidio del Rio Grande was an outpost on the Mexican side of the river; it is apparently not now marked by any major Mexican placename.

The indefinite Texas or Rio Grande type locality of *Heterodon nasicus* credited to Gen. Churchill is here restricted to the Río Grande approximately 4.3 air miles (7 km) southwest El Indio or approximately 19 air miles (30.6 km) downstream from Eagle Pass, Maverick County, Texas. Several other USNM Churchill specimens with Rio Grande oriented localities suggest an extended bivouac and/or that Presidio del Rio Grande may have been a designated shipping point.

Other species of reptiles (USNM) credited to Churchill and the same circumstances of collection as the holotype of *Heterodon nasicus* are listed below (no such amphibian species listed in Yarrow 1883 or Cope 1889). These specimens obtained in late September-early October, 1846 may have been taken on either side of the river (Presidio Crossing area downstream from Eagle Pass, see text), or from anywhere between San Antonio and the Río Grande.

*Holbrookia maculata*.—"several specimens from Texas " (Baird & Girard 1852b:344).

*Phrynosoma cornutum*.—"Rio Grande, west of San Antonio" (Cope 1900:435).

*Phrynosoma modestum*.—"Brought from the valley of the Rio Grande west of San Antonio" (Baird & Girard 1852a:69); "Brought from the Rio Grande, west of San Antonio (Girard in Baird & Girard 1852b:365); Rio Grande, west of San Antonio (Cope 1900:439).

*Cnemidophorus perplexus*.—"on the Rio Grande west of San Antonio, (Texas,)" (Baird & Girard 1852c:128); "Texas" (Yarrow 1883:50; Cope 1900:574 [as *C. tessellatus perplexus*]); two specimens of *C. inornatus* (Maslin et al. 1958:343), paralectotypes of *C. perplexus* (= *C. neomexicanus*, Taylor & Walker 1996).

*Churchilla bellona* (= *Pituophis melanoleucus*).—"on his march along the Rio Grande in 1846" (Baird & Girard 1852a:70); "on his march to Mexico, on the left bank of the Rio Grande, at the crossing near Presidio del Norte, in 1846" (Baird & Girard 1852b:351); "Rio Grande" (Baird & Girard 1853:68 as *Pituophis bellona*); the Baird & Girard 1852b reference to Presidio del Norte, which refers to Ojinaga and the Presidio-Ojinaga river crossing farther north on the Rio Grande, is considered an error and mistranscription for Presidio del Rio Grande.

*Eutainia dorsalis*.—"Rio Grande, Texas" (Baird & Girard 1853:32); this italicized locality (perhaps reflecting shipping point origin) is elaborated in text as "collected between Monclova, Mexico, and the Rio Grande"; name currently applied to upper Rio Grande population of *Thamnophis sirtalis*.

*Eutainia vagrans* (= *Thamnophis* sp.?)."—"South of Rio Grande, N. Mexico" (Baird & Girard 1853:36).

*Elaps tristis* (= *Micrurus fulvius*).—"Rio Grande, W of San Antonio" (Baird & Girard 1853:23); "Rio Grande" (Yarrow 1883:82 [same specimen, USNM 1123, also listed under *E. euryxanthus*]; Cope 1900:1122 [as *E. fulvius*]).

*Crotalophorus edwardsii* (= *Sistrurus catenatus*).—"S. Bank of Rio Grande" (Baird & Girard 1853:15).

Geiser (1948:272) also noted that Churchill collected fishes for Baird at the crossing of the Rio Grande. Pool (1975:96, 136, map) noted Eagle Pass as established in 1850 next to Fort Duncan (sanctioned in 1849, first buildings in 1850). Thus, Eagle Pass was not a recognized placename in 1846 at the time of Churchill's Rio Grande crossing.

## NEOTYPE DESIGNATION

As an adjunct to Eckerman's revisionary work (1996) and his recognition of *Heterodon nasicus nasicus* in the Eagle Pass/Río Grande area, which is near populations of *H. nasicus kennerlyi* to the north and south along the Río Grande, it would appear prudent to designate a neotype. Since the holotype of *H. nasicus* is lost, USNM 1249 is here designated as neotype. Both the lost holotype and USNM 1249 are applicable to *H. nasicus nasicus* (nine or more azygous scales and separation of postnasal and oculars by loreals) rather than *H. nasicus kennerlyi* (seven or less azygous scales, and contact of postnasal and oculars). Baird (1859b:18), Yarrow (1883:140), and Cope (1900:777) listed USNM 1249 from Eagle Pass, Texas, credited to A. Schott. Arthur C.V. Schott, employed as a scientific collector, was associated with the activities of the U.S.-Mexican Boundary Survey. At the time of the collection of USNM 1249, probably in late 1851/early 1852, Schott was attached to a party headed by Lieut. Nathaniel Michler of the Corps of Topographical Engineers that was sent to survey the Río Grande from Fort Duncan (= Eagle Pass) (Pool 1975:96, 101, 111) south to Laredo (Goetzmann 1965:183).

Baird (1859a:14) recorded "Eagle Pass, Texas" for a specimen of *Heterodon nasicus* illustrated on Plate XXVIII, Fig. 43 (dorsal, ventral, side [left], and snout view of head; rostral scale outline, scale rows on left side of body; and anal plate area). The depicted loreal configuration (large lower abutting on supralabials, and small upper loreal separating nasal and orbitals) and disposition of the numerous 12 or 13 azygous scales agree with that of the designated neotype, USNM 1249. The head scalation of Baird's illustrations and USNM 1249 were compared by Gotte (pers. comm.) who wrote that after "a scale by scale comparison of the scales on top of the head I am convinced that it [USNM 1249] is the model for the drawing and that the drawing is accurate." This 1859 Baird report consists only of illustrations (line drawings of scalation features) of many species of snakes depicted on 13 plates (contemplated text of the Report on Reptiles not published [as noted on p. 11 and 13]). A notation in the Explanation of the Plates (p. 13) reads "The figures have, as far as possible, been taken from the type specimens of the species, especially those described [presumably as new] in the catalogue of serpents in the museum of the Smithsonian Institution, (1853,) to which the page column refers"; however, as previously noted the description of *H. nasicus* in this catalog (Baird & Girard 1853) is based on USNM 1285.

In addition to the illustrations in Baird (1859a) noted above, the neotype of *Heterodon nasicus* (USNM 1249) is a female, in good condition with a body and tail length of about 572+87 mm, 142 ventrals and 36 subcaudals. The 12 azygous scales separate the frontal and prefrontals, and two loreals separate the postnasal and oculars (both sides of head). The blotched pattern on the body and tail is faded (about 28 right and left dorsal blotches on body). The venter is patterned with about an equal mixture of pale and dark colors.

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HABITAT ASSOCIATIONS  
OF THE SMALL-MAMMAL COMMUNITY  
IN THE GRAND PRAIRIE OF NORTH-CENTRAL TEXAS

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**Abstract.**—Small mammals and vegetation were sampled in 12 subhabitats on a ranch in Johnson County in north-central Texas from September 1995 through May 1996 for the purpose of understanding the habitat affinities and community structure of native rodent species in the Grand Prairie. Principal components analyses of both vegetation and mammal data reduced the 12 subhabitats to three habitat groups: grasslands, upland woodlands and lowland woodlands. Three species of rodents (*Sigmodon hispidus*, *Baiomys taylori* and *Reithrodontomys fulvescens*) achieved their highest densities in the grasslands transects. Two other species (*Peromyscus pectoralis* and *P. attwateri*) were captured in their highest densities in the upland woods habitats; however, *P. pectoralis* occurred in a wide variety of habitats. *Neotoma micropus* and *Peromyscus maniculatus* were minor components of the rodent community.

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The Grand Prairie region of north-central Texas encompasses approximately 6.5 million acres (2.3 million hectares) of grassy slopes, low escarpments, and wooded drainages, and is bounded to the east and west by the Eastern Cross Timbers and the Western Cross Timbers, respectively (Gould 1975; Coburn 1985). This region, extending nearly as far north as the Red River and southward into Travis County, comprises all or parts of 22 counties (Hayward et al. 1992). Blair (1950) included the Grand Prairie as the westernmost of several distinctive prairies in the Texan biotic province, which he viewed as a broad ecotone between the mesic Austroriparian forests of eastern Texas and the drier grasslands of the Kansan and Balconian provinces to the west.

Few papers on the small-mammal fauna of the Grand Prairie have been published. Dalquest & Horner (1984:13) excluded the Grand Prairie in their study of mammals of north-central Texas. Schmidly's (1983) treatment of eastern Texas mammals extends only as far west as the Balcones Fault Zone, which abuts the Cross Timbers and Prairies region. The white-ankled mouse (*Peromyscus pectoralis*) is documented from the northernmost Grand Prairie in Cooke County (Kilpatrick & Caire 1973). Baccus et al. (1971) acknowledged the Grand Prairie as

a region which the northern pygmy mouse (*Baiomys taylori*) had invaded in its continuing northward expansion of range; this species is also recorded from Bosque County, near the center of the Grand Prairie (Hart 1972). Cleveland et al. (1984) reported the woodland vole (*Microtus pinetorum*) from western Hill County. Severinghaus & Goran (1982) noted the occurrence of 13 species of mammals in Coryell and Bell counties near the southern extent of the Grand Prairie.

Field studies conducted at the Klondike Ranch in extreme southern Johnson County have provided an opportunity to contribute further to the knowledge of the mammalian fauna of this physiographic region. From September 1995 through May 1996, small-mammals were sampled and vegetational data was collected from the community for the purposes of developing an inventory of the mammalian fauna and a better understanding the habitat affinities and community structure of native rodent species in the Grand Prairie.

#### MATERIALS AND METHODS

*Study site.*—Klondike Ranch (latitude N 32° 10', longitude W 97° 30') is located at the southern tip of Johnson County, Texas, approximately 13 km west of Rio Vista. Most of this approximately 400 hectare ranch is situated on gently rolling upland (ca. 234 m elevation). The remainder of the site consists of terraces and bottomland associated with the Brazos River (ca. 156 m elevation). Most of the relief between upland and lowland segments of the site is abrupt, consisting of a limestone bluff and associated slopes. The rimrock of the bluff, an exposure of Edwards Limestone, is overlain by clays and rock of the Weno Formation (Keyes 1977). The slopes below the bluff and most of the lowlands represent members of the Comanche Peak Limestone and Walnut Clay Formations, with sandy alluvium adjacent to the river.

Grassy habitat, predominantly coastal bermuda grass (*Cynodon dactylon*), surrounds two small ponds located in the upland portion of the ranch. The majority of the upland, however, supports extensive woodland, primarily juniper (*Juniperus ashei*), live oak (*Quercus virginiana*), hackberry (*Celtis reticulata*), cedar elm (*Ulmus crassifolia*), and scaly bark oak (*Quercus durandii*). Soils in this upland area are of the Aledo-Bolar complex, which are well-drained soils formed in interbedded limestone and marl and are gently sloping 2-8° (Coburn 1985). The canyon is more mesic and sheltered than other areas. Many of the

plant species in the canyon are similar to those of the higher elevations; however, the understory in the canyon is better developed, with hog plum (*Prunus rivularis*) and an abundance of poison ivy (*Rhus toxicodendron*). Soils in the canyon and on the slopes below the bluff are of the Bolar-Aledo complex. These are moderately deep, well-drained, loamy soils formed in interbedded limestones and calcareous marls. As described by Coburn (1985), these soils achieve maximum slopes of 20%, but soils of this series were found on slopes up to about 60%.

Several soil series characterize the site from the base of the slope beneath the bluff to the river's edge. Clay loams of the Sunev and Seawillow series (sloping  $\leq 5\%$ ) extend from the base of this slope and grade into the sandy alluvial soils nearer the river (Yahola-Gaddy sandy loam; Coburn 1985). Trees in the riparian forest are primarily hickories and pecans (*Carya* sp.), willow (*Salix nigra*), and cottonwood (*Populus deltoides*). The terrace above the bottomwoods serves as a haymeadow. The ecotone between the bottomwoods and the hayfield is dominated by roughleaf dogwood (*Cornus drummondii*) and peppervine (*Ampulopsis arborea*).

*Field methods.*—Sampling transects, 50 m in length, were placed in 12 general habitats: four in wooded settings in the uplands, four in grassy situations, three in and near the riparian woodlands, and one in the canyon. For sampling of rodents, Sherman live traps were baited with rolled oats and set at approximately 5 m intervals along these transects; the number of traps set ranged from 30 to 50 per transect. Rodent sampling was conducted for a total of seven nights from September 1995 through March 1996; the total trapping effort was 1,260 trapnights (Table 1). Data regarding the small-mammal community were expressed both as the number of individuals captured per 50 trap nights of sampling effort and as the percentage of the community represented by each species for that transect. Most of the rodents captured were preserved as skin-and-skull specimens and deposited in the Baylor University Department of Biology collection of vertebrates.

In May 1996, vegetational surveys were conducted along the 12 transects to obtain quantitative information on ground cover, stem count, and woody biomass. The cover analysis procedure used was modified from Daubenmire (1959) and has been used successfully in previous study of habitat associations of small mammals (Wilkins 1995). At the

5, 15, 25, 35, and 45 m points along each transect, a rectangular frame of 0.1 m<sup>2</sup> (0.5 m by 0.2 m) was placed on the ground. Cover was estimated for different classes of vegetation at several heights within the frame. Each vegetation class was assigned a value from 0 to 6 on the basis of the estimated amount of cover provided by that plant category: 0 = 0% cover, 1 = 1-5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-95%, and 6 = 96-100%. Cover provided by dead material was assessed at 5, 10, 25, and 50 cm above the surface, whereas cover by Johnson grass, by bermuda grass, and by other grasses was estimated at 25 and 50 cm. Cover by forbs was determined at 5, 10, and 25 cm, and cover by woody vegetation was evaluated at 50 cm and at 1 m and higher.

Stem counts and biomass of woody plants were assessed at the 10, 20, 30, 40, and 50 m points along each transect (Kershaw 1964). At these points, a circle of 1 m radius was described on the ground. All stems of each woody species present within the circle were counted and their basal diameters were measured at approximately 3 cm above the ground by using a diameter-at-breast-height (dbh) tape. For stems too small to be measured with the dbh tape, diameter was recorded as 0.5 cm. Biomass is expressed as cm<sup>2</sup> of cross-sectional area of stem biomass per m<sup>2</sup> of ground surface for each species of woody plant. Stem count is expressed as number of individuals present in a circle of 1 m<sup>2</sup> radius (i.e., individuals per 3.14 m<sup>2</sup>) for each woody species.

*Analytical methods.*—Statistical analyses were conducted by using the Statistical Analysis System (SAS Institute 1985). Descriptive statistics were generated for each data set for each transect. Exploratory analyses were conducted by principal components analyses (PCA) to determine similarities among the 12 transects, with the intent of recognizing fewer general habitats than the original dozen. PCA was conducted for each of the three sets of plant data (cover, stem count, biomass) separately, then for these data sets combined. Similarly, PCA was conducted separately, then combined, for the two sets of mammal data. The final PCA combined all vegetative and mammal data into one analysis. The Spearman correlation procedure was then used to evaluate associations between each species of rodent sampled and the various vegetation features characterizing each transect.

## RESULTS

*Vegetative description of transects.*—General vegetational descriptions of the 12 habitats studied follow: The "haymeadow woods" transect

extended through woods lying between the Brazos River and the hay-meadow, in an area regrowing from prior clearing. Willow, hickory and pecan saplings, with diameters of 1-3 cm, were common. Undergrowth of *Smilax* (greenbrier) and peppervine was dense, and leaf litter covered approximately 39% of the surface at 5 cm. The tree canopy above 1 m shaded approximately 60% of the ground surface; this was due mostly to mature trees rooted outside of the sampling plots. Enough light penetrated to allow growth of some clumps of grasses and forbs.

The "haymeadow edge" transect passed through a transition zone between the haymeadow and the riverine woods. Coverage at 5 cm by dead material was high (89%). Forbs covered almost 20% of the ground, but cover was <10% at other heights. Tree species were uncommon along this transect, with roughleaf dogwood, pecan and cottonwood present. Of these, pecan was dominant, with stems occupying around 50 cm<sup>2</sup>/m<sup>2</sup> of ground. There was an understory of vines, including greenbrier, peppervine and grape; however, they collectively occupied only 1.2 cm<sup>2</sup>/m<sup>2</sup>.

The "riverside woods" transect was placed within about 15 m of the river. Canopy coverage was about 40%. A well-developed understory of forbs was present. Cottonwood saplings were the dominant woody species, yet they occupied only about 5 cm<sup>2</sup>/m<sup>2</sup>. There were dense clumps of greenbrier and peppervine, with a biomass of approximately 1.5 cm<sup>2</sup>/m<sup>2</sup>.

Several transects were set in situations associated with the bluff overlooking the haymeadow and river. The "rimrock" transect ran along the rim of the bluff. Soil here was shallow to absent, in places revealing bare slabs of limestone. Vegetation was sparse, with a few clumps of juniper, but no understory, either alive or dead. Several patches of prickly pear cactus (*Opuntia*) were scattered along the transect.

The "grass by rimrock" transect was located approximately 3 m from the edge of the bluff rim. Ground cover consisted primarily of grasses (approximately 50% coverage; height of about 0.5 m). Other vegetation present included a few forbs and dead herbaceous material, but woody plants were absent.

The "woods below rimrock" transect was situated just below the

abrupt 3-5 m drop-off from the rim. This transect paralleled the rock face on a wooded slope of about 45°. There was a well-developed layer of leaf litter, but neither grasses nor forbs were present. A diverse mix of woody plants grew on this slope. Stems of greenbrier, poison ivy and *Bumelia lanuginosa* (gum elastic) made up the shrub understory, together occupying about 0.6 cm<sup>2</sup>/m<sup>2</sup>. Juniper was the dominant tree, both in number and in biomass, occupying 18.49 cm<sup>2</sup>/m<sup>2</sup>, but was mixed with small (< 4 cm basal diameter) scaly bark oaks. Hackberry, ash and Shumard oak (*Quercus shumardii*) were present at < 1 stem/m<sup>2</sup>.

A similar habitat was found along the "canyon rim" transect. There was only a thin litter layer, made mainly of juniper needles, that was interrupted by small patches of grasses and forbs. This area was dominated by a few mature junipers (17.67 cm<sup>2</sup>/m<sup>2</sup>) intermixed with many young cedar elms, ashes, and scaly bark oaks.

The "canyon" transect supported many of the same tree species as the canyon rim; however, the junipers present in the canyon were saplings rather than mature. Cedar elms in the canyon were mature, occupying 117 cm<sup>2</sup>/m<sup>2</sup>. The canyon supported a dense shrub layer, specifically hog plum, *Bumelia*, and poison ivy.

The "juniper woodlands" transect was located in the uplands of the western side of the ranch. The soil was thin and gravelly. Junipers were the most-common tree (biomass of 7.92 cm<sup>2</sup>/m<sup>2</sup>). There was virtually no understory. Toward the eastern end of this transect, there was a transition from woodland into a more-complex community, including some young Shumard oaks and greenbrier.

The "riverside upland" transect was placed along the lower reaches of the slope beneath the rimrock where it approached the river. This area is best described as a transition zone from the upland woodland communities to the riverside woodlands. Mature juniper and cedar elm were co-dominant woody species, each occupying around 17 cm<sup>2</sup>/m<sup>2</sup>. Hickory and hackberry saplings were mixed with Shumard oak and ash, none of which filled > 0.1 cm<sup>2</sup>/m<sup>2</sup>. The understory was dominated by greenbrier.

The "grassy slope" transect was located slightly upslope from the "grass by rimrock" site. Soil was deeper on the grassy slope and a more-complex community of mainly herbaceous species was present.

The ground was consistently covered with grasses and dead plant material and occasionally punctuated with small junipers and some small shrubs, poison ivy and sumac.

The "grass by tank" transect was set around the pond in a field that was occasionally mowed. Three-quarters of the ground surface was covered by dead grass material. Some live bermuda grass had grown up through the duff layer. No woody plants were present, making this an area of extremely low vegetative diversity.

*Analyses of vegetative data.*—For the combined vegetative data set, 10 principal components were required to account for all of the variation within the data set (Fig. 1a). The first two principal components accounted for 30% of the variation. On principal component I, strongly positive coefficients were found for cover provided by dead material at 5 cm (0.26) and 10 cm (0.23), Johnson grass at 25 cm (0.21), and forbs at 25 cm (0.24). The most highly weighted woody species were hickory (0.19) and holly (0.19). Strongly negative coefficients were identified for woody cover at 1 m (-0.22), poison ivy (-0.25), cedar elm (-0.21), and hog plum (-0.20). This suggests a division between grassland and woodland communities. For principal component II, strongly positive coefficients were found for cover provided by woody vegetation at 50 cm (0.35), forbs at 10 cm (0.27), and ash trees (0.31). Large negative coefficients were found for dead material at 5 cm (0.26), 10 cm (-0.23), and 25 cm (-0.16). This indicates a division between a grassland community and an early-successional-stage woodland community.

Of the three individual vegetative data sets, the cover analysis was the most effective discriminator among transects (Fig. 1b). Over 95% of the variation was accounted for by the first six axes, and the first two accounted for 54% of the variation. The strongest positive coefficients for principal component I were for dead material at 5 cm (0.42) and 10 cm (0.43), bermuda grass at 25 cm (0.29), and Johnson grass at 25 cm (0.32). The most strongly negative coefficients were for woody plants at 50 cm (-0.21) and 1 m (-0.38). The strongly positive coefficients of principal component II were for forbs at 5 cm (0.43), 10 cm (0.45), and 25 cm (0.41) and woody at 50 cm (0.34). The negative coefficients were for bermuda grass at 25 cm (-0.21) and 50 cm (-0.21).

Full variation of the stem-count data set was accounted for by nine principal components (Fig. 1c). The first two components described

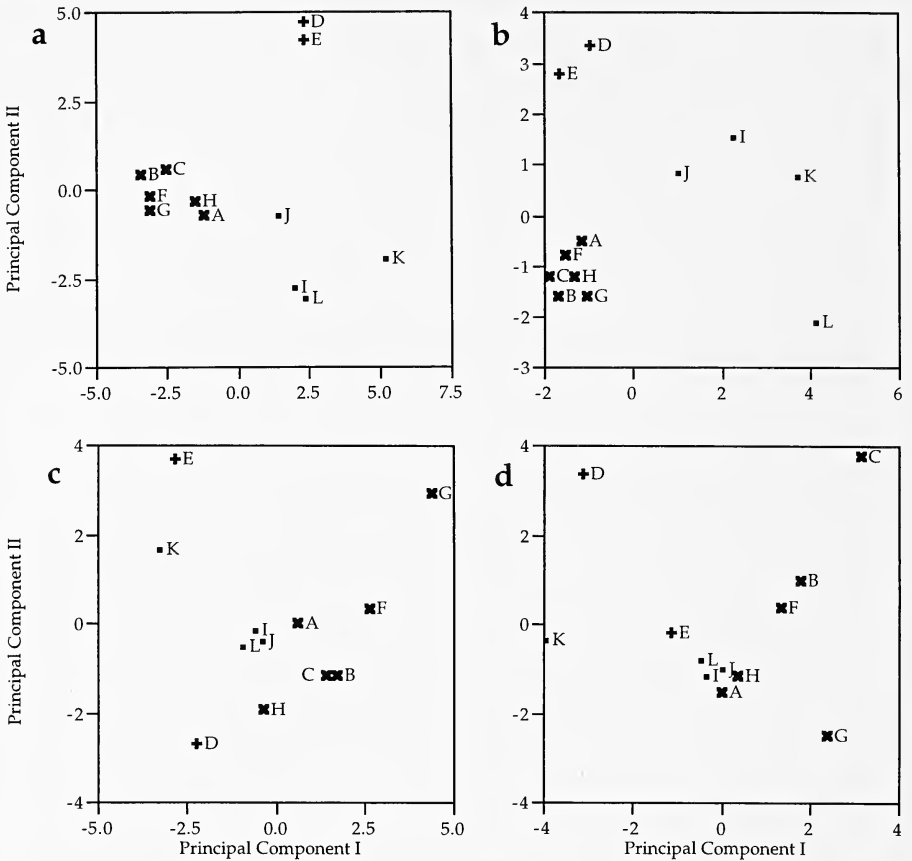


Figure 1. Relationships of sampling transects based on principal components analyses of vegetative data: (a) composite data set, (b) cover, (c) counts of woody stems, and (d) biomass of woody stems. Letters in plots of principal components I and II indicate transects: A, juniper woods; B, riverside upland; C, canyon; D, riverside woods; E, haymeadow woods; F, canyon rim; G, woods below rimrock; H, rimrock; I, grassy slope; J, grass by rimrock; K, haymeadow edge; L, grass by tank. Symbols indicate habitat groupings: upland woodlands indicated by "x", lowland woodlands by "+", and grasslands by a filled square.

38% of the variation. For principal component I, strongly positive coefficients were for juniper (0.31), Shumard oak (0.29) hackberry (0.33), and scaly bark oak (0.34). Strongly negative coefficients were identified for pecan (-0.20), roughleaf dogwood (-0.20), peppervine (-0.25), cottonwood (-0.16), and grape (-0.20). As the former group of plants was common near the river while the latter was present at higher elevations, these results suggest a division between upland and lowland woodlands. Principal component II had strongly positive coefficients for peppervine (0.37), pecan (0.37), hickory (0.16), and willow (0.38),



while negative coefficients were strong for holly (-0.20), cottonwood (-0.16) and American elm (-0.23).

Analysis of woody biomass revealed similar relationships among transects (Fig. 1d). Nine principal components were required to account for at least 95% of the variation, with the first two explaining 36% of the variation. Principal component I again seemed to distinguish between lowland and upland woodland communities. Strongly positive coefficients were found for hackberry (0.32) and cedar elm (0.25), species found at the higher elevations, while negative coefficients were identified for roughleaf dogwood (-0.37), cottonwood (-0.30), and pecan (-0.29), which occur near the river. Transects with a low diversity of woody species clustered near the center of the graph. Strongly positive coefficients for principal component II were indicated for ash (0.46) and cedar elm (0.25), while strongly negative coefficients were found for both Shumard oak (-0.22) and scaly bark oak (-0.21).

Based upon the contrasts found among the three single data sets, the transects were divided into three broad groups: grasslands, upland woodlands, and lowland woodlands. Membership of a transect in a group was determined by the clusters found on the graph of the merged data set (Fig. 1a). The grass near rimrock, grass by slope, grass by tank, and haymeadow edge transects were associated in a group hereafter referred to as the "grasslands" habitats. Similarities among them are a high level of ground cover and minor presence of woody plants. The riverside upslope, canyon, canyon rim, woods below rimrock, rimrock and juniper woodlands transects all clustered together as "upland woodlands" habitat. They shared a thick canopy and little ground cover, as well as similar tree species. The riverside woods and the haymeadow woods transects, which are both located along the river, were paired due to moderate ground cover, a thick canopy, and presence of forbs and grasses. This group is referred to as the "lowland woodlands" group.

*Description of small-mammal communities.*—Seven species of rodents were trapped during the survey (Table 1): *Peromyscus pectoralis* (white-ankled mouse), *P. attwateri* (Texas mouse), *P. maniculatus* (deer mouse), *Reithrodontomys fulvescens* (fulvous harvest mouse), *Baiomys taylori* (northern pygmy mouse), *Sigmodon hispidus* (hispid cotton rat) and *Neotoma micropus* (southern plains woodrat). Each of these species was trapped in at least one of the grassland transects (Table 1). At the

Table 1. Results of small-mammal trapping along 12 transects at Klondike Ranch, Johnson County, Texas, 1995-96. Indicated for each species of rodent sampled are its relative density (number caught per 50 trap nights) and the proportion (percentage) of the community represented by that species.

Density or proportion and transect	Number of trap nights	Species						
		<i>Peromyscus atwateri</i>	<i>Peromyscus pectoralis</i>	<i>Peromyscus maniculatus</i>	<i>Reithrodontomys fulvescens</i>	<i>Baiomys taylori</i>	<i>Sigmodon hispidus</i>	<i>Neotoma micropus</i>
Relative Density								
Grass by tank	160	0	0.31	0.31	0.63	0.31	0.63	0
Grass by rimrock	30	0	1.67	0	0	1.67	1.67	1.67
Grassy slope	90	0	0	0	0.56	0.56	0	0
Haymeadow edge	140	0.36	0.71	0	0.36	1.79	2.5	0
Haymeadow woods	60	0	0	0	0	0	0	0
Riverside woods	120	0.83	0.56	0.28	0	0.28	0	0
Canyon rim	130	2.31	1.54	0	0	0	0.38	0
Riverside upland	88	2.27	1.7	0	0	0	0	0
Juniper woods	128	2.34	0	0	0	0	0	0
Canyon	130	0	3.85	0	0	0	0	0
Woods below rimrock	147	0	5.1	0	0	0	0	0
Rimrock	37	0	8.11	0	0	0	0	1.35
Proportion of Community								
Grass by tank	160	0	14.3	14.3	28.6	14.3	28.6	0
Grass by rimrock	30	0	25	0	0	25	25	25
Grassy slope	90	0	0	0	50	50	0	0
Haymeadow edge	140	6.3	12.5	0	6.3	31.3	43.8	0
Haymeadow woods	60	0	0	0	0	0	0	0
Riverside woods	120	42.9	28.8	14.3	0	14.3	0	0
Canyon rim	130	54.6	36.4	0	0	0	9.1	0
Riverside upland	88	57.1	42.9	0	0	0	0	0
Juniper woods	128	100	0	0	0	0	0	0
Canyon	130	0	100	0	0	0	0	0
Woods below rimrock	147	0	100	0	0	0	0	0
Rimrock	37	0	85.7	0	0	0	0	14.3

"grass by tank" transect, the two dominant species were *R. fulvescens* and *S. hispidus*, captured at a rate of 0.63 animals per 50 trap nights, while *P. maniculatus*, *P. attwateri* and *B. taylori* each were captured at a rate of 0.31 animals per 50 trap nights. At the "grass by rimrock" site, the rodent community was equally divided between *P. attwateri*, *B. taylori*, *S. hispidus* and *N. micropus*, with each captured at a rate of 1.67 per 50 trap nights. This was one of only two transects where *N. micropus* was captured. At the "grassy slope" transect, only *S. hispidus* and *B. taylori* were captured. Five species were trapped at the "hay-meadow edge" transect, the predominant two being *S. hispidus*, 2.5 captures per 50 trap nights, and *B. taylori*, 1.79 captures per 50 trap nights. Others present at the "haymeadow edge" were *P. pectoralis*, *P. attwateri* and *R. fulvescens*. This was the only occurrence of *P. attwateri* in a grassland transect.

The lowland-woods habitats were sparsely inhabited by ground-dwelling rodents. No animals were trapped in the "hay meadow woods" transect. At the "riverside woods" transect, *P. attwateri* was caught most frequently, at 0.83 per 50 trap nights, followed by *P. pectoralis*, *P. maniculatus* and *B. taylori* (Table 1).

The upland woods habitats were dominated by *P. attwateri* and *P. pectoralis*, with one or the other of these species making up over half of the community at each transect. At the "canyon rim" transect, *P. pectoralis* was captured at a rate of 1.54 per 50 trap nights, *P. attwateri* at 2.31 per 50 trap nights, and *B. taylori* at 0.38 per 50 trap nights. These two *Peromyscus* species were caught in approximately the same proportions at the "riverside upland" transect. *P. attwateri*, at 2.34 per 50 trap nights, was the only species captured at the "juniper woodland" transect. *P. pectoralis* was the only species at the "canyon" and "woods below rimrock" transects, and made up 85% of the community at the "rimrock" transect. The only other rodent species at the "rimrock" transect was *N. micropus* (Table 1).

*Statistical analyses of small-mammal data.*—In the principal components analysis of relative-density data, five components accounted for 95% of the variation among the small-mammal data (Fig. 2a). For principal component I, strong positive coefficients were present for *B. taylori* (0.60), *S. hispidus* (0.57) and *R. fulvescens* (0.36). Negative coefficients were recorded for *P. attwateri* (-0.30) and *P. pectoralis* (-0.18). Strongly positive coefficients for principal component II were

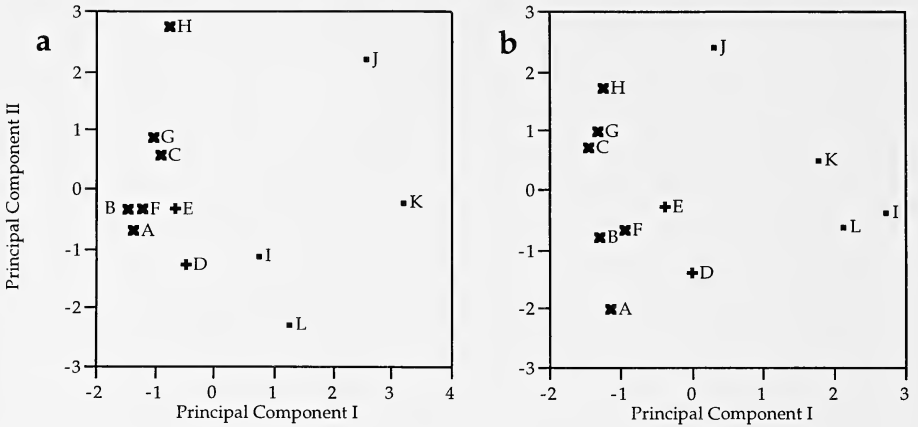


Figure 2. Relationships of sampling transects based on principal components analyses of mammal data: (a) relative density as number caught per 50 trap nights, (b) proportion of the community represented by each species. Letters in plots of principal components I and II indicate transects: A, juniper woods; B, riverside upland; C, canyon; D, riverside woods; E, haymeadow woods; F, canyon rim; G, woods below rimrock; H, rimrock; I, grassy slope; J, grass by rimrock; K, haymeadow edge; L, grass by tank. Symbols indicate habitat groupings: upland woodlands indicated by "x", lowland woodlands by "+", and grasslands by a filled square.

noted for *P. pectoralis* (0.55) and *N. micropus* (0.56), while negative coefficients were found for *P. maniculatus* (-0.41) and *R. fulvescens* (-0.40). This pattern suggests a division between the grassland and woodland communities.

A data set consisting of the proportions of the community made up of each species was analyzed similarly. Five components explained 95% of the variation (Fig. 2b). For the first principal component, strong positive coefficients were again found for *B. taylori* (0.57), *S. hispidus* (0.38) and *R. fulvescens* (0.51), and negative coefficients were presented for *P. attwateri* (-0.25) and *P. pectoralis* (-0.40). The second principal component agreed with that of the first analysis: Strong positive coefficients were found for *P. pectoralis* (0.40) and *N. micropus* (0.60), while negative coefficients were found for *P. maniculatus* (-0.30), *P. attwateri* (-0.57) and *R. fulvescens* (-0.13). The results of these analyses of small-mammal data confirmed the sorting of the transects into three groups.

*Correlation analyses.*—Comparison of the densities of small mammals with vegetative features of the habitats in which they were captured revealed many significant ( $P \leq 0.05$ ) correlations: With regard to vegetative cover, densities of *R. fulvescens* correlated with dead material

at 5 cm ( $r = 0.699$ ), at 10 cm ( $r = 0.704$ ), and at 25 cm ( $r = 0.664$ ), bermuda grass at 25 cm ( $r = 0.776$ ) and 50 cm ( $r = 0.631$ ), and Johnson grass at 25 cm ( $r = 0.748$ ). *Reithrodontomys fulvescens* densities correlated inversely with woody cover at 50 cm ( $r = -0.614$ ) and at 1 m and higher ( $r = -0.657$ ). Density of *B. taylori* was also significantly correlated with dead material at 5 cm ( $r = 0.758$ ), at 10 cm ( $r = 0.795$ ), and at 25 cm ( $r = 0.868$ ), and Johnson grass at 25 cm ( $r = 0.593$ ). *Baiomys taylori* also was associated with forbs at 5 cm ( $r = 0.591$ ) and negatively correlated with woody cover at 50 cm ( $r = -0.593$ ) and at 1 m and above ( $r = -0.779$ ). *Sigmodon hispidus* was correlated with bermuda grass at 25 cm ( $r = 0.678$ ), Johnson grass at 25 cm ( $r = 0.704$ ), and negatively correlated with woody at 1 m and higher ( $r = 0.630$ ). *Peromyscus pectoralis* was negatively associated with forbs at 5 cm ( $r = -0.644$ ) and at 10 cm ( $r = -0.717$ ). The analysis of community proportion with cover revealed the same significant associations with a few exceptions. Additionally, *B. taylori* was associated with dead material at 50 cm ( $r = 0.634$ ), but was not associated with Johnson grass at 25 cm. *Peromyscus pectoralis* also was associated with woody cover at 1 m and higher ( $r = 0.578$ ), and *P. maniculatus* was associated with bermuda grass at 50 cm ( $r = 0.674$ ).

Correlation analysis of stem counts with proportion of mammalian community revealed significant associations between *R. fulvescens* and sumac ( $r = 0.631$ ), and of *P. pectoralis* with both *Bumelia* ( $r = 0.607$ ) and cedar elm ( $r = 0.684$ ). Density of *P. pectoralis* correlated significantly with stem counts of ash ( $r = 0.698$ ) as did that of *P. maniculatus* with number of American elm stems ( $r = 0.604$ ).

Comparisons of mammal densities with plant biomass demonstrated significant associations of *P. pectoralis* with ash ( $r = 0.640$ ) and juniper ( $r = 0.613$ ), as well as for *P. maniculatus* with American elm ( $r = 0.604$ ). A negative correlation occurred between *B. taylori* and juniper ( $r = -0.703$ ). Community proportion composed by *P. pectoralis* correlated significantly with ash ( $r = 0.813$ ), *Bumelia* ( $r = 0.607$ ), hackberry ( $r = 0.687$ ) and juniper ( $r = 0.600$ ). Community proportion composed by *B. taylori* again correlated negatively with juniper ( $-0.656$ ), whereas that for *R. fulvescens* correlated with greenbrier ( $r = 0.631$ ).

## DISCUSSION

*Diversity of habitats and rodent communities present.*—Analyses of vegetational data indicated that the 12 habitats could be reduced to three

general habitat types: upland wooded habitats, wooded habitats associated with the Brazos River, and the various grassy habitats (whether located in upland or lowland areas). The predominant plant species in upland woods were juniper, Shumard oak, scaly bark oak, ash and hackberry. Lowland woods comprised stands of woody species such as cottonwood, pecan and dogwood. Woody plants were generally absent from the grassland transects, instead being dominated by bermuda grass, Johnson grass and assorted native grasses and forbs.

Concordantly, analyses of the small-mammal data yielded the same three groups of transects. The predominant rodent species in the upland woods habitats were the Texas mouse (*Peromyscus attwateri*) and the white-ankled mouse (*P. pectoralis*); the southern plains woodrat (*Neotoma micropus*) occurred only in the uplands. In the grasslands situations, cotton rats (*Sigmodon hispidus*), fulvous harvest mice (*Reithrodontomys fulvescens*) and pygmy mice (*Baiomys taylori*) were the primary rodent species. The lowland woods habitats were sparsely inhabited by rodents; the community here generally included species found in the other habitats, but this was the only setting in which deer mice (*P. maniculatus*) were captured.

*Habitat preferences of various rodent species.*—Correlation analyses identified various vegetational features that characterize habitats preferred by the various rodent species. These habitat correlations generally agree with those previously published (Schmidly 1983; Davis & Schmidly 1994; Wilkins 1995). For example, abundance of cotton rats, harvest mice and pygmy mice generally correlated positively with amount of dead herbaceous material and grasses, but negatively with most measures of woody material.

The ecological interaction of *Peromyscus attwateri* and *P. pectoralis* that was observed in this study corroborate some published accounts while contradicting others. In accordance with findings of Baccus & Horton (1984) and Etheredge et al. (1989), *P. pectoralis* was found at its greatest densities in association with slopes and limestone outcrops (six transects), though this species also was present in three of the four grassland transects. As suggested by Brown (1964) and Garner (1967), juniper was also found as a significant component of the habitats in which *P. attwateri* was found at the greatest densities. Etheredge et al. (1989) observed that these two species have highly similar niches, but that when they coexist, *P. attwateri* is a habitat generalist while *P.*

*pectoralis* is a specialist whose niche is contained within that of *P. attwateri*. In contrast, this study found *P. pectoralis* in a greater array of habitats (9 of 12 transects; Table 1) than *P. attwateri* (5 of 12 transects). In three of the four transects where they co-occurred (riverside woods, canyon rim, riverside upland), density of *P. attwateri* was greater than for *P. pectoralis*. The only habitat in which *P. attwateri* occurred but from which *P. pectoralis* was absent was the juniper woods, where *P. attwateri* achieved its greatest density. These findings suggest that *P. pectoralis* is the generalist and *P. attwateri* the specialist, and that neither species' niche locally is included entirely in that of the other.

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CHANGES IN THE BREEDING BIRD COMMUNITY  
OF SUBTROPICAL EVERGREEN FOREST IN THE LOWER  
RIO GRANDE VALLEY OF TEXAS, 1970s-1990s

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**Abstract.**—Breeding-bird censuses conducted in 1994-1996 at the Santa Ana National Wildlife, southern Texas were compared to those done on the same site in 1973-1978. The study site was described as subtropical evergreen forest in the 1970s, but is now thorn-forest with remnant evergreen forest. Canopy cover was incomplete and lower and trees were smaller and more densely packed in 1994-1996 than in 1973-1978. Seven bird species typical of thorn-forest and thorn-scrub, which were not present in 1973-1978, made up 20% of the breeding bird community in 1994-1996. Species tolerant of thorn-forest and thorn-scrub made up 76.0% of the breeding bird community in 1994-1996, compared to 54.6% in 1973-1978. Three forest bird species disappeared from the study site. Water management will be needed if subtropical evergreen forest and its bird community are to be restored.

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Habitat alteration has caused declines of many breeding birds in the Lower Rio Grande Valley (LRGV) of Texas during the 20th century, particularly in forested habitats (Oberholser 1974). The construction of Falcon Dam in 1953 reduced flooding frequency, and a combination of freezes and droughts since are thought to have caused a shift from riparian plant and bird species to those typical of thorn-forest and thorn-scrub (Fleetwood 1967; Lonard & Judd 1991). For example, Red-billed Pigeon (*Columba flavirostris*), Tropical Parula (*Parula pitiayumi*) and Hooded Oriole (*Icterus cucullatus*), once common members of the riparian breeding bird community (Davis 1940) are rare today in the LRGV.

The avifauna of subtropical evergreen forest dominated by Texas ebony (*Pithecellobium ebano*) was studied by Gehlbach et al. (1976) and Gehlbach (1987). Nesting Red-billed Pigeons, Rose-throated Becards (*Pachyramphus aglaiae*) and Altamira Orioles (*Icterus gularis*) were among a diverse subtropical breeding bird community in the 1970s. Subtropical evergreen forest occurs on well-drained floodplain soil (Cottam & Trefethen 1968), often on a terrace above the riparian woodland dominated by Mexican ash (*Fraxinus berlandieriana*), cedar elm (*Ulmus crassifolia*), and sugar hackberry (*Celtis laevigata*) (cf. Rupert 1997). Once widespread at Santa Ana National Wildlife Refuge (SANWR), both evergreen forest and riparian forest are now widely

replaced by Tamaulipan thorn-forest and thorn-scrub in much of the refuge (Fleetwood 1967; Gamel 1997). In such habitats, smaller trees and shrubs such as mesquite (*Prosopis glandulosa*), Wright's acacia (*Acacia wrightii*), Texas persimmon (*Diospyros texana*), lotebush (*Ziziphus obtusifolia*), crucifixion thorn (*Koeberlinia spinosa*) and snake eyes (*Phaulothamnus spinescens*) are common (Vora 1990; scientific names are from Lonard et al. 1991). Since SANWR forests are thought to have been affected by freezes and by long-term lack of flooding, the goal of this study was to determine if changes had occurred in the breeding bird community of evergreen forest and its habitat since the 1970s.

### STUDY SITE

The study was conducted at Santa Ana National Wildlife Refuge (SANWR), Hidalgo Co., Texas, in the middle region of the LRGV. Most of SANWR is now covered by thorn-forest (3-6 m in height), thorn-scrub (<3 m in height), and riparian (deciduous) forest (8-13 m tall). In the 1970s, the present 8 ha study site was part of a closed-canopy subtropical evergreen forest of mature Texas ebony, tepehuaje (*Leucaena pulverulenta*), and anacua (*Ehretia anacua*), with an average tree height of 16.1 m (Gehlbach 1987). Gehlbach (1987) describes an abrupt transition to thorn-forest (brush) at the southern and western edges of the study site. A mixture of riparian forest, thorn-forest and wetlands bordered the study site to the north and east. This forest was the only remaining mature Texas ebony/tepehuaje stand on SANWR by the late 1960s, according to Fleetwood (1967).

### METHODS

Bird censusing and vegetational analysis were conducted in the same 8-ha study site studied by Gehlbach during 1973-1978 (Gehlbach et al. 1976; Gehlbach 1987). Bird censuses were done as outlined by the International Bird Censusing Committee (Svenson & Williamson 1970). Censusing was started at dawn and was usually completed by 11:00. Each year, 10-14 censuses were conducted during during late February-late July 1994-1996 (Brush in 1994, Cantu in 1995-1996). Using composite species maps, the number of territories occupied by each species was determined.

The density and species list of birds in 1994-1996 was compared with Gehlbach's (1987) data. Since Gehlbach gave no density for

White-winged Dove, the density from the similar evergreen forest in Gehlbach's study site along the Rio Corona, Mexico was used. Further details of the study are in Cantu (1996).

Vegetation was studied using James & Shugart's (1970) method of quantitative habitat description. Tree density, dominance, frequency, and percent canopy cover were determined with this method, as were shrub density, and percent ground cover, using ten 0.05 ha plots randomly chosen to conduct the vegetation quantification study.

## RESULTS

*1994-1996 breeding bird community.*—Twenty-five bird species which maintained at least one territory in at least one year were recorded; 19 averaged at least one territory per year (Table 1). The number of territories per species averaged 6.1 (Table 2).

Two species, White-winged Dove (averaging 41 territories) and Olive Sparrow (averaging 19.5 territories) made up more than one third of the breeding bird community. Mourning Dove, Plain Chachalaca, White-tipped Dove, Golden-fronted Woodpecker, White-eyed Vireo and Long-billed Thrasher averaged 7-15 territories per year. The eight species listed above made up 67% of all territories.

*Changes in the breeding bird community.*—Three of the most abundant species during 1994-1996, Mourning Dove, White-eyed Vireo and Long-billed Thrasher, did not breed on the study site in 1973-1978. Four other species, absent in 1973-1978 (Northern Cardinal, Northern Mockingbird, Common Ground-Dove and Greater Roadrunner), occurred in smaller numbers during 1994-1996 (Table 1). The two most abundant species during 1994-1996, White-winged Dove and Olive Sparrow, increased by 850% from 1973-1978. Species which invaded the study site since the 1970s made up 20% of the breeding pairs in 1994-1996.

Red-billed Pigeon, Rose-throated Becard, and Altamira Oriole maintained territories or nested on the study site during 1973-1978 but not during 1994-1996. Of these only the Altamira Oriole had averaged at least one territory per year during 1973-1978. Altamira Orioles wintered in the northern section of the study site, but moved out by early March of 1994-1996. Singing individual Red-billed Pigeons were observed on or near the study site in March and May 1995 but did not remain. Rose-throated Becard was not detected during 1994-1996.

Table 1. Average territories/8 ha for two studies of breeding bird communities at Santa Ana National Wildlife Refuge, southern Texas. Species names are listed as in the A.O.U. Checklist of North American Birds, 6th edition, including 41st supplement). Asterisks indicate species confirmed as breeding on the study site. Breeding habitat was derived from Parker et al. (1996), with scrub used broadly to include low thorn-forest and thorn-scrub.

BIRD SPECIES	1973-1978	1994-1996	HABITAT
Black-bellied Whistling-Duck ( <i>Dendrocygna autumnalis</i> )	0.2*	0.3	Forest
Red-shouldered Hawk ( <i>Buteo lineatus</i> )	0.0	0.2*	Forest
Plain Chachalaca ( <i>Ortalis vetula</i> )	5.2*	10.8*	Forest, edge
Red-billed Pigeon ( <i>Columba flavirostris</i> )	0.2*	0.0	Forest
White-winged Dove <sup>1</sup> ( <i>Zenaida asiatica</i> )	3.0*	35.0*	Forest, scrub
Mourning Dove ( <i>Zenaida macroura</i> )	0.0	13.2*	Scrub, forest
Common Ground-Dove ( <i>Columbina passerina</i> )	0.0	0.3	Scrub
White-tipped Dove ( <i>Leptoptila verreauxi</i> )	1.5*	12.3*	Forest, edge
Yellow-billed Cuckoo ( <i>Coccyzus americanus</i> )	1.4*	3.0*	Forest
Greater Roadrunner ( <i>Geococcyx californianus</i> )	0.0	0.3	Scrub
Groove-billed Ani ( <i>Crotophaga sulcirostris</i> )	0.6*	2.3*	Scrub
Buff-bellied Hummingbird ( <i>Amazilia yuacatanensis</i> )	0.6*	0.3	Forest, edge
Golden-fronted Woodpecker ( <i>Melanerpes aurifrons</i> )	3.6*	8.3*	Scrub, forest
Ladder-backed Woodpecker ( <i>Picoides scalaris</i> )	0.9*	5.0*	Scrub, forest
Brown-crested Flycatcher ( <i>Myiarchus tyrannulus</i> )	1.2*	5.0*	Forest, scrub
Great Kiskadee ( <i>Pitangus sulphuratus</i> )	0.4*	3.5*	Forest, scrub
Couch's Kingbird ( <i>Tyrannus couchii</i> )	2.3*	5.3*	Forest, scrub
Rose-throated Becard <sup>2</sup> ( <i>Pachyramphus aglaiae</i> )	+*	0.0	Forest, edge
Green Jay ( <i>Cyanocorax yncas</i> )	2.0	4.0	Forest
Tufted Titmouse ( <i>Baeolophus bicolor</i> )	1.1*	4.7*	Forest
Carolina Wren ( <i>Thryothorus ludovicianus</i> )	0.6*	1.0	Forest
Northern Mockingbird ( <i>Mimus polyglottos</i> )	0.0	1.0	Scrub, edge
Long-billed Thrasher ( <i>Toxostoma longirostre</i> )	0.0	7.8*	Scrub, edge
White-eyed Vireo ( <i>Vireo griseus</i> )	0.0	5.5	Scrub, forest
Northern Cardinal ( <i>Cardinalis cardinalis</i> )	0.0	2.8	Scrub, forest
Olive Sparrow ( <i>Arremonops rufivirgatus</i> )	3.2*	17.7*	Forest, scrub
Bronzed Cowbird ( <i>Molothrus aeneus</i> )	2.0	3.0*	Forest, scrub
Altamira Oriole ( <i>Icterus gularis</i> )	1.5*	0.0	Forest
TOTAL NUMBER OF TERRITORIES	31.3	152.6	

<sup>1</sup> Estimated using density given by Gehlbach et al. (1976) for similar habitat along the Rio Corona, Mexico.

<sup>2</sup> One female nested on site in 1977, no density given.

Table 2. Characteristics of vegetation and breeding bird communities of an 8-ha study site at Santa Ana National Wildlife Refuge in 1973-1978 (Gehlbach 1987) and 1994-1996 (this study). Canopy cover in 1973-1978 was estimated conservatively from Gehlbach's description of a "continuous canopy". See Table 1 for list of birds using scrub habitat.

	SUBTROPICAL EVERGREEN FOREST	THORN FOREST/REMNANT EVERGREEN FOREST
<u>Vegetation</u>		
Canopy cover	90%	47%
Mean canopy height (m)	16.1	6.1
Tree density (n/ha)	161	396
Mean DBH (cm)	29.0	17.9
<u>Birds</u>		
Total number of species	20	25
Number of scrub species	9	16
Species diversity	2.66	2.66
Territories/species	1.56	6.10
% terrs. non-passerine	54.6	59.8
% terrs. scrub species	54.6	76.0

The breeding bird community in 1994-1996 had a 30% higher species richness and a 388% higher number of territories than the 1973-1978 community. 16 species known to use thorny habitats held 76% of the territories in 1994-1996, an increase of 21.5% since 1973-1978 ( $G = 5.539$ ,  $df = 1$ ,  $P = 0.019$ ). The percent of territories held by non-passerines increased by 5.2% over 1973-1978 ( $G = 0.267$ ,  $df = 1$ ,  $P = 0.267$ ). Bird species diversity was the same during both time periods (Table 2).

*Plant communities in 1994-1996 versus 1973-1978.*—With a broken canopy that averaged about 10 m lower than in the 1970s, the study site during 1994-1996 is best described as thorn-forest with limited evergreen forest remnants (Table 2). Tree density was almost 2.5 times greater than in 1973-1978, but trees averaged much smaller in diameter at breast height (DBH). Dead trees (species unknown) were the most abundant type of woody plant. There was a dense shrub layer averaging 14,180 stems per ha in 1994-1996. Gehlbach et al. (1976) photographed a very open understory and shrub layer on the Rio Corona study site, but shrub density on the Rio Grande site was not reported (Gehlbach 1987). Epiphytic ball-moss (*Tillandsia recurvata*) and Spanish moss (*T. usneoides*) occurred on some of the large trees, although they were not as dense as in other forests at SANWR.

## DISCUSSION

Both the bird and plant communities have changed markedly between the 1970s and the 1990s in a study site at SANWR. The bird community is now more typical of Tamaulipan thorn-forest, due to the loss of some forest bird species such as Altamira Oriole and the arrival of several new bird species typical of thorn-forest and thorn-scrub, such as Long-billed Thrasher, Northern Cardinal and White-eyed Vireo (Rappole & Blacklock 1994; Parker et al. 1996). Bird species typical of low thorn-scrub, such as Greater Roadrunner, Common Ground-Dove and Northern Mockingbird, have invaded parts of the study site with the lowest vegetation (Cantu 1996).

The greater density of trees and the existence of a denser understory has allowed many of the bird species already present to increase. The marked increase in the number of White-winged Doves is probably due to the growth of thorny trees (particularly Texas ebonies) of shorter stature, which are preferred as nest sites (Cottam & Trefethen 1968). Olive Sparrows nest in low, dense vegetation and forage mainly under a dense understory (Brush 1998b). Other increasing species such as Plain Chachalaca, White-tipped Dove and Golden-fronted Wood-pecker nest or forage regularly in dense habitat (Rupert 1997). Although nocturnal birds were not included in this study, Gamel (1997) showed at least three territories of Elf Owls (*Micrathene whitneyi*) on the study site during 1995-1996, while Gehlbach (1987) reported only a partial territory on the scrubby edge of the forest.

Species which are currently numerous on the site are generally common in thorn-forest and thorn-scrub in southern Texas (Rappole & Blacklock 1994), and some occur commonly in suburban habitat in the LRGV (Oberholser 1974; Gorena 1995). Total bird density now approaches that reported by Davis (1940) who studied birds in a riparian forest at SANWR which had a dense understory and shrub layer. The higher number of territories per species in 1994-1996 is higher than in most temperate and tropical forests (Gehlbach et al. 1976) but is similar to some southwestern riparian forests (Rosenberg et al. 1991).

A more subtle change on the study site was the continued decline of

a few bird species, which were already uncommon in the 1970s. Tropical species depending on tall trees or closed-canopy forest appear to be the most threatened. Red-billed Pigeons may require tall riparian forests, as they now are mainly restricted to such areas in the Lower Rio Grande Valley (Brush 1998c).

The disappearance of the Altamira Oriole from the study site is harder to explain, since they forage frequently in scrubby or successional areas and 3-4 pairs nested at SANWR during 1994-1996 (Brush 1998a). The loss of tall Texas ebony and tepehuaje, thought to be preferred nesting sites (Pleasants 1993), and parasitism by Bronzed Cowbirds (Brush 1998a) may both have played a role. Brood parasitism may be the more important factor, as Couch's Kingbird (*Tyrannus couchii*), another species nesting in tall trees (Rupert 1997), has increased on the study site since the 1970s. Couch's Kingbirds chase Bronzed Cowbirds from the nesting territory, eject cowbird eggs, and are rarely parasitized (Carter 1986; Clotfelter & Brush 1995).

The state-threatened Rose-throated Becard probably no longer nests in Texas, although it still occurs rarely during the non-breeding season (Texas Ornithological Society 1995). It may depend on the existence of open woodland with tall trees near water (Sutton 1949). Expected continued declines in these forest species due to habitat loss or fragmentation will result in lower regional diversity.

The death of many large trees has apparently allowed invasion by plant species more tolerant of drier conditions such as brasil, spiny hackberry, lotebush and colima to invade the study site. Although Texas ebony and anacua are still common, they are represented mainly by individuals of shorter stature (Cantu 1996). Large trees apparently killed by severe freezes generally have not recovered, although a few show limited resprouting from the base. Tepehuaje, in particular, has been eliminated as a canopy species on the study site. In contrast, some individual tepehuajes already exceed 12 m in height in wetter areas along the Rio Grande or agricultural canals, indicating the potential for rapid recovery if moisture is available. Vegetative succession may be occurring very slowly, but in the absence of adequate soil moisture the new climax may be Tamaulipan thorn-forest (Vora 1990).

Why has the plant community changed so drastically in some ways? Both Fleetwood (1967) and Gehlbach (1981) attribute some of the important changes to long-term lack of flooding since the construction of Falcon Dam in 1953. Death of large trees has been noted in several areas at both SANWR and Bentsen-Rio Grande Valley State Park, particularly during severe droughts (Brush 1998a). Although freezes may have an important short-term effect, areas which are near the river or are in particularly low floodplains in old oxbows (*resacas*) of the Rio Grande still contain closed-canopy riparian or evergreen forests today (Castillo 1997; Brush 1998c). Water management, in which water is pumped into floodplain forest, has shown promising early effects on germination of tree seedlings (Castillo 1997). This practice may need to be continued and expanded to restore large tracts of sub-tropical evergreen and riparian forests and their avian communities in the Lower Rio Grande Valley of Texas.

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## AN ADAPTIVE TOKEN PASSING PROTOCOL ON A STAR LAN

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**Abstract.**—The IEEE 802.5 is the standard protocol for token ring local area networks (LAN's) currently in use in many areas. It is popular because of its many advantages, including reliability, simplicity of hardware, ease of use with various transmission media, efficiency at heavy load and flexibility in link length and number of stations attached to the network. One of its drawbacks, however, is its inefficiency at moderate and light loads. At light loads, a station with a packet to send must wait, on average, half a round trip propagation delay time to receive the free token. This paper uses the star topology implementation of the token ring network to develop a new protocol with improved performance characteristics at moderate and light loads. This is accomplished by having stations notify the central station when they have data to send. The central station sends the token only to these stations. It is shown that, for the new protocol, all performance characteristics have a significant improvement.

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The IEEE 802.5 (ANSI/IEEE 1989) standard has become a widely accepted protocol for token ring local area networks. A major part of its success is due to the advantages it offers over other network architectures. These advantages include its reliability and simplicity of hardware interfacing the transmission medium to the stations electronics. Since each station provides an active tap to the transmission medium, there is great flexibility in the length of the link between stations. This also means more stations can be attached to the transmission medium without deterioration of the signal. The token ring has been remarkably adaptable to the available transmission medium. It has been implemented using shielded twisted wire pair, coaxial cable, and fiber optic cable, all with great success.

A token ring is a ring whose media access control (MAC) protocol is based on a short control frame, called a token, which circulates around the ring. A station that wants to transmit data on the medium must wait for a free token. When a free token arrives at the station, the token is changed to busy and the station transmits it, followed by its data packet. When the station has transmitted its packet and receives the first part of its data packet back on the network, it releases a new free token. The free token circulates around the ring until it reaches another station that has data to send.

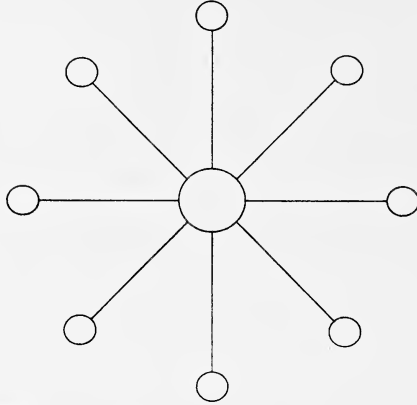


Figure 1. A star topology.

One of the drawbacks of the token ring is its inefficiency at moderate and light loads. At light load conditions, only a few of the stations on the ring have data to send. By the token ring protocol, however, the token circulates through all stations, regardless of whether or not a station has data to transmit. A station having data to transmit must wait for the free token to pass through all stations, even those that do not have data to transmit. It is this aspect of the protocol that is the cause of the inefficiency, the delay associated with the token passing through idle stations to get to an active station.

Many token ring networks are actually implemented using a star topology (Stallings 1993). This paper proposes a new protocol for the star topology token ring LAN. The new protocol is not subject to the same delays as the topological. This paper has sections devoted to the following major topics: the new protocol for the star topology LAN, performance analysis for the new protocol, simulation results, and conclusions.

### THE STAR PROTOCOL

The star LAN looks topologically like a wheel without a rim. It consists of a central controller and any number of other stations, each connected directly to the central controller. Figure 1 shows a LAN in a star topology. The central controller has separate hardware to interface with each of the other nodes. This means it is capable of communication with any station at any time, even simultaneously.

### THE BASIC STAR PROTOCOL

It is assumed that there are a total of  $M$  stations on the network but only  $N$  of them are active at any time. The protocol works as follows.

A station that does not have data is inactive. When it becomes active and joins the ring it sends a "request to send data" token to the central station. The central station keeps a bit vector of active nodes. When it receives the request token, it sets the bit for the appropriate node in the vector and sends the transmit token to the station during the next token pass. When a node has no more data, it leaves the ring by returning the transmit token without a data packet. Thus, stations do not have to wait for the transmit token to pass through idle stations. This clearly overcomes the drawback of the standard protocol under any load conditions.

There is extra processing required of the central station. This processing can be performed concurrently with the transmission of data packets, thus not adding any further time delays.

A token transmission cycle consists of sending the transmit token to a data station followed by the data transmission. The following depicts a token transmission cycle:

$$T_i \text{ Data}_i$$

$T_i$  denotes the token for station  $i$  and  $\text{Data}_i$  denotes the data for station  $i$ . The following depicts a complete transmission cycle:

$$T_1 \text{ Data}_1 \quad T_2 \text{ Data}_2 \quad \dots \quad T_n \text{ Data}_n$$

#### PRIORITY STAR PROTOCOL

The above protocol can be used, with minor modification, in a ring with multiple priority levels. In a multiple priority environment, the "request" token contains a priority field with enough bits to cover the number of priority levels. When a node sends a request token, it also sets the priority bits to indicate the priority of its data. The central controller sends the transmit token first to stations with the highest priority, then to the stations with the second highest priority level, and so on.

A token transmission cycle consists of sending the transmit token to a data station followed by the data transmission. In the priority protocol, the transmit token is sent to the stations in order of priority. A token transmission cycle was given earlier.

#### TOKEN FORMAT

Since proposing different tokens for different purposes, this section is devoted to describing a new token format for the new star protocol.

The transmit token is the following eight bit pattern:

**0 1 1 1 1 1 1 0**

For the request data token, bits are changed in the middle of the pattern to obtain the following token:

**0 1 0 1 1 1 1 0**

To accommodate multiple priority levels, three bits are dedicated to allow for up to eight levels of priority. The priority data token is:

**0 1 0 1 P<sub>2</sub> P<sub>1</sub> P<sub>0</sub> 0**

P<sub>i</sub> denotes priority i.

The above tokens account for all services offered by the protocols proposed above.

### PERFORMANCE ANALYSIS

This section compares the waiting time delay of an equivalent topological ring to the topological star. It then compares the propagation and transmission time performance of the two protocols. The results show the proposed star protocol has significantly better performance characteristics in both cases.

The following notations are used in the ensuing discussion:

r	time to pass the free token from one station to the next
x	message transmission time
x'	mean message transmission time
M	total number of stations on the network
N	number of active stations on the network
T	token rotation time
T'	mean token rotation time
$\lambda$	Poisson arrival rate of messages to each station
$\rho$	traffic intensity at each station = $\lambda T'$
$V_T^2$	variance of the token rotation time
$C_b^2$	square of coefficient of variation of token rotation time
D'	mean message delay
W'	mean time spent waiting in queue
Q'	mean queue length

In Sethi & Sadyam (1985), the authors provide a performance analysis of token rings that gives analytical results for two message length distributions, constant and exponential. Results are provided for both token rotation time and delay for a limited-to-one service discipline. Both performance issues are addressed in subsequent sections.

### TOKEN ROTATION TIME

The above authors (Sethi & Sadyam 1985) analyze a token ring with  $M$  total stations. The number of active stations,  $N$ , during a given token rotation is assumed to have a binomial distribution. The authors derive results, assuming both constant and exponential message lengths.

### CONSTANT MESSAGE LENGTH

For a constant message length assumption, the following results are presented for a LAN in which the token visits all stations (Sethi & Sadyam 1985).

For the token rotation time:

$$T_R = (M r)/(1 - M \lambda x)$$

for traffic intensity:

$$\rho_R = (M \lambda r)/(1 - M \lambda x)$$

for the square of the variance of token rotation time:

$$V_{TR}^2 = M \rho (1 - \rho) x^2$$

and for the square of coefficient of token rotation time:

$$C_{bR}^2 = [\rho (1 - \rho) x^2] / [M (r + \rho x)^2]$$

In all the above equations, the number of stations plays a significant role because the token must pass through all  $M$  stations even if only a few stations are active.

For a star that is operating as a token ring, only  $N$  stations in the network will be active, where  $N < M$ . By the new star protocol, the token will pass only to the  $N$  active stations. Thus, the following equations apply by simple substitution of the number of active stations.

For token rotation time:

$$T_s = (N r)/(1 - N \lambda x)$$

for the traffic intensity:

$$\rho_s = (N \lambda r)/(1 - N \lambda x)$$

for the square of the variance of token rotation time:

$$V_{TS}^2 = N \rho (1 - \rho) x^2$$

and for the square of the coefficient of token rotation time:

$$C_{bR}^2 = [\rho (1 - \rho) x^2] / [M(r + \rho x)^2]$$

To ease the mathematical manipulation, the simplifying assumption that  $(1 - N \lambda x) \approx (1 - M \lambda x) \approx 1$  is made.

The ratio of mean token rotation time of the ring to the star is:

$$T_R/T_S = M/N$$

the ratio of traffic intensities is:

$$\rho_R/\rho_S = M/N$$

the ratio of square of variance of token rotation time is:

$$V_{TR}^2/V_{TS}^2 = M/N$$

and for the square of the coefficient of token rotation time is:

$$C_{bR}^2/C_{bS}^2 = N/M$$

As the number of active stations decreases, the new star protocol shows increasing performance characteristics for parameters related to token rotation time. This performance gain carries over to exponentially distributed message lengths, as shown in the next subsection.

#### EXPONENTIALLY DISTRIBUTED MESSAGE LENGTHS

The following analytical results are found for the token rotation time for a token ring with exponentially distributed message lengths (Sethi & Saydam 1985).

For mean token rotation time:

$$T_R' = (M \rho) / (1 - M \lambda x')$$

for traffic intensity:

$$\rho_R = (M \lambda r) / ((1 - M \lambda x')$$

for the square of the variance of mean token rotation time:

$$V_{TR}^2 = M \rho (2 - \rho) (x')^2$$

and for the square of the coefficient of the mean token rotation time:

$$C_{bR}^2 = [\rho (2 - \rho) (x')^2] / [M (r + \rho x')^2]$$



Once again, the total number of stations,  $M$ , on the ring has a significant impact on performance. For the new star protocol, only  $N$  stations are active so only  $N$  stations need to see the token. The following results hold for the new star protocol by simple substitution of the number of active stations.

For the mean token rotation time:

$$T_s' = (N r)/(1 - N \lambda x')$$

for traffic intensity:

$$\rho_s = (N \lambda r)/(1 - N \lambda x')$$

for the square of the variance of mean token rotation time:

$$V_{TS}^2 = N \rho (2 - \rho) (x')^2$$

and for the square of the coefficient of mean token rotation time:

$$C_{bs}^2 = [\rho (2 - \rho) (x')^2]/[N (r + \rho x')^2]$$

Forming the ratios of ring values to star values and making the simplifying assumption  $(1 - M \lambda x') \approx (1 - N \lambda x') \approx 1$ , the following results are obtained:

$$T_R'/T_s' = M/N$$

$$\rho_R/\rho_s = M/N$$

$$V_{TR}^2/V_{TS}^2 = M/N$$

and

$$C_{bR}^2/C_{bs}^2 = N/M$$

#### DELAY ANALYSIS

Analytical results are also derived for mean waiting time, mean message delay, average number of messages in the individual queue, mean number of messages in all queues, and ring utilization (Sethi & Saydam 1985).

The results of time spent in queue are:

$$W' = ((1 + C_b^2) T')/(2 (1 - \rho))$$

for mean message delay:

$$D' = W' + x'$$

for mean queue length:

$$Q' = ((1 + C_b^2) \rho) / (1 - \rho)$$

for mean number of messages in queues in the system:

$$Q_s' = [((1 + C_b^2) \rho) / (2(1 - \rho))] + \lambda x'$$

and for ring utilization:

$$U_R = 1 - [1 - M \lambda r / (1 - M \lambda x)]^M$$

All these equations except the last hide their dependence on the number of stations in the network. To compare the performance of the ring to the star, equations are substituted for ring and star, respectively from the last section, and the ratio is formed. The results are as follows.

For mean queue length:

$$Q_R' / Q_s' = M / N$$

for mean waiting time:

$$W_R' / W_s' = M / N$$

for the mean message delay:

$$D_R' / D_s' = M / N$$

and for ring utilization:

$$U_R / U_s = [1 - (1 - \lambda_R)^M] / [1 - (1 - \rho_s)^N]$$

Since  $\rho_R < \rho_s$ ,  $(1 - \rho_R)^M < (1 - \rho_s)^N$ , the result is:

$$U_R < U_s$$

For all parameters related to delay or queue length, the new star protocol is superior to the traditional token ring protocol.

Only a limited-to-one service discipline was analyzed. The entire preceding analysis intentionally overlooked another factor contributing to delay, the packet transmission time. On a topological ring, the packet transmission time is the time to traverse, on average, half the links on the ring. On a topological star, packet transmission time is the time to traverse only the links to active stations.

### SIMULATION RESULTS

A small simulator was constructed for both the existing and the adaptive protocols. The simulator was run to compare the token rotation times (TRT) for the two protocols for fixed size message lengths.

Table 1. Token rotation times. Times are given in milliseconds.

	Protocol					Existing
	Adaptive					
Stations	11	21	27	41	47	50
TRT	03.7	07.0	09.0	13.7	14.4	16.7

Multiple runs were made for the adaptive protocol based on the number of active stations. Runs were made using an arrival rate of 0.75 messages per second arriving to the system. The service rate was normalized to one. The arrival of messages was assumed to be balanced evenly across the number of active stations. Table I indicates the results of simulations for both protocols.

As Table 1 shows, the token rotation time for the adaptive protocol is much lower than for the existing protocol. Since delay is a function of token rotation time, it can be expected that delay will also be lower for the adaptive protocol than for the existing protocol.

### CONCLUSIONS

This paper presented several new star protocols designed for a token ring operating on a topological star. The new protocols include a basic star protocol and enhanced star protocol implementing a multiple priority scheme for data transmission.

The performance of the traditional token ring protocol was then compared to the new star protocol for both token rotation time and message delay. For all parameters, the new star protocol showed superior performance.

Simulations were run for both protocols to compare token rotation time. The results indicate that the adaptive protocol offers time delays that are significantly smaller than the existing protocol.

Future research follows two directions. First is an analysis of the star protocol for both gated and exhaustive service disciplines. Then it is desirable to see if the performance gains can carry over and the protocol adapted for integrated services, adding voice stations to the network.

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A PRELIMINARY LIST OF LARGER FUNGI  
FROM KINGWOOD FOREST NORTHEAST  
OF HOUSTON, TEXAS

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**Abstract.**—A preliminary survey of the larger fungi occurring in Kingwood Forest of southeast Texas is presented. A total of 152 species in 18 different families are reported. The habitat and frequency of occurrence of each species is given. A review of additional regional studies is also presented.

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Reports on the fungal flora of the state of Texas include Theirs (1959a; 1959b; 1959c), Lewis et al. (1981), Lewis & MacGraw (1984), Singer et al. (1990) and Rodham & Lewis (1994). Metzler & Metzler (1992) recently listed 120 agarics and allied fungi in their field guide. Considering the vast and diverse geographical situations, vegetational types and climatic conditions of Texas, a much more complex and varied fungal composition is expected to be present. For example, Miller (1993) described a new species of *Cortinarius* from southeast Texas and Abraham & Loeblich (1993) has reported *Gymnopilus palmicola* Murr. colonizing the adventitious roots of the ornamental palm *Sabal palmetto* (Wal.: Lod. & Schul.) from Galveston. This complexity could be attributed to the introduced fungal elements of diverse origin (such as African, European and South American) interacting with the indigenous fungal components, often introduced along with their hosts such as pine, eucalyptus or palm.

Although there are several forests in and around the city of Houston such as Kingwood, Friendswood, Woodlands, etc., fungi occurring in these forests have not been studied and are therefore poorly understood. Most of these forests are semi-inhabited and subjected to considerable pressures of various nature. Human activities such as extension of residential areas, construction of roads, swimming pools, recreational activities, etc., exert increasing pressures on both flora and fauna. Extensive pressures of abiotic and edaphic nature especially from the ever increasing industrial pollution could lead to the elimination of several useful fungi and bring others to the verge of extinction. No serious attempts have been made to study the fungal resources which directly contribute to the good forest standing of this region. This study

was undertaken to survey the fungal flora of Kingwood Forest and document the macro fungi in relation to the tree communities (where the interaction is often either mycorrhizal or parasitic). It is intended that this information on occurrence, distribution and frequency of species would be of benefit in formulating future conservation measures.

### METHODS AND SPECIES LIST

This study was conducted during the months of June and July 1993 in Kingwood Forest, a mixed deciduous coniferous forest situated 25 miles NE of the city of Houston, Texas. A total of 250 collections were made and 152 specimens were identified to their specific level. A broad spectrum of the fungal composition of Kingwood Forest is presented. The species are arranged in alphabetical order within families following Singer (1986). The habitat of each species is given as duff, dung, lignicolous and soil (or combinations thereof). The frequency of occurrence of each species is given as infrequent, common or abundant (and widespread). All specimens are deposited with the holdings of the Mycological Herbarium of Texas Southern University (MHTSU).

<u>SPECIES</u>	<u>MHTSU</u> <u>HEBARIUM #</u>	<u>HABITAT</u>	<u>RELATIVE</u> <u>FREQUENCY</u>
<b>Family Agaricaceae</b>			
<i>Agaricus campestris</i> (Fr.)	1020	soil/duff	common
<i>Agaricus micromegathus</i> Peck	1062	soil/duff	infrequent
<i>Agaricus meleagris</i> J. Shaffer	1070	duff/soil	infrequent
<i>Agaricus rhoadsii</i> Murril	1088	soil/duff	infrequent
<i>Agaricus sylvaticus</i> (Vitt.) Fr.	1003	duff/soil	common
<i>Agaricus xanthodermoides</i> Murr.	1121	soil/duff	common
<i>Agaricus langei</i> (Moll.) Moll.	1039	soil/duff	infrequent
<i>Agaricus lanipes</i> (Moll.:Schaff.) Sing.	1046	duff/soil	infrequent
<i>Agaricus subperonatus</i> (Lange) Sing.	1117	soil/duff	infrequent
<b>Family Amanitaceae</b>			
<i>Amanita bisporigera</i> Atk.	1129	soil/duff	infrequent
<i>Amanita brunnescens</i> Atk.	1042	soil/duff	infrequent
<i>Amanita caesaria</i> (Scop.) Pers.	1140	soil/duff	common
<i>Amanita citrina</i> (Schaeff.) S.F. Gray	1026	duff/soil	common
<i>Amanita flavaconia</i> Atk.	1031	duff/soil	abundant
<i>Amanita farinosa</i> Schw.	1090	duff/soil	infrequent
<i>Amanita rubescens</i> (Pers.:Fr.) S.F.Gray	1004	duff/soil	common
<i>Amanita spissa</i> (Fr.) Quel.	1116	soil/duff	common
<i>Amanita porphyria</i> (A. & S.:Fr.) Sear.	1150	soil/duff	infrequent
<i>Amanita pantherina</i> (DC.:Fr.)Sec.	1044	soil/duff	common
<i>Amanita vaginata</i> (Bull.:Fr.)Vitt.	1077	soil/duff	common
<i>Amanita verna</i> (Bull.)Lamk.	1018	duff/soil	infrequent
<i>Amanita lividia</i> S.F. Gray	1079	soil/duff	infrequent
<i>Amanita fulva</i> (Sch.) Seyot	1134	soil/duff	infrequent
<i>Amanita pubescens</i> Schw.	1111	soil/duff	infrequent

**Family Bolbitiaceae**

<i>Agrocybe pediades</i> (Fr.)Fayod	1081	soil/duff	common
<i>Agrocybe vervacti</i> (Fr.) Singer	1002	duff/soil	common
<i>Agrocybe semiorbicularis</i> (Bull.) Fayod	1035	soil/duff	common
<i>Bolbitius vitellinus</i> (Pers.:Fr.)Fr.	1038	soil/duff	common
<i>Conocybe aurea</i> (J. Schaeff.) Hongo	1132	soil/duff	common
<i>Conocybe tenera</i> Wat.	1009	duff/soil	common
<i>Conocybe semiglobata</i> (Kunher)			
Kunher & Watlng	1072	duff/soil	common
<i>Conocybe glabra</i> (Murril) Wat.	1011	soil/duff	infrequent

**Family Boletaceae**

<i>Boletus communis</i> Fr.	1126	soil/duff	infrequent
<i>Boletus erythropus</i> (Fr.) Kromb.	1087	soil/duff	common
<i>Boletus badius</i> Fr.	1029	soil/duff	infrequent
<i>Boletus piperatus</i> Fr.	1016	duff/soil	infrequent
<i>Boletus reticulatus</i> Peck	1078	soil/duff	infrequent
<i>Boletus pulverulentus</i> Opat.	1071	soil/duff	infrequent
<i>Boletus impolitus</i> Fr.	1124	soil/duff	infrequent
<i>Chalciporus piperatus</i> (Bull.:Fr.)Singer	1101	duff/soil	infrequent
<i>Gyroporus cynescens</i> (Fr.) Quel.	1073	soil/duff	common
<i>Leccinum Scabrum</i> Smith & Theirs	1145	soil/duff	infrequent
<i>Leccinum abellum</i> (Peck.) Singer	1025	soil/duff	infrequent
<i>Tylopilus tabacinus</i> (Peck.) Singer	1099	duff/soil	abundant
<i>Suillus tomentosus</i> (Kauff.)Singer,			
Snell & Dick	1037	soil/duff	common
<i>Suillus frostii</i> Murr.	1055	soil/duff	common
<i>Suillus lakei</i> (Murr.) Smith & Theirs	1092	duff/soil	infrequent
<i>Austroboletus gracilis</i> (Pk.) Wolfe.	1119	soil/duff	infrequent
<i>Xerocomus spadiceus</i> (Fr.) Quel.	1013	soil/duff	infrequent

**Family Cantharellaceae**

<i>Cantharellus cibarius</i> Fr.	1057	soil/duff	common
<i>Cantharellus cinnabarnius</i> Schw.	1122	soil/duff	abundant

**Family Coprinaceae**

<i>Coprinus sterquilinus</i> (Fr.)Fr.	1125	soil	infrequent
<i>Coprinus plicatus</i> (Curt.:Fr.) Fr.	1142	soil	infrequent
<i>Coprinus lagopus</i> Karst.	1006	soil	infrequent
<i>Leucocoprinus fragillissimus</i>			
(Berk. & Curt.) Pat	1034	soil/duff	common
<i>Leucocoprinus birnbaumii</i> (Corda) Singer	1097	soil/duff	common
<i>Copelandia cynescens</i> (B. & Br.) Sacc.	1007	soil	infrequent
<i>Panaeolus semiovatus</i> (Sow.:Fr.) Lundell	1067	duff/soil	common
<i>Panaeolus accuminatus</i> Quel.	1033	soil	common
<i>Panaeolus sphinctrinus</i> (Fr.) Quel.	1128	soil	common
<i>Psathyrella conodolleana</i> (Fr.) Maire	1014	soil/duff	common
<i>Psathyrella gracilis</i> (Fr.) Quel.	1131	duff/soil	infrequent
<i>Psathyrella campanulatus</i> (Bull.:Fr.) Quel.	1017	soil/duff	infrequent
<i>Psathyrella conopilus</i> (Fr.) Pears. & Dennis	1036	soil/duff	infrequent

**Family Cortinariaceae**

<i>Gymnopilus penetrans</i> (Fr.:Fr.) Murr.	1093	lignicolous	infrequent
<i>Inocybe fastigiata</i> (Schaeff.:Fr.) Quel.	1027	soil	common
<i>Inocybe maculata</i> Bond.	1005	soil	infrequent
<i>Inocybe geophylla</i> (Sow.:Fr.) Kummer	1115	soil	infrequent
<i>Galerina</i> sp.	1032	lignicolous	infrequent
<i>Hebeloma testacea</i> (Batsch.:Fr.) Quel.	1113	soil/duff	infrequent
<i>Crepidotus applanatus</i> (Pers.:Pers.) Kum.	1041	lignicolous	infrequent
<i>Pholiota curvipes</i> (Fr.) Quel.	1075	lignicolous	infrequent
<i>Pholiota graminis</i> (Quel.) Singer	1063	lignicolous	infrequent
<i>Hypoloma fasciculare</i> (Huds.:Fr.) Karst	1015	lignicolous	infrequent

**Family Entolomataceae**

<i>Entoloma mougeotii</i> (Fr.) Hesler	1008	soil/duff	common
<i>Entoloma nitidum</i> Quel.)	1051	duff/soil	common
<i>Entoloma strictus</i> (Pk.) Sacc.	1052	duff/soil	infrequent
<i>Entoloma serrulatum</i> (Fr.) Hes.	1069	duff/soil	infrequent

**Family Gomphidiaceae**

<i>Chroogomphus vinicolor</i> O.K. Miller	1021	duff/soil	common
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**Family Lentinellaceae**

<i>Lentinus strigosus</i> (Schew.) Fr.	1001	lignicolous	abundant
<i>Lentinus lepideus</i> (Fr.:Fr.)Fr.	1010	lignicolous	common
<i>Lentinus tigrinus</i> (Bull.:Fr.)Fr.	1082	lignicolous	infrequent
<i>Panellus stipticus</i> (Fr.) Karst.	1012	lignicolous	infrequent
<i>Pleurotus ostreatus</i> (Fr.) Kummer	1019	lignicolous	common
<i>Pleurotus platypus</i> (Cke. & Mass.) Sacc.	1028	lignicolous	infrequent

**Family Lepiotaceae**

<i>Lepiota americana</i> Peck	1089	soil	common
<i>Lepiota procera</i> (Fr.) S.F. Gray	1022	soil/duff	common
<i>Lepiota humei</i> Murr.	1068	duff/soil	infrequent
<i>Lepiota clypeolaria</i> (Bull.:Fr.) Kummer	1091	soil/duff	infrequent
<i>Chlorophyllum molybdites</i> (Fr.) Sacc	1074	soil/duff	abundant
<i>Leucoagaricus naucus</i> (Fr.) Singer	1076	soil	infrequent

**Family Pluteaceae**

<i>Pluteus cervinus</i> (Schaeffer.Fr.)Kummer	1105	lignicolous	common
<i>Pluteus burserae</i> Singer	1065	soil	infrequent

**Family Russulaceae**

<i>Lactarius controversus</i> (Fr.:Fr.)Fr.	1023	soil/duff	infrequent
<i>Lactarius hygrophoroides</i> Beck & Curt.	1030	soil	infrequent
<i>Lactarius corrugis</i> Peck	1110	soil/duff	infrequent
<i>Lactarius indigo</i> (Schw.) Fr.	1024	soil/duff	abundant
<i>Lactarius maculatipes</i> Burl.	1056	soil	infrequent
<i>Lactarius piperatus</i> (Fr.) S.F. Gray	1102	soil/duff	infrequent
<i>Lactarius volemus</i> (Fr.) Orton	1106	duff/soil	infrequent
<i>Russula brevipes</i> Peck.	1135	duff/soil	abundant
<i>Russula emetica</i> group	1107	soil	common
<i>Russula foetens</i> (Fr.) Fr.	1096	soil/duff	common
<i>Russula laeta</i> Moll.	1084	soil	infrequent
<i>Russula maculata</i> Quel.	1054	soil/duff	infrequent

**Family Schizophyllaceae**

<i>Schizophyllum commune</i> Fr.	1047	lignicolous	common
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**Family Strophariaceae**

<i>Stropharia ambigua</i> (Pk.) Zel.	1043	soil	abundant
<i>Psilocybe coprophila</i> (Bull.:Fr.) Kummer	1085	duff	common
<i>Psilocybe semilanceata</i> (Batsch:Fr.) Quel.	1114	duff/soil	infrequent

**Family Tricholomataceae**

<i>Armillaria mellea</i> (Fr.) Karst.	1058	lignicolous	common
<i>Collybia butyracea</i> (Fr.) Quel.	1123	soil/lignicolous	common
<i>Collybia dryophylla</i> (Fr.) Kummer	1094	lignicolous/soil	infrequent
<i>Collybia gibba</i> (Pers.) Kummer	1053	soil/duff	infrequent
<i>Collybia personata</i> (Fr.) Orton	1048	soil	common
<i>Clitocybe flaccida</i> (Fr.) Quel.	1080	soil	infrequent
<i>Mycena clavicularis</i> (Fr.) Gillet	1130	soil	infrequent
<i>Mycena pura</i> (Fr.) Kummer	1133	soil	infrequent
<i>Mycena overholtsii</i> Smith & Solheim	1136	soil	infrequent
<i>Mycena maculata</i> Karst	1149	soil/duff	common
<i>Mycena tintinabulum</i> (Fr.) Quel	1138	duff/soil	common



<i>Mycena polygramma</i> (Bull.) S.F. Gray	1156	soil	infrequent
<i>Hohenbulla peltaloides</i> (Berk) Singer	1127	lignicolous/duff	common
<i>Panus crinitus</i> (L.: Fr.) Singer	1059	lignicolous	infrequent
<i>Trocholoma portentosum</i> (Fr.) Quel	1155	soil/duff	infrequent
<i>Tricholomopsis</i> sp.	1139	soil	infrequent
<i>Melanoleuca psuedoluscina</i> Bon.	1157	soil	infrequent
<b>Family Polyporaceae</b>			
<i>Clavulina coniculata</i> Fr.	1159	duff/lignicolous	common
<i>Coltricia perennis</i> Fr.	1147	lignicolous	common
<i>Cerrena unicolor</i> Fr.	1141	lignicolous	common
<i>Daedaleopsis confragosa</i> (Bolt.) Schroet	1153	lignicolous	common
<i>Daedaleopsis tricolor</i> Fr.	1143	lignicolous	infrequent
<i>Fomes formentarius</i> (Fr.) Kick	1146	lignicolous	common
<i>Fomitopsis pinicola</i> (Fr.) Kar.	1148	lignicolous	common
<i>Ganoderma applanatum</i> (Fr.: Wall.) Pat.	1152	lignicolous	common
<i>Ganoderma tsugae</i> Murr.	1152A	lignicolous	abundant
<i>Gleophyllum sepiarium</i> (Fr.) Karst	1086	lignicolous	infrequent
<i>Hydnum imbricatum</i> Fr.	1154	lignicolous	infrequent
<i>Hydnum repandum</i> L.	1158	lignicolous	infrequent
<i>Lenzites betulina</i> (L.) Fr.	1040	lignicolous	common
<i>Mycorrhaphium adustum</i> (Wild.) Karst.	1100	lignicolous	infrequent
<i>Polyporus brumalis</i> Fr.	1103	lignicolous	common
<i>Stereum unicolor</i> (Wild.) S.F. Gray	1104	lignicolous	infrequent
<i>Trametes versicolor</i> (L) Pil.	1060	lignicolous	abundant
<i>Trametes gibbosa</i> (Pers.) Fr. (L.)	1108	lignicolous	common
<b>Family Gasteromyceae</b>			
<i>Bovista radicata</i> Berk. & Curt	1049	soil/duff	common
<i>Geastrum saccatum</i> Fr.	1050	duff/soil	infrequent
<i>Geastrum pectinatum</i> Pers.	1095	soil/duff	infrequent
<i>Lycoperdon pyriforme</i> Schaeff.:Fr.	1118	duff/soil	common
<i>Lycoperdon perlatum</i> Pers.	1098	duff/soil	infrequent
<i>Scledroderma citrinum</i> Pers.	1109	duff/soil	common
<i>Scledroderma veruucosum</i> Bull.:Pers.	1061	duff/soil	infrequent
<i>Pisolithus tinctorus</i> (Pers.) Cok. & Couch	1083	duff/soil	abundant

## DISCUSSION

The structure and composition of the Kingwood fungal flora is similar to Florida and neighboring states as well as the Big Thicket area of east Texas (see Lewis et al. 1981; 1984). Most of the mycorrhizal species, especially members of Amanitaceae, Boletaceae, Cortinariaceae and Russulaceae, are an integral part of this ecosystem which also contributes to a comparatively stable and balanced species diversity in Kingwood Forest in spite of increasing environmental pressures.

The mycorrhizal species outnumber other agarics in this study. Members of the Ascomycetes are not absent in Kingwood Forest but their poor representation in this study is probably due to the time and season of the collection. These as well as other fungi belonging to the Gasteromycetes and Polyporaceae probably do occur in the area but were neither collected nor encountered. Additional studies of the area are needed to verify the presence of these additional species.

*Amanita flavaconia*, *A. livida* and *A. pubescens* and several members of Boletes and members of Russulaceae are of temperate origin while the impact of the tropical and Mexican (South American) species are obvious in the increasing numbers of *Pleurotus*, *Panus*, *Lentinus* and allied species. Most of the agarics collected are temperate in habitat which appear similar in all respects to their European counterparts. Therefore certain connections could be drawn with fungal floras of neighboring continents. Some species such as *Laccaria laccata*, *Volvariella speciosa* (and closely related members *V. bombycina* and *Pholiota squarrosa*) as well as some of the brown-spored members of Cortinariaceae and the whole spectrum of white-spored Tricolomataceae would be expected to occur in this area of east Texas.

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A COMPARISON OF TRACE ELEMENT CONCENTRATIONS  
IN *CORBICULA* SP. (BIVALVIA: CORBICULIDAE)  
AND SEDIMENT FROM THE CONCHO RIVER BY  
ENERGY DISPERSIVE X-RAY FLUORESCENCE

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**Abstract.**—Asian clams (*Corbicula* sp.) and river sediment were collected from the Concho River approximately 1 kilometer west of Paint Rock, Texas. Both animal tissue and sediment were analyzed for concentrations of P, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Hg, Se, Br, Rb, and Sr using energy dispersive x-ray fluorescence spectroscopy (EDXRF). Differences in elemental concentrations between bivalve tissue and sediment were determined. Of the sixteen elements tested, concentrations of P, Ni, Cu, Zn, Se, and Br were higher in the bivalve tissue. Ti, V, Cr, Mn, Fe, Hg, Rb, and Sr concentrations were higher in the sediment. There was no significant difference between the concentration of K in the clam and the sediment. A statistical comparison of Ca concentration could not be determined because the concentration of this element in both the animal and the sediment was frequently greater than the instrument could measure (10,000 ppm).

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There have been many studies conducted that have used bivalve mollusks to determine the presence and bioavailability of elements in heavily industrialized regions. The most commonly studied elements are the heavy metals Mn, Cu, Zn, Cd and Pb (Havlik & Marking 1987). Also, most studies have concentrated on the quantification of elements already known or suspected to be present. Relatively few studies have been conducted as a qualitative and quantitative analysis of several elements at a single site. A major problem that must be confronted by researchers seeking to determine the presence or absence of elements in a particular environment is time required for laboratory analysis. Atomic absorption is the technique most commonly used to detect the presence of trace quantities of elements. This technique, while sensitive to dilute concentrations, is time consuming because only one element can be studied at a time. Sample preparation is often a time consuming endeavor when using atomic absorption.

This study was an effort to determine the concentrations of several elements, in sediment and *Corbicula* sp., in a timely manner. Furthermore, the authors made statistical comparisons of those concentrations to determine the possible use of *Corbicula* sp. as a bioindicator in the Concho River.

## METHODS AND MATERIALS

*Organisms and sediment.*—*Corbicula* sp. used in this study were taken from a mussel sanctuary in Concho County, Texas. The mussel sanctuary is a segment of the Concho River (Section 1421 of the Colorado River watershed) that is the protected habitat of the Texas pimpleback (*Quadrula petrina*) and is excellent habitat for Asian clams. Eighteen clams were collected from a 0.25 m<sup>2</sup> area in water 0.5 m deep. The clams were taken from a population whose density exceeds 2200/m<sup>2</sup> (Howells et al. 1996). Because the absolute ages of bivalves are difficult to assess (Neves & Moyer 1988), and weight is generally relative to age for a given population, all tissue analyzed came from adult bivalves with a mean weight of 21.78 g ± 3.99 g. All of the specimens were collected, placed on ice for approximately 20 hours, weighed, and then frozen until samples were prepared for analysis. Sediment used in this study was collected by scraping a rinsed, 250 mL Nalgene bottle through the substrate at the clam collection site at a depth of approximately 1 cm.

*Tissue preparation.*—Frozen clams were allowed to thaw for 15 minutes to facilitate excision of the soft tissue from the valves and a gill was removed. Gill tissue was analyzed because, according to Naimo (1995), gills are more likely to contain traces of metal than other tissues. A plastic spatula and plastic forceps were used instead of metal to avoid possible sample contamination. A single gill was macerated with a PTFE pestle and a glass mortar to break the connective tissue between the cells. Triplicate samples were prepared from each gill by transferring 5 µL of macerated gill material to each of three Formvar (Ladd Research Industries, Inc., Burlington, VT) films with a micropipet. Formvar films were prepared from a 2% Formvar solution in 1,2-dichloroethane, by placing single drops of the Formvar solution onto the surface of distilled water. The resulting thin film was then attached onto a 35 mm carousel slide by raising the slide through the film from beneath the water's surface. The final step in sample preparation was to fix, with glue, a second Formvar film over the sample so that each of the triplicate samples of gill material was between two thin layers of Formvar. The samples were allowed to dry for 30 minutes at ambient temperature before being analyzed.

*Sediment preparation.*—Sediment and water in the collection bottle were mixed with a vortex mixer. After the large particles settled and most of the sample water was decanted, the top layer consisted of sand

and clay sized particles with some organic matter. Five  $\mu\text{L}$  of the top layer was pipetted onto each of nine Formvar films. The Formvar film preparation and the remaining steps of sediment preparation are exactly like those in the tissue preparation steps.

*Instrumentation.*—All tissue and sediment samples were analyzed with a Philips Electronic Instruments PV9550HP energy dispersive x-ray fluorescence spectrometer interfaced with an EDAX PV9800 analyzer system. Primary x-rays were generated at an Rh target and were filtered with a thin-foil Rh filter. All samples were irradiated for 1000 live seconds with a 35kV x-ray tube potential and a 30 mA current. For all of the elements reviewed here, except Hg, the net intensities of fluorescent x-rays ( $I_E$ ), which were proportional to the mass of each element in the sample, were obtained by subtracting the background radiation and integrating the  $K_\alpha$  and  $K_\beta$  peaks at their respective energies in keV. Because the primary x-rays from a Rh target are not energetic enough to elicit  $K_\alpha$  and  $K_\beta$  lines from Hg, the net intensities of fluorescent x-rays of Hg were obtained by subtracting the background radiation and integrating the  $L_\alpha$  and  $L_\beta$  peaks respectively. The intensity of 1% of the Compton scatter peak ( $I_{cs}$ ), which was proportional to the mass of the sample, was integrated in a window of 19.80 keV to 20.40 keV. The ratio of the net intensity of fluorescent x-rays to the intensity of 1% of the Compton scatter peak, ( $I_E/I_{cs}$ ), was later used to assess the concentration of each element.

Calibration curves of each element in this report are stored on the hard-drive of the EDAX PV9800. Each calibration curve was determined by the standard method of plotting the  $I_E/I_{cs}$  ratios of a given element versus the known concentrations of that element. Minimum detection limits were determined by subtraction of blank values from each measured elemental concentration. In this study, the concentrations of P, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Hg, Se, Br, Rb, and Sr in *Corbicula* sp. and sediment were determined from the stored calibration curves in the EDAX PV9800. All concentrations are reported in parts per million (ppm) dry weight.

## RESULTS

Concentrations, in ppm dry weight, from each of the three spectra were averaged, tested for outliers using the American Society for Testing Materials  $T_n$  test, and recorded as a single concentration. The concentration of each element, from each animal, was then averaged, tested for outliers, and is reported in Table 1.

Table 1. Average concentrations (ppm) of elements in sediment and *Corbicula* sp determined by EDXRF. Also recorded in this table is the number (n) of animals used to determine the mean concentration of each element in that animal. (\*) Denotes elements with higher concentrations in bivalve tissue.

Element	Concentration in ppm dry weight		n
	Sediment	<i>Corbicula</i> sp.	
*P	0	0.9 ± 0.2	17
K	0.4	0.3 ± 0.1	18
Ca	**	**	
Ti	760	0.0 ± 0.0	18
V	60	8 ± 7	18
Cr	20	4 ± 5	18
Mn	260	18 ± 8	16
Fe	**	240 ± 130	16
*Ni	0	3 ± 2	18
*Cu	17	120 ± 31	18
*Zn	49	230 ± 70	17
Hg	7.0	0.0 ± 0.0	18
*Se	0.53	8.3 ± 2.4	18
*Br	20	52 ± 12	18
Rb	53	2.4 ± 2.7	18
Sr	450	99 ± 30	18

\*\* > 1.0 X 10<sup>4</sup> ppm

The single sample of sediment was used to produce nine spectra. The concentrations of each element, from each of the nine spectra were averaged, tested for outliers using the  $T_n$  test, and reported in Table 1.

Sixteen one-tailed t-tests were performed at a confidence interval of 0.05 to determine if the difference in the concentration of each element was significant between the sediment and the *Corbicula* sp. Results of the t-tests suggest that this bivalve significantly concentrates P, Ni, Cu, Zn, Se, and Br above levels of those elements in the sediment.

## DISCUSSION

Of the 16 elements tested for in this study, only P, Ni, Cu, Zn, Se, and Br were bioconcentrated by *Corbicula* sp. It is interesting to note that *Corbicula* sp. contained no Ti or Hg in the gills even though there were significant levels of both in the sediment. Though more research in this area is necessary to draw conclusions, the alkali and alkali-earth metals in this study may very well be regulated by active transport

mechanisms in the *Corbicula* sp. All of the Group I and Group II metals did have measurable concentrations in the animals. Other elements, most notably transition metals that were not concentrated in *Corbicula* sp., are seldom available as free metal ions in basic waters such as those which flow over the limestone bed of the Concho River and may not have been bioavailable to the *Corbicula* sp. Bioavailability and subsequent accumulation are dependent upon many biological and physical factors, some of which have not yet been identified (Elder & Collins 1991). Therefore, it is only suggested that *Corbicula* sp. be considered as a bioindicator in the Concho River for the elements that this study has found the animal to bioconcentrate.

As a method of determining elemental concentration in tissue and sediment, EDXRF should be considered. Though not as sensitive as some other methods, it is sufficiently sensitive when analyzing tissue from animals that are known to concentrate the elements in question. EDXRF is certainly time efficient when compared to more common methods since many elements can be evaluated at once. In this study, sample preparation was also kept to a minimum since weighings of tissue and sediment were unnecessary to obtain concentration values. Sample preparation is also simplified because EDXRF can evaluate any chemical form of a given element.

#### ACKNOWLEDGMENTS

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REPRODUCTIVE STAGE AND OSMOREGULATION ABILITY  
OF THE ESTUARINE CLAM *RANGIA CUNEATA*  
(BIVALVIA: MACTRIDAE)

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**Abstract.**—Osmoregulation experiments were conducted on *Rangia cuneata*, over a range of salinities from <0.5 ppt to 20 ppt, to determine if the reproductive stage and high gonadal biomass affected osmoregulation ability and survival. Clams in the ripe and partially spent stages with 27% gonadal biomass and in the ripe stage with 39% gonadal biomass were not able to osmoregulate at <0.5 and 1 ppt salinities, with 100% mortality after 48 and 72 hours, respectively. Clams in the partially spent stage, in the process of cytolyzing unspawned gametes, with 30% gonadal biomass survived the entire 360-hour experiment. These data indicate the energy cost of being iteroparous, resulting in high gonadal biomass, and of osmoregulation, in combination, resulted in the observed mortalities.

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Three osmoregulation experiments were conducted on the estuarine clam *Rangia cuneata* (Gray) to determine if the lack of ability to osmoregulate could have been responsible for observed mortalities during previous laboratory exposures (McConnell & Harrel 1995) to non-lethal concentrations of copper (20-25  $\mu\text{g/L}$ ). Copper exposures were conducted during September 1991 and December 1992 at salinities <0.5 ppt and resulted in 100% mortality in both control and exposure groups. During both experiments clams were in the ripe reproductive stage (Cain 1973; 1975; Jovanovich & Marion 1989) and gonadal biomass was 49% of total biomass during September and 39% during December. This suggested that the clams were unable to osmoregulate due to high reproductive energy demand.

#### MATERIALS AND METHODS

During October and December 1992 and February 1993 clams, were collected from Fence Lake, located between the Intracoastal Waterway and the Gulf of Mexico in Sea Rim State Park, Jefferson County, Texas and osmoregulation experiments were conducted. Only clams between 40-60 mm were used to minimize size or age differences. Before each experiment, 200 clams were collected and maintained for 24 hours in the laboratory in Fence Lake water. Clams were then transferred to a large

holding tank containing a mixture of reagent grade type II water (APHA-AWWA-WPFC 1989; Peltier & Weber 1985) and Instant Ocean<sup>®</sup> to form the desired salinity, and maintained for an additional 48 hours. Hopkins et al. (1973) found no differences in osmoregulatory abilities between *R. cuneata* exposed to natural estuarine water and Instant Ocean<sup>®</sup> water. Ten specimens were then placed into each glass jar containing five liters of reagent grade type II water and Instant Ocean<sup>®</sup> to form salinities of <0.5, 1, 5, 10, 15, and 20 ppt, with duplicates of each salinity. Two controls, of the same salinity as Fence Lake at the time of collection, were also established. Salinities of control groups were 12 ppt for October, 7 ppt for December, and 5 ppt for February. All experiments were conducted at  $22^{\circ}\text{C} \pm 2$ . Salinity and temperature were measured with a YSI Model 33 S-C-T meter.

Hemolymph osmolalities were determined on two clams from each salinity at time zero, every 4 to 12 hours until hour 24, every 12 to 24 hours until hour 72, and periodically thereafter until 100% mortality occurred or 360 hours passed. Hemolymph was extracted from the heart ventricle with a 10  $\mu\text{L}$  capillary pipette drawn to a fine point and osmolalities were determined using a Wescor 5500 vapor pressure osmometer. Salinity standards and blanks were introduced periodically to verify instrument accuracy.

To determine average percent gonadal biomass, eight clams were removed from their shells and weighed to the nearest 0.001 g wet weight. Gonadal and somatic tissues were separated under a stereo-scopic microscope by dissection, blotted dry, weighed, then dried for 24 hours at  $90^{\circ}\text{C}$ . Dry tissue was then weighed to determine somatic tissue biomass, gonadal tissue biomass, and percent gonadal biomass. Reproductive stages were determined microscopically as defined by Cain (1973; 1975) and Jovanovich & Marion (1989).

## RESULTS

During osmoregulation experiments, average percent gonadal biomass and reproductive stages were: 27% and ripe-partially spent in October, 39% and ripe in December, and 30% and partially spent with cytolysis of unspawned gametes occurring in February. During October and December the gonads were orange in color and had a hard solid consistency. During February, when cytolysis was occurring, the gonads were pale yellow to white and had a soft mushy consistency. Osmolalities for the same salinity for the same experiment were within 2% of each other.

During all experiments, clams in  $<0.5$  ppt (13 mOsm/L) and 1 ppt (29 mOsm/L) salinities were active osmoregulators and reached equilibrium hemolymph osmolality of about 100 mOsm/L in 4 to 18 hours (Figs. 1, 2 and 3). However, during October and December, 100% mortality occurred after 48 hours in  $<0.5$  ppt salinity and after 72 hours in 1 ppt salinity (Fig. 4). During February, clams exposed to these salinities survived the entire 360-hour experiment (Figs. 3 and 4). During all experiments, clams exposed to 5 ppt salinity (145 mOsm/L) were slight osmoregulators and hemolymph osmolality varied from 134 to 205 mOsm/L, with no mortalities. During the 10 and 20 ppt salinity exposures clams were osmoconformers and all survived the 360-hour experiments (Figs. 1, 2, 3 and 4).

### DISCUSSION

*Rangia cuneata* possesses the osmoregulatory mechanisms of both freshwater and marine bivalves and can adapt to salinities ranging from freshwater to 20 ppt (Hopkins et al. 1973; Saintsing & Towle 1979; Otto & Pierce 1981a; 1981b; Deaton 1981). Hopkins et al. (1973) estimated that a minimum energy requirement of 2.0% of the metabolic energy would be required for osmoregulation at salinities of 3 ppt or lower. This assumed that the energy expense would equal the thermodynamic minimum. Thus, this number would be higher during periods of rapid osmotic adjustment. Additionally, energy allocations for general metabolism, growth, and reproduction can affect survival during stressful times (Begon et al. 1990; Harrel 1993). The most stressful period in the life of adult *R. cuneata* may occur during the ripe and partially spent reproductive stages, when gonadal biomass is greater than 25% of total biomass. Thus, this may have resulted in the observed mortalities at low salinities during the October and December experiments, when energy demands for normal metabolism, maintenance of gametes, and osmoregulation were high.

In February, clams were partially spent, cytolysis of gametes was occurring, and no mortalities occurred. The cytolysis of gametes may represent a period of lower relative energy demand. Thus, more energy can be allocated for osmoregulation. Also, some of the cytolysed gametes may have been phagocytized providing more energy for osmoregulation. Jovanovich & Marion (1989) reported that phagocytic cells were common in the lumina of follicles of spent *R. cuneata*.

During the October and December experiments, some clams that were moved from control salinities (12 ppt and 7 ppt, respectively) into

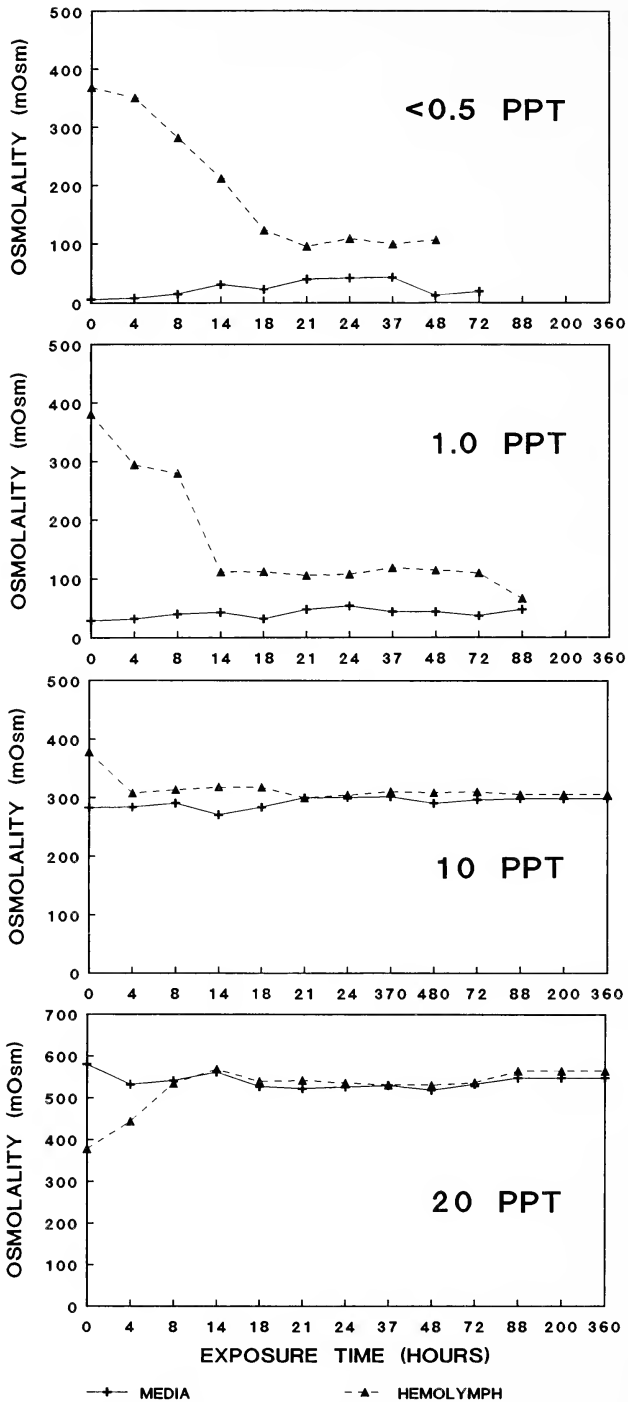


Figure 1. Osmolality of *Rangia cuneata* hemolymph and the exposure medium during the October 360-hour experiment. When lines end 100% mortality had occurred.

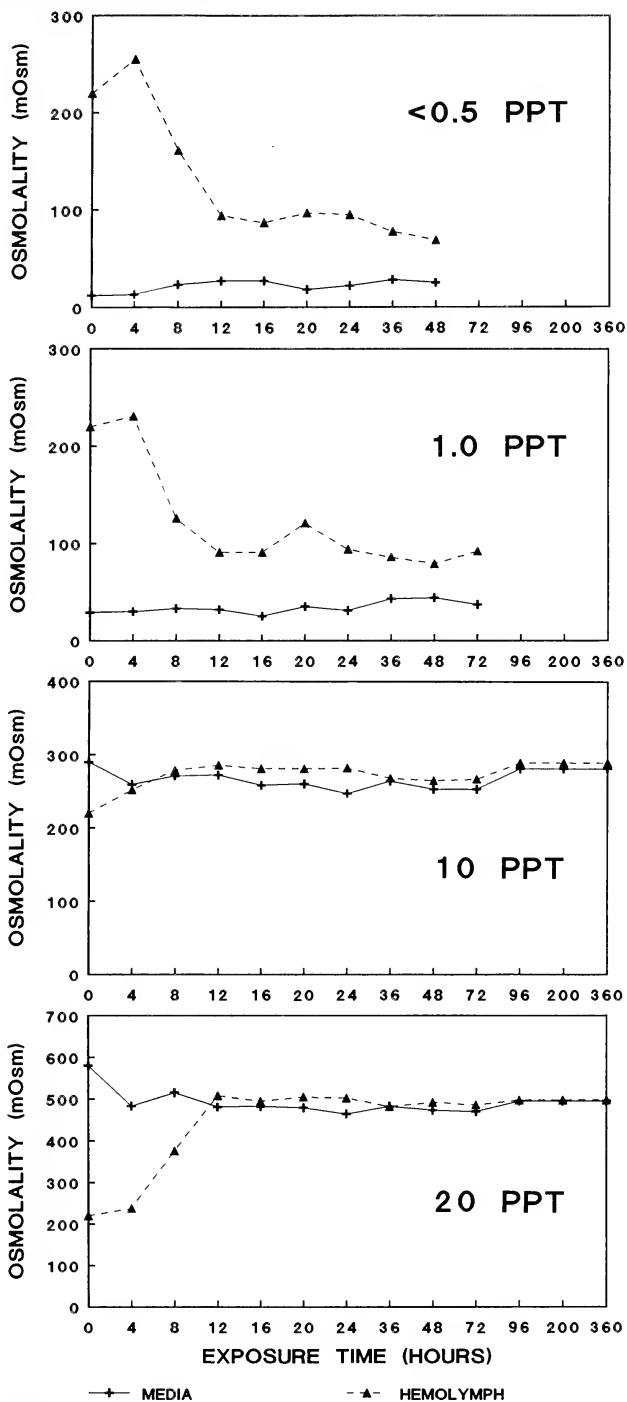


Figure 2. Osmolality of *Rangia cuneata* hemolymph and the exposure medium during the December 360-hour experiment. When lines end 100% mortality had occurred.

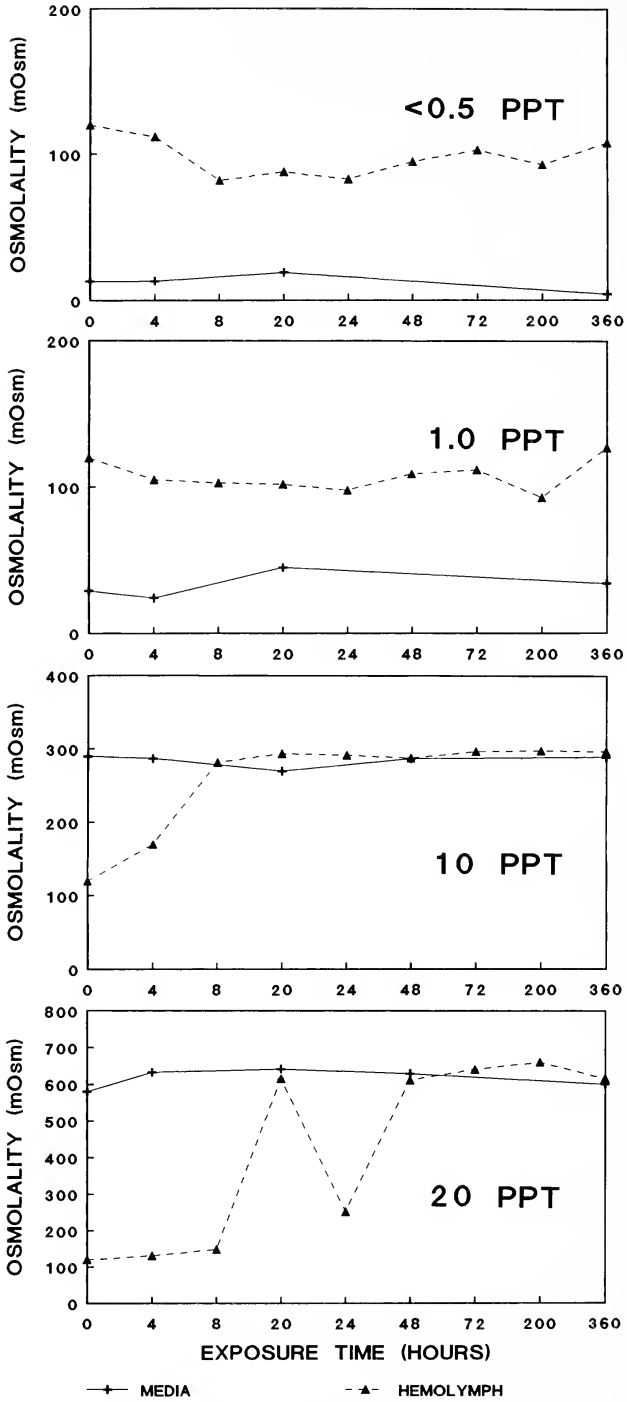


Figure 3. Osmolality of *Rangia cuneata* hemolymph and the exposure medium during the February 360-hour experiment.

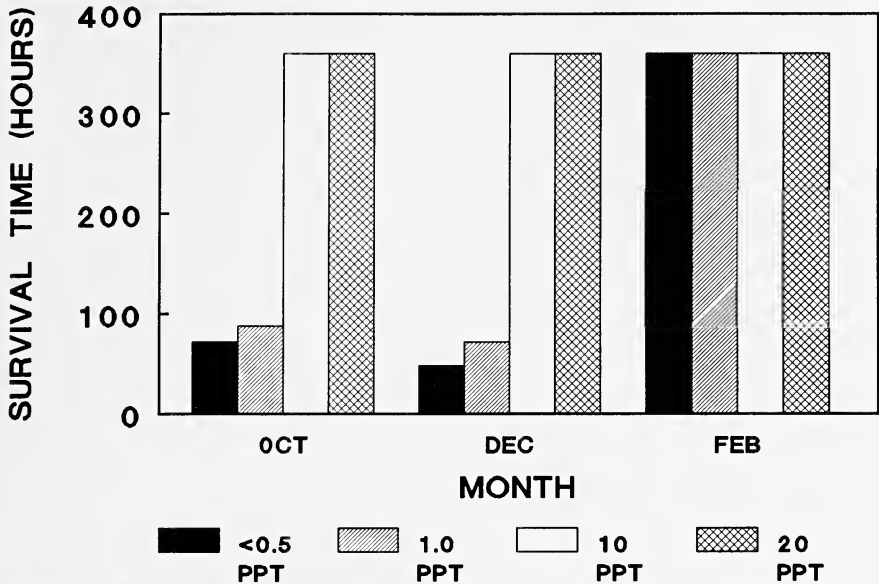


Figure 4. Survival times of *Rangia cuneata* exposed to <math><0.5</math>, 1, 10, and 20 ppt salinities during the osmoregulation experiments.

<math><0.5</math>, 1, and 20 ppt salinities spawned after 24 to 48 hours. The clams moved into the <math><0.5</math> and 1 ppt salinities were osmoregulators and all died. The clams moved into the 20 ppt salinity were osmoconformers and all survived the 360-hour experiment.

In February, six clams maintained in 20 ppt salinity water were placed directly into <math><0.5</math> ppt water and six clams maintained in <math><0.5</math> ppt water were placed directly into 20 ppt water. These manipulations resulted in no mortalities, indicating that the lower acclimation salinity in February was not a factor in survival and that energy for osmoregulation was not a problem during cytolysis of gametes.

At higher salinities (10 ppt or higher), *R. cuneata* is an osmoconformer; conserving energy for reproduction, growth, and survival during stressful times. However, the energy cost of being iteroparous and more frequent spawning, as occurs at less stable higher salinity sites, seems to be more stressful than the cost of constant osmoregulation, as occurs at low salinity sites. This may explain the phenomenon of larger and older *R. cuneata* living in fresher waters along a salinity gradient as reported by Ladd (1951), Gunter (1961), Pfitzenmeyer & Drobeck (1964) and Harrel (1993). Individuals living in fresher waters are less often subjected to salinity changes required to induce spawning (Cain 1973; 1975; Hopkins et al. 1973), thus more energy would be available for growth and survival, increasing longevity. Clams located where

salinity changes frequently induce spawning would have less energy for growth and survival, resulting in shorter longevity.

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LOTIC FRESHWATER MUSSELS (FAMILY UNIONIDAE)  
OF THE ANGELINA AND DAVY CROCKETT  
NATIONAL FORESTS OF EAST TEXAS

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**Abstract.**—Ten streams located in the Davy Crockett National Forest and five streams located in the Angelina National Forest of eastern Texas were surveyed during 1995 and 1996 to determine species composition and abundance of freshwater mussels. Nine species of freshwater mussels were collected from 10 of the study streams; five of the streams contained no mussels or only shells of dead specimens. Species found as well as their abundance and habitats are discussed.

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Among the 52 species of freshwater mussels (Family Unionidae) reported in Texas, 17 are listed as threatened, endangered, or of special concern (Williams et al. 1993) and one is listed as federally endangered. B. A. Steinhagen Reservoir and the adjacent Neches and Angelina rivers, located in eastern Texas, still support one of the most abundant and diverse populations of freshwater mussels remaining in Texas (Howells 1996). The 65,360-hectare Davy Crockett National Forest (DCNF) lies in the Neches and Trinity river basins with the Neches River serving as the eastern boundary (Fig. 1). The 62,422-hectare Angelina National Forest (ANF) lies in the Neches River basin, and on the north and south sides of Sam Rayburn Reservoir, an impoundment of the Angelina River (Fig. 2). These forests, along with most of eastern Texas, are characterized by slow-moving streams with normally dependable volumes of water (Neck 1986). Very little research has been conducted on the majority of these streams. This study establishes base-line data of the bivalve fauna of the small streams located in the Davy Crockett and Angelina National Forest.

#### STUDY AREA

Fifteen previously unclassified streams, which are tributaries of either the Neches or Angelina rivers, were sampled during this study. Ten streams located in the DCNF selected by the U.S. Forest Service (USFS) for study included: (1) Alabama Creek, fourth-order stream, southeast corner of DCNF, with sand-gravel-detritus substrate, sampled 9 June 1996 at the terminal end of Forest Road (FR) 523 in forest compartment 101; (2) Austin Branch, third-order stream, northwest corner of DCNF,

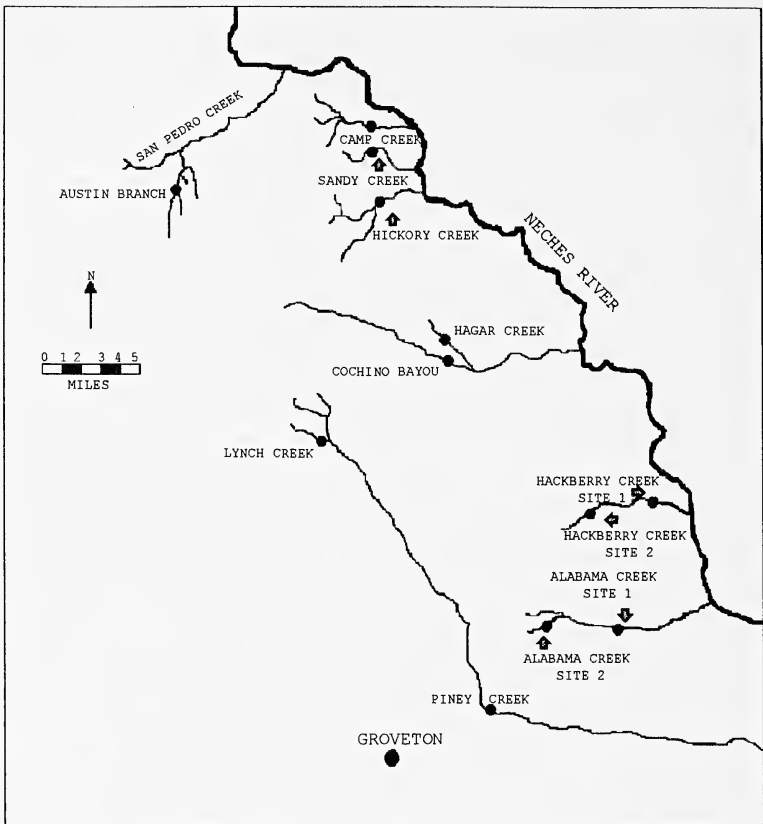


Figure 1. Map showing location of the study streams within the Davy Crockett National Forest, Texas.

with deep-shifting sand substrate, sampled 3 September 1996 at the terminal end of FR 544-A in forest compartment 2; (3) Camp Creek, third-order stream, northern DCFNF, deep, shifting sand substrate, sampled 10 September 1995 beginning at FR 511 bridge; (4) Cochino Bayou, fifth-order stream, central portion of DCFNF, loam-detritus substrate, sampled 1 October 1995 at FR 582 bridge; (5) Hackberry Creek, third-order stream, eastern DCFNF, sampled 1 October 1995 at two locations (a) Farm to Market Road 2262 bridge, clay overlain by thick sand substrate, erosion due to cattle activity, (b) Farm to Market Road 2501 bridge, hard pan clay substrate with occasional sandbars; (6) Hagar Creek, third-order stream, central portion of DCFNF, loam-detritus substrate with outcroppings of pebbles and small cobble, sampled 24 September 1995 at FR 582 bridge in forest compartment 50; (7) Hickory Creek, fourth-order stream, northern DCFNF, loam-detritus substrate,

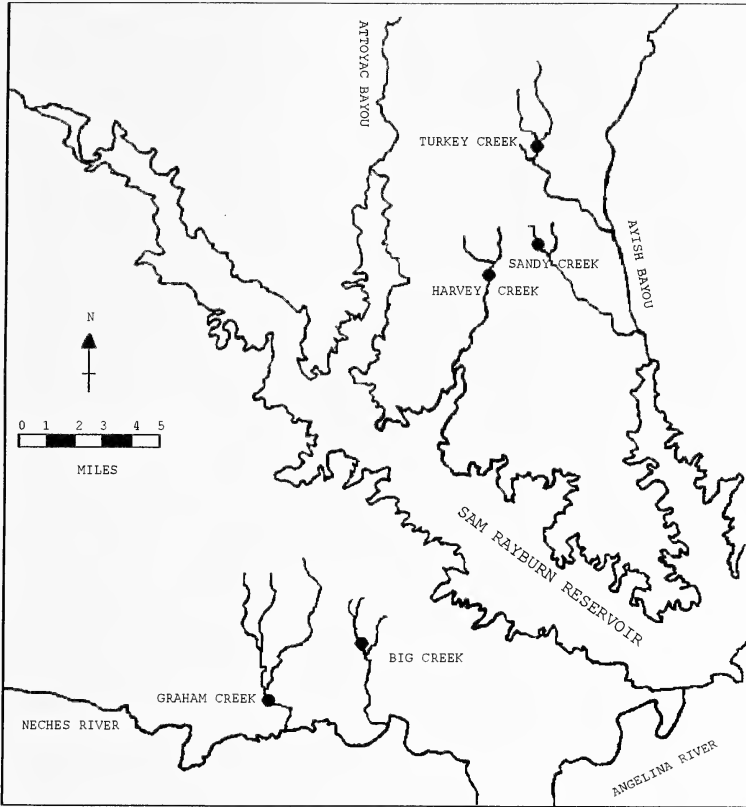


Figure 2. Map showing location of the study streams within the Angelina National Forest, Texas.

sampled 10 September 1995 at FR 511 bridge; (8) Lynch Creek, intermittent first-order stream, western DCNF, sand-loam substrate, sampled 28 November 1994 at Farm Road 2781 bridge in forest compartment 70; (9) Piney Creek, fourth-order stream, drains large portion of southeastern DCNF, loam-clay substrate, sampled 9 July 1995 beginning at FM 2262 bridge near forest compartment 98; (10) Sandy Creek, first-order stream, northern DCNF, loam-sand substrate, sampled 10 September 1995 at FR 307 bridge near forest compartment 17.

Five previously-unclassified streams were chosen for study from ANF. These included: (1) Big Creek, third-order stream, southern ANF, clay substrate, sampled 25 June 1995 at Forest Road 303 bridge near compartment 94; (2) Graham Creek, fourth-order stream, southwestern ANF, pocketed clay substrate with silt deposits in the pockets, sampled 25 June

Table 1. Species and number of individuals of freshwater mussels (family Unionidae) collected from the Davy Crockett National Forest, Texas.

Species	Alabama	Austin	Camp	Cochino	Hackberry	Hagar	Hickory	Lynch	Piney	Sandy
<i>Glebula rotundata</i>	0	0	0	0	0	0	2	0	4	0
<i>Lampsilis hydiana</i>	0	0	0	2	0	0	0	0	23	0
<i>Lampsilis teres</i>	0	0	0	0	0	0	0	0	1	0
<i>Ligumia subrostrata</i>	0	0	0	3	0	0	0	0	0	0
<i>Pyganodon grandis</i>	0	0	0	0	0	0	0	0	4	0
<i>Toxolasmus</i>										
<i>texasensis</i>	0	0	0	2	0	0	0	0	0	0
<i>Unionmerus declivus</i>	4	0	0	0	1	6	0	0	55	0
<i>Unionmerus</i>										
<i>terralasmus</i>	0	0	0	1	1	0	0	0	0	0
<i>Villosa lienosa</i>	0	0	0	0	0	0	0	0	20	1

1995 at Forest Road 314 bridge near forest compartment 98; (3) Harvey Creek, second-order stream, northeast ANF, loam-clay substrate, sampled 8 October 1995 at Forest Road 319 bridge, (4) Sandy Creek, first-order stream, northeast ANF, sand-detritus substrate, sampled on 18 June 1995 at Forest Road 307 bridge near forest compartment 18; (5) Turkey Creek, third-order stream, northeast ANF, gravel substrate, sampled 8 October 1995 at terminal end of Forest Road 342.

#### MATERIALS AND METHODS

Each study stream was sampled for two hours by wading and hand-collecting. During sampling, representative specimens of each species found were placed in large, plastic containers containing 95% ethyl alcohol. All other mussels were counted, identified, and immediately returned to the substrate. Dead mussels or shells collected were placed in labeled plastic bags. Upon transport back to the laboratory, preserved specimens were placed in wide-mouth glass jars with fresh 95% ethyl alcohol. Shells from dead mussels were cleaned, dried, and sprayed with a nonglossy acrylic sealer to prevent the epidermis from flaking. Specimens were identified to species using various taxonomic keys (Pennak 1989; Cummins & Mayer 1992; Vidrine 1993). Voucher specimens have been deposited in the Stephen F. Austin State University Invertebrate Museum, Nacogdoches, Texas.

#### RESULTS

One hundred ninety one individuals representing nine unionid species and numerous *Corbicula fluminea* (Family Corbiculidae) were collected from the streams of the Davy Crockett and Angelina National Forest (Tables 1 & 2). Ten of the streams sampled contained living mussel populations, while five did not. Live mussels were not found in Austin

Table 2. Species and number of individuals of freshwater mussels (family Unionidae) collected from the Angelina National Forest, Texas.

Species	Big	Graham	Harvey	Sandy	Turkey
<i>Glebula rotundata</i>	0	0	0	0	0
<i>Lampsilis hydiana</i>	0	14	0	0	0
<i>Lampsilis teres</i>	0	0	0	0	0
<i>Ligumia subrostrata</i>	4	0	0	0	0
<i>Pyganodon grandis</i>	0	0	0	0	0
<i>Toxolasmus texasensis</i>	3	0	0	10	0
<i>Unio merus declivus</i>	15	0	0	11	0
<i>Unio merus tetralasmus</i>	0	0	0	3	0
<i>Villosa lienosa</i>	0	0	0	0	0

Branch, Camp Creek and Lynch Creek in the Davy Crockett National Forest, nor in Harvey Creek and Turkey Creek in the Angelina National Forest. It is believed that the substrate, hydrology, or both, of these streams were not suitable to support freshwater mussels.

Piney Creek (DCNF) contained the greatest number of mussel taxa with six species present. *Unio merus declivus* was the most abundant species and accounted for 51% of the live mussels collected from this stream. This species was also the most abundant throughout the survey area and accounted for 48% of the mussels counted, and was found in six of the streams sampled. *Lampsilis hydiana* was the second most abundant species in Piney Creek and accounted for 21% of the mussels counted. *Lampsilis hydiana* was also the second most abundant species found throughout the survey area and accounted for 21% of the mussels counted, and was collected from four of the streams sampled. The third most abundant species in Piney Creek was *Villosa lienosa* which accounted for 19% of the mussels found. *Glebula rotundata*, *Pyganodon grandis* and one living individual of *Lampsilis teres* were also collected from Piney Creek. This was the only stream in which these three species were present.

Cochino Bayou (DCNF) contained the second greatest number of mussel taxa with five species present. *Lampsilis hydiana* was the most abundant species collected from this stream and accounted for 50% of the mussels counted. *Ligumia subrostrata* and *Toxolasmus texasensis* were second in abundance with each accounting for 19% of the mussels counted. One individual of *Unio merus tetralasmus* was also collected from this stream.

Big and Sandy creeks (ANF) contained three species each. *Unio merus declivus* was the most abundant species collected from both streams

accounting for 68% and 46%, respectively, of the mussels collected. *Toxolasmus texasensis* was also collected from both of the streams and was the second most abundant species in Sandy Creek accounting for 42% of the mussels counted. In addition, four individuals of *L. subrostrata* were collected from Big Creek and three individuals of *U. tetralasmus* were collected from Sandy Creek.

No living unionids were collected from the initial study site of Hackberry Creek. However, one living individual of *U. tetralasmus* and one living individual of *U. declivus* were collected from a second study site.

Alabama, Hagar, Hickory and Sandy creeks (DCNF), as well as Graham Creek (ANF) each contained only one living species of unionid. *Unio merus declivus* was the only living species collected from Alabama and Hagar creeks. *Villosa lienosa* was the only species collected from Sandy Creek, while Graham and Hickory creeks only contained *L. hydiana*.

The exotic mussel *Corbicula fluminea* was collected from two of the streams sampled. It was found in abundance in Hickory Creek and occasionally in Cochino Bayou.

#### DISCUSSION

Piney Creek and Cochino Bayou contained the greatest number of mussel species of any of the study streams. Twidwell et al. (1992) identified Piney Creek as the South Central Plains ecoregion's least impacted stream. Also, an ichthyological study conducted by Kelly (1995) found that these two streams had the highest Index of Biotic Integrity (IBI) scores of the ten study streams in the Davy Crockett National Forest. The IBI is a broadly-based biological index which has been shown to be able to assess many man induced stresses, such as sewage (Leonard & Orth 1986), siltation (Berkman & Rebeni 1987), and many other chemical effects. The freshwater mussel's reproductive strategy includes a parasitic relationship with a fish host. Upon release from the female mussel, the glochidia must attach to a suitable host species within a few hours or days, depending on the mussel species, or they will die. Mussel literature indicates only three North American unionids may transform to the juvenile stage without any host, and another species uses a salamander. Therefore, in order to sustain a healthy and diverse mussel population, healthy fish populations are essential.

The streams of the national forest were dominated by mussel species which are able to tolerate low flow conditions often found in small streams. *Unio merus declivus* and *U. tetralasmus* are able to withstand dewatering and drought (Neck & Metcalf 1988). *Unio merus declivus* was frequently found in less than 5 cm of water and occasionally above the water line. *Ligumia subrostrata* is sometimes found with *Unio merus* in small, shallow water bodies where other mussels may not occur (Howells et al. 1996).

Hackberry Creek (DCNF) is a prime example of the greatest threat to East Texas mussel populations. East Texas has fragile sandy soils and even minor modification of terrestrial vegetation results in extensive sand deposition in streams and rivers (Howells 1996). This deposition smothers existing mussels and creates an unsuitable substrate for future mussel populations. The initial sample site on Hackberry Creek was located in an area where the stream ran through pasture land and cattle activities had removed most of the vegetation from the stream banks. The water was highly stained from cattle waste and the substrate consisted of clay overlain with a thick layer of sand. No mussels were found at this site. The second site sampled on Hackberry Creek was located upstream from the cattle activity and had vegetated banks, clear water, and a hard pan clay substrate with occasional small sandbars. Live specimens of *U. declivus* and *U. tetralasmus* were collected from this location.

Piney Creek contained several mussel species generally not found in small East Texas streams. *Glebula rotundata* is typical of lower reaches of rivers just above brackish waters and is rarely found this far inland (Howells 1996). It is interesting to note that individuals with white nacre and individuals with purple nacre were both taken from this stream.

*Pyganodon grandis* was also another species not typical of small streams. This species does well in impoundments generally preferring no-flow conditions (Oesch 1984). It was found in larger pools of Piney Creek.

#### CONCLUSIONS

This study would indicate that the small streams of the Angelina and Davy Crockett National Forest still support freshwater mussel populations. However, this study was intended to provide initial base-line data for future studies. Further study of these streams and other streams in this area are needed to further document the freshwater mussel species present.

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## GENERAL NOTES

THE ANTS (HYMENOPTERA: FORMICIDAE)  
OF THE CADDO LAKE REGION OF NORTHEAST TEXAS**Carl R. Turner and Jerry L. Cook***304 University, Suite 207, Marshall, Texas 75670 and**Department of Entomology, Texas A&M University**College Station, Texas 77843-2475*

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Caddo Lake, located in northeast Texas, represents a unique bald-cypress ecosystem. It is unique not only in its origin but also in its distribution of cypress trees which are found throughout much of the lake. Caddo is the only natural lake in the state of Texas and one of the largest natural lakes in the South. This study was undertaken to determine a preliminary listing of the ant species of the islands and cypress trees in this unique habitat.

## MATERIALS AND METHODS

Specimens were collected from April through August of 1996. Only those ants that could be reached from a boat were collected from cypress trees. Specimens were collected with forceps and placed in 70% isopropyl alcohol. Specimens were later dry mounted, identified and deposited with the Texas A&M Insect Collection (Voucher No. 622).

## RESULTS AND DISCUSSION

Thirteen species of ants were collected. Table 1 lists the species and location(s) where they were collected. There are likely more species present on the islands and cypress trees than reported in this study. Sampling was limited to that part of the trees that could be reached from a boat and collection was only done during daylight hours. There is a good possibility of additional ant species living high in the cypress trees or species that are active only at night.

Nine species of ants were found on six isolated cypress trees while only seven species were found at ten collecting sites on the islands. On the cypress trees in water, one tree had four species, three trees had two species and two trees had one species. *Tapinoma sessile* was collected from a cavity in a cypress tree. One cypress tree located on the bank

Table 1. Ant species collected and collection sites.

	Number of Sites
<b>Cypress trees in water:</b>	
<i>Crematogaster lineolata</i> (Say)	2
<i>Crematogaster clara</i> Mayr	1
<i>Aphaenogaster lamellidens</i> Mayr	1
<i>Camponotus pennsylvanicus</i> (DeGeer)	1
<i>Camponotus rasilis</i> Wheeler	3
<i>Leptothorax schauimi</i> Roger	1
<i>Tapinoma sessile</i> (Say)	1
<i>Solenopsis invicta</i> Buren	1
<i>Pheidole</i> sp.	1
<b>Island collections:</b>	
	<b>Collection Site(s)</b>
<i>Crematogaster lineolata</i> (Say)	Cypress tree, fallen log & forest floor
<i>Aphaenogaster fulva</i> Roger	Cypress tree & forest floor
<i>Camponotus pennsylvanicus</i> (DeGeer)	Cypress tree (2 sites), hickory tree & forest floor
<i>Camponotus americanus</i> Mayr	Fallen log
<i>Leptothorax pergandei</i> Emery	Fallen log
<i>Solenopsis invicta</i> Buren	River bank & forest floor
<i>Formica</i> sp.	Fallen log (2 sites)

of an island had three species while one cypress tree in the interior of the island had only one species.

The only known report of an ant species living on cypress trees isolated in water is that of *Crematogaster vermiculata* Emery which has been found only when associated with bald cypress (Buren 1968). Most of the species found have not been reported on such cypress trees to the authors' knowledge. Two species of ants, *Aphaenogaster lamellidens* and *Camponotus rasilis* are not found in the checklist of Texas ants by Wheeler & Wheeler (1985). It should be noted, however, that Creighton (1950:389) reported *Camponotus rasilis* from Texas.

The number of ant species collected on the cypress trees was unexpected. Although three species of ants were collected from a cypress tree on the bank of an island, the cypress trees isolated in water appear to have more life on them such as dragonflies, spiders and insects than do the trees on the islands. Further investigation into the diversity of life on these trees and their ecology would be of great interest.

#### ACKNOWLEDGMENTS

The authors would like to thank Ed Riley for his help with the ant specimens.

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UNUSUAL NESTING OF THE MUSCOVY DUCK  
*CAIRINA MOSCHATA* IN NORTHEASTERN MEXICO

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The distribution of the Muscovy Duck *Cairina moschata* extends from southern Texas (Hidalgo, Starr and Zapata counties), and northeastern and central Mexico, south through Central America and South America to northern Argentina and southern Bolivia (Leopold 1959; Gomez-Dallmeier & Cringan 1989; Howell & Webb 1995; TOS 1995). The species nests at heights of 3-20 m in tree hollows, between palm leaves, in artificial nest boxes (Johnsgard 1975; Cruz-Nieto 1991) and rarely in rushes on the ground (Phillips 1923).

This note documents an unusual nesting of the Muscovy Duck (at the western boundary of the species' distribution) in a crevice in the wall of a cave in northeastern Mexico. The limestone cave (40 m wide by 20 m high by 35 m deep) has an east-facing entrance in a cliff about 100 m above Mexico Highway 85 and is located 3 km southwest of Ciudad Mante, Tamaulipas near the small village of El Abra (22°36'33''N, 99°01'27''W).

On 15 February 1997, a male and 11 female Muscovy Ducks were observed perched on crevices in the walls of the cave. One female was observed on the floor of the cave brooding a group of 15-18 down-covered ducklings. The species is not known to construct a nest. However, below one large crevice in the cave wall (about 15 m above the cave floor) a large pile of down had collected, likely fallen from a

nesting site above. Since the typical clutch size is 6-9 eggs (Phillips 1923; Sibley 1967; Woodyard 1982), the large numbers of ducklings under the care of a single female probably resulted from "dump nesting".

The species has an incubation period of 35 days (Johnsgard 1975). Therefore, the eggs had to have been laid in the cave in late December or early January. Rojas (1954) suggested that the nesting season begins in December; however, Leopold (1959) disagreed, finding little evidence for a fixed breeding season in Mexico. More recently, Cruz-Nieto (1991) reported the nesting season extended from May to September.

Hoffman (1992) reported that Muscovy ducklings leave the nest and follow their mother to water several days after hatching. Due to the hazardous rock covered hillside and highway that separated the nestlings from the nearest water (a small stream), it is unlikely that they would survive the passage. No evidence exist indicating the survival of this brood. Research on the Green Parakeet *Aratinga holochlora* by Eitniear & Aragon-Tapia (1997) has been ongoing for several years at the cave with no previous evidence of Muscovy duck roosting or nesting. This occurrence was likely an opportunistic nesting because of the lack of large trees in the forest nearby that would have provided suitable nesting cavities (Woodyard & Bolen 1984).

#### ACKNOWLEDGMENTS

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UTILIZATION OF CAVE SWALLOW NESTS  
BY THE CAVE MYOTIS, *MYOTIS VELIFER*,  
IN CENTRAL TEXAS

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It is known that *Myotis velifer* utilize the nests of cliff swallows, *Petrochelidon pyrrhonota* (cf. Buchanan 1958) and occasionally barn swallows, *Hirundo rustica* (cf. Jackson et al. 1982). However, there has been no record of this bat species using cave swallow, *Petrochelidon fulva*, nests as roosting sites. Buchanan (1958) attributed the use of cliff swallow nests by *M. velifer* to be prompted by a decrease in air temperature, especially when air mass direction was from the northwest. Recent studies of *M. velifer* roosting habits (Pitts & Scharninghausen 1986) suggest utilization of swallow nests by this species all year, except when temperatures are below freezing. This observation by Pitts & Scharninghausen is contrary to the previous explanation provided by Buchanan (1958).

In December 1995, several specimens of *Myotis velifer* were found occupying two types of swallow nests (cliff and cave) located 5.1 miles N of Georgetown in Williamson County, Texas. These nest-dwelling bats were discovered inside several boxed culverts along Interstate 35. This is the first record of *M. velifer* using cave swallow nests. Nests of

cave swallows are similar to those of barn swallows, being cup-shaped but having a heavy protruding lip with higher sides. The higher sides and heavier lip associated with the cave swallow nest may provide protection against the wind currents that traverse the underground culverts, thus stabilizing the micro-environment around the bat. Not all observed nests were structurally identical and considerable variation in terms of bat occupancy was observed even when multiple nests were only a few feet apart. In many cases, two to three bats were found in a single nest. Observations also revealed that all nests were occupied by male bats. This sex bias may be seasonal in that more males were found in the culverts at this time.

Extensive surveys of bats occupying similar box culverts along Interstate 45 in Leon and Madison counties, Texas, revealed several nests of barn, cave, and cliff swallows. Although *M. velifer* was not observed in these culverts, two other vespertilionid bats (*Pipistrellus subflavus* and *Myotis austroriparius*) were common (Walker et al. 1996). During the entire time that these two species were being studied, there was no record of either species utilizing swallow nests. From these observations, it appears that unlike these two species, *M. velifer* demonstrates a preference for swallow nests when roosting in culverts. Therefore, the use of swallow nests by *M. velifer* may provide a means of controlling micro-environment requirements not important to the other two species.

#### ACKNOWLEDGMENTS

We would like to thank Dr. Keith Arnold for identifying the swallow nests. Thanks also go to Jody K. Sandel for helping the senior author become familiar with bat culvert studies in the field.

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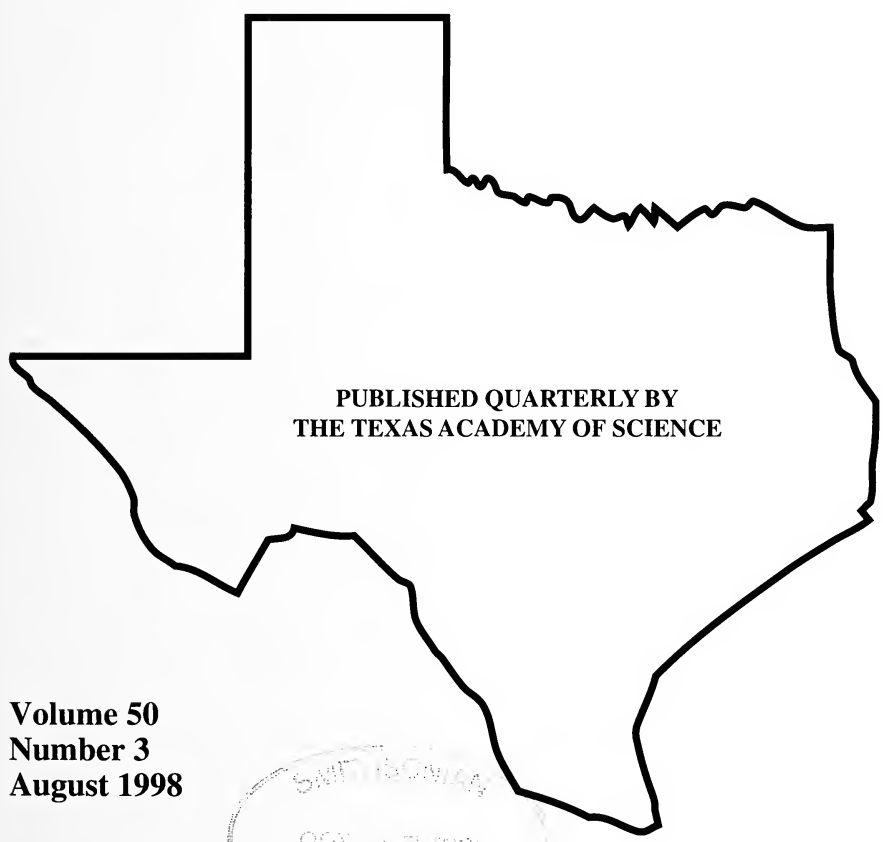
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## HYPERBARIC OXYGEN AND HYPOXIA ALTER PRODUCTION OF NITRIC OXIDE BY J774 MURINE MACROPHAGES

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**Abstract.**—This study examined changes in production of nitric oxide (NO) by cultured macrophages (M $\Phi$ ) exposed to increased oxygen tensions during changes in hyperbaric oxygen (HBO) treatments. Experiments were conducted using the murine macrophage cell line J774 which was stimulated with gamma-interferon ( $\gamma$ -INF) and lipopolysaccharide (LPS). Control cells, incubated in  $pO_2 = 20\text{mmHg}$  or  $40\text{mmHg}$ , produced significantly ( $P \leq 0.0001$ ) less NO ( $49.2 \pm 2.6\%$  and  $41.1 \pm 2.1\%$  respectively) than did cells exposed to 95% room air (RA), 5%  $CO_2$ . Cells incubated in  $pO_2 = 20\text{mmHg}$  or  $40\text{mmHg}$ , and intermittently exposed to HBO (12 PSiG) significantly ( $P \leq 0.0001$ ) increased production of NO when compared to their untreated control cells ( $148.75 \pm 18\%$  and  $101.75 \pm 6.3\%$  respectively). Cells incubated in 95% RA and treated with HBO showed little increase in production of NO ( $11.4 \pm 11.4\%$ ) when compared to control cells. There was no significant difference in production of NO when comparing HBO-treated cells that were incubated with different oxygen tensions; however, cells that were incubated with  $pO_2 = 20\text{mmHg}$  and treated with HBO produced slightly more NO ( $13.5 \pm 9.2\%$ ) than did HBO-treated cells that were incubated with 95% RA. These data show that (1) J774 murine M $\Phi$  exposed to relatively low oxygen tensions, produce less NO than do those which are incubated in 95% room air, and that (2) production of NO is augmented when these cells are intermittently exposed to HBO.

---

Improvements in rates of healing of wounds and bacterial clearance in infected tissue during intermittent exposure to hyperbaric oxygen (HBO) have been documented (Thom 1989; 1992). Increased bacterial clearance may partly stem from changes in production of nitric oxide (NO) by resident macrophages (M $\Phi$ ) in wounds while exposed to increased oxygen tensions that occur during HBO treatments. This study was undertaken to determine changes in the production of nitric oxide (NO) in the murine M $\Phi$  cell line J774 which was stimulated with gamma-interferon ( $\gamma$ -INF) and lipopolysaccharide (LPS), and exposed to HBO.

*Therapeutic use of hyperbaric oxygen.*—Since the early 1960's, there has been a resurgence in the interest of the use of hyperbaric oxygen (HBO) therapy as a clinical modality and of research into its mechanisms

of action. Its use has increased world-wide, and treatments using HBO for some conditions number well into the thousands each year in the United States alone (Thom 1989). Clinical administration of HBO is a prescribed treatment and is achieved through the intermittent use of single-patient (monoplace), or multi-place hyperbaric chambers where the patient breathes 100% O<sub>2</sub> while the pressure of the chamber is increased to a point that is higher than one ATA (atmosphere absolute or 760mmHg) (Thom 1992).

The primary effects of HBO treatment include diminution of gas bubbles, which exist as arterial or venous emboli, and an increase in alveolar pO<sub>2</sub>. However, clinical implications for the use of HBO are varied because resulting physiological effects of HBO go beyond simple elevation of arterial and tissue oxygen tension (pO<sub>2</sub>) and diminution of the size of gas bubbles. Changes in oxygen tensions alter vascular tone and perfusion (Thom 1989). Oxygen alone is bactericidal to certain organisms and works synergistically with aminoglycoside antibiotics (Mader et al. 1987; Davis & Hunt 1988; Park et al. 1992). Other work suggests that HBO is beneficial in the treatment of polymicrobial sepsis, although not necessarily because of increased antibacterial activity (Thom 1986).

The use of HBO therapy as a clinical modality is periodically reviewed and approved under the auspices of the Undersea and Hyperbaric Medical Society (UHMS). Approved indications for clinical HBO administration include; treatment of decompression sickness, carbon monoxide poisoning, clostridial myonecrosis (gas gangrene), crush injuries, compartment syndrome, or other injuries resulting in acute traumatic ischemia. Other approved uses include treatment of exceptional blood-loss anemia, refractory osteomyelitis, soft tissue radionecrosis, osteoradionecrosis, thermal burns, and treatment of non-healing wounds (Thom 1992).

Certain aspects of the immune system may ultimately be affected by their supply of oxygen (Knighton et al. 1986; Davis & Hunt 1988; Grim et al. 1990; Park et al. 1992). Bitterman et al. (1994) reported rapid alterations in CD4/CD8 T-cell ratios in peripheral blood after a standard HBO treatment protocol in both human and murine subjects. Their murine model also exhibited significant changes in CD4/CD8 T-cell ratios in lung, lymph node and splenic tissues. Increased oxygen

tensions have demonstrated an effect on activity, killing ability and other functions of polymorphonuclear leukocytes and other white blood cells in several models (Mader et al. 1980; Mader et al. 1987; Davis & Hunt 1988; Smith & Mohideen 1991; Park et al. 1992; Scannell et al. 1993). Alterations in tissue oxygen tensions have also demonstrated effects on adhesion of neutrophils to endothelial cells (Ginis et al. 1993), cellular proliferation in vitro (Grant et al. 1992; Shapiro et al. 1994), expression of vasoactive and growth factors such as endothelin-1 (Knighton et al. 1983; Kourembanas et al. 1991; Kourembanas 1993) and expression of endothelial constituted nitric oxide synthase (McQuillan et al. 1994).

Results of research have illustrated the positive effects of HBO on rates of healing of wounds as well as on bacterial clearance within infected tissue. It has been demonstrated that wounded tissue is characteristically hypoxic (Davis & Hunt 1988) and that bactericidal efficiency of polymorphonuclear leukocytes and other white cells is diminished in hypoxic conditions (Park et al. 1992). Administration of HBO increases the oxygen tension in infected or ischemic tissue by a simple increase in oxygen gradient at the capillary-tissue level (Davis & Hunt 1988). Most investigators theorize that the increase in bacterial clearance may be largely due to the return of normal function to hypoxic resident white cells, predominantly, polymorphonuclear leukocytes (Mader et al. 1980; 1987; Davis & Hunt 1988; Thom 1989).

*Macrophages.*—In a review, Park et al. (1992) reported that the ability of murine macrophages to kill opsonized *Escherichia coli* was not impaired by 'anaerobiosis' ( $pO_2 < 5\text{mmHg}$ ). More recent research indicates that hypoxia may induce the production of tumor necrosis factor- $\alpha$  in a human macrophage model (Scannell et al. 1993).

Prolonged exposure to high concentrations of oxygen adversely affects function of macrophages. Continuous exposure to as little as 40% oxygen decreases phagocytosis. A decrease in bacterial killing ability has also been reported during prolonged exposure to hyperoxia. This may be due to a decrease in the respiratory burst, demonstrated by a decrease in NADPH-oxidase activity as well as a decrease in the generation of  $O_2^-$  and  $H_2O_2$  (Park et al. 1992).

In contrast, an increase in the activity of macrophages in wounds may coincide with intermittent hyperbaric treatment. Park's review cited

research that indicated that there was no decrease in adherence or inhibition of phagocytosis during brief exposure to hyperoxia (100% O<sub>2</sub>, 1 to 3 ATA, for two hours). Another study, cited by Park, indicated an increase in bacterial clearance during a two-hour exposure to HBO. Another model demonstrated an increase in production of H<sub>2</sub>O<sub>2</sub> with corresponding changes in bacterial and fungal cytotoxicity with only brief exposures to 100% O<sub>2</sub> at normobaric pressures (Smith & Mohideen 1991).

*Cell line.*—The murine macrophage cell line, J774, originally arose from a tumor of a female BALB/c mouse in 1968, and possesses the cytologic, adherence and phagocytic characteristics typical of macrophages (Ralph et al. 1975). In recent years, these and other macrophages have been shown to produce nitric oxide (NO) when stimulated with certain cytokines, such as  $\gamma$ -INF and/or LPS (Stuehr & Marletta 1985; Ding 1988; Nathan 1992; Boudard 1994; Fujihara et al. 1994; Fujihara et al. 1994). Because of these characteristics, this cell line was selected for use in this study.

*Nitric oxide.*—Production of NO by macrophages is the result of activation of an inducible form of the enzyme, nitric oxide synthase (i-nos), which, in the presence of dissolved O<sub>2</sub> and the reduced form of nicotinamide adenine dinucleotide (NADPH), cleaves the terminal guanidino nitrogen from the amino acid L-arginine yielding NO, NADP, and L-citrulline (Moncada et al. 1991; Nathan 1992; Marletta 1994).

Production of NO is inhibited by the analog of L-arginine, N<sup>G</sup> monomethyl-L-arginine (LN<sup>MMA</sup>) (Hibbs et al. 1987; Adams et al. 1991; Moncada et al. 1991). Production of NO may be assessed by determining concentrations of its oxidized product, nitrite, in supernatant of culture medium (Ding et al. 1988; Archer 1993). Nitric oxide is a small, gaseous, paramagnetic, toxic and unstable radical in its unoxidized form (Moncada et al. 1991; Archer 1993). It is highly effective in destruction of many bacteria, fungi and cancerous cells (Hibbs et al. 1987; Lorsbach et al. 1993). It plays an important role in macrophage-mediated immunity and clearing of invading microorganisms, including yeasts, helminths, trophozoites and mycobacteria (Lin & Chadee 1992; Nathan 1992).



*Purpose of study.*—The purpose of this study was to investigate the possibility that exposure to, (1) decreased oxygen tensions or, (2) intermittent hyperbaric oxygen or, (3) a combination of both, could alter production of NO by J774 murine macrophages. The hypotheses were, (1) J774 macrophages incubated in  $pO_2 = 20\text{mmHg}$  or  $40\text{mmHg}$  will produce less NO than will cells incubated in  $pO_2 = 150\text{mmHg}$ , and that, (2) intermittent HBO will increase production of NO by these cells.

## MATERIALS AND METHODS

*Hyperbaric chamber.*—A small steel chamber was constructed from a steel pipe nipple (10.2 by 35.6 cm). After being appropriately cleaned for use with oxygen (Wellborn & James, pers. comm.), one end was permanently sealed with a threaded steel cap. The other end was fitted with a threaded steel cap containing a thick rubber O-ring to allow for repeated removal and replacement of the cap while providing a tight seal. Appropriately sized holes were drilled and tapped for placement of brass valves and pipe fittings. All fittings, valves and tubing were also carefully cleaned so that they could be safely used with oxygen. A metal bracket was constructed and mounted on the chamber to assure stability. To maintain the necessary temperature ( $37^\circ\text{C}$ ) during HBO treatments, the chamber was situated within an incubator. Gasses used for purging and compression were humidified and heated prior to entry into the chamber. A small humidifier was constructed from a section of PVC pipe. Again, appropriately sized holes were drilled and tapped to allow insertion of brass fittings, valves and copper tubing. This humidifier was then filled with distilled water and was situated within a small water-bath set at  $37^\circ\text{C}$ .

*Cell culture.*—The murine macrophage cell line, J774, was purchased from American Type Culture Collections (ATCC). Cells were incubated in Dulbecco's modified Eagle's medium (DMEM) (HEPES conversion, Sigma) with gentamicin ( $0.05\text{mg/mL}$ ), penicillin ( $100\text{U/mL}$ ), streptomycin ( $0.1\text{mg/mL}$ ), amphotericin B ( $0.25\mu\text{g/mL}$ ) and 10% heat inactivated fetal calf serum (FCS) ( $56^\circ\text{C}$ , 30 min, Sigma). Culture medium was endotoxin tested to  $0.03\text{ ng/mL}$  (*Limulus* ameocyte lysate assay) by the manufacturer. The cells were incubated in 75-mL plastic culture flasks (Falcon) in a humidified incubator containing 5%  $\text{CO}_2$  at  $37^\circ\text{C}$ .

Upon confluence of cells, which was determined by microscopic examination, the supernatant was removed from several arbitrarily chosen culture flasks and replaced with 10 to 12 mL of complete (10% FCS) DMEM (4°C) for 5 to 7 min. The plates were scraped with a cell scraper (VWR Scientific); and the cells and supernatant were collected and pooled. Each pooled aliquot was randomly assigned an experiment number. All cells for each individual experiment, (n=4) regardless of incubation conditions and HBO treatment, were drawn from this aliquot to diminish differences incurred during counting. Live cell density (number of live cells/mL medium) was determined using trypan blue exclusion (Sigma). The cells were plated into six, 24-well culture dishes (Falcon) (one well each per experimental group), at  $1 \times 10^6$  live cells/well. The cells were incubated (37°C, 5% CO<sub>2</sub>) for 2.5 to 3 hours to allow the cells to adhere to the wells. The medium was replaced with complete, warm (37°) DMEM (2mL/well) containing 100 U/mL mouse  $\gamma$ -INF (recombinant (*E. coli*), Boehringer) and 10 ng/mL LPS (*E. coli*, 0111:B4, Sigma). Concentrations approximating these have proven to be effective for induction of production of NO by the J774 macrophages and other murine macrophage cell lines (Granger et al. 1993; Boudardet al. 1994; Fujihara et al. 1994). The plates were returned to 37°C and were continually exposed to one of three humidified gas mixtures (Bailey Oxygen and Tool Company, Bryan, Texas). Gas mixtures, were, (1) 2.8% O<sub>2</sub>, 92.2% N<sub>2</sub>, and 5% CO<sub>2</sub>; (2) 6.0% O<sub>2</sub>, 89% N<sub>2</sub> and 5% CO<sub>2</sub>; or (3) room air and 5% CO<sub>2</sub>. After 8 to 12 hours, the cells which were to be treated with HBO were sealed within the hyperbaric chamber, and the HBO treatment protocol was initiated. Control cells were continually exposed to their selected gas mixtures without HBO for the duration of the 48-hour treatment protocol.

*Hyperbaric treatment of cells* (Figure 1).—Hyperbaric treatments consisted of a 10-minute purge with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> at 6 to 9 liters/minute. This was done to eliminate N<sub>2</sub> from the chamber and insured physiologic CO<sub>2</sub> levels during HBO treatment. The chamber was then pressurized with 100% O<sub>2</sub> (without CO<sub>2</sub>) to 12 PSiG for 1.5 hour. After decompression, the cells were immediately returned to their respective humidified gas mixtures at 37°C. This procedure was repeated twice daily for two days. Control cells for each group did not receive hyperbaric treatments and remained incubated at 37°C while continually exposed to the gas mixture assigned to each respective group.

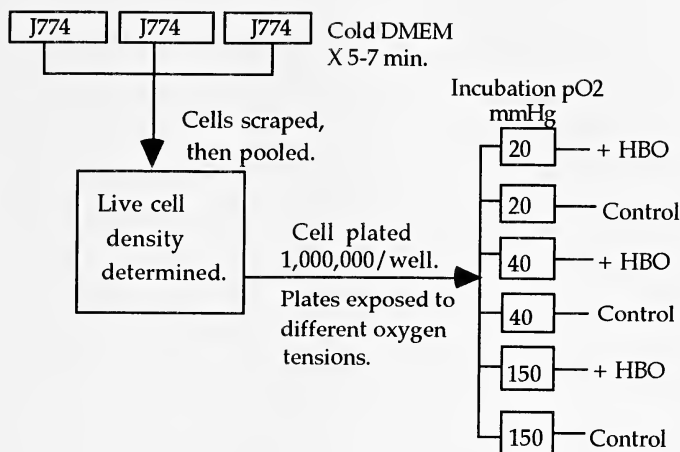


Figure 1. General schemata of cell collection, pooling, and designation of treatment.

*Nitrite assay.*—The concentration of nitrite in the supernatant of culture medium of treated and untreated cells was spectrophotometrically measured using the previously described Griess reaction (Ding et al. 1988; Boudard et al. 1994). Supernatants were collected at the end of the 48-hour trial; placed in 15-mL conical tubes (Falcon) and centrifuged at 400 g x 10 min (4°C) to remove cells from the medium before the assay was performed. The medium (150  $\mu$ L/well) was pipetted into 96-well micro assay plates (Falcon) and incubated for 10 minutes with an equal volume of Griess reagent. Griess reagent consisted of a mixture of equal parts of 1% sulfanilamide (Sigma) and 0.1% N-1-naphthylethylene diamine dihydrochloride (Sigma) in 5% H<sub>3</sub>PO<sub>4</sub> (Sigma). After 10 minutes, the absorbance of each well at 562nm was read using a kinetic micro plate reader (Molecular Devices) operating with SoftMax software (version 2.01).

*Experimental design and statistical analysis.*—Two plates of cells were randomly assigned to one of three incubation conditions. One plate of each pair was assigned to receive HBO treatment. The other plate served as a control (no HBO). After the 48-hour treatment regimen, the optical densities of supernatant from medium of treated and untreated cells were assayed spectrophotometrically as described. A standard curve was created using complete DMEM combined with known amounts of sodium nitrite. Concentrations of nitrite, which

served as the dependent (response) variables, were determined by relating the optical densities of supernatant from principal samples to the standard curve. These concentrations were analyzed by regression analysis appropriate for completely randomized design using the PC-SAS software package. A Duncan's Multiple Range Test was used to determine significance ( $\alpha = 0.05$ ;  $\beta = 0.80$ ) of the independent variables (incubation conditions and HBO). The effects of HBO on production of NO within each incubation condition were evaluated. The effects of incubation conditions within HBO treated and control (no HBO) plates were also evaluated. The percent of change of production of NO between treated and control cells within each incubation group was also analyzed for significance. The percent of change between control and treated cells was calculated using the formula:

$$\text{percent change} = \frac{\text{nitrite } \mu\text{M/mL (treated cells)} - \text{nitrite } \mu\text{M/mL (untreated cells)} \times 100}{\text{nitrite } \mu\text{M/mL (untreated cells)}}$$

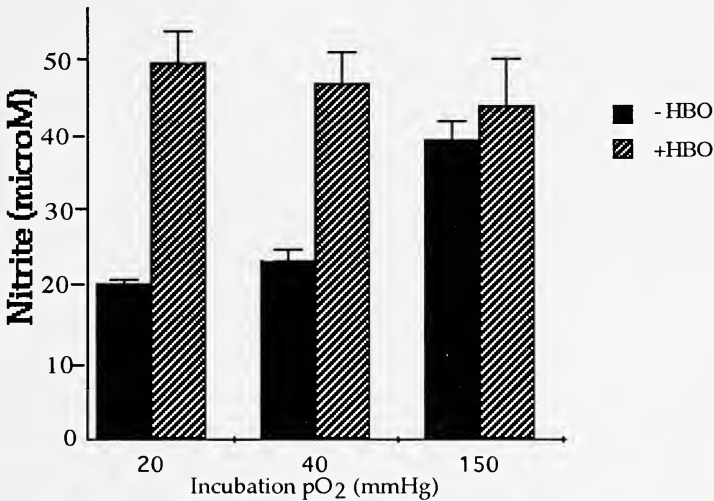
## RESULTS

Stimulated J774 macrophages that were incubated with  $pO_2 = 20\text{mmHg}$  or  $pO_2 = 40\text{mmHg}$  produced significantly ( $P \leq 0.0001$ ) less nitrite, ( $49.2 \pm 2.6\%$  and  $41.1 \pm 2.1\%$  respectively) than did cells incubated in 95% room air ( $pO_2 = 150\text{mmHg}$ ). However, HBO-treated cells exposed to  $pO_2 = 20\text{mmHg}$ , or  $40\text{ mmHg}$  exhibited a significant increase ( $P \leq 0.0001$ ) in production of nitrite when compared to their control counterparts ( $148.75 \pm 18\%$ , and  $101.75 \pm 6.3\%$  respectively).

In contrast, HBO-treated cells that were continually exposed to  $pO_2 = 150\text{mmHg}$  exhibited non-significant change in production of nitrite when compared to their untreated controls ( $11.37 \pm 11.4\%$ ). Effects of incubation oxygen tensions produced no significant difference ( $P > 0.05$ ), among HBO treated groups. However, these treated cells incubated with  $pO_2 = 20\text{mmHg}$ , consistently produced more NO ( $13.5 \pm 9.2\%$ ) than did treated cells that were incubated at  $pO_2 = 150$  although this amount was not significant ( $P = 0.44$ ) (Table 1) (Fig. 2, 3 and 4).

Table 1. Mean concentrations ( $\mu\text{M}$ ) and percent change of nitrite in culture supernatant of cells treated with HBO and/or incubated in  $p\text{O}_2 = 20\text{mmHg}$ ,  $40\text{mmHg}$  or  $150\text{mmHg}$  ( $n=4$ ). (Data from each experiment rounded to nearest whole number before analysis) (+ Not significantly different from each other)

$p\text{O}_2$	20	40	150
+HBO	$49.7 \pm 4.3$	$47.2 \pm 4.1$	$+44.2 \pm 6.4$
-HBO	$20.0 \pm 0.7$	$23.2 \pm 1.6$	$+39.5 \pm 2.7$
% Change	$148.7 \pm 18.3\%$	$101.7 \pm 6.3\%$	$11.4 \pm 11.4\%$



### Effects of HBO on Nitrite Concentrations

Figure 2. Concentrations of nitrite of HBO treated (+HBO) and control cells. Cells were exposed to different oxygen tensions ( $p\text{O}_2 = 20, 40$  or  $150\text{mmHg}$ ). Experimental groups received a 1.5 hr HBO treatment at 12 PSiG twice daily for two days ( $n=4$ ).

### DISCUSSION

During the last decade, the importance of NO within biological systems has been recognized. It is a potent regulator of vascular tone, a neuronal messenger and a mediator of microbial killing. Its production is dependent upon the cellular enzyme nitric oxide synthase, NADPH, the amino acid L-arginine and dissolved oxygen (Nathan 1992).

### Percent Change of Nitrite Concentrations

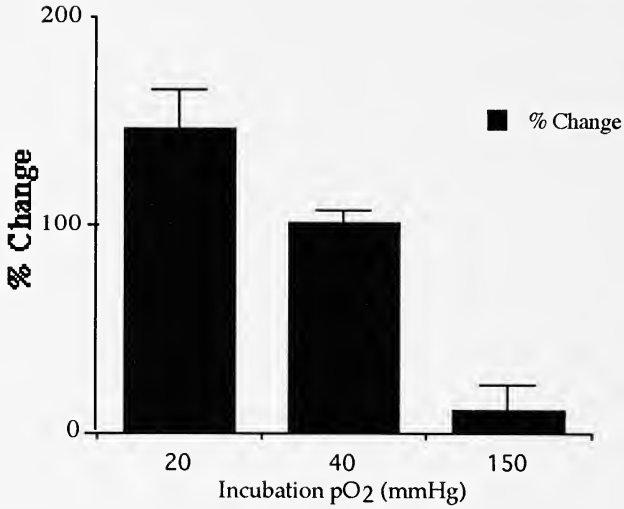


Figure 3. Representation of percent change of concentrations of nitrite between HBO treatment and control cells within different incubation conditions. ( $pO_2 = 20\text{mmHg}$ ,  $40\text{mmHg}$  or  $150\text{ mmHg}$ ) ( $n=4$ ).

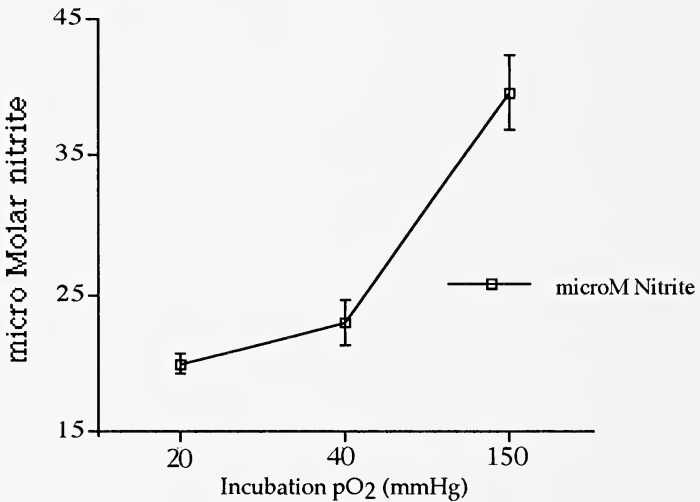


Figure 4. Change in production of nitrite in untreated cells. Values for  $pO_2 = 20$  and  $40\text{mmHg}$  are 49.2 and 41.1% less than those for  $pO_2 = 150\text{mmHg}$  respectively ( $n=4$ ).

Hypoxia is characteristic of nearly all wounds and infected tissue (Davis & Hunt 1988). Treatment with HBO increases tensions of

oxygen in these tissues restoring function to fibroblasts, endothelial cells and resident white cells. Effects of HBO on rates of bacterial clearance within infected wounds and other tissue has been largely attributed to restoration of function of polymorphonuclear leukocytes in wounds. Models investigating rates of phagocytosis and oxidative killing have demonstrated increases in cellular function during periods of intermittent HBO (Smith & Mohideen 1991).

One of the primary effects of HBO is a simple increase in the amount of dissolved oxygen within the tissue. Although the production of NO requires dissolved oxygen, few, if any, investigations have considered the possibility that production of NO by macrophages within wounds or other tissues may be altered due to decreased oxygen tensions, or that intermittent restoration of oxygen in these areas may supply sufficient intracellular oxygen to restore production of NO and thus augment the bactericidal efficiency of these macrophages.

The model used in this study was developed using the murine macrophage cell line, J774, because it has demonstrated the ability to produce NO when stimulated with  $\gamma$ -INF and/or LPS. Propagation of a cell-line in lieu of procurement and processing of macrophages from live mice was chosen because of benefits in cost, ease of maintenance, and the ease and speed with which cells could be harvested and plated. Also, use of this cell-line assured homogeneous populations of macrophages, thus eliminating stimulatory or inhibitory effects from other subsets of leukocytes.

Considerable research has been conducted which evaluates cellular mechanisms in disease *in vitro*. Unfortunately, involvement of oxygen and its availability for these mechanisms is often overlooked. Incubation of cells is most often carried out in a humidified atmosphere of 95% room air and 5% CO<sub>2</sub>. The resultant pO<sub>2</sub> is near 150mmHg. This partial pressure was confirmed during this study by analysis of both incubator gas, and culture medium. This tension (pO<sub>2</sub> = 150mmHg) is well above what would be expected in any terrestrial mammalian tissue under normal situations. Therefore, an attempt to compare results from studies that utilize models to study oxygen-dependent mechanisms *in vitro*, the production of NO for example, to cellular activity *in vivo*, should be done with prudence and taken in context.

Until recently, the  $pO_2$  in healthy tissue was thought by most to be 40 to 45mmHg. Advances in technology and measuring techniques have helped to produce data which indicate that the actual oxygen tension in tissue is even lower than previously believed and a reevaluation of current concepts of tissue  $pO_2$  has been suggested (Weiner 1994). Therefore, the hypoxic state of wounds and infected tissue (relative to normally oxygenated healthy tissue) could possibly approximate anoxia.

As previously proposed, for certain cellular mechanisms, oxygen may serve as the rate-limiting factor under physiologic conditions. Further study should elucidate effects at more realistic oxygen tensions. Mixtures of gasses used in this model were not intended to drive the cellular  $pO_2$  to near anoxic levels, only to provide for an environment that was hypoxic relative to standard incubator conditions, and that approximates more closely oxygen tensions in vivo.

*Verification of gas tensions and normal physiologic pH.*—Except for the 100% oxygen used to compress the hyperbaric chamber, all gasses utilized in this study contained 5%  $CO_2$ . Concentrations of  $CO_2$  and pH of medium were confirmed to be within normal physiological limits by blood-gas analysis. Potential changes in pH occurring during HBO treatment were minimized by the 10-minute purge with 95% oxygen and 5%  $CO_2$  gas mixture before compression of the chamber, as well as the addition of bicarbonate and HEPES to the culture medium. Normal physiologic pH and  $pCO_2$ , as well as increased  $pO_2$ , were verified by blood-gas analysis of culture medium that was collected after exposure to a standard 1.5 hour HBO protocol.

*Interpretation of data.*—In this model, concentrations of nitrite significantly decreased in supernatant collected from cells that were exposed to low oxygen tensions when compared to those that were incubated in 95% room air. These data suggest that, (1) macrophages that were incubated in low oxygen tensions produced diminished amounts of i-nos enzyme, resulting in decreased production of NO, or that, (2) there were potentially equal or greater amounts of i-nos produced by these 'hypoxic' macrophages, but due to a lack of dissolved substrate ( $O_2$ ), the enzyme was unable to function.

Cells that were exposed to a  $pO_2 = 20$  or 40mmHg and then were intermittently exposed to HBO exhibited a marked increase in



concentration of nitrite in the culture medium. However, upon calculation of percent change, cells incubated in 95% room air ( $pO_2 = 150\text{mmHg}$ ), had less overall change than did cells incubated in  $pO_2 = 20$  and  $pO_2 = 40\text{mmHg}$ . All cells treated with HBO produced nearly equal amounts of nitrite when groups incubated within different oxygen tensions were compared. In fact, cells incubated in the lower  $pO_2$  conditions, consistently, although not significantly, produced higher concentrations of nitrite. These data suggest that, (1) exposure to intermittent HBO does increase production of NO by J774 murine macrophages incubated in  $pO_2 = 20$  or  $40\text{mmHg}$ , (2) macrophages that were exposed to lower oxygen tensions may have produced the i-nos enzyme in equal or possibly greater amounts than did those exposed to 95% room air, and that (3) decreased production of NO by the untreated cells was due to a lack of dissolved  $O_2$  to serve as a cofactor and not necessarily due to a decrease in the production of i-nos.

### CONCLUSION

In conclusion, this study demonstrates that (1) the murine macrophage cell line, J774, produces less nitric oxide when incubated in low  $pO_2$  conditions, (2) these changes are not necessarily due to a decrease in production of i-nos, rather, that at oxygen tensions that more closely replicate conditions in vivo, dissolved  $O_2$  may serve as the rate-limiting factor in the enzymatic reaction among i-nos, NADPH and L-arginine, and (3) intermittent exposure to HBO increases production of NO by J774 murine macrophages incubated in  $pO_2 = 20\text{mmHg}$  and  $pO_2 = 40\text{mmHg}$ . Therefore, alterations in bacterial clearance in infected tissue noted during HBO therapy may be due, in part, to more efficient production of NO by macrophages residing in wounds or other areas where the partial pressure of oxygen is normally low or has dropped due to one or more pathological processes.

If, after further investigation, this theory holds true, there may be increased implications for the use of HBO as an adjunctive treatment for infectious diseases beyond those for which its use is currently accepted.

### ACKNOWLEDGMENTS

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NAUTILOIDS FROM THE CRETACEOUS (CAMPANIAN)  
OZAN FORMATION, TAYLOR GROUP,  
FANNIN COUNTY, TEXAS

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**Abstract.**—Two nautiloid species, *Cimomia ozani* new species and *Eutrephoceras dekayi* (Morton), are described from the Campanian Ozan Formation of the Taylor Group in northeast Texas. The fossil specimens were collected from a glauconitic biomicrite bed exposed in the North Sulphur River channel in Fannin County. These are the first nautiloids described from the Taylor Group.

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Nautiloids are low in richness and abundance in all Cretaceous strata of Texas (Kummel 1953; 1956; Hanger 1989), and the Upper Cretaceous is particularly barren. Stephenson (1941) described only one nautiloid species from the Navarro Group. The nautiloids in this report are the first ever described from the Taylor Group. Testimony to Kummel's (1953) observation on the limited richness and abundance of Texas Cretaceous nautiloids is the fact that over five decades of collecting of the Taylor Group in the northeast Texas region by East Texas State University (now Texas A&M University at Commerce) faculty and students, among others, has produced only the seven specimens described here.

Outcrops along the North Sulphur River channel in Fannin County, Texas, expose marl and limestone strata of the Ozan Formation. A single 0.3 meter thick bed of glauconitic biomicrite is very fossiliferous and contains both reddish, ironstone casts and black, phosphatic casts and molds. To date, this bed has yielded a fauna that includes thousands of specimens of ammonites, gastropods, oysters, other bivalves, and disarticulated vertebrate remains. It is interpreted as a condensed section (Echols 1984) based upon: occurrence of ammonites from four mid-continent ammonite range zones, undulatory bottom surface, bioturbated surface, and abundant ironstone and phosphate concretions. Correlation via ammonites with the midcontinent suggests that the condensed bed accumulated slowly (at least two million years) from the mid Early Campanian to the early Late Campanian.

All fossils are from the North Sulphur River Channel, approximately one quarter mile east (downstream) from the FM 2990 bridge, 1.8 miles north of its junction with highway 34, near the town of Ladonia, Texas, in Fannin County. All specimens are deposited in the collections of the University of Texas at Austin (ET).

## SYSTEMATIC PALEONTOLOGY

Order Nautiloidea Agassiz 1847  
Family Hercoglossidae Spath 1927  
Genus *Cimomia* Conrad 1866  
*Cimomia ozani*, new species  
Figure 1a

*Diagnosis.*—*Cimomia* with pronounced hyponome deflection, faint transverse ribbing, and very slight sutural sinuosity, consisting of a broad ventral and lateral lobe.

*Description.*—Nautiliconic shell; subglobular shape; involute; preserved as ironstone casts with some shell material; maximum diameter varies from 4.70 cm to 6.31 cm; holotype diameter = 5.41 cm; number of camerae preserved varies from 6 to 9 per one half volution; hyponome deflection from horizontal; maximum deflection = 6.75 mm; very faint transverse ribbing where shell is preserved; suture with broad and shallow ventral saddle, shallow, rounded lateral lobe; small rounded saddle near umbilicus.

*Material examined.*—Three specimens; ET5503 (holotype), ET5520 and ET1747.

*Etymology.*—This species is named for the Ozan Formation along the North Sulphur River in Fannin County, Texas.

*Discussion.*—*Cimomia ozani* differs from all other Cretaceous species of the genus in the very slight sinuosity of the suture. The phylogeny of nautiloids is still highly speculative, but evolutionary trends of increasing sinuosity through the Cretaceous within a lineage of *Eutrophoceras* to *Cimomia* have been hypothesized (Kummel 1964). The Eocene species, *C. vaughani* from southwestern Texas (Gardner 1923) is very similar to *C. ozani*, with the exception of a more sinuous suture, supporting the hypothesized trend.

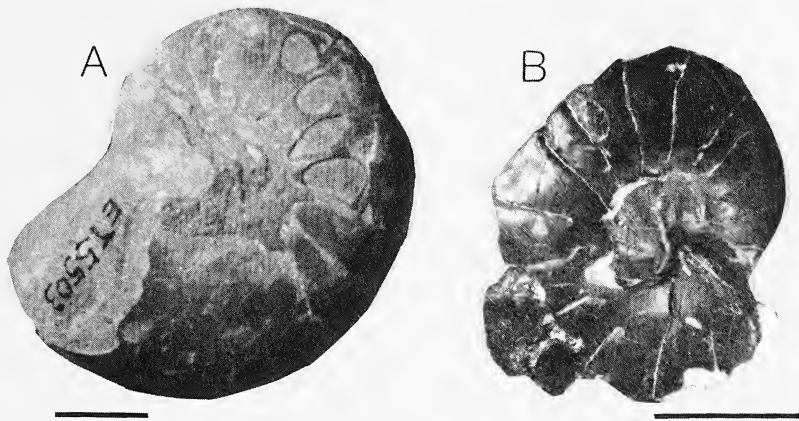


Figure 1. (A) *Cimomia ozani*, new species, holotype (ET5503). (B) *Eutrephoceras dekayi* (Morton 1834) (ET5605). Bars = 1 cm.

Family Nautilidae de Blainville 1825

Genus *Eutrephoceras* Hyatt 1894

*Eutrephoceras dekayi* (Morton 1834)

Figure 1b

*Description*.—Crushed and distorted phosphate casts consisting of varying numbers of camerae, up to 19 per 1 volution; subglobular shape; involute; maximum diameter = 5.06 cm; faint transverse ornament, up to sixteen ribs per five mm; transverse ribbing maintained on all camerae where visible; evident hyponomal deflection, deflection from horizontal = 4.01 mm; umbilical diameter = 0.2 mm; umbilical shoulders rounded; siphuncle faintly visible at 0.5 times the venter to dorsum distance; widely spaced sutures, with broad ventrolateral saddle; broad lateral and ventral lobe.

*Material examined*.—Four specimens; ET5504, ET1762, ET5505 and ET5605.

*Discussion*.—The relatively straight suture plus the umbilical size and shape clearly assign these to *E. dekayi*. Hyatt (1894) suggested that *E. dekayi* had no surface ornamentation, though Whitfield (1892) clearly shows transverse ornament. Much of the confusion probably arises

because, as Whitfield (1892) noted, the type specimen consists of only part of one chamber with very little shell material. *Eutrephoceras perlatus* (Morton 1834) from the Cretaceous Prairie Bluff Chalk of Alabama is similar in size and suture, but is not an umbilicated species, as are the Ozan Formation individuals. Stephenson (1941) erected the species, *E. planoventer*, for specimens that were flattened on the venter, but they fall within the range of variation of *E. dekayi* throughout North America (Miller & Garner 1962) and within the Ozan Formation (this report). Thus, the same species is present in both Taylor and Navarro groups.

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## RECOVERY OF MIOCENE TERRESTRIAL MICROVERTEBRATES FROM THE FLEMING FORMATION IN EAST TEXAS

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**Abstract.**—Small mammal teeth similar to those recovered from pedogenic-nodule-rich conglomerates in the upper Fleming Formation in western Louisiana have been recovered from nodule-bearing conglomerate at an east Texas locality near Coldspring, which had yielded mammals of the Cold Spring Local Fauna, strengthening its correlation with the Louisiana faunas. The new Texas specimens, which include rodent teeth, insectivore or chiropteran tooth fragments and fish teeth, are the first record of cricetid (*Copemys*) and heteromyid rodents from the Cold Spring Local Fauna.

One goal of vertebrate paleontological work in the Miocene of western Louisiana has been correlation with the long-established sequence of Miocene vertebrate sites in eastern Texas, studied by Wilson (1956), Patton (1969), Quinn (1955) and Prothero & Manning (1987), among others. Schiebout (1997a) summarized this correlation effort, pointing out the fact that the lack of small vertebrates at most of the classic east Texas sites represents a major bar to comparison with the western Louisiana sites, where small vertebrates are the main fossils recovered (Schiebout 1994; 1996; Schiebout et al. 1996). The western Louisiana Miocene fauna shows some affinities to the Burkeville Local Fauna, although Schiebout concluded that (based on large vertebrates) it is closer to the overlying Cold Spring Local Fauna (Schiebout 1997a). Recovery of terrestrial microvertebrate specimens from the Cold Spring sites, which previously has lacked them, has become a priority.

### STUDY AREA

Burkeville, Texas is approximately 10 km west of the Texas/Louisiana boundary. Coldspring, Texas is approximately 135 km west of the boundary, in San Jacinto County south of Lake Livingston and is clearly stratigraphically higher in the Fleming outcrop than beds near Burkeville.

Work by Anderson (1958) recognized conglomerate layers rich in calcium carbonate nodules at some of the classic Cold Spring sites. Detailed locality data on sites visited by LSU field crews is on file at the LSU Museum of Natural Science, Vertebrate Paleontology Section.

Two visits by LSU field crews in 1996 to the vicinity of site 2 of Anderson (1958), approximately 2.4 km (1.5 miles) north of Coldspring, Texas, revealed that some of the old vertebrate fossil quarries are still visible, although partially filled and grassed. The level of human occupation of the region has risen, and the sites are now in a pasture. The exposures in which Anderson measured section are likewise overgrown, but the LSU field crew recovered a boulder of conglomerate in a creek adjacent to the quarries, along which Anderson had measured section (Anderson 1958).

#### METHODS AND RESULTS

The microvertebrates from the western Louisiana sites are recovered by acid dissolution and screening from conglomerates rich in pedogenic nodules (Schiebout et al. 1998). Wherever these conglomerates have been found in the Castor Creek Member of the Fleming Formation in western Louisiana, they have proved productive of teeth of terrestrial vertebrates, freshwater fish teeth and bone fragments.

The boulder of conglomerate recovered from the Anderson site 2 near Coldspring was a poorly sorted, subrounded granule to pebble grade conglomerate with a sand and mud matrix (Hinds, pers. comm.). It was rich in nodules (Figs. 1 & 2), usually rounded and many showing septarian features, similar to nodules interpreted as pedogenic in the western Louisiana fossiliferous conglomerates (Schiebout 1994: Figs. 3-5; Jones 1995; Schiebout 1997b: Photographs 10-13). When treated with dilute acetic acid and screened, approximately 10 kg of this Coldspring conglomerate has yielded seven teeth of the cricetid rodent *Copemys*, a single tooth of a heteromyid rodent, and insectivore or chiropteran tooth fragments, along with fish teeth and fragments of larger vertebrate teeth and bones. The *Copemys* falls within the size range seen at the Fort Polk Miocene sites. It is a genus previously unknown from the Cold Spring Local Fauna, for which heteromyids, insectivores, and chiropterans have also not been recorded previously (Stevens, pers. comm.).

#### DISCUSSION

Initial study of the Coldspring micromammal fauna confirms the possibility that the western Louisiana sites may be as young as the Cold Spring Local Fauna, in that the fossils recovered are similar both to them and to specimens from older Burkeville Local Fauna sites in east Texas, Town Bluff and Trinity River (Dorsey 1977), from which specimens were recovered by screening sandy material (Jacobs, pers. comm.). It seems likely that larger samples of microvertebrates from

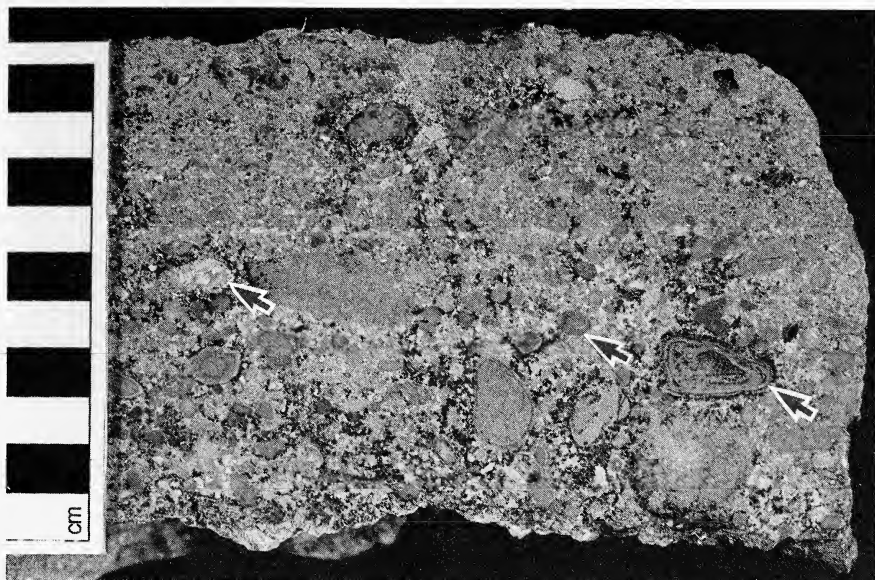


Figure 1. Sawed surface of the Coldspring conglomerate boulder which is being dissolved to yield microvertebrates. It has been sprayed with glossy, clear enamel to enhance contrast. Arrows indicate some of the soil-formed carbonate nodules.

Cold Spring levels in Texas will reveal faunal differences from the Burkeville levels, similar to differences in the large mammals used in developing the sequence of Local Faunas (Wilson 1956), but it is also possible that some of the common small mammals may have been stratigraphically long ranging in the Gulf coast of the Miocene. Schiebout's summary of correlation of the western Louisiana sites with east Texas sites, prior to the recovery of Coldspring micromammals, gives a figure showing the sequence of Fleming Formation Local Faunas (Schiebout 1997a: Fig. 5).

Efforts to extend the biostratigraphic and geographic range of research on the microvertebrates from nodule-rich conglomerates in the Miocene of the Gulf Coast also include work in Louisiana on members underlying the productive Castor Creek Member of the Fleming Formation (Hinds 1998). Detailed mapping in the Fort Polk area of western Louisiana is currently underway by LSU researchers, who are alert for the conglomerates, especially in the clay-rich units. The Fleming in Louisiana is considered to consist of six members, with clay-rich and sand-rich members alternating (Rogers & Callandro 1965). The clay-rich members, from stratigraphically lowest to highest, are the Lena, Dough Hills, and the Castor Creek. Turcan et al. (1996: Table 1) working with

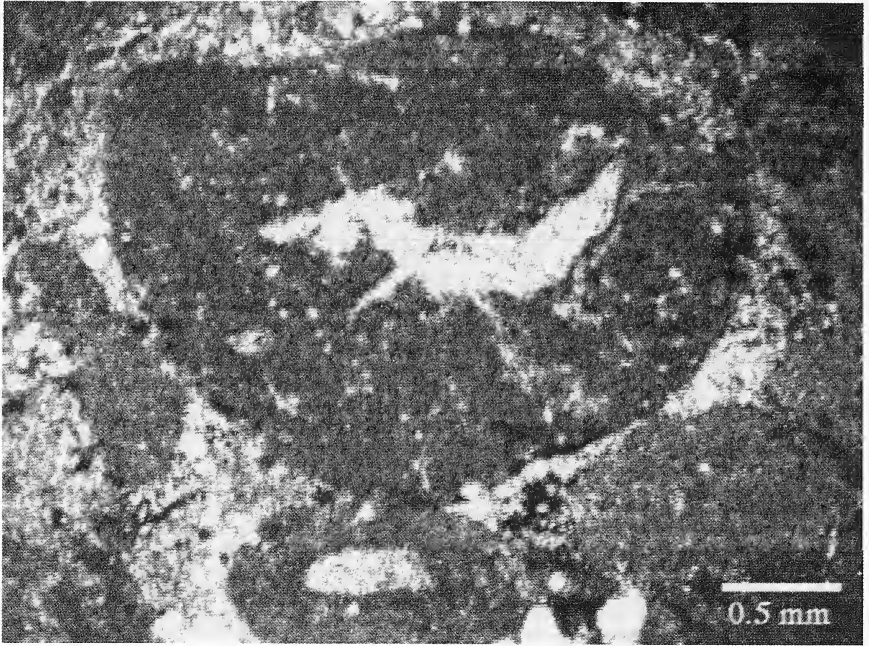


Figure 2. Thin section from the boulder shown in Figure 1, under plain light. View centers on a carbonate nodule showing septarian structure, produced by slower drying of its center compared to the exterior. The nodules tend to be rounded, probably reflecting a combination of original shape, softness and reworking. Digital image by Xiaogang Xie.

data from electric logs included beds outcropping near Burkeville, Texas and the Castor Creek Member in western Louisiana in their contiguous Burkeville aquiclude. The current geological mapping should help clarify whether the clay-rich and sand-rich zones are localized and interfingering deposits, or are actually relatively continuous from western Louisiana through east Texas.

The fossiliferous conglomerates in western Louisiana occur in fluvial mudstones and have been attributed to reworking of soils and concentration of debris including animal remains, during drops in base level in which rivers entrenched and the floodplains were subject to increased erosion (Schiebout 1994: 679; Jones et al. 1995). Smith & Kitching (1997) reported a bed of pedogenic nodule conglomerate rich in terrestrial vertebrate remains, including skulls and partial skeletons of cynodonts, in floodplain deposits of Lower Jurassic age in South Africa, which they likewise attributed to a regional base level drop.

One purpose of this study is to record occurrences of these conglomerates in both east Texas and Louisiana in the hope that scientists whose work takes them to areas of Fleming Formation outcrop will notice conglomerates, alert us, and confer about sending a sample for trial processing for microvertebrates in our bulk acid lab. Fossiliferous conglomerates range from 10-25 cm thick in western Louisiana (Jones et al. 1995). The nodules produce a very distinctive speckled appearance (Fig. 1). Very worn bone fragments are the fossils most likely to be observed on conglomerate outcrop surfaces, with the small teeth usually represented only by very rare bits of shiny enamel. The teeth of terrestrial vertebrates from these conglomerates are usually between one and two mm in maximum crown length. The rocks vary in degree of cementation by calcium carbonate. Some can be crumbled by hand and others required a jackhammer to break for transport to the laboratory. They tend to be the most resistant rocks in their intervals, so they can be recognized in very small outcrops.

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FORAGING FLIGHTS, REPRODUCTIVE SUCCESS  
AND ORGANOCHLORINE CONTAMINANTS IN CATTLE EGRETS  
NESTING IN A RESIDENTIAL AREA OF BRYAN, TEXAS

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**Abstract.**—This study was designed to determine reproductive success, habitat use and foraging trips of Cattle Egrets (*Bubulcus ibis*) nesting in the small city of Bryan, Texas and to compare residues of persistent organochlorine compounds in eggs of Cattle Egrets from recent years with data collected in the past. Mean clutch size and reproductive success of egrets nesting in Bryan in 1993 and 1994 were not different from the state average reported for egrets in non-urban habitats. Cattle egrets flew non-randomly in and out of the colony predominantly alone or in pairs and foraged mostly in pastures with cattle at 10-15 km around the colony. The only organochlorine residues found at detectable levels were DDE and PCBs. Mean DDE levels in eggs were mostly at near background levels and were significantly lower than those reported for Cattle Egrets in the 1970s.

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The range of Cattle Egrets (*Bubulcus ibis*) has been expanding in North America for nearly 50 years (Crosby 1972; Telfair 1994). In addition to being successful in exploiting different types of foraging habitats (Siegfried 1978; Mora 1992), Cattle Egrets have reproduced well and apparently have not been affected by agricultural pesticides and other contaminants (Mora 1991; Telfair 1994). Cattle Egrets have established breeding colonies near residential areas in small cities (Telfair & Thompson 1986). By the mid-1980s, over 20 heronries were documented near residential areas in Texas (Telfair & Thompson 1986).

The reproductive success and ecology of Cattle Egrets nesting in agricultural and non-urban environments have been well documented (Siegfried 1972a; 1972b; Telfair 1983; Arendt & Arendt 1988; Mora 1991; 1992). However, the ecology and reproductive success of Cattle Egrets nesting in urban environments are not as well documented (Telfair 1983). Cattle Egrets feed primarily on insects (grasshoppers, crickets, flies and moths), spiders and frogs in pastures with cows (Heatwole 1965; Siegfried 1972b; Telfair 1983) or in irrigated agricultural areas (Mora 1992). Why Cattle Egrets nest near residential and heavy traffic areas in towns is largely unknown. Some plausible

explanations are predator avoidance and availability of a conveniently located central place for foraging (because the surrounding landscape near small urban areas may include wetlands, reservoirs, pastures and agricultural fields), that may lead to increased reproductive success.

The objectives of this study were: (1) to determine reproductive success of Cattle Egrets nesting in an urban environment; (2) to document foraging trips and habitat use, particularly as they might be influenced by the location of the colony near a residential area; and (3) to compare residues of some persistent organochlorine compounds in eggs of Cattle Egrets collected recently with those collected in previous years in Texas. Cattle Egrets and other wading birds have been proposed as potential indicators of environmental contaminants (Custer et al. 1991) and could be used to infer contaminant loads in other species.

## METHODS

*Study site.*—Cattle Egrets began nesting in the city of Bryan in 1990 (Arnold pers. comm.). The colony was established on a 5 ha tract of private property of the CITGO and Arco companies (Klesel pers. comm.) located between Palasota Drive, Groesbeck and Bittle Lane streets (30° 39' N, 96° 22' W). Nest-site vegetation consisted primarily of honey mesquite (*Prosopis glandulosa*), hackberry (*Celtis reticulata*), winged elm (*Ulmus alata*), water oak (*Quercus nigra*) and eastern red cedar (*Juniperus virginiana*). The nesting colony apparently was undisturbed during its first three years.

*Nest observations and egg collection.*—Observations of the colony during this study were initiated in 1993. Cattle Egrets arrived at the site in mid-April and began immediately to build nests. In May 1993, 10 eggs, one per nest, were collected from full clutches of haphazardly selected nests for the analysis of organochlorine pesticides and polychlorinated biphenyls (PCBs). Four of these eggs were randomly selected for chemical analyses because of funding limitations. These 10 nests, plus an additional 20 nests, were marked for further observations of reproductive success. Observations of these nests were conducted twice a week throughout the reproductive season, until chicks were about three weeks old. At every inspection, the number of eggs and chicks per nest were recorded.

In 1993, the Bryan heronry consisted of about 1,500 Cattle Egret nests with a few nests (<50) of Snowy Egrets (*Egretta thula*) and three Little Blue Herons (*E. caerulea*). In the fall of 1993, most trees and shrubs where the colony had been established were bulldozed, leaving



only a few bigger trees at the site.

In 1994, Cattle Egrets returned to the same site also around mid-April and settled in the few remaining trees in the area. These trees, however, were insufficient in number, and most birds occupied the adjacent area on the west side of Groesbeck St. In 1994, the number of Cattle Egrets nesting in the area increased significantly from 1993, and about 2,000 pairs were estimated in the former CITGO area alone. By late May, the number of nests on the north side of Groesbeck St. had increased to about 6,000 pairs, and by mid-June, there were about 9,000 nesting pairs. Thus, the total number of nests estimated in 1994 was approximately 11,000 pairs. In 1994, nine Cattle Egret eggs from separate nests were collected for organochlorine analyses. Again, only four eggs were actually analyzed because of funding limitations. Also, an additional 20 nests were marked to evaluate reproductive success. As in the previous year, the nests were checked twice a week for the duration of the breeding period, until the chicks reached approximately three weeks of age. In addition to Cattle Egrets nesting in this area, there were also a few nests of Great Egrets (*Ardea alba*), Snowy Egrets, Little Blue Herons and White Ibises (*Eudocimus albus*). In the fall of 1994, the site where Cattle Egrets nested was again cleared of all shrubs and small trees, leaving only a few tall, older trees in the property.

Additional observations of 68 nests at the colony were conducted during July and August, 1994, to record behavior of chicks and parents at the nest. An average of eight haphazardly selected nests were observed three times a week for about 10 minutes each. Observations at the nest began when an individual bird arrived at the nest. Information recorded included number of chicks on the nest, presence of an adult with the chick, whether food was brought to the chicks in that period, direction of adult arrival, number of chicks fed, whether the arriving bird took over the nest, and whether the partner departed and the direction of departure.

*Feeding flights and habitat use.*—Feeding flight directions and foraging areas of Cattle Egrets were recorded in 1994. The direction and number of birds departing or arriving at the colony, individually or in groups, were recorded twice a week, for 15 minutes at each of four colony corners (north, south, east and west). Egrets were considered part of a group when they were flying in the same direction and were within approximately  $\leq 10$  m of their closest neighbor.

Surveys of foraging habitat use areas were conducted two times per week during July and August, 1994. The surveys were conducted along

roads of Brazos, Burleson, Robertson and Grimes counties, within a 25 km radius from the colony. Upon location of cattle egrets, the location, habitat type (pasture or other agricultural crop, standing water, trees), activity (foraging with cattle or behind tractors, resting), number of birds, number of cattle (if present), and distance and direction from the colony were recorded.

*Chemical analyses.*—Eight eggs were analyzed for organochlorine pesticides and polychlorinated biphenyls (PCBs) at the Geochemical and Environmental Research Group, Texas A&M University, College Station, Texas, by methods previously described (McLeod et al. 1985; Brooks et al. 1989). Briefly, 0.5 g of the egg homogenate was mixed with anhydrous sodium sulfate and extracted with methylene chloride. The extract was then concentrated and run through a silica gel/alumina column, and further purified with HPLC to reduce matrix interferences. The extract was then concentrated to 1 ml and analyzed by glass capillary gas chromatography with a Hewlett Packard 5890A GC, with split/splitless injection,  $^{63}\text{Ni}$  electron capture detector, and a DB-5 (30 X 0.25 mm i.d.) fused silica capillary column (J&W Scientific). Spike recoveries were above 80%, and variation between duplicates was within 15%. Confirmation by GC/mass spectrometry was performed in two samples.

*Statistical analysis.*—Differences in clutch size, number of young per nest and concentrations of DDE and PCBs in eggs were determined by Mann-Whitney U tests. Differences in departures and arrivals to the colony among four directions were determined by ANOVA of ranks. Significant differences among means were determined by Tukey's multiple comparison procedures. All statistics were performed with the use of SAS software (Statistical Analysis Systems).

## RESULTS

*Reproductive success.*—In 1993, mean clutch size of Cattle Egrets was significantly smaller ( $P < 0.05$ ) than in 1994 (Table 1). However, the mean number of young fledged per successful nest, young per nest attempt (Table 1), and the ratio of clutch size/brood size (1.3 and 1.4 respectively) were similar between years. The size of the nesting colony was more than five times greater in 1994 than in 1993.

*Feeding flights and food delivery.*—On average, over 500 birds were observed departing from or arriving at the colony during each 60-min morning observations during the peak of the breeding season in 1994. Approximately 75% of the departing flights were to the north, significantly different ( $P < 0.001$ , Table 2) from other directions. The

Table 1. Clutch size and reproductive success of Cattle Egrets nesting in a residential area in Bryan, Texas, 1993 and 1994.

Year	Nests	Clutch Size $\pm$ <i>SD</i>	% Successful Nests <sup>1</sup>	Young/successful Nest $\pm$ <i>SD</i>	Young/nest Attempt <sup>2</sup>
1993	30	2.93 $\pm$ 0.58 a <sup>3</sup>	86.7	2.19 $\pm$ 0.63 a	1.9 $\pm$ 0.96 a
1994	28	3.29 $\pm$ 0.60 b	100	2.36 $\pm$ 0.56 a	2.36 $\pm$ 0.56 a

<sup>1</sup> A successful nest was considered when at least one chick was raised to near fledging or up to 3 weeks of age.

<sup>2</sup> A nest attempt was considered when a nest had two or more eggs and either was successful in producing young or was abandoned.

<sup>3</sup> Means sharing same letter are not significantly different.

Table 2. Mean number of arrival and departure flights (individuals and groups) of Cattle Egrets at the colony in Bryan, Texas, 1994<sup>1</sup>.

Direction	Departure Flights $\pm$ <i>SD</i>	Arrival Flights $\pm$ <i>SD</i>
S	61 $\pm$ 45	21 $\pm$ 7
E	51 $\pm$ 37	33 $\pm$ 18
N	405 $\pm$ 140	5 $\pm$ 8
W	23 $\pm$ 10	27 $\pm$ 10

<sup>1</sup> Average of 6 observations for 15 min at each direction during July 1994.

pattern for arrivals was somewhat different than departures, and arrivals from the east were significantly greater than those from the north ( $P < 0.05$ ).

The flock size of most departing and arriving flights of Cattle Egrets at the colony in Bryan was either one (75%) or two (15%) birds per flock. The proportions of arrivals and departures of individual birds or groups was similar during the 2-3 week period. Thus, approximately 90% of the total number of flights were single individuals or pairs. Clearly, the proportion of birds or groups departing or arriving at the colony decreased as group size increased (Table 3).

Approximately 90% of the 68 nests observed for food delivery had one foraging partner returning to the nest with food. Ten percent of the remaining observed adults returned to the nest without food, but most (5 of 7) of them did not have any hatchlings or chicks to feed at the nest. Most birds returned from the north (85%) and south (15%). The birds returning with food to the nest fed an average of 1.34 chicks/nest immediately after their return, a slightly lower figure than the number of chicks available/nest. However, the number of young/nest was not significantly different from the number of young fed/nest (paired t-test,

Table 3. Proportion (%  $\pm$  SD) of arrivals and departures, individually or in groups, of Cattle Egrets nesting in the City of Bryan, Texas, 1994.

Number in group	Arrivals	Departures
1	74.4 $\pm$ 5.7	73.4 $\pm$ 10.5
2	13.6 $\pm$ 1.9	16.9 $\pm$ 6.5
3	5.8 $\pm$ 3.2	4.9 $\pm$ 1.8
4	2.7 $\pm$ 1.8	1.6 $\pm$ 0.9
5	1.3 $\pm$ 1.0	1.0 $\pm$ 0.6
>5	2.3 $\pm$ 2.7	2.3 $\pm$ 2.0

$P > 0.05$ ). After the arriving birds fed their young, they took over the nest from the partner about half the times. The replacement frequency was greater (75% of the times) before, but lower (23% of the times) after July 22, when most chicks were able to move around branches near the nest and required less protection by their parents. This takeover of the nest by the arriving adult coincided with the proportion of adults staying at the nest. That is, parent birds were observed with chicks at the nest 35% of the times; however, the proportion was 50% before, and only 17% after July 22. Approximately 38% of the birds arriving with food departed the colony immediately after food delivery. Arriving and departing flights by the same individual increased significantly (75%) by late July-early August, when most of the chicks were at least three weeks old and required less protection by their parents.

*Habitat use.*—Approximately 345 Cattle Egrets per day were observed foraging in groups of 10-200 individuals. The average group size was 63. Forty-two percent of the groups were foraging near cattle, 39% were near standing water, 14% were by the roadside or in ungrazed pastures, and the remaining 5% were behind tractors. Foraging groups were observed at distances of 4 to 25 km from the colony; however, most birds (67%) were observed at 10-15 km from the colony. Egrets were observed foraging in all directions from the colony; however, this study did not have enough field observations to determine if there was a preference toward a specific side of the colony, as might be indicated by the feeding flight and foraging observations.

*Organochlorines.*—Except for DDE (a metabolite of DDT) and PCBs, other organochlorine residues were non-detected or near detection limits. Low levels of hexachlorobenzene (HCB), hexachlorocyclohexane (HCH), oxychlorane, trans-nonachlor, p,p'-DDD, and p,p'-DDT were also reported in a few samples. DDE residues were relatively low and not different between eggs collected in 1993 and 1994 (Table 4).

Table 4. DDE and PCBs ( $\mu\text{g/g}$  ww) in Cattle Egret eggs from a colony established in a residential area in Bryan, Texas, 1993 and 1994.

Year	N	% Moisture $\pm$ SD	% Lipid $\pm$ SD	DDE $\pm$ SD	PCBs $\pm$ SD
1993	4	81.1 $\pm$ 0.4	7.1 $\pm$ 1.4	0.071 $\pm$ 0.08	0.065 $\pm$ 0.10
1994	4	78.5 $\pm$ 1.3	6.9 $\pm$ 1.6	1.50 $\pm$ 2.81	0.031 $\pm$ 0.02

However, one egg from 1994 had about 70 times more DDE (5.72  $\mu\text{g/g}$  ww) than the rest of the eggs (mean=0.08  $\mu\text{g/g}$  ww, n=7, Table 4). This high DDE value in one egg increased the mean by 20-fold in 1994. Residues of PCBs also were similar between Cattle Egret eggs collected in 1993 and 1994 and in all cases were below 0.1  $\mu\text{g/g}$  ww (Table 4). The egg with the high DDE residue also had elevated concentrations of p,p'-DDD (0.135  $\mu\text{g/g}$  ww) and p,p'-DDT (1.28  $\mu\text{g/g}$  ww) compared to the other eggs.

#### DISCUSSION

*Nesting and reproductive success.*—The Cattle Egret colony that settled in a residential area of Bryan, Texas in 1990, increased more than 5 times within a three year period. This colony became an attraction and a public health concern among area residents as well as city and state officials (several articles in *The Eagle*, Vol. 120, July 1994, Bryan-College Station, Texas). Reproductive success of Cattle Egrets nesting near a residential area in Bryan, Texas, during 1993 and 1994 was not different from reproductive success of other colonies settled in non-urban environments (see Telfair 1993 for a review). Mean clutch size and number of fledglings per nest at the Bryan colony in 1993 and 1994 were similar to the average in Texas of 2.81 eggs and 2.45 fledglings, respectively (Telfair 1993). Thus, selection of urban habitats for nesting by Cattle Egrets did not result in increased or decreased reproductive success relative to the state average. The differences in clutch size between 1993 and 1994 may be an artifact of year to year variability, because almost all nesting conditions were similar for both years. Based on nest observations during this study, bird behavior at this colony was not different from that reported from other colonies (Siegfried 1972a; Telfair 1983).

*Feeding flights and habitat use.*—Directional observations of feeding flights at the egret colony in residential Bryan were limited to a distance of less than 100 m because of obstruction of view by nearby homes in

each direction. It is likely that the egrets followed a specific path or direction within town as they departed, but eventually took a different direction for foraging once they were outside of town. This may explain why observations of departures were predominantly to the north, whereas arrivals were mostly from the east. Many pastures were available in the northeast direction, and the predominance of flights in one direction suggests that egrets were not flying randomly in all directions. Similar observations were found in a colony located in the middle of agricultural fields in northwest Mexico (Mora 1997b).

Cattle Egrets at the colony in Bryan flew primarily alone or in pairs. These results agree with other studies of Cattle Egret colonies in Virginia where egrets were also observed flying primarily alone (Erwin 1984). In contrast, previous observations in irrigated agricultural areas in the Mexicali Valley of Baja California, Mexico, indicated that Cattle Egrets flew predominantly in groups of two and three individuals (Mora 1997b). The differences in observations may be due to the location of the heronries relative to the distribution and density of food resources. In the case where birds flew in groups, the colony was located in the middle of surface-irrigated fields where density of prey can be high (Mora 1992; 1997b). The colony in Bryan was situated near residential homes and egrets had only pastures to feed in. Lack of abundant concentrated prey, as are often found in irrigated fields, may influence whether egrets depart in groups rather than alone. Prey densities in pastures with cattle were not determined in this study.

Cattle Egrets foraged primarily in pastures distributed around the colony at a distance of 10-15 km. These observations agree with most observations of Cattle Egrets foraging in Texas (Telfair 1983; 1993). The foraging group sizes observed were smaller than mean group sizes observed in surface-irrigated fields in northern Mexico (Mora 1992), which could be correlated with prey densities or prey abundance.

*Organochlorines.*—DDE and PCB residues were low in egret eggs, except for one sample which had DDE at levels ( $5.72 \mu\text{g/g ww}$ ) that could be of concern for species that may prey on Cattle Egrets (e.g. Peregrine Falcons, *Falco peregrinus*). DDE levels as low as  $1 \mu\text{g/g ww}$  in the prey of raptors could result in detrimental effects on their reproduction (Enderson et al. 1982). The detection of p,p'-DDD and p,p'-DDT in the sample with high DDE suggests acquisition from an area where DDT may have been used recently. Cattle Egrets migrate to Mexico and Latin America where DDT is still used for malaria control (Lopez-Carrillo et al. 1996), and more potential exposure in the

wild is possible. However, recent studies have shown no differences in concentrations of DDE among birds collected in the southwestern United States and Mexico (Mora 1997a). The DDE levels in eggs of Cattle Egrets from the Bryan Colony were lower than the mean levels reported in previous years in eggs from Cattle Egrets in Texas. Mean DDE levels (3.5 ppm ww) in Cattle Egret eggs with thin shells collected in 1973 in Ellis County, Texas (Telfair 1983), were only 2.2 times higher than the mean observed in 1994, but nearly 50 times higher than the mean observed in 1993 in the Bryan colony. Thus, the 1993 data probably reflect better the decreasing trend in DDE levels observed in birds from Texas (Mora 1995). PCBs were very low and almost at background levels suggesting no local PCB source for breeding birds in the Bryan area.

### CONCLUSIONS

Cattle Egrets nesting in a residential area in the city of Bryan had similar nesting and reproductive success as colonies nesting in non-urban environments in Texas. Colony size in 1994 was much larger than the average colony size for the state. Feeding flights were non-random, and foraging habitat use was similar to that displayed by egrets nesting in non-urban habitats in Texas. Contaminants in eggs collected in 1993 and 1994 indicated that, for the most part, acquisition of DDE and PCBs was low in the area. Concentrations were not sufficiently high to cause negative effects on birds or other wildlife. Advantages of nesting in urban environments for Cattle Egrets remain undetermined.

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## GAUSS AND THE REGULAR HEPTODECAGON

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**Abstract.**—This note outlines the proof and provides a set of diagrams for the division of a circle into 17 equal arcs.

Karl Friedrich Gauss (1777-1855) at the age of 18 gave a ruler-compass construction of dividing a circle into 17 equal arcs. This note consists of an outline of his proof and a set of diagrams which systematically give the construction.

## QUADRATIC EQUATIONS

Let

$$ax^2 + bx + c = 0, \quad a \neq 0 \quad (1)$$

be a quadratic equation with real coefficients. Consider a rectangular coordinate system (Fig. 1). Let A and B, on the  $x$ -axis, correspond to  $x_1$  and  $x_2$ , respectively, where  $x_1$  and  $x_2$  are the roots of (1). Let  $OU = 1$  and  $OD = \frac{c}{a}$ . Then

$$(OU)(OD) = \frac{c}{a} = x_1x_2 = (OA)(OB). \quad (2)$$

This implies that the four points, U, D, A and B are on circle (1). Since M, the midpoint of AB, corresponds to

$$\frac{1}{2}(x_1 + x_2) = -\frac{b}{2a}, \quad (3)$$

one can construct the circle which passes through U, D, A and B. Therefore the roots of (1) can be constructed (Amir-Moéz 1987).

## THE SEVENTEEN ROOTS OF UNITY

Consider the equation

$$z^{17} - 1 = 0. \quad (4)$$

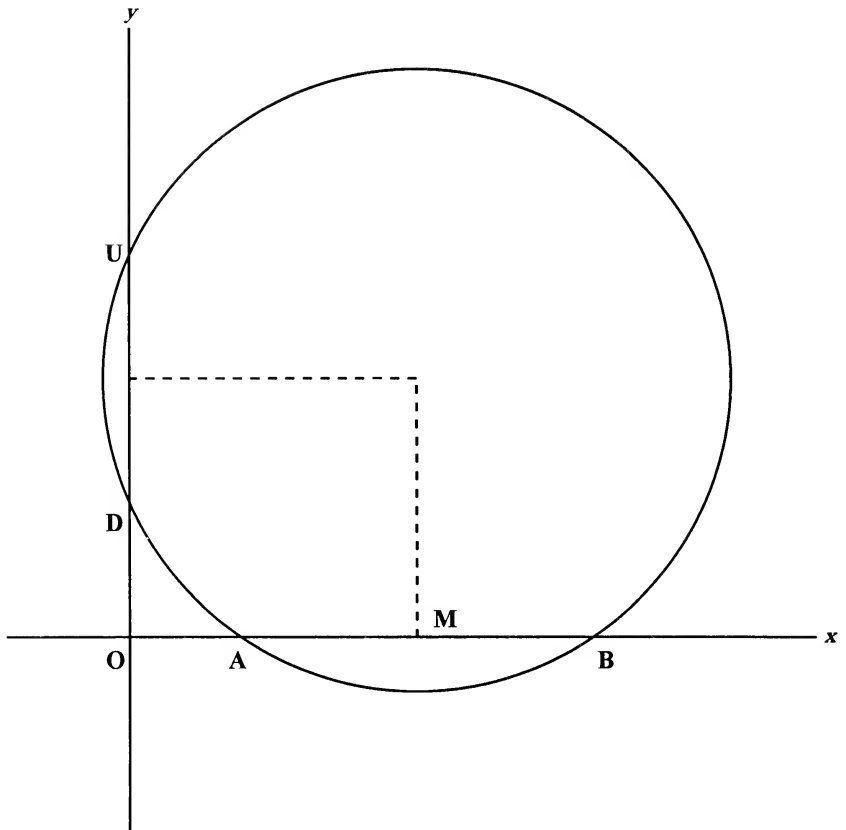


Figure 1. A rectangular coordinate system.

One can easily factor (4) into

$$(z - 1)(z^{16} + z^{15} + \dots + z^2 + z + 1) = 0 \quad (5)$$

Let  $z_0 = 1$  and  $z_1 = z$  (Fig. 2). Observe that

$$z_k = z^k, \quad k = 1, \dots, 16 \quad (6)$$

and

$$z_k = z^{*}_{17-k}, \quad k = 9, \dots, 16 \quad (7)$$

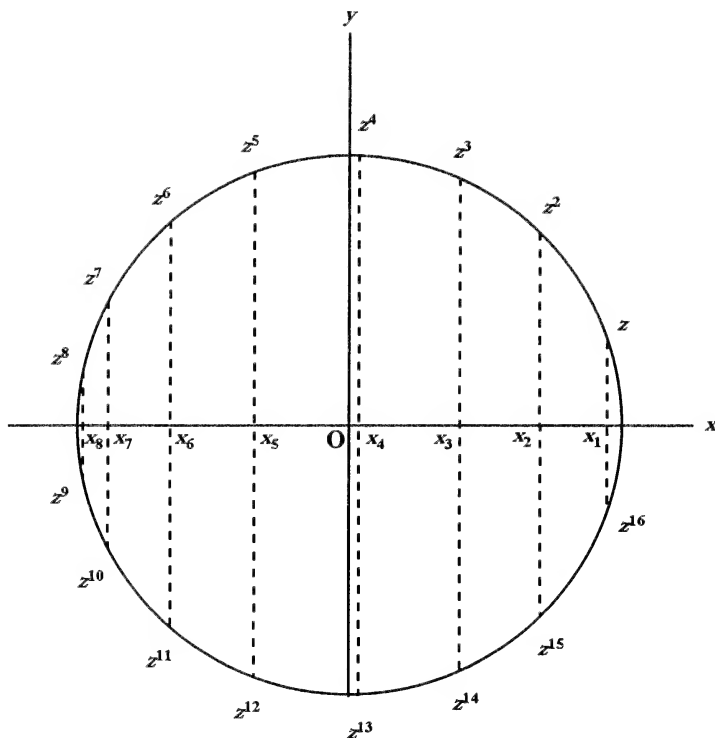


Figure 2. Assigning  $z_0 = 1$  and  $z_1 = z$ .

where, for example,  $z^*$  is conjugate of  $z$ . This implies that

$$z_h + z_{17-h}^* = 2 \cos \frac{2(\pi + h\pi)}{17}. \tag{8}$$

Then

$$z_h = x_h + iy_h \tag{9}$$

and

$$z_h + z_{17-h}^* = 2x_h, \quad h = 9, \dots, 16. \tag{10}$$

One can write (10) as:

$$z_k + z_{17-k}^* = 2x_k, \quad k = 1, \dots, 8. \tag{11}$$

(Dickson 1952).

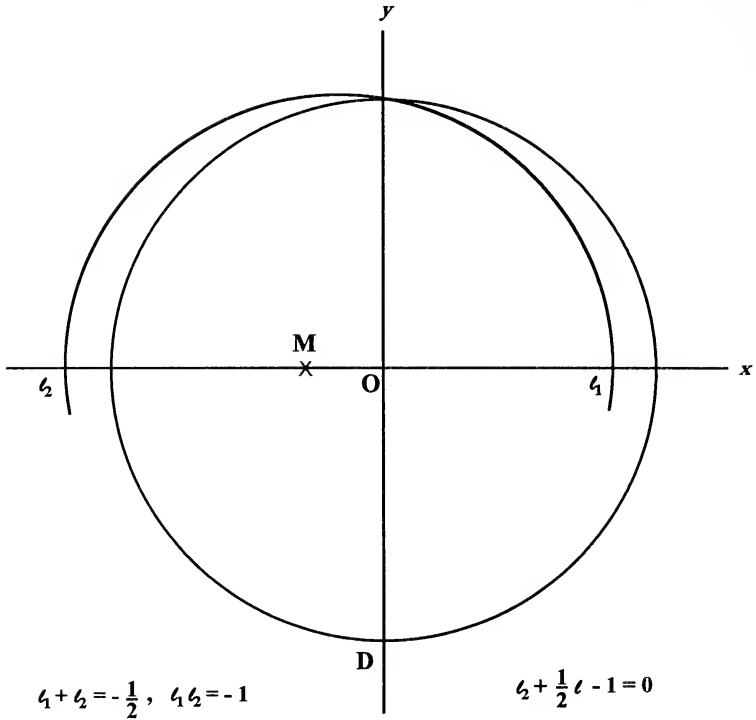


Figure 3. Construction of the roots of equation (16).

### THE RULER-COMPASS CONSTRUCTION

Gauss has considered

$$\begin{aligned}
 l_1 &= \frac{1}{2} \{ (z + z^{16}) + (z^2 + z^{15}) + (z^4 + z^{13}) + (z^8 + z^9) \} \\
 &= x_1 + x_2 + x_4 + x_8
 \end{aligned}
 \tag{12}$$

and

$$\begin{aligned}
 l_2 &= \frac{1}{2} \{ (z^3 + z^{14}) + (z^5 + z^{12}) + (z^6 + z^{11}) + (z^7 + z^{10}) \} \\
 &= x_3 + x_5 + x_6 + x_7
 \end{aligned}
 \tag{13}$$

Note that

$$l_1 + l_2 = -\frac{1}{2}, l_1 l_2 = -1.
 \tag{14}$$

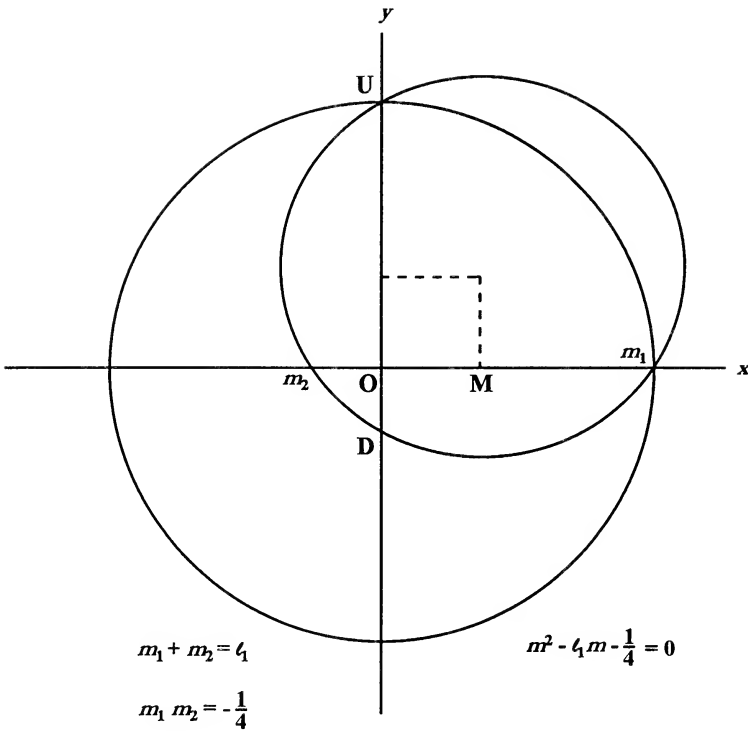


Figure 4. Construction of  $m_1$  and  $m_2$ .

Thus  $\ell_1$  and  $\ell_2$  satisfy the quadratic equation

$$\ell^2 + \frac{1}{2} \ell - 1 = 0. \tag{15}$$

One can construct the roots of (15) (Fig. 3).

Next let

$$m_1 = \frac{1}{2} [(z + z^{16}) + (z^4 + z^{13})] = x_1 + x_4 > 0, \tag{16}$$

and

$$m_2 = \frac{1}{2} [(z^2 + z^{15}) + (z^8 + z^9)] = x_2 + x_8, \tag{17}$$

$$m_1 + m_2 = x_1 + x_2 + x_4 + x_8 = \ell_1, \tag{18}$$

and

$$m_1 m_2 = -\frac{1}{4}. \tag{19}$$

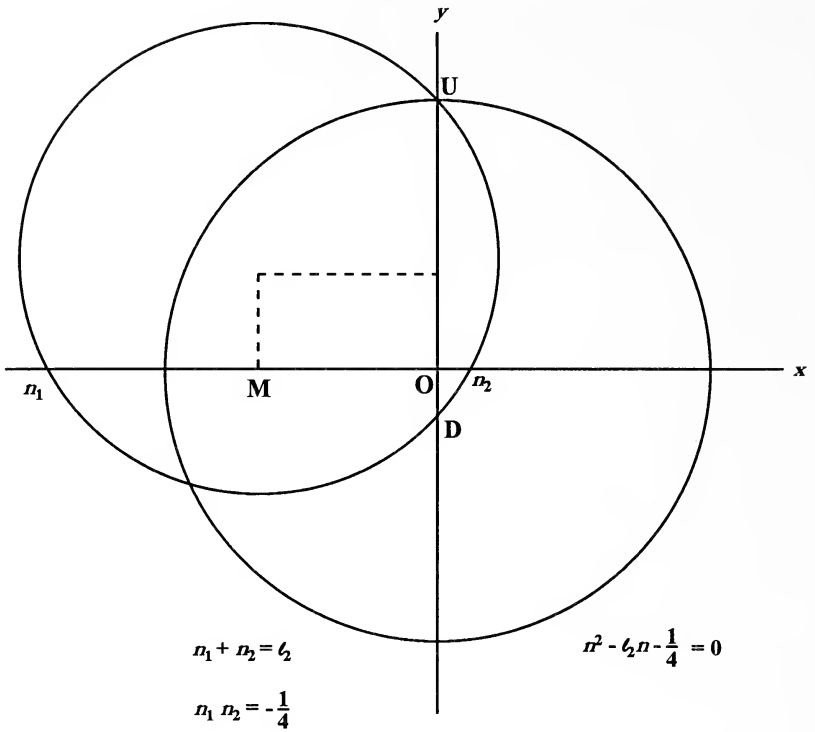


Figure 5. Construction of the roots of equation (24).

So  $m_1$  and  $m_2$  satisfy the quadratic equation

$$m^2 - \ell_1 m - \frac{1}{4} = 0. \tag{20}$$

By carrying  $\ell_1$  from (Fig. 3),  $m_1$  and  $m_2$  can be constructed (Fig. 4). The reader may examine the figure against Figures 1 and 3.

Now

$$n_1 = \frac{1}{2} [(z^3 + z^{14}) + (z^5 + z^{12})] = x_3 + x_5, \tag{21}$$

and

$$n_2 = \frac{1}{2} [(z^6 + z^{11}) + (z^7 + z^{10})] = x_6 + x_7. \tag{22}$$

Note that

$$n_1 + n_2 = x_3 + x_5 + x_6 + x_7 = \ell_2, \text{ and } n_1 n_2 = -\frac{1}{4}. \tag{23}$$

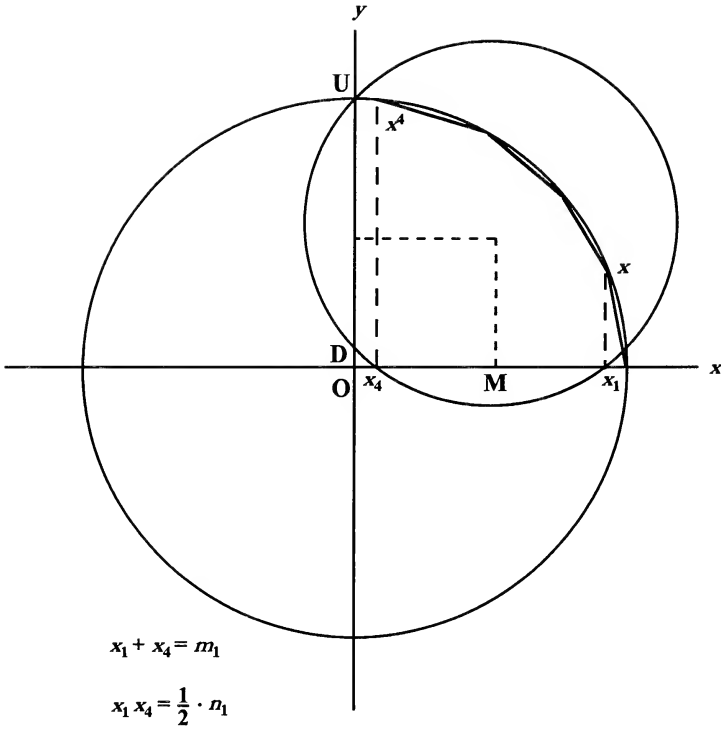


Figure 6. Construction of the roots of equation (28), the final result.

Therefore  $n_1$  and  $n_2$  satisfy the quadratic equation

$$n^2 - \ell_2 n - \frac{1}{4} = 0. \tag{24}$$

Constructing the roots of (24), one obtains (Fig. 5).

Finally note that

$$x_1 = \frac{1}{2} (z + z^{16}) \text{ and } x_4 = \frac{1}{2} (z^4 + z^{13}). \tag{25}$$

So

$$x_1 + x_4 = \frac{1}{2} (z + z^4 + z^{13} + z^{16}) = m_1 \tag{26}$$

and

$$x_1 x_4 = \frac{1}{4} (z^5 + z^{14} + z^3 + z^{12}) = \frac{1}{2} n_1. \tag{27}$$

Therefore  $x_1$  and  $x_4$  satisfy the quadratic equation

$$x^2 - m_1x + \frac{1}{4}n_1 = 0. \quad (28)$$

Constructing roots of (28) gives the final result (Fig. 6). Note that  $z$  and  $z^4$  can be obtained from  $x_1$  and  $x_4$ . The rest of the construction is quite clear.

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CHROMOSOMAL VARIATION IN THE SCRUB MOUSE  
*AKODON MOLINAE* (RODENTIA: SIGMODONTINAE)  
IN CENTRAL ARGENTINA

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**Abstract.**—A cytogenetic study of 34 specimens of the scrub mouse *Akodon molinae* from nine localities in La Pampa and San Luis provinces of central Argentina revealed  $2n = 43$  in 18 specimens,  $2n = 44$  in eight and  $2n = 42$  in eight individuals. This variation results from a Robertsonian polymorphism involving chromosome pair 1. Information on the distribution and biogeography of *Akodon molinae* is provided for central Argentina.

**Resumen.**—En un estudio citogenético de 34 ejemplares del ratón pajizo *Akodon molinae* provenientes de nueve localidades de las provincias de La Pampa y San Luis en el centro de Argentina, se encontró  $2n = 43$  en 18 especímenes,  $2n = 44$  en ocho y  $2n = 42$  en ocho individuos. Esta variación se debe a un polimorfismo Robertsoniano en el par 1. Se incluye información sobre la distribución y biogeografía de *Akodon molinae* para el centro de Argentina.

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The scrub mouse, *Akodon molinae* Contreras 1968, is one of the most studied sigmodontine rodents in South America (Myers 1988). Molecular (Apfelbaum & Blanco 1984; Apfelbaum & Reig 1989), ecological (Ojeda 1989) and cytogenetical (Bianchi et al. 1969; 1973; 1976; 1979a; Bianchi & Merani 1980; Wittouck et al. 1995) information has been described. In central Argentina, this widespread species inhabits Espinal forests dominated by caldén, *Prosopis caldenia*. It also occurs in scrub vegetation of halophytic shrub communities, Monte Desert habitats, borders of grasslands and cultivated fields, and deforested areas where there is adequate shrub cover.

*Akodon molinae* is difficult to distinguish morphologically from other members in the *A. varius* species group to which it belongs (Myers 1989), and cytogenetics can provide key characters for identification. *Akodon molinae* has a variable karyotype with a diploid number ranging from  $2n = 42$  to 44. In the  $2n = 42$  form the first pair of autosomes is represented by two large metacentrics, which are represented by four large subtelocentrics in the  $2n = 44$  cytotype. The  $2n = 43$  is the most common variant and the first pair of chromosomes consists of two sub-

metacentrics and a large metacentric. The rest of the autosomes are acrocentric except for the smallest pair which is metacentric. The X chromosome is a large acrocentric and the Y chromosome is a small acrocentric (Bianchi et al. 1973). This polymorphism was first reported by Bianchi et al. (1969), and subsequently described in greater detail (Bianchi et al. 1971; 1973; 1979a; Wittouck et al. 1995).

*Akodon dolores*, described by Thomas (1916), a related if not conspecific form, shares chromosomal banding patterns and morphology with *A. molinae* and both have high values of genetic similarity, based on protein electrophoresis (Bianchi et al. 1979b; Apfelbaum & Blanco 1984; Myers 1989). The basic karyotype of *Akodon dolores* ( $2n = 34$ ) consists of five pairs of large metacentric or submetacentric chromosomes, eleven pairs of acrocentric chromosomes of decreasing size, and a pair of small metacentrics. The X chromosome is an acrocentric of medium size and the Y chromosome a small acrocentric. Additionally, the first five pairs of banded chromosomes can present polymorphisms producing individuals with  $2n = 34-40$  diploid numbers (Kiblski et al. 1976; Wittouck et al. 1995). The geographic distribution of both, based on published records, do not overlap (Apfelbaum & Blanco 1984). Moreover, at the type locality of *Akodon dolores* specimens had the karyotype originally described as belonging to *A. molinae* (cf. Wittouck et al. 1995). In this report the specific name (epitax) "*molinae*" is applied to the  $2n = 42-43-44$  forms. Herein, additional cytogenetic data for *Akodon molinae* are reported from other areas of central Argentina for which this information has not been published.

#### MATERIAL AND METHODS

Thirty-four specimens of *Akodon molinae* were collected from nine localities in San Luis and La Pampa provinces of central Argentina. In most cases, the yeast stress method (Lee & Elder 1980) was used to obtain a higher mitotic index. Specimens were subjected to the standard procedure of in-vivo colchicine induced mitotic arrest for obtaining chromosomes from bone marrow. Slides were produced by dropping the cell suspension from a 50-60 cm height into a large drop of distilled water on the surface of the slide (Baker et al. 1982). Chromosome slides were Giemsa stained, observed and photographed and the diploid number and chromosomal morphology were determined for each specimen based on ten spreads.

*Material examined.*—All voucher specimens were prepared as standard study skins and skulls or fluid-preserved and are housed in the collections of Texas Tech University Museum (TTU), Lubbock, Texas;

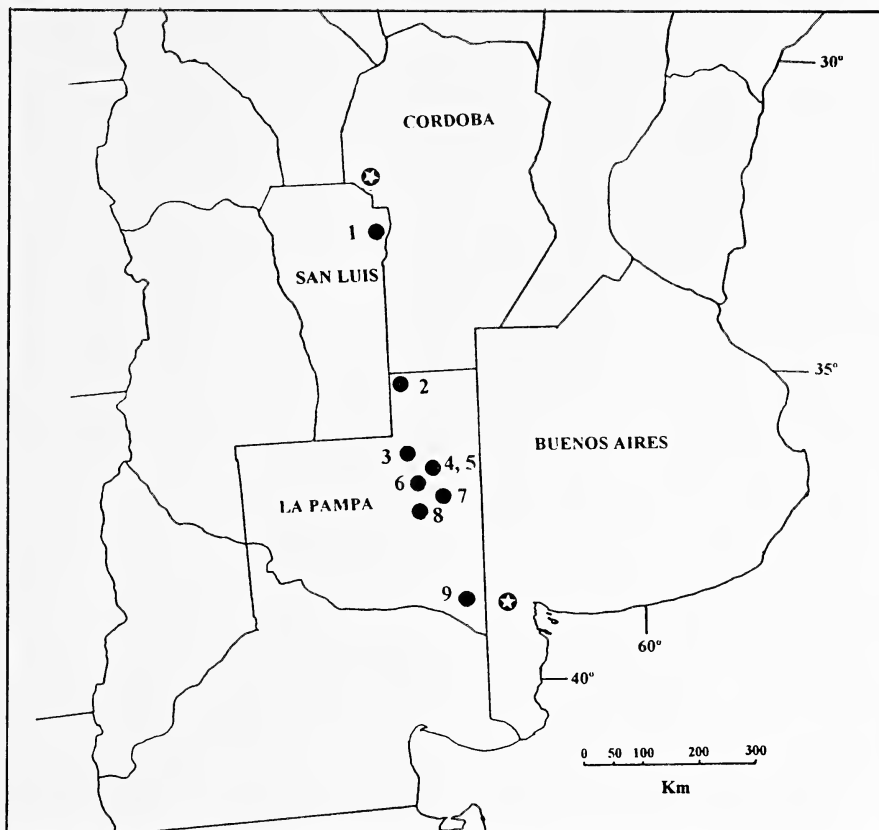


Figure 1. Map of central Argentina depicting collecting localities. Numbers are referenced in the text. Stars represent the type localities of *Akodon dolores* (in Córdoba) and *A. molinae* (in Buenos Aires).

the mammal collection of the Universidad Nacional de Río Cuarto (UNRC), Río Cuarto, Córdoba, Argentina; and the collection of La Pampa Vertebrate Survey (RVP, Plan de Relevamiento de los Vertebrados de la Provincia de La Pampa), deposited in the Museo Provincial de Historia Natural, Santa Rosa, La Pampa, Argentina. Vegetation information for habitats where *Akodon molinae* specimens were collected in La Pampa province follows Cano et al. (1980). Collection localities with numbers in parenthesis are referenced to Figure 1.

San Luis Province: Chacabuco Department: Rincón de Papagayos (Site 1): One female (RVP 245). General vegetation of the area are Chaco Serrano transition forests with palms.

La Pampa Province: Rancul Department: 15 km SW Chamaicó, Loma Loncovaca (Site 2): One female (RVP 246), open Espinal forests of caldén (*Prosopis caldenia*). Toay Department: ca. 45 km NW of Santa Rosa, Estancia El Pincén (Site 3): One female (TTU 64404), open



Figure 2.  $2n = 42$  *Akodon molinae* karyotype. Estancia Los Toros, Toay Department, La Pampa, Argentina (TTU 64401, male).

Espinal forests of caldén; 10 km SW of Santa Rosa, Chacra La Lomita (Site 5): One male (TTU 66518) and one female (TTU 66517), crops with pastures and linear habitats along fences with piquillín (*Condalia microphylla*), molle (*Schinus* sp.), and caldén; 12 km NNE of Naicó, Estancia Los Toros (Site 6): Eight males (TTU 64393, TTU 64395, TTU 64397, TTU 64399, TTU 64401, TTU 64402, TTU 64403, RVP 247) and eight females (TTU 64394, TTU 64392, TTU 64396, TTU 64398, TTU 64400, TTU 66519, UNRC 163, UNRC 164), young ("renoval") caldén forests and pastures; Parque Luro (Site 7): Four males (RVP 248, RVP 249, UNRC 165, UNRC 166) and two females (RVP 250, UNRC 167), open Espinal forests of caldén. Capital Department: Laguna Don Tomás, Santa Rosa (Site 4): Two females (TTU 64391, UNRC 168), floodable area with semihalophytic shrubs. Utracán Department: 10 km W Quehué, Estancia Los Molinos (Site 8): Two males (TTU 64405, TTU 64406), caldén forests and mixed shrublands of *Larrea divaricata*, *Condalia microphylla* and *Chiquiraga erinacea*. Caleu Caleu Department: 40 km N Anzoategui, Almacén El 52 (Site 9): Two males (TTU 64388, TTU 64390) and one female (TTU 64389), mixed shrubland composed mainly of *Condalia microphylla*, *Larrea divaricata* and *Acantholippia seriphioides* with *Prosopis caldenia* forests.

## RESULTS AND DISCUSSION

Eight of the specimens examined had the  $2n = 42$  chromosomic variant (Fig. 2). The majority ( $n = 18$ ) possessed the heterozygous  $2n = 43$  karyomorph (Fig. 3a), and the  $2n = 44$  cytotype was present in eight individuals (Fig. 3b). These were distributed in the localities sampled as follows: Rincón de Papagayos (1 = 42), Loma Loncovaca (1 = 42), Estancia El Pincén (1 = 43), Laguna Don Tomás (2 = 43), Chacra La Lomita (1 = 42; 1 = 43), Estancia Los Toros (2 = 42; 7 =



Figure 3. Pair 1 chromosome polymorphism. a.  $2n = 43$  (UNRC 163). b.  $2n = 44$  (TTU 64402). Estancia Los Toros, Toay Department, La Pampa, Argentina.

43; 7 = 44), Parque Luro (2 = 42; 4 = 43), Estancia Los Molinos (1 = 42; 1 = 43), Almacén El 52 (2 = 43; 1 = 44). Previously, different proportions of these karyomorphs have been reported. For Chasicó, Buenos Aires Province, the type locality of *A. molinae*, a total of 18 specimens possessed the  $2n = 42$  karyotype, 14 had the  $2n = 43$ , and one had the  $2n = 44$  form (Bianchi et al. 1969; 1971; 1973). More recently Wittouck et al. (1995) reported three animals with 42, nine with 43, and nine with 44 chromosomes for Yacanto and Villa Dolores, Córdoba Province.

At Estancia Los Toros, all three cytotypes were present in proportions expected of a random mating population ( $\chi^2 = 0.015$ ,  $p = 0.01$  for accordance with Hardy-Weinberg equilibrium).

*Akodon molinae* is restricted to the south and west part of the range of the *A. dolores/molinae* complex, which is located in the Espinal forests and with some localities (not karyologically studied) inside the Monte Desert. The distribution of the *A. dolores* karyomorphs is restricted to the east of the Cordobean Sierras, except for specimens reported for Catamarca Province (Bianchi et al. 1979b). A careful survey may show the extent of the geographic distribution of these forms.

#### ACKNOWLEDGMENTS

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REPRODUCTION IN THE BLACKNECK GARTER SNAKE,  
*THAMNOPHIS CYRTOPSIS* (SERPENTES: COLUBRIDAE)

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**Abstract.**—Reproductive tissue was examined from 120 museum specimens of *Thamnophis cyrtopsis* from Arizona, New Mexico, Texas and the Mexican state of Sonora. This species of garter snake appears to follow a seasonal reproductive cycle in which maximum spermiogenesis occurs in summer-autumn. Females with enlarged follicles or developing embryos were found March-June. The mean clutch size for 25 females was  $8.4 \pm 4.3$  SD (range 3-24). The maximum clutch size of 24 is apparently a new record for this species. Neonates were found June to August.

The blackneck garter snake *Thamnophis cyrtopsis* occurs from south-east Utah to Guatemala and from central Texas to central and southern Arizona from sea level to around 2700 m (Stebbins 1985). The biology of this species is summarized by Webb (1980). There are only anecdotal reports on reproduction in this species (Sabath & Worthington 1959; Viitt 1975; Tennant 1984; Stebbins 1985; Jones 1990; Degenhardt et al. 1996). The purpose of this paper is to provide information on the seasonal ovarian and testis cycles of *T. cyrtopsis* and to report additional clutch sizes.

MATERIALS AND METHODS

A sample of 120 specimens of *Thamnophis cyrtopsis* (64 females) Mean Snout-Vent Length, SVL =  $508.6 \text{ mm} \pm 74.1 \text{ SD}$ , range 378-705 mm; (56 males) Mean SVL =  $441.2 \text{ mm} \pm 64.3 \text{ SD}$ , range 338-677 mm from Arizona, New Mexico, Texas and Sonora, México was examined from the herpetology collections of Arizona State University, Tempe and the University of Arizona, Tucson. Counts were made of enlarged follicles ( $> 6 \text{ mm}$  diameter), oviductal eggs or embryos. The left testis, epididymis, vas deferens and part of the kidney were removed from males; the left ovary was removed from females for histological examination. Tissues were embedded in paraffin and cut into sections at  $5 \mu\text{m}$ . Slides 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. Epididymides and vasa deferentia were examined for sperm. Slides of kidney sexual segments were examined for secretory activity.

Kidneys and vasa deferentia were not available for examination from some road-killed males.

*Material examined.*—The following specimens of *Thamnophis cyrtopsis* were examined from the herpetology collections of Arizona State University, Tempe (ASU) and the University of Arizona, Tucson (UAZ).

ARIZONA: APACHE COUNTY, 2 specimens (ASU 903-904); COCHISE COUNTY, 7 specimens (ASU 24232, UAZ 34524, 39933, 41604, 42342, 42478, 43802); GILA COUNTY, 6 specimens (ASU 2245, 2340, 2365, UAZ 30947, 35976, 44766); GRAHAM COUNTY, 11 specimens (ASU 7012, 7015, 7018, 7772, 7776-7778, 7784, 13293, 22465, UAZ 45889); GREENLEE COUNTY, 3 specimens (UAZ 26499, 42711-42712); MARICOPA COUNTY, 8 specimens (ASU 1364, 2208, 8844, 9163, 13889-13890, UAZ 37037, 43170); MOHAVE COUNTY, 1 specimen (ASU 27845); NAVAJO COUNTY, 2 specimens (ASU 3160, UAZ 35975); PIMA COUNTY, 35 specimens (UAZ 26483, 26487, 26490-26493, 26496, 26498, 26500, 26504, 26506-26508, 26512-26513, 26523-26524, 26528, 26531, 26533, 26542, 26548, 26567, 26569, 27274, 32925, 41597-41598, 41600, 41603, 41605, 41607, 42713, 44976, 47141); PINAL COUNTY, 3 specimens (ASU 906, 2388, 27391); SANTA CRUZ COUNTY, 13 specimens (UAZ 26511, 26517, 26526, 26560, 26565, 28602, 35534, 36072, 40501, 41590, 41594, 47324, 49999); YAVAPAI COUNTY, 2 specimens (ASU 950, UAZ 39927). NEW MEXICO: GRANT COUNTY, 1 specimen (UAZ 26509); HIDALGO COUNTY, 2 specimens (UAZ 32771-32772) TEXAS: JEFF DAVIS COUNTY, 1 specimen (UAZ 41819); VAL VERDE COUNTY, 1 specimen (UAZ 42256).

MÉXICO: SONORA, 22 specimens (UAZ 26687, 26690, 26692, 28066, 28138, 32626, 38944-38947, 39284, 39297, 42359, 45133, 45135, 45137, 45139-45140, 45994, 46459, 46461, 46671).

## 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*). In the regressed testes, seminiferous tubules contained spermatogonia and Sertoli cells. In recrudescence, there was renewal of spermatogenic cells characterized by spermatogonial divisions; primary and secondary spermatocytes and spermatids may have been present. In spermiogenesis, metamorphosing spermatids and mature sperm were present. Males undergoing spermi-



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

Month	N	Regressed	Recrudescence	Spermiogenesis
March	2	2	0	0
April	6	5	0	1
May	10	3	3	4
June	9	1	3	5
July	12	1	2	9
August	5	0	1	4
September	8	0	2	6
October	3	0	0	3
November	1	0	0	1

genesis were found April-November; regressed testes were found March-July (Table 1). Males with testes in recrudescence were found May-September. The smallest spermiogenic male measured 338 mm SVL. To avoid bias from including immature males in my analysis, males smaller than this size were excluded from the study. At least part of each monthly male sample was undergoing spermiogenesis April-November. Epididymides of all spermiogenic males contained sperm. Sperm were present in the vasa deferentia during all months (March-November) indicating *T. cyrtopsis* has the potential of breeding throughout the year. However, because 11/27 (41%) males had regressed testes in spring and 10/12 (83%) of September-November males were undergoing spermiogenesis (Table 1), the male cycle appeared to fit the postnuptial type of breeding pattern (Saint Girons 1982; Seigel & Ford 1987) with maximum spermatogenic activity in late summer and fall and mating occurring the following spring utilizing sperm stored overwinter in the vasa deferentia. One must also consider the possibility of some *T. cyrtopsis* mating occurring during autumn. Fall matings have been reported in three species of *Thamnophis* (*T. atratus*, *T. ordinoides*, *T. sirtalis*) and suggested to occur in two additional species (*T. radix* and *T. elegans*) (see Rossman et al. 1996). Kidney sexual segments were enlarged and contained secretory granules in 23/26 (88%) of males undergoing spermiogenesis, 5/6 (83%) males with regressed testes and 2/4 (50%) of males with recrudescence testes. Mating coincides with hypertrophy of the kidney sexual segment (Saint Girons 1982).

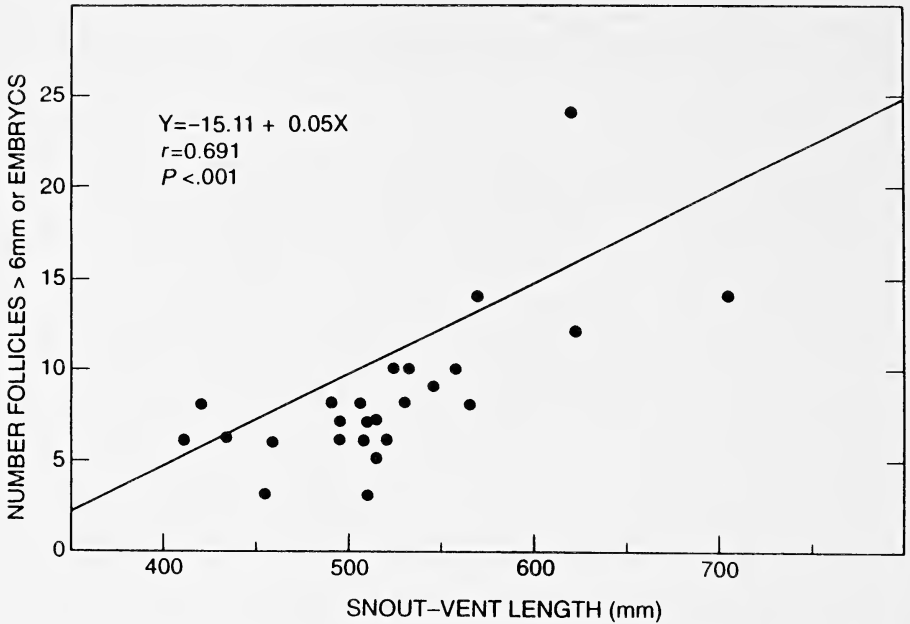


Figure 1. Linear regression of enlarged follicles (> 6 mm) or developing embryos on snout-vent length for 25 *Thamnophis cyrtopsis* from Arizona and Sonora, México, including two Arizona specimens from Vitt (1975).

The smallest reproductively active female (follicles > 6 mm diameter) measured 412 mm SVL. All females smaller than 412 mm SVL were excluded from the study to avoid bias from including immature females in my analysis of the ovarian cycle. Mean clutch sizes for 25 females, including clutches of 6 and 10 from Vitt (1975), averaged  $8.4 \pm 4.3$  SD (3-24 range). There was a significant positive correlation between female body size and enlarged follicles > 6 mm, or embryos ( $P < .001$ ) (Fig. 1). Other litter sizes from the literature were from Arizona and Texas: (Arizona) eight young were born from one female on 3 July (Vitt 1975) and four females gave birth, 29 June (14 young), 11 July (19 young), 17 July (21 young), 19 July (22 young) (Jones 1990); (Texas) seven young were born from one female on 14 August (Sabath & Worthington 1959) and six young were born from one female on 14 July (Tennant 1984). Young of *T. cyrtopsis* were born the second week of July in New Mexico (Flehart 1967). Stebbins (1985) reported a range of about 7-25 young. Apparently, the litter of 25 young attributed in the literature to *T. cyrtopsis* was actually from *T. eques* (see Degenhardt et al. 1996). Thus, the clutch of 24 reported herein may represent the largest clutch known for *T. cyrtopsis*.

Table 2. Monthly distribution of conditions in seasonal ovarian cycle of *Thamnophis cyrtopsis*. Values shown are the numbers of females exhibiting each of the four conditions.

Month	N	Inactive	Yolk deposition	Enlarged follicles (> 6 mm diameter)	Developing embryos
March	5	1	1	2	1
April	17	4	0	12	1
May	6	1	0	0	5
June	10	7	1	0	2
July	12	12	0	0	0
August	3	3	0	0	0
September	8	8	0	0	0
October	1	1	0	0	0
November	2	2	0	0	0

The female reproductive cycle appears to follow that of other species of *Thamnophis* (see Rossman et al. 1996) in having prenuptial or Type 1 secondary vitellogenesis (*sensu* Aldridge 1979) with females entering hibernation with small, previtellogenic follicles. Secondary vitellogenesis (yolk deposition) occurs in spring. Females with enlarged follicles > 6 mm diameter or developing embryos were found March-June (Table 2). The presence of some inactive females during the period when gravid females were found (March-June) suggests that not all mature females produce litters each year. Data from Table 2 indicate 23/38 (61%) of females from March-June produced litters. It is not known if the one March and one June female *T. cyrtopsis* that were undergoing yolk deposition would have produced litters. This figure (61%) is within the range of gravid females (46-100%) for six species of *Thamnophis* in Seigel & Ford (1987). Data on reproductive frequency for other species of *Thamnophis* suggests most females reproduce each year, although some females may not breed every year (Rossman et al. 1996). This appears to be the case for females of *T. cyrtopsis*.

Neonates were found in June to August. The date of birth, size of neonates at birth and growth rates likely show geographic and yearly variation.

#### CONCLUSIONS

*Thamnophis cyrtopsis* appears to follow a seasonal reproductive cycle in which maximum spermiogenesis occurs in late summer-autumn.

Sperm is stored overwinter in the vasa deferentia. Mating presumably takes place in spring with the possibility of some occurring in autumn. Gravid females were found March-June. Mean clutch size was  $8.4 \pm 4.3$  SD (range 3-24). The maximum clutch size of 24 may represent a new record for this species.

#### ACKNOWLEDGMENTS

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## A COMPARISON OF TRAP EFFECTIVENESS FOR REPTILE SAMPLING

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**Abstract.**—Comparisons of reptile populations require that sampling techniques provide unbiased estimates across habitats and seasons. Use of multiple techniques are frequently suggested to fully sample reptile communities. The effects of multiple sampling techniques on measurements and analyses of population attributes are limited. This study compared measurements of abundance based upon drift fences equipped with pitfall and funnel traps to plywood artificial structures in two habitats in the Chihuahuan Desert. Drift fence arrays produced higher abundance estimates for *Cnemidophorus marmoratus* ( $P < 0.001$ ) than artificial structures. Artificial structures attracted more *Uta stansburiana* on uplands compared to arroyos ( $P = 0.045$ ) but this result is biased because of an inability to distinguish individual animals with plywood artificial habitat. Provision of water under plywood artificial habitats had no measurable effect on capture rate. Within drift fence arrays, pitfall traps captured more *C. marmoratus* and *C. inornatus* than funnel traps. *Cnemidophorus marmoratus* seemed to be susceptible to pitfall trapping during spring ( $P = 0.058$ ). *Uta stansburiana* were susceptible to funnel trapping during spring, but had equal catchability for all other season x method combinations ( $P = 0.012$ ). Plywood artificial structures produced biased abundance estimates for *U. stansburiana* among habitats. Funnel traps and drift fences alone would produce a seasonal bias for *U. stansburiana* whereas pitfall traps and drift fences alone produced a seasonal bias for *C. marmoratus*.

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There are numerous techniques available for surveying herpetofaunal communities (Campbell & Christman 1982; Vogt & Hine 1982; Heyer et al. 1994). Capture biases between species are often related to trapping techniques (Gibbons & Semlitsch 1981). Thus, it is recognized that no single technique is sufficient for successful detection of all species within a community (Storm & Pimental 1954; Gibbons & Semlitsch 1981; Campbell & Christman 1982; Vogt & Hine 1982; Dodd 1991; Rice et al. 1994). For this reason, several techniques are frequently applied simultaneously in various combinations (i.e., pitfalls, time or area constrained searches, artificial habitats, and funnel traps). With so many techniques, even empirical comparisons between studies require an understanding of relative method effectiveness.

Direct comparisons of technique effectiveness are limited. Gibbons & Semlitsch (1981) compared drift fences equipped with pitfall traps to

hand collecting in South Carolina. Campbell & Christman (1982) compared drift fence arrays equipped with pitfalls and funnels to pitfall traps alone (without drift fences) and to time- and area-constrained searches in Florida. They also compared drift fences differing in configuration and total fence length. Vogt & Hine (1982) compared drift fence materials in Wisconsin. Bury & Corn (1987) compared four combinations of drift fences, pitfalls, and funnels in Oregon and Washington. Interestingly, comparisons in desert ecosystems are few despite the importance of herpetofauna in these areas. Rice et al. (1994) conducted a pilot study in New Mexico comparing several trapping/detection methods, but there were few animals and sampling time was limited. A general conclusion of these investigations is that drift fences provide the best results for the effort expended (Gibbons & Semlitsch 1981).

Drift fences are frequently used in combination with pitfall traps or funnel traps, alone or in combination. Vogt & Hine (1982) found that funnel traps captured more lizards than pitfall traps in Wisconsin. Except for this study, the relative effectiveness of pitfall traps and funnel traps when used with drift fences is not known.

Frequently, the goal of research efforts is measurement of population (density, etc.) or community (diversity, etc.) characteristics. Comparisons of such characteristics between habitats is the basis for management action. The ability of sampling methods to produce unbiased estimates of population characteristics is critical for habitat-based comparisons.

This study compared the effectiveness of drift fences equipped with pitfall traps and funnels to plywood artificial structures in two habitats associated with foothills of the Chihuahuan Desert. Also the effectiveness of plywood artificial structures with and without addition of supplemental water was compared. Finally, the relative contribution of pitfall and funnel traps associated with drift fences to the total capture efficiency was investigated during the spring and fall of 1994.

#### MATERIALS AND METHODS

Factors influencing reptile captures and surveys in two habitats associated with foothills of the Sacramento Mountains were studied in the Tularosa Valley of southern New Mexico. The area encompassed approximately 126 km<sup>2</sup> of desert/montane ecotone. Elevation ranged from 1255-1585 m. Rainfall during the sampling period was 0.94 cm

below normal (19.33 cm actual vs. 20.27 cm expected) (National Oceanic and Atmospheric Administration 1994). Habitats comprised arroyos and uplands as identified by Jorgensen (1996). Sampling sites were located in arroyos and on adjacent uplands (eight arroyo, eight upland) in eight drainages (blocks). Sampling was conducted with drift fence arrays equipped with pitfall and funnel traps, and plywood artificial structures. Drift fence sampling was conducted in each area whereas artificial structure sampling was conducted in only four of the drainages. Sampling was conducted from 18 March to 26 September, 1994 except that funnel traps were removed during the month of July to prevent mortality caused by hyperthermia.

Drift fence arrays were comprised of three permanently mounted 7.6 m fences (25.6 cm high) separated by 120° (Jones 1986) meeting at a common proximal center where a pitfall bucket (depth = 29 cm, diameter = 21 cm) was placed. Pitfall buckets were also placed at distal ends of each fence. Fencing was constructed of aluminum or galvanized steel flashing. Funnel traps (Imler 1945; Fitch 1987) were placed along each side of each fence midway between the center and the ends. Funnels were cylindrical and were constructed from two 1.9 liter (1/2 gal) black plastic flower pots with the bottoms cut out and metal window screen. Different pieces of window screen were stapled to each flower pot and formed into a cylinder. Window screen was also used to form funnel entrances at the end of the cylinder opposite the flower pot. The completed trap was formed by placing one flower pot within the other. Pitfall buckets and funnel traps were shaded with small pieces of plywood.

Artificial structure sampling units were comprised of four quarter-sheets of plywood laid flat against the substrate and placed in the same general area. Two sheets were placed on relatively sandy substrata and two sheets were placed on relatively stony substrata. The substrate under one plywood sheet on each substrate type was wetted with approximately 0.5 liter of water, twice per week throughout the study period.

Drift fence arrays equipped with pitfall and funnel traps were compared to artificial structures in arroyos and on adjacent uplands in four drainages (blocks). The number of unique animals (marked with toe clips) captured by drift fences was compared to the number of sightings at artificial structures (animals located underneath the structures that

were sighted when the structures were lifted or that ran as the structure were approached). The analysis is biased in favor of artificial structures because multiple sightings of the same animal were possible. This analysis was used because it accurately reflects a recognized bias with artificial structures; it was frequently impossible to capture sighted animals and thus ensure that they were only counted once. The analysis is further biased in favor of artificial structures because funnel traps were removed from drift fences during the month of July to prevent mortality due to hyperthermia. A 2-way *ANOVA* was used for randomized blocks, treatments being habitat (arroyo vs. upland) and trapping method (drift fences vs. artificial structures).

The ability of supplemental water to influence artificial structure use in arroyos and on adjacent uplands was investigated in four drainages (blocks). Data analyzed were total animals sighted. A 2-way *ANOVA* was used for randomized blocks, treatments being habitat (arroyo vs. upland) and water supplement (supplemented vs. not supplemented).

Total captures were compared between pitfall and funnel traps located within the same drift fence array (block) during two seasons (spring [18 March 30 June] and fall [1 August 26 September]). Data analyzed were number of animals per 100 drift fence nights of effort. The data were analyzed with 2-way *ANOVA* for randomized blocks, treatments being season (spring and fall) and trap type (pitfall or funnel).

Analyses were conducted with SPSS for Windows (Norusis 1993) and Statistix (Analytical Software 1991).

## RESULTS

*Drift fence arrays vs. artificial structures.*—Drift fence arrays caught more *Cnemidophorus marmoratus* ( $P < 0.001$ ) than artificial structures attracted (Table 1). Artificial structures attracted more *Uta stansburiana* on uplands than in arroyos (F-test  $P = 0.045$ , LSD test  $P = 0.05$ ) (Table 1). Provision of water under plywood artificial habitats had no measurable effect on site attraction for 3 common lizard species (Table 2).

*Pitfall vs. funnel traps within drift fence arrays.*—Pitfall traps captured more *C. marmoratus* and *Cnemidophorus inornatus* than funnel traps located within the same drift fence array (Table 3). Also, *C. marmoratus* seemed to be especially susceptible to pitfall trapping during



Table 1. Relative abundance of reptiles for two methods (under plywood artificial habitat [Ply-total sightings] or drift fence array system [Ary-number of unique individuals]) of capture in two Chihuahuan Desert habitats. Values accompanied by the same superscript in the same row are not significantly different.

Method	Method		se	P
	Ply	Ary		
<i>Cnemidophorus marmoratus</i>	1.0	20.4	1.28	0.000
<i>Crotaphytus collaris</i>	0.8	0.3	0.49	0.451
<i>Sceloporus undulatus</i>	0.3	0.8	0.32	0.302
<i>Uta stansburiana</i>	11.5	10.4	1.87	0.680
<i>Hypsiglena torquata</i>	0.6	0.3	0.33	0.449

Habitat x Method Interaction	Habitat				se	P
	Arroyo		Upland			
	Ply	Ary	Ply	Ary		
<i>Cnemidophorus marmoratus</i>	0.3	20.3	1.8	20.5	1.81	0.737
<i>Crotaphytus collaris</i>	0.0	0.5	1.5	0.0	0.63	0.150
<i>Sceloporus undulatus</i>	0.5	1.5	0.0	0.0	0.46	0.302
<i>Uta stansburiana</i>	4.0 <sup>b</sup>	9.0 <sup>b</sup>	19.0 <sup>a</sup>	11.8 <sup>ab</sup>	2.64	0.045
<i>Hypsiglena torquata</i>	0.0	0.5	1.3	0.0	0.47	0.098

spring, although the season x method interaction failed to achieve significance by a small amount ( $P=0.058$ ) (Table 3) (*C. marmoratus* were equally abundant during spring and fall [Jorgensen 1996]). *Uta stansburiana* were more susceptible to funnel trapping during spring, but had equal catchability for all other season x method combinations (Table 3).

## DISCUSSION

Plywood artificial structures attracted fewer *C. marmoratus*, the active forager, than drift fence arrays captured (1.0 vs. 20.4) (Table 1). Samples from plywood artificial habitats indicated there were more *U. stansburiana*, the sit-and-wait forager, on uplands compared to arroyos (Table 1). This result contrasts with the drift fence result from the same study sites that displays no such effect (Table 1) and with Jorgensen (1996) who also found no such effect sampling over two years in the same study area.

Site supplementation with water did not alter capture rates for plywood artificial structures (Table 2). Addition of water did not confound sampling when rainfall occurred. Rather, supplemental water was additive to rainfall.

Table 2. Relative abundance (total sightings) of lizard species under watered (+) and unwatered (-) plywood artificial habitats during 1994, in two habitats.

	Water		se	P
	+	-		
<i>Cnemidophorus marmoratus</i>	0.5	0.5	0.14	1.000
<i>Sceloporus undulatus</i>	0.1	0.1	0.13	1.000
<i>Uta stansburiana</i>	5.5	6.0	1.19	0.774

Water x Habitat Interaction	Habitat				se	P
	Arroyo		Upland			
	+	-	+	-		
<i>Cnemidophorus marmoratus</i>	0.0	0.3	1.0	0.8	0.20	0.252
<i>Sceloporus undulatus</i>	0.3	0.3	0.0	0.0	0.19	1.000
<i>Uta stansburiana</i>	2.0	2.0	9.0	10.0	1.69	0.774

Table 3. Relative abundance (captures per 100 drift fence nights) of lizard species captured in pitfall (Pit) and Funnel (Fun) traps within drift fence arrays during spring and fall, 1994. Values accompanied by the same superscript in the same row are not significantly different.

	Pit	Fun			se	P
<i>Cnemidophorus marmoratus</i>	14.7	7.9			1.38	0.002
<i>Cnemidophorus exsanguis</i>	0.5	1.4			0.36	0.120
<i>Cnemidophorus inornatus</i>	1.3	0.4			0.26	0.024
<i>Cophosaurus texanus</i>	1.2	0.0			0.45	0.076
<i>Sceloporus undulatus</i>	0.4	0.7			0.33	0.529
<i>Uta stansburiana</i>	5.3	8.7			1.05	0.031

Season x Method Interaction	Season				se	P
	Spring		Fall			
	Pit	Fun	Pit	Fun		
<i>Cnemidophorus marmoratus</i>	22.4	11.7	7.0	4.2	1.95	0.058
<i>Cnemidophorus exsanguis</i>	0.7	1.6	0.4	1.1	0.51	0.749
<i>Cnemidophorus inornatus</i>	1.5	0.3	1.1	0.4	0.37	0.515
<i>Cophosaurus texanus</i>	1.5	0.0	0.9	0.0	0.63	0.638
<i>Sceloporus undulatus</i>	0.5	1.3	0.2	0.0	0.47	0.281
<i>Uta stansburiana</i>	4.4 <sup>b</sup>	12.0 <sup>a</sup>	6.1 <sup>b</sup>	5.5 <sup>b</sup>	1.49	0.012

Pitfall and funnel traps used in conjunction with drift fences complement each other in that each catches more individuals of at least one species (Table 3). Pitfall traps captured more *C. marmoratus* and *C. inornatus* than did funnel traps, whereas funnel traps captured more *U. stansburiana* (Table 3). Interestingly, funnel traps were especially effective for *U. stansburiana* during spring (Table 3). This suggests that if only funnel traps were used with drift fences for *U. stansburiana*, seasonal effects would be detected where none exist. Thus, drift fences

equipped with only funnel traps are biased for seasonal sampling, at least for *U. stansburiana*.

Density and correlated measures of abundance, although imperfect, are frequently the basis for interpreting ecological significance (Brown 1995) and undertaking management action (Anderson & Gutzwiller 1996). Therefore, it is important that biases inherent in sampling techniques be identified so that they can be controlled through experimental design. Otherwise, interpretations of results will be flawed with potential adverse consequences for species and habitat.

### CONCLUSION

It is concluded that drift fence arrays equipped with both pitfall and funnel traps are necessary for sampling reptile populations, at least in mixed desert scrub upland and arroyo habitats. Plywood artificial structures were ineffective and biased for sampling reptile populations in this study area. Further, plywood artificial habitat sampling is biased based upon the fact that it is difficult, or even impossible, to distinguish individual animals.

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## GENERAL NOTES

RECENT RECORDS OF THE RIVER OTTER (*LUTRA CANADENSIS*)  
ALONG THE TEXAS GULF COAST

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The North American river otter (*Lutra canadensis*) formerly ranged throughout most of the eastern portion of Texas from the coast west to approximately the 98th Meridian (Bailey 1905). Schmidly (1983) places otter occurrence east of the Balcones Fault Zone, which separates the Gulf coastal plains from the upland plateaus and plains of central and western Texas. River otters are presently known only from the eastern one-fourth of the state in major watersheds (Davis & Schmidly 1994). Otters have most likely been extirpated from the Panhandle, north-central and southern Texas (Davis & Schmidly 1994). Historically, river otter presence was indicated as far south as Cameron County (Schmidly 1984; Davis & Schmidly 1994).

A significant decline in the Texas river otter population in the early 1920s was attributed to over-harvest for the fur trade and caused the state to suspend otter trapping from 1927-1950 (Brownlee 1977; Bartnicki & Boone 1989). Declines in otter populations in Texas due to hunting and trapping have recently been noted as early as the 1830s (Weniger 1997). Current river otter populations are considered to be increasing in some areas (Brownlee 1977; Toweill & Tabor 1982), but limited to the Pineywoods, Post Oak Savannah, and Gulf Prairie and Marsh regions of the easternmost quarter of the state (Brownlee 1977; Foy 1984; Schmidly 1984; Bartnicki & Boone 1989).

Boone (1983) indicated otter presence was not recorded south of the Trinity River system. Coastal river otter populations have been reported as restricted to the area from eastern Galveston County northeast to

Orange County (Bartnicki & Boone 1989). Available information on the current range of *L. canadensis* does not indicate it to occupy the Texas Gulf Coast any farther south than the Bolivar Peninsula. Current maps of river otter distribution in Texas do not depict river otter presence in Matagorda County. Over the past four years, river otters (single and in groups) have been sighted on 22 different instances along the coastal waters of Galveston and Matagorda counties (see Sighting Records). This constitutes a possible southern extension of the present range of the river otter of at least 150 km along the Texas Gulf Coast.

Several opportunistic sightings were made on Galveston Island and in the Galveston Ship Channel (GSC) in Galveston County, and near the Intracoastal Waterway (ICWW) on the Clive Runnells Family Mad Island Marsh Preserve (MIMP) in Matagorda County. Two of the Galveston County sightings were made while conducting bottlenose dolphin (*Tursiops truncatus*) surveys for the Marine Mammal Research Program (MMRP), Texas A&M University at Galveston; other sightings were reported by local residents, colleagues and fishermen. The MIMP is approximately 13 km southwest of Matagorda and 14.5 km northeast of Palacios in Matagorda County at 28°40'N 96°05'W. Sightings in the MIMP were collected by staff of the Nature Conservancy of Texas during routine preserve monitoring. Sightings not made directly by the authors were confirmed by verifying the observers' descriptions.

The following is a listing of the records reported herein. Data are grouped by county and in chronological order. Specific arrangement of these data are: County; Specific locality; Date Observed; Numbers observed; Type of evidence (sighting, specimens, photo); Deposition of evidence (if available).

*Sighting records.*—**Galveston County:** GSC between the Pelican Island Bridge and Pennzoil Sulfur Dock, across from Texas A&M University at Galveston (TAMUG) campus; 8 and 27 Aug. 1994; 4 individuals; sightings and photographs; photos held at MMRP, TAMUG. Residential boat slips/canals in Jamaica Beach (10 km W of Galveston on FM 3005, Galveston Island at 29°22'28"N 94°58'47"W); 9 Sept. 1994; 1 individual; sighting. GSC at Del Monte Foods dock (Pier 18); 23 Nov. 1994; 1 individual; sighting. TAMUG ship dock at Pelican Island; 23 Nov. 1994; 1 individual; sighting. GSC at Texas Sea Port Museum/Elissa dock (Pier 21); 11 Feb. 1995; 1 individual; sighting. Hwy. 146 at Loop 197 bridge in a wastewater discharge

canal, Texas City, TX; 27 March 1995; 2 individuals; sighting. Galveston Island Causeway (southbound at Galveston Island); 19 Aug. 1995; 3 carcasses; photographs; photos held at MMRP, TAMUG. Residential boat slips/canals in Jamaica Beach (10 km west of Galveston on FM 3005, Galveston Island at 29°22'28"N 94°58'47"W); 7 Oct. 1995; 1 individual; sighting. Spanish Grant Subdivision (off Stewart Rd. 1.6 km west of nine mile road on Galveston Island); 10 Jan. 1996; 2 individuals; sighting. Bolivar Peninsula (approximately 1.6 km from the ferry landing); 2 April 1996; 1 individual; sighting. Spanish Grant Subdivision (off Stewart Rd. 2.9 km west of Nine Mile Road on Galveston Island); 10 April 1996; 1 carcass; pelt at Texas Cooperative Wildlife Collection (TCWC 53282; total length of 114 cm). Hwy. 146 (approximately 10 km from I45N), Texas City; 16 July 1996; 1 carcass; sighting. GSC at Del Monte Foods dock (Pier 18); 8 Dec. 1996; 1 individual; sighting. TAMUG ship dock at Pelican Island; 8 Dec. 1996; 1 individual; sighting. Bolivar Peninsula (0.40 km from ferry landing) 13 Nov. 1997; 1 carcass; sighting.

**Matagorda County:** Irrigation ditch along N. Road of MIMP; 30 Nov. 1991; 1 individual; sighting. Canal on MIMP; 28 Jan. 1994; 2 individuals; sighting. ICWW near the Mad Island Cut outlet to W. Matagorda Bay on MIMP; 8 Feb. 1994; 2 individuals; sighting. West Branch Slough Crossing on MIMP; 4 April 1995; 1 individual; sighting. N. Slough Crossing on MIMP; 11 Sept. 1995; 2 individuals; sighting. MIMP; 28 Feb. 1996; 2 individuals; sighting.

It was not possible to determine if the records were of different individuals or multiple sightings of a small number of animals. It is believed that the sightings in Galveston County made on 8 and 27 August 1994 represent the same group, based on location of the sighting (only a 13 m difference in location) and size and number of the animals.

While Melquist & Dronkert (1987) noted that food resources are usually responsible for range expansion or significant movements by otters, the results of the sightings reported here may indicate not only a possible range expansion but an increase in the Texas river otter population. Considering that trapping and hunting of otters is not as prevalent as it once was, these sightings appear to support the premise that otters may still be recovering from serious population decimation that began many years ago.

In spite of this possible recovery, otters inhabiting industrial and/or heavily trafficked areas such as the Galveston Ship Channel and surrounding areas, are subject to additional risks. The sighting made on 2 Apr. 1996 in Galveston County was of a river otter running across the road at night. All six carcasses noted for Galveston County were found on roadways. Four carcasses were recovered. Of these, only one (TCWC 53282) was in salvageable condition. Currently, automobile traffic and habitat destruction appear to be the worst threats to otters (Toweill & Tabor 1982; Foy 1984; Melquist & Dronkert 1987; Ehrhart 1995).

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ATYPICAL INFECTION OF ADULT  
*MACDONALDIUS SEETAЕ* KHANNA, 1933  
 (NEMATODA: FILARIATA) IN A TRANS-PECOS RAT SNAKE,  
*BOGERTOPHIS SUBOCULARIS* (SERPENTES: COLUBRIDAE)

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The genus *Macdonaldius* was established by Khanna (1933) for the type species *Macdonaldius seetae* Khanna 1933, which was collected from the portal vein of the colubrid snake, *Coluber melanoleucus*. Since it's description, eight more species have been described from Squamata hosts (four from snakes, four from lizards); and one from the order Testudines. Two of these species: *Macdonaldius carinii* Vaz & Pereira 1935, from snakes of Brazil; and *Macdonaldius pflugfelderi* Frank 1964, from *Physignathus lesueurii* of Australia, were transferred to the genus *Oswaldofilaria* Travassos, 1933 by Sonin & Baruš (1968). The following are considered valid species by Sonin (1968): *M. seetae* Khanna, 1933; *Macdonaldius andersoni*, Chabaud & Frank 1961; *Macdonaldius grassii* (Caballero 1954) Chabaud & Frank 1961; *Macdonaldius oschei*, Chabaud & Frank 1961; *Macdonaldius innisfailensis* (Mackerras 1962) Frank 1964; *Macdonaldius colimensis* Telford, 1965; and additionally, *Macdonaldius mackiewiczzi* Chattervati, 1985, which has subsequently been described.

The purpose of this note is to report adult *M. seetae* in the colubrid

snake *Bogertophis subocularis* (syn. *Elaphe subocularis*) for the first time, and additionally, to report this helminth being located atypically within a host.

One Trans-Pecos rat snake, *B. subocularis* (Brown 1901), (Serpentes: Colubridae) was received from a herpetological dealer on 21 December 1994, which was reported to be wild-caught from southwestern North America. It had died shortly after its arrival at the dealer, was frozen and maintained at  $-20^{\circ}\text{C}$  until examined for parasites, then deposited in the Carnegie Museum of Natural History (Section of Amphibians and Reptiles, Pittsburgh, Pennsylvania; Collection No. CM-147,466). The snake's internal organs did not appear to have undergone any obvious autolytic change.

Four adult specimens (one male, three females) of *M. seetae* (Filariata: Diplotriaenoidea) were collected from the surface of the host's mesentery on 24 June 1997. No specimens were found in any of the large abdominal vessels. Helminths were placed in 10% buffered formalin, transferred to 70% ethyl alcohol and studied as wet mounts in glycerin. Voucher *M. seetae* were deposited in the United States National Parasite Collection (USDA, Beltsville, Maryland; USNPC No. 87587). Morphological comparisons were made to the original descriptions of all recognized species, and to the following voucher and paratype specimens from the USNPC: *M. andersoni* (No. 34786); *M. colimensis* (No. 82139); *M. grassii* (No. 85041); and *M. seetae* (No. 34786).

The specimens were very filariform, whitish in color, and their morphological characteristics consistent with those described for the genus by Khanna (1933). The male specimen measured 25.3 mm long by 0.21 mm wide at its greatest width. The posterior end was tightly coiled in four spiral turns, and possessed five pairs of papillae: two pairs preanal, one of which is directly anterior to the anus; and three pairs postanal. The postanal papillae are positioned accordingly: one pair proximal (nearly perianal), one pair mesal, and one pair distal. Two pairs of amphids were located at the terminal end of the tail, one pair positioned slightly ventral and the other slightly dorsal. The right spicule was stout, and measured 0.11 mm long. The left spicule was long, slender, and possessed a filariform terminal end. No gubernaculum could be distinguished, nor could an exact measurement be determined for the left spicule. The female specimens measured

45.18-50.75 mm (mean 47.43) long by 0.27-0.37 mm (mean 0.31) wide at the greatest width. The undivided esophagus measured 0.758-0.808 mm (mean 0.775) long. The vulva possessed a single sphincter and was situated post-esophageal, 1.25-1.59 mm (mean 1.38) from the anterior end.

*Macdonaldius* is quite similar in its morphological characteristics between species and exhibits considerable intraspecific variation. Telford (1965) studied three endemic species from Colima, Mexico and considered the following characteristics taxonomically significant for species determination: total body length, arrangement of male caudal papillae, appearance of the distal tip of the left spicule, vulvar sphincter morphology, and comparative ratio's of esophageal length and distance of vulva from anterior end. Based on these taxonomic characteristics, the present specimens were identical to the *M. seetae* specimens described by Khanna (1933) with the exception of total length. However, this reduction in size may have resulted because of the specimens aberrancy within the host.

Hull & Camin (1959) redescribed this species as a result of numerous specimens being collected from two common bullsnakes, *Pituophis catenifer sayi* from Texas, which allowed a more thorough description of the species. The authors discussed a group of adanal papillae which they considered to be present in all male specimens of this species. They indicated that Khanna (1933) may have overlooked these papillae in the original description of *M. seetae*, since these are difficult to observe unless the specimen is properly oriented. Curiously, these papillae were not observed on the present male specimen either, even after numerous positioning attempts. The same positioning techniques, however, did allow this group of adanal papillae to be observed in the male *M. seetae* specimens loaned from the USNPC.

Of the *Macdonaldius* species which infect snakes (including *M. seetae*), all adults have been recovered from within the large abdominal vessels (renal, hepatic-portal, portal and post vena cava) and hepatic sinusoids (Khanna 1933; Hull & Camin 1959; Chabaud & Frank 1961; Telford 1965). *Macdonaldius grassii* and *M. innisfailensis* have been reported from the peritoneum of *Sceloporus ferrariperezi* (Iguanidae) and the subperitoneal tissues of *Physignathus lesueurii* (Agamidae) respectively (Caballero 1954; Mackerras 1962). Adult specimens of *M. seetae* have apparently never been reported from a host's mesentery.

The microfilaria of *M. seetae* have recently been reported from a

wildcaught *B. subocularis* (as *Elaphe subocularis*) from El Paso County, Texas (Smith 1997). The collection of adult *M. seetae* from this host species supports Smith's (1997) determination that this onchocercid occurs in the Trans-Pecos rat snake.

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RECORDS OF SPECIES AND RANGE EXTENSIONS  
OF MAMMALS IN TAYLOR COUNTY, TEXAS

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Taylor County is located in the Rolling Plains of Texas, with an isolated outcropping of the Edwards Plateau in the southern half of the county. Located within the Kansan Biotic Province (Blair 1950), Taylor County is bordered by Jones, Shackelford, Callahan, Coleman, Runnels, Nolan and Fisher counties. Taylor County is an area of semi-arid climate with an average precipitation of approximately 23 inches per year. The range of precipitation is 9-48 inches per year and the average growing season is 225 days.

The three trapping areas examined for this study were: Tye in the NNW corner of the county, Abilene in the NE portion of the county and Abilene State Park in the center portion of the county. Other trapping areas were examined less frequently to complete the geographic survey of the county.

The Tye collecting site (3.3 miles west of Tye) consists of cultivated plains with dominant soil being Rowena Clay Loam (USDA 1976). The trapping was done on uncultivated land bordering the Union-Pacific railroad tracks and I-20. In this area, there are scattered mesquite trees (*Prosopis juliflora*), prickly pear (*Opuntia occidentalis*), yucca (*Yucca yucca*), and various grasses. Thornton & Lee (1996) reported that this area is dominated by bearded grass (*Andropogon* sp.) and buffalo grass (*Buchloe dactyloides*). Moreover, curly mesquite (*Hilaria belangeri*) and hairy tridents (*Erioneuron pilosum*) are present, as well as two species of three-awn (*Aristida purpurea* and *Aristida wrightii*).

Abilene State Park is a preserve which includes areas of mixed grass plains, riparian habitat, as well as an outcropping of the Edwards Plateau. The main soil type found in this area is Shep Loam (USDA 1976). The plains areas are dominated mostly by several species of grama (*Boutelous* sp.) and several species of bearded grass (*Andropogon* sp.). There are dense stands of Juniper (*Juniperus einchotii*), mesquite and scattered prickly pear. Along the riparian areas there are stands of pecan (*Carya illoinensis*). In the hills (isolated Cretaceous, Edwards

Plateau outcropping), post oak (*Quercus stellata*) and live oak (*Quercus virginiana*) are the dominant species.

Abilene, a city of 107,000, lies in the drainage plain of three creeks. Consequently, there are areas of thick riparian growth found along the drainage and flood plains of the creeks.

Previous records of Taylor County represent only a part of the mammalian fauna now documented and observed to live there (Jones & Jones 1992; Davis & Schmidly 1994). Widespread species such as *Procyon lotor* serve as an example of common mammals that were left unrecorded by previous surveys (Jones & Jones 1992; Davis & Schmidly 1994). This survey of Taylor County is important because it fills a gap among recent surveys conducted in the nearby counties of Coke, Stonewall and Tom Green (Simpson & Maxwell 1989; Boyd et al. 1997; Ruhl & Stangl 1997). All specimens documented in this study are deposited with the holdings of the Abilene Christian University Natural History Collection (ACUNHC).

*Spermophilus variegatus*.—This specimen was collected on a median, in an area of hills, on Loop 322, near a small pond, at the base of the Kirby Reservoir dam. The habitat is consistent with that reported by Davis & Schmidly (1994). This specimen represents a range extension for the species. This animal was recorded in Coke County to the southwest (Simpson & Maxwell 1989). Boyd et al. (1997) reported that Tom Green County may be the northernmost limit of common occurrence. The Taylor County specimen was found in the furthest northeastern corner of the county (ACUNHC 417) in the city of Abilene. This record may indicate a larger population in the isolated hills in the southern portion of Taylor County.

*Sciurus niger*.—The eastern fox squirrel is very abundant due to the many riparian habitats in the city of Abilene and in Abilene State Park. There are specimens reported from Tom Green and Coke counties, but there are no records for the species west of Tom Green (Simpson & Maxwell 1989; Boyd et al. 1997). The specimens acquired in this survey are from the city of Abilene (ACUNHC 104, 137).

*Reithrodontomys fulvescens*.—This species is common in areas of cleared brush and the stream-side habitats of Elm Creek in Abilene State Park. There are no specimens recorded from Coke or Tom Green

counties, but there is one reported from Runnels County to the south and Fisher, Jones and Stonewall counties to the north (Boyd et al. 1997; Ruhl & Stangl 1997). Moreover, there are specimens from Callahan County to the east collected as part of this study and Goetze et al. (1995). The specimens collected in Taylor County are from 9 mi SW of Abilene (ACUNHC 191), Abilene State Park (ACUNHC 88, 184), and 3.3 mi W of Tye (ACUNHC 164).

*Reithrodontomys montanus*.—This specimen (ACUNHC 182) was collected 3.3 mi W of Tye in a habitat that was dominated by sotol (*Yucca* sp.). *Reithrodontomys montanus* appears to be uncommon in Taylor County. There are records from the surrounding counties of Coke, Tom Green and Stonewall (Simpson & Maxwell 1989; Boyd et al. 1997; Ruhl & Stangl 1997).

*Peromyscus maniculatus*.—This is the most common *Peromyscus* in Taylor County, although *Peromyscus attwateri* was recorded from Taylor County (Goetze et al. 1995). The deer mouse was found in a variety of habitats and in all the localities sampled. For example, in Abilene State Park, the deer mouse was found in the hills, along Elm Creek, and in cleared brush habitat. In Coke and Tom Green counties, the deer mouse was found to be uncommon (Simpson & Maxwell 1989; Boyd et al. 1997). The specimens reported in this study are ACUNHC 85, 86, 94, 95, 160, 161, 162 and 165.

*Baiomys taylori*.—The northern pygmy mouse has a preference for grassy areas such as those found along railroad and highway right of ways, and are often found in association with *Sigmodon hispidus* (cf. Davis & Schmidly 1994). It has been shown that the pygmy mouse has been extending its range north and west (Hall 1981; Davis & Schmidly 1994). A former western boundary shows that this species occurs only as far west as McCulloch County, Texas (Hall 1981). The species has been found in Coke and Tom Green Counties (Simpson & Maxwell 1989; Boyd et al. 1997). The specimens listed in this study are from 3.3 mi W of Tye (ACUNHC 146 and 147) and the city of Abilene (ACUNHC 154).

*Neotoma albigula*.—This specimen represents an eastern range extension record from those taken in Coke and Stonewall counties (Simpson & Maxwell 1989; Ruhl & Stangl 1997). These animals (ACUNHC 153, 181) were found in a cleared brush habitat in Abilene

State Park. *Neotoma micropus* also occurs in Taylor County. However, the two *Neotoma* species do not occur sympatrically. *Neotoma micropus* prefers open grassland habitat near Tye, whereas *N. albigula* prefers brush piles surrounded by trees.

*Urocyon cinereoargenteus*.—Records from Coke and Tom Green counties, as well as sightings in Callahan County, show that this species was to be expected as part of the Taylor County mammal fauna (Simpson & Maxwell 1989; Boyd et al. 1997). One of the specimens (ACUNHC 197) reported in this study was salvaged on the Abilene city highway loop and the other (ACUNHC 128) from 5 mi SW of Abilene.

*Bassariscus astutus*.—Davis & Schmidly (1994) reported this species as widespread in Texas and in the counties surrounding Taylor. One specimen was collected in the city of Abilene (ACUNHC 136).

*Procyon lotor*.—Both photographic records and specimens of this species (ACUNHC 91, 92) were obtained from Abilene State Park. The species is abundant in Taylor County and in surrounding counties (Davis & Schmidly 1994).

*Mephitis mephitis*.—One specimen (ACUNHC 260) was collected from the city of Abilene along Cedar Creek. There is also a photographic record of this species in Abilene State Park. Moreover, there are numerous sightings from Taylor and surrounding counties (Davis & Schmidly 1994).

*Taxidea taxus*.—One badly damaged specimen (ACUNHC 420) was documented with photography and an incomplete specimen from 21 mi SSW of Abilene along highway 277. With no additional records, this species seems to be rare in Taylor County. The badger is perhaps becoming rare because of the scarcity of prairie dog town habitats.

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## NOTEWORTHY RECORDS OF MAMMALS FROM CENTRAL AND SOUTH TEXAS

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While conducting research on mammals in central and south Texas, seven species of mammals were collected which represent first records from four different counties. Documentation of the occurrence of these species will aid researchers in biogeographical studies of the mammals of Texas. Information concerning species distributions is taken from Davis & Schmidly (1994). Mammalian subspecies designations follow Hall (1981) and Jones & Jones (1992). Whenever possible, specific localities of capture or observation are given in Universal Transverse Mercator (UTM) coordinates. Measurements given in text are in millimeters. Voucher specimens are housed in the Texas A&M University - Kingsville Animal Research Collection (TAIC).

*Dasyus novemcinctus mexicanus* Peters.—The right manus and partial carapace of a nine-banded armadillo (TAIC 1619) was collected from alongside of U.S. Highway 77, 2 mi N, 1 mi W of Sarita (UTM 14 6627661E, 3021614N) in Kenedy County on 9 July 1996. This specimen represents the first record of the nine-banded armadillo in Kenedy County. The surrounding habitat was open, mesquite brushland south of Los Olmos Creek. No external measurements or reproductive data could be obtained from this specimen.

A male armadillo (TAIC 1617) was collected in Webb County, 1 mi W of Laredo (UTM 14 455679E, 3048078N) on 23 March 1996. External measurements of this individual were total length, 763; tail length, 312; length hind foot, 95; ear length, 40. The nine-banded armadillo resides in mesquite brushland habitats and riparian areas adjacent to the Rio Grande in South Texas. This is the first record of this species from Webb County.

*Perognathus merriami merriami* J. A. Allen.—A specimen of Merriam's pocket mouse (TAIC 1611) was obtained 4 mi N, 1 mi E of Sarita in Kenedy County (UTM 14 619244E, 3017739N) on 25 July 1996. This specimen represents the first report for this county. The pocket mouse was captured along Los Olmos Creek in tidal flat vegetation dominated by sea ox-eye, gulf cord grass, and mesquite. The specimen, a young female, evinced no reproductive activity.

*Chaetodidus hispidus hispidus* Baird.—The hispid pocket mouse has an almost ubiquitous distribution within Texas. However, records are lacking from many counties within the range of *C. hispidus*. On 25 July 1996, two specimens of *C. hispidus* (one female, TAIC 1612, and one male TAIC 1613) were obtained at the same location and habitat as described in the species account of *P. merriami*. Both hispid pocket mice were in reproductive condition. The male had scrotal testes and the female carried nine embryos. The report of these specimens verifies the presence of *C. hispidus* throughout South Texas.

*Canis latrans texensis* Bailey.—Davis & Schmidly (1994) reported the coyote as an ubiquitous species in Texas, however, not all occurrences in the state are verified by specimens. On 16 March 1996, the upper canines from a fragmented coyote skull (TAIC 1620) were collected along Los Olmos Creek, 3 mi N, 0.5 mi W of Sarita in Kenedy County (UTM 14 618329E, 3017062N). No prior documentation of *C. latrans* exists for

## Kenedy County.

*Procyon lotor fuscipes* Mearns.—Museum specimens of the raccoon are absent from the northern Cross Timbers and southern Rolling Plains regions of Texas. On 14 July 1996, a skull was salvaged from a male raccoon (TAIC 1618) found 2 mi S, 3 mi E of Mullin in Mills County (14 530104E, 3491227N). The raccoon was collected from a highway right-of-way through live oak and juniper pasturelands. Standard external measurements, taken along with the skull, are: total length, 796, tail length, 214, length hind foot, 122; ear length, 62. The presence of *P. lotor* in Mills County fills a distributional gap of this species on the northeastern Edwards Plateau and western Cross Timbers regions.

*Mephitis mephitis varians* Gray.—Records of the striped skunk are absent in an area of south Texas which includes Duval County in the north and, from west to east, Zapata, Jim Hogg, Brooks and Kenedy counties. Most of the land in these counties is privately owned, and public access is limited. As a result, records of mammals from this area have been sporadically reported. On 14 March 1996, the right manus was salvaged from a striped skunk (TAIC 1616) 5 mi S of Sarita in Kenedy County (14 619593E, 3001852N). The skunk was badly decomposed; external measurements and reproductive data were impossible to obtain. The report of this species aids in defining the distribution of *M. mephitis* in South Texas.

*Conepatus mesoleucus mearnsi* Merriam.—The lower jaws were salvaged from a hog-nosed skunk (TAIC 1615) 6 mi N, 1 mi E of Lampasas in Lampasas County (14 569278E, 3443980N) on 14 July 1996. This specimen assists in defining the range of *C. mesoleucus* within Central Texas. The nearest records of *C. mesoleucus* are from Brown, McLennon and Travis counties. This specimen was collected adjacent to a live oak pasture containing bluestem grasses. Rocky hills vegetated with juniper trees also were nearby.

## ACKNOWLEDGMENTS

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\* \* \* \* \*

## OBSERVATIONS OF BREEDING SITE FIDELITY OF GREEN-TAILED TOWHEES (*PIPILO CHLORURUS*) IN CENTRAL NEW MEXICO

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The movement of migratory birds in relation to the sites where they breed has been of interest to ornithologists for decades; the observation was made long ago that adult birds in some species will return to breeding sites used the previous year (Greenwood & Harvey 1982). Although many species have been studied in this regard, data on the degree of philopatry is still lacking in many passerine birds. Little natural history information has been reported (Ehrlich et al. 1988) for the green-tailed towhee, *Pipilo chlorurus*, which winters in Mexico and the southwestern United States, and breeds in the montane and high plateau regions of the western United States (Norris 1968). This report describes observations confirming that between-year fidelity to territory occurs in *Pipilo chlorurus*.

Birds were captured in mist-nets in the Sandia Mountains at a site located approximately 15 km northeast of Albuquerque, New Mexico, at an altitude of 2900 m. The netting area was a cleared corridor,

approximately 50 m in width, cut through a forest of mixed conifers (dominant genera: *Pseudotsuga*, *Abies*) and aspen (*Populus*). The corridor itself consisted of various grasses and mixed shrubs (dominant genera: *Ribes*, *Lonicera*, *Rubus*). Nine green-tailed towhees were captured along approximately 300 m of this corridor from 20 July to 23 July 1991. Each individual was banded with a U.S. Fish & Wildlife Service numbered aluminum band, and a single colored plastic band, each on opposite legs. Weight was determined using a Pesola scale, and measurements were taken of the wing chord and tarsus. Molt status, eye color, and presence/absence of brood patch were also recorded. The plumage and eye color of juvenile *P. chlorurus* are distinct from adults, and were used to identify three of these birds as juveniles (the presence of a yellow gape around the mouth was also used in two of these cases). There was a difference among adults in the status of a brood patch, being highly distinct in some individuals. Although *P. chlorurus* adults are sexually monomorphic in plumage, each adult with a brood patch was classified as female; this is based partly on the fact that in other towhees, only females have brood patches and brood the young (Ehrlich et al. 1988).

During the first two weeks in May 1992, a visual survey was conducted of the birds in this area. Of several individuals of *P. chlorurus* observed over the entire area, three adults were identified that had been banded the year before. Binoculars were used to identify each of these birds and to confirm both the presence of a band on each leg and the color of the plastic band. Each was observed singing (indicative of males) from a prominent perch on a shrub within 20 m of the location where they were captured the previous spring. All three of these birds had been identified as "male" when originally captured based on the lack of a distinct brood patch. The wing chord measures of these birds (BLUE: 79 mm, LT. BLUE: 79 mm, PURPLE: 80 mm) and the body weights (BLUE: 27 g, LT. BLUE: 31 g, PURPLE: 40 g) are consistent with the dimensions for males reported by Morton (1991) from late season juveniles. When originally captured, there was no evidence that these birds were breeding locally, but the observation of all three singing in the area suggests that they had returned to this locality to establish breeding territories.

Between-season site fidelity in *P. chlorurus* has been noted once previously by Allan R. Phillips, as reported by Norris (1968), in

northern Arizona; however, this account did not specify the sex or whether the bird was territorial. Although the observations reported here include no return data on females, these findings do suggest that *P. chlorurus* adult males have a strong tendency to return to a previous breeding site. It appears to be the rule in birds for the female to be the predominant dispersing sex from breeding sites, with the only notable exceptions being in geese and ducks (Greenwood 1980). The juveniles that were captured probably fledged in the area, as the date of netting in this study corresponds to the absolute earliest that Morton (1991) caught juveniles undergoing postfledging dispersal (mean date: 11 Aug).

Greenwood & Harvey (1982) note that little is known about the relative importance of various ecological factors that influence whether a bird returns to a breeding site from year to year. However, in some species (e.g. the bobolink, *Dolichonyx oryzivorus*) the level of reproductive success in the previous year appears to influence the likelihood of an individual returning to a particular breeding site (Gavin & Bollinger 1988). The fact that at least male *P. chlorurus* show strong breeding site fidelity suggests the potential for interesting research questions into the ecological and natural history factors influencing site fidelity in this species, and whether *P. chlorurus* exhibits differential migration distances among populations such as occurs in the White-crowned Sparrow (*Zonotrichia leucophrys*) reported by Cortopassi & Mewaldt (1965).

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## THE WETLAND VEGETATION OF CADDO LAKE

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**Abstract.**—Caddo Lake, located along the boundary of northeastern Texas and northwestern Louisiana, contains extensive areas of relatively undisturbed forested wetlands and has been designated a wetland of international importance under the Ramsar Convention. Vegetation and water levels from 30 plots were sampled. Aerial photographs of the 3000 ha Caddo Lake State Park and Wildlife Management Area were used to map the park into 119 survey areas, each representing a distinct patch of vegetation. Both data sets were separately subject to Two Way Indicator Species Analysis (TWINSPAN) classification and Detrended Correspondence Analysis (DCA) ordination. Water levels were strongly correlated with the first axis of a DCA ordination of the 30 plots which arranged plots along a flooding gradient from mesic natural levees and terraces to cypress swamps. These results were corroborated by analysis of data from the 119 survey areas and were used to identify six community types: Rich Mesic Slopes, Mesic Bottomland Ridges, Bottomland Oak Flats, Cypress-Water elm Swamps, Closed-Canopy Cypress Swamps and Deep water Cypress Swamps. These communities are comparable to other wetland communities described for the region.

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Forested wetlands in the southern USA have suffered extensive losses since the time of European settlement (Mitsch & Gosselink 1993). Most remaining areas exist as isolated fragments of less than 100 ha (Gosselink et al. 1990). Between 1940 and 1980, the national rate of loss of forested wetlands in the USA was 2.8 million ha per year with most of the loss occurring in the South (Abernathy & Turner 1987). In eastern Texas, only an estimated 37% of the presettlement riparian forests still existed in 1980 (Frye 1987). Although much of the loss is due to conversion to agriculture, water resource projects are also a threat; over 240,000 ha of Texas bottomland forest were destroyed by impoundments such as Lake of the Pines, Toledo Bend Reservoir and Sam Rayburn Reservoir (Frye 1987).

Caddo Lake, located in northeastern Texas and northwestern Louisiana, is associated with 3000 ha of contiguous forested wetlands on public lands (Taylor et al. 1996) and additional wetlands on private lands. It provides an intact forested wetland landscape on a scale seldom seen today in the South. Consequently, Caddo Lake is recognized as a wetland habitat of international importance by the Ramsar Convention, an international convention named after Ramsar, Iran, the place of its adoption (Navid 1989; Davis 1994). Caddo lake, Catahoula

Lake in Louisiana, and the Okefenokee Swamp in Georgia are the only three Ramsar wetlands in the southern USA excluding peninsular Florida (US Fish and Wildlife Service 1996).

There have been many hydrologic, geomorphic and vegetation studies of bottomlands and swamps in the South including Chambless & Nixon (1975), Thompson (1980), Bedinger (1981), Mathies et al (1983), Miller (1990), Nixon et al. (1990), Shankman & Drake (1990), Shankman (1991), Barrett (1995) and Crouch & Golden (1997). However, in spite of Caddo Lake's importance as a major wetland landscape, only limited non-published information about its wetland vegetation (Hine & Nixon 1992; Sheffield 1995; MacRoberts 1979) is available. This paper provides a quantitative description and gradient analysis of the wetland plant communities of Caddo Lake, focusing on the public lands of the Caddo Lake State Park and Wildlife Management Area.

#### STUDY AREA

Caddo Lake lies between Mooringsport, Louisiana, and Karnack, Texas at approximately 32° 42' 38" N, 94° 8' 25" W along Big Cypress Bayou, a tributary of the Red River. It is in the Pineywoods vegetation area of Hatch et al. (1990), and the Southeastern Mixed Forest Province of Bailey et al. (1994). The climate is humid-subtropical with a mean annual low temperature of 11°C, an average high temperature of 22°C, an annual mean of 116.8 cm of rainfall, and negligible snowfall (Larkin & Bomar 1983).

Historically called Ferry Lake, Caddo Lake is a drowned floodplain, one of several lakes that formed around the year 1800 as a result of a 120 km-long series of log jams (the Great Raft) that blocked the main channel of the Red river (Barrett 1995). The Raft forced water into side channels of the Red River and caused water to back up into tributaries, forming Caddo Lake and the now extinct Sodo Lake. Steam boats entered Caddo Lake via side channels of the Red River, contributing to the commerce of the time (Dahmer 1988). Removal of the Great Raft in 1873 drained Sodo Lake and caused Caddo Lake to slowly recede until a dam built in 1914 near Mooringsport, Louisiana, stabilized the lake and preserved the cypress and bottomland wetlands of the formerly natural lake (Dahmer 1988; Barrett 1995). The extent of the alteration of the original hydrologic regime and consequent influence on vegetation is unknown, although sedimentation rates were higher prior to the break up of the Raft and dam construction as the result of inflow of sediment-rich water from the Red River (Barrett 1995).

Caddo Lake, including its wetlands, covers roughly 10,7200 ha at capacity of 51.36 m above mean sea level, but there is considerable seasonal variation in water levels and in the area actually flooded (USGS 1:2400 Topographic map; A.I.D. Associates 1993; US Army Corps of Engineers 1994b). The eastern portion of Caddo Lake, much of which lies in Louisiana, is the deepest and consists largely of open water (Barrett 1995). The western portion, in Texas, is the focus of this study. It is a "freshwater delta" (Barrett 1995) occurring on drowned stream channels, point bar deposits, natural levees and lacustrine deposits. These features provide for a diversity of communities including ponds, cypress swamps, hardwood flats and mesic communities.

### METHODS

Two transects, perpendicular to Cypress Bayou, were randomly located along a 1.5 km baseline in the Caddo Lake State Park and Wildlife Management Area. The transects, with a combined length of 1500 m followed a moisture and elevation gradient from a mesic natural levee (53.4 m above sea level) through a bottomland oak flat, to a cypress swamp (50.6 m above sea level). Thirty points, each defining the center of a series of nested plots, were located at 50 m intervals along the transects. The samples, a modified and simplified form of the "Whitaker method" (Shmida 1984), included a series of nested plots. Each point defined a 1000 m<sup>2</sup> circular plot, a 500 m<sup>2</sup> circular plot, a 100 m<sup>2</sup> circular plot, a 3.16 m by 3.16 m (10 m<sup>2</sup>) plot, and a 1 m<sup>2</sup> plot. Plot centers were permanently marked with tagged aluminum stakes. Inundated plots were marked by recording the distance and azimuth from a nearby tagged reference tree.

Diameter at 1.5 m (breast) height (dbh) was recorded for each tree greater than 10 cm dbh in the 500 m<sup>2</sup> plot and the number of stems of shrubs and saplings less than 10 cm dbh but greater than 1m high was tallied for species in the 100 m<sup>2</sup> plot. Ground layer species (herbaceous species and woody plants less than 1 m tall) were assigned "occurrence ranks" based on their occurrence in different sized plots. Species occurring in 1 m<sup>2</sup> plots were assigned an occurrence rank of "five" while those found in the 10 m<sup>2</sup> plot but not in the 1 m<sup>2</sup> plot received a rank of "four", and those occurring in the 100 m<sup>2</sup> but not the smaller plots received a rank of "three". Species not found in the 100 m<sup>2</sup> plot but observed within a radius of approximately 18m (1000 m<sup>2</sup> area) of the plot center were given a rank of "two" if three or more individuals or colonies were encountered and a rank of "one" if only one or two individuals were encountered or if plants were found only in one or two

small, localized colonies. Percentage cover was also estimated for all ground cover species in the 10 m<sup>2</sup> plot. Species not found in the 10m<sup>2</sup> plot but in the larger sample area were given a "trace" coverage of 0.1%. The coverage, projected on the ground, and occurrence of the epiphyte *Tillandsia usneoides* (L.) L. was included with the ground layer.

The depth of water covering inundated plots or the depth to water in a shallow pit on non-flooded plots was measured for each plot on 6 December 1996, a time when the majority of the plots were inundated and the water level at a nearby gauging station (Tall Pines Lodge) was observed at 52.03 m above mean sea level. A level and sighting-stick were used to measure the elevation of two plots that were more than 1m above the water. Since the gauging station was relatively close (< 1000 m) to the plots and negligible current was observed, variation in the elevation of the water surface or "head" was considered to be negligible. The elevation of each plot was estimated by subtracting the depth of the plot below water from the water level elevation (52.03 m).

Since a goal of this study was to survey and map the plant communities of the entire Caddo Lake State Park and Wildlife Management Area, a survey methodology was devised that would facilitate a rapid survey of the area, but still obtain data suitable for quantitative analysis. Polygons, each representing a distinct patch of vegetation, were drawn on January 1993 color infrared aerial photographs covering the Caddo Lake State Park and Wildlife Management Area. During the summer and fall of 1994 and 1995, 119 of these areas were visited and a representative portion of each area roughly 1 ha in size was surveyed. All overstory species (> 10 cm diameter at 1.5 m (breast) height (dbh), mid story species (< 10 cm dbh but > 1 m high), and ground layer (herbaceous plants and woody plants < 1m high) were identified and ranked on a five-point abundance scale within their respective stratum. Notes on hydrology, soils, and wildlife also were recorded. In addition to providing a survey of most of the Park, these data provided an independent data set with which to corroborate the results of the transect plot data.

Voucher specimens were deposited in the herbarium of Stephen F. Austin State University (ASTC). Nomenclature follows Hatch et al. (1990) and Kartesz (1994) for species not found in Hatch et al. (1990).

*Data analysis.*—Databases were compiled for both the transect and survey data sets. Overstory and ground layer data from both data sets were separately subjected to Detrended Correspondence Analysis (DCA) ordination (Hill 1979a; Hill & Gauch 1980) and Two Way Indicator Species Analysis (TWINSPAN, Hill 1979b): TWINSPAN is a hierarchi-

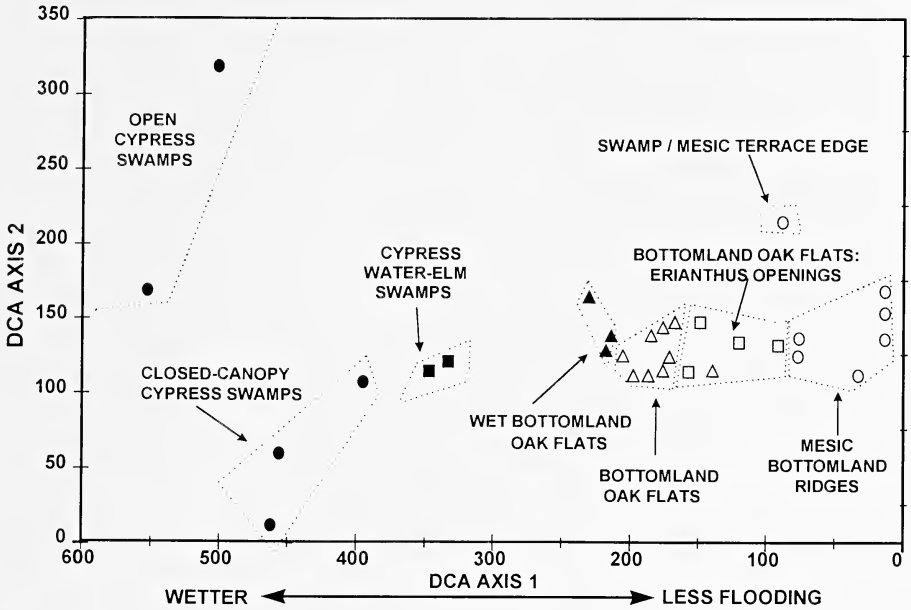


Figure 1. A DCA ordination based on ground layer occurrence ranks for 30 plots along a transect in the Caddo Lake state Park and Wildlife Management Area. Symbols represent a classification of the plots based on ground layer TWINSpan except that solid triangles represent overcup oak sites which were not classified separately by TWINSpan but were distinguishable from other bottomland oak flats (open triangles) on the basis of DCA. Dotted lines represent a final community type classification based on TWINSpan and DCA of both the ground layer and overstory strata. Since the direction of an ordination axis is merely an artifact of how the computer read the matrix, axis 1 has been reversed to facilitate visual comparison with the other ordination figures.

cal, divisive polythetic classification which classifies samples into groups with similar species composition. Input data for each species in each sample included log-transformed density (recommended by Harcombe et al. 1993) for the transect overstory, occurrence ranks for the transect ground layer, and abundance ranks for the survey data. Linear regression (Steele & Torrie 1980; Ludwig & Reynolds 1988) was used to relate DCA scores to water levels for the transect data set. Field notes were used to relate a DCA of the 119 survey sites to a flooding gradient and to other observed environmental factors.

DCA and other ordination techniques summarize complex, multivariate, samples-by-species data sets by arranging samples objectively along several axes on the basis of their species composition. Vegetation samples can be graphed as a scatter diagram based on ordination scores, in which points that are near each other represent samples with similar species composition while points distant from each other represent

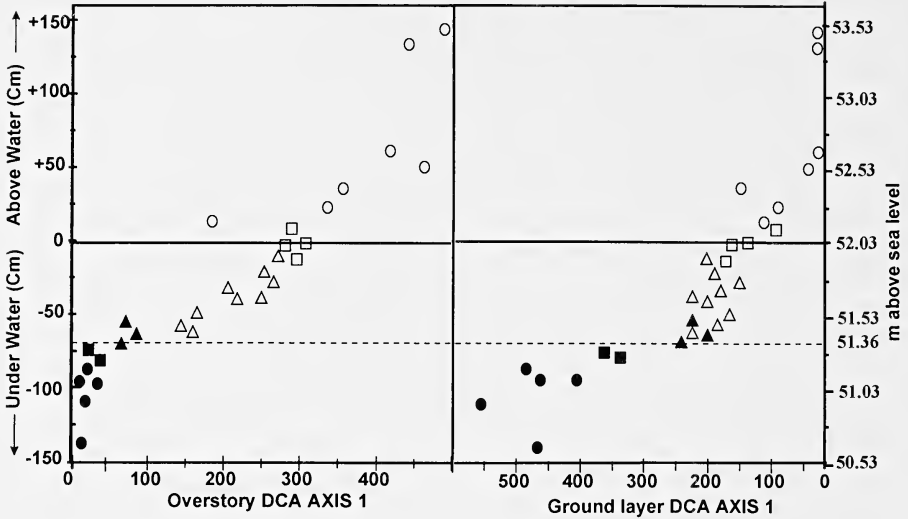


Figure 2. The relationship between water levels measured on 6 December, 1996, and both overstory and ground layer composition as expressed by the first axis of a DCA ordination. Overstory  $r^2 = 0.84$ ,  $p < 0.01$ , Ground flora  $r^2 = 0.74$ ,  $p < 0.01$ . The solid line indicates the water level at the time of sampling, 52.03 m above sea level. The dotted line indicates the normal pool elevation of Caddo Lake, 51.36 m above sea level. Since the direction of an ordination axis is merely an artifact of how the computer read the matrix, axis 1 for the ground layer ordination has been reversed to facilitate visual comparison with the other ordination figures. Symbols represent a classification of the plots based on ground layer TWINSpan. Solid triangles represent overcup oak sites which were not classified separately by ground layer TWINSpan but were distinguishable from other bottomland oak flats (open triangles) on the basis of DCA.

samples with dissimilar species composition (Hill & Gauch 1980; Gauch 1982; Jongman et al. 1995). Ordination axes are generally interpreted as being gradients in species composition reflecting an underlying environmental gradient such as one of flooding or nutrients, (Gauch 1982; Ludwig & Reynolds 1988).

TWINSpan and DCA results for both the overstory and ground layer, field notes and observations of aerial photographs were used to classify the 119 survey samples into community types. Since plant communities generally change continuously along environmental gradients, (Gleason 1926; Gauch 1982) all classifications are somewhat artificial; arbitrary decisions occasionally had to be made among adjacent types when there was lack of agreement between results for overstory, ground flora, TWINSpan and DCA. Ordination results were usually given preference over TWINSpan results for these decisions. The classification was corroborated by comparison of the

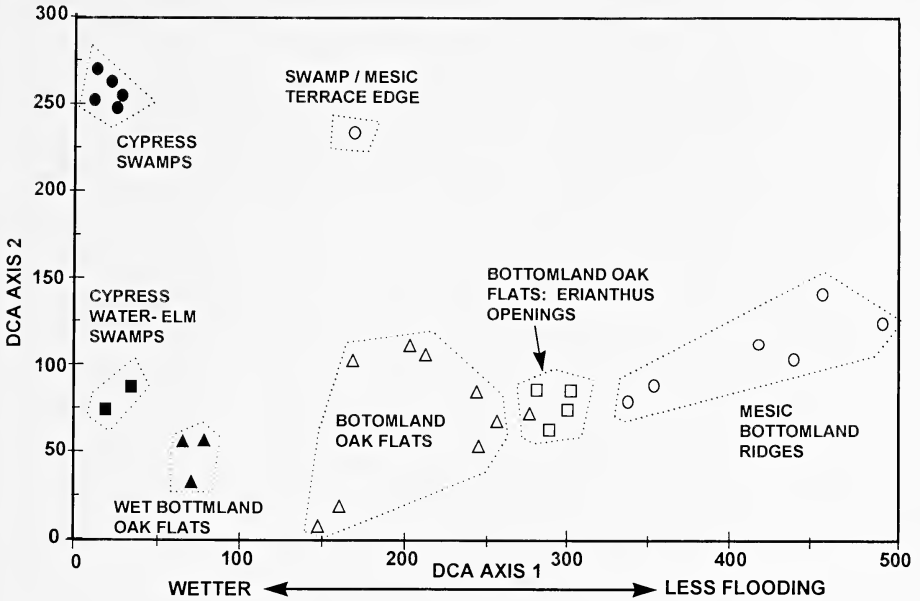


Figure 3. A DCA ordination based on Log-density of overstory overstory of 30 plots along a transect in Caddo Lake state Park and Wildlife Management Area. Symbols represent a classification of the plots based on TWINSpan of ground layer species except that solid triangles represent overcup oak sites which were separated from other bottomland oak flats (open triangles) on the basis of DCA and overstory TWINSpan. Dotted lines represent a final community type classification based on TWINSpan and DCA of both the ground and overstory strata.

community descriptions with the results from the transect data set and was used along with aerial photographs to develop a map of the wetland vegetation of Caddo Lake State Park and Wildlife Management Area and adjacent areas.

DCA ordines species simultaneously with samples (Hill & Gauch 1980; Hill 1979a). Species occurring nearby on an ordination tend to occur in the same type of samples, while distant species are generally found in different types of samples. Likewise TWINSpan classifies species into groups on the basis of the sites they occur in (Hill 1979b; Gauch 1982). TWINSpan and DCA results were used to classify Caddo Lake species into "ecological species groups": groups of species which respond similarly to environmental gradients and tend to occur together on similar types of sites (Muller-Dombois & Ellenberg 1974; Barnes et al. 1982). Presence of several members of a group is a strong indication of a site's ecological conditions and community type. For ground layer species, only those listed as community indicators or strong

Table 1: Mean basal area ( $m^2/ha$ ) and absolute density (stems/ha, in parentheses) for each tree species > 10 cm dbh encountered in 29 plots along a transect from the Caddo Lake State Park and Wildlife Management Area. The 30<sup>th</sup> plot, a heterogeneous plot spanning a mesic slope and a cypress swamp (See Figure 4) was omitted from the tabulation. Samples are classified into community types on the basis of ground layer TWINS PAN and DCA while species are classified into groups on the basis of overstory TWINS PAN. MB = Mesic Bottomland Ridges, BFE = Bottomland Oak Flats: *Erianthus* openings, BFW = Bottomland Oak Flats: Willow oak-dominated, BFO = Bottomland Oak Flats: Overcup oak-dominated, CW = Cypress-Water elm Swamps, CS = Closed-canopy Cypress Swamps, and OC = Open Cypress Swamps.

	MB	BFE	BFW	BFO	CW	CC	OC
<i>Acer rubrum</i> L.	0.38 (43)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Celtis laevigata</i> Willd.	0.05 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Nyssa sylvatica</i> Marsh.	1.52 (43)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Pinus taeda</i> L.	11.80 (47)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Quercus nigra</i> L.	7.01 (80)	1.20 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Quercus pagoda</i> Raf.	1.48 (13)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Sassafras albidum</i> (Nutt.) Nees	0.18 (7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Vaccinium arboreum</i> Marsh.	0.04 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Betula nigra</i> L.	0.69 (17)	0.92 (28)	0.40 (6)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Carya aquatica</i> (Michx.f.) Nutt.	0.08 (7)	0.48 (12)	0.64 (9)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Crataegus opaca</i> Hook. & Arn.	0 (0)	0.12 (8)	0.20 (11)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Liquidambar styraciflua</i> L.	3.93 (110)	3.44 (92)	3.32 (66)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Quercus phellos</i> L.	1.59 (20)	13.50 (116)	11.51 (183)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Diospyros virginiana</i> L.	0.11 (3)	0 (0)	0.05 (3)	0.21 (20)	0 (0)	0 (0)	0 (0)
<i>Gleditsia aquatica</i> Marsh.	0 (0)	0 (0)	0 (0)	0.72 (27)	0.88 (40)	0 (0)	0 (0)
<i>Quercus lyrata</i> Walt.	1.32 (17)	1.61 (36)	9.10 (51)	17.46 (153)	1.45 (10)	0 (0)	0 (0)
<i>Salix nigra</i> Marsh.	0 (0)	0 (0)	0 (0)	1.55 (7)	0 (0)	0 (0)	0 (0)
<i>Planera aquatica</i> Marsh.	0 (0)	0 (0)	0.11 (9)	5.74 (173)	7.41 (360)	0 (0)	0 (0)
<i>Taxodium distichum</i> (L.) L. Rich	0 (0)	0 (0)	2.27 (23)	2.79 (60)	43.41 (480)	58.01 (450)	19.08 (170)
TOTAL	30.18 (413)	21.27 (296)	27.59 (361)	28.47 (440)	53.15 (890)	58.01 (450)	19.08 (170)



preferentials (occurred in at least 57% of the samples for the group) during one of the divisions of TWINSPAN were included in the ecological species groups.

## RESULTS

*Gradient analysis.*—The first axis of a DCA ordination of the 30 transect plots based on the occurrence rank of ground layer species (Figure 1) was strongly correlated with water levels on the plots ( $R^2 = 0.74$ ,  $p < 0.01$ , Figure 2). The ordination reflected a gradient from well-drained mesic sites on a natural levee along Cypress Bayou (high first DCA axis ordination scores) to seasonally flooded oak-dominated flats (intermediate scores) to semi-permanently inundated water elm and cypress swamps (low scores). TWINSPAN classified the plots into five groups (the symbols in Figures 1 and 2) and showed close agreement with the DCA arrangement (Figure 1).

Mesic sites (open circles in Figure 2) were above 52.03 m above sea level and were not inundated at the time of measurement. Three subtypes of Bottomland Oak Flats were evident (Figure 1): somewhat open-canopied sites on slight mounds with a dense ground layer dominated by *Erianthus strictus* Baldw. (DCA score 87-161), intermediate sites with a closed willow oak (*Quercus phellos* L.) canopy and an abundance of *Carex jorii* Bailey (DCA score 162-206), and wetter sites with an overcup oak (*Q. lyrata* Walt.) dominated overstory and a sparse ground layer (DCA score  $> 207$ ). The *Erianthus* oak sites (open squares, Figures 1 and 2) largely occurred between 51.9 and 52.1 m above sea level. The other Bottomland Oak Flats (triangles) occurred below 51.9 m with the overcup oak-dominated stands (solid triangles, Figure 2) tending to occupy the lowest sites at about 51.36-51.5 m. Two types of swamps were also evident along the first DCA axis: Cypress Water-elm Swamps (solid squares of Figures 1 and 2), had a dense sub-canopy of water elm (*Planera aquatica* (Walt.) J.F. Gmel. and some soil-rooted ground flora such as *Brunnichia ovata* (Walt.) Shinners and *Saururus cernuus* L., while cypress swamps (solid circles) were pure stands of bald cypress (*Taxodium distichum* (L.) L. Rich.) dominated by floating and submersed plants on the surface layer. Swamps occurred below the normal pool elevation of Caddo Lake (51.36m above sea level), with cypress swamps generally in deeper water than Cypress Water-elm Swamps (Figure 2). The second DCA axis was related to variation among cypress swamps: Open-canopied stands with a dense surface layer of *Nuphar luteum* (L.) Sibth. & Sm, *Nelumbo lutea* (Willd.) Pers., had high second axis scores while closed-canopy stands, their surface layer dominated by Lemnaceae species and *Egeria densa* Planch. had low scores (Figure 1).

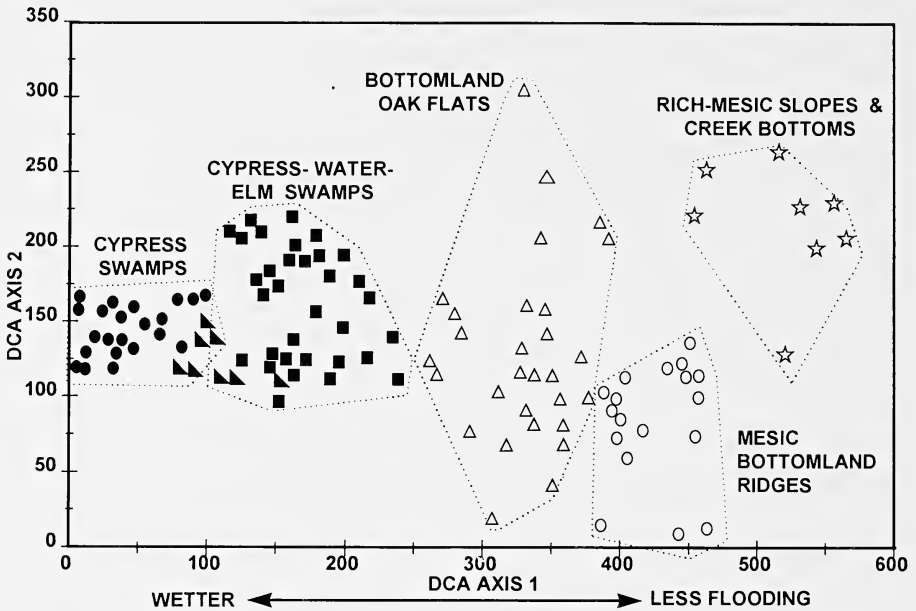


Figure 4. A DCA ordination of 119 vegetation survey sites based on ground layer abundance ranks from Caddo Lake State Park and Wildlife Management Area. Symbols represent a TWINSpan classification of the samples while lines represent a community-type classification derived from TWINSpan and DCA of both the overstory and ground layer.

A DCA ordination of the 30 transect plots on the basis of log-transformed density of overstory trees provided an arrangement of samples much like that of the ground layer ordination (Figure 3). As with the ground layer, first axis DCA scores were strongly correlated with water levels ( $R^2 = 0.84$ ,  $p < 0.01$ , Figure 2). Wet Bottomland Oak Flats with their overcup-oak dominated canopy were more distant from the other bottomland hardwood samples than for the ground layer ordination. The overstory ordination arrangement also corresponded closely with the ground-layer TWINSpan classification, represented by the symbols displayed in Figures 1, 2 and 3).

Mean overstory density (Table 1) for mesic and Bottomland Oak Flat communities was generally in the range of the 300-600 stems/ha that was reported for the Big thicket (Marks & Harcombe 1981). Maximum density was observed among the Cypress-Water elm Swamps, while Open-canopied Cypress Swamps in deeper water showed the lowest density (Table 1). Excluding the open *Erianthus* sites, mean basal area for the bottomland Oak Flats (28  $m^2/ha$ , Table 1) was similar to the 29  $m^2/ha$  reported on equivalent sites from the Big Thicket (Marks &

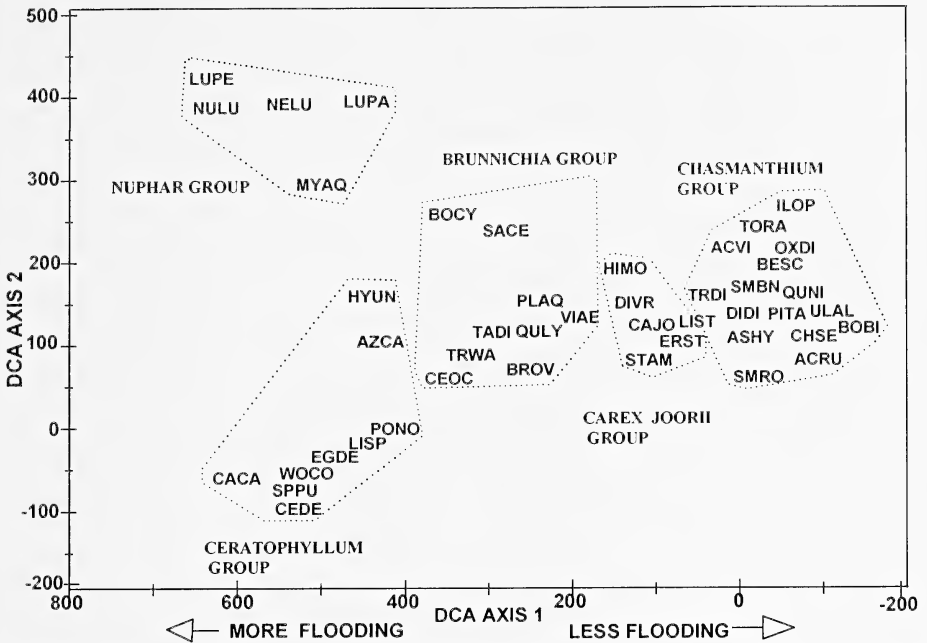


Figure 5. A DCA ordination of 136 ground layer species from 30 plots along a transect at the Caddo Lake State Park and Wildlife Management Area. Dotted lines represent ecological species groups, based on TWINSpan and DCA into which the species have been classified. Groups are named after a representative species that has both a high frequency of occurrence in the community types characterized by the group and a high fidelity to the communities. Only 44 species with strong indicator preference are displayed. Species codes, which are formed from the scientific names of each species, are listed in Table 2. Since the direction of an ordination axis is merely an artifact of how the computer read the matrix, the first DCA axis has been reversed to facilitate visual comparison with the other ordination figures.

Harcombe 1981), but was lower than that reported from other southeastern bottomland forests (Robertson et al. 1978). Basal area was higher for the swamps (Table 1), in part because of buttressing of the stems. However, only one Closed-canopy Cypress Swamp sample (90 m<sup>2</sup>/ha) approached the 130 m<sup>2</sup>/ha reported for the cypress/tupelo stand described in Marks & Harcombe (1981).

The first axis of a DCA ordination of the 119 survey areas based on ground layer species arranged samples in a pattern similar to that of the transect data set (Figure 4). As with the transect data, the ordering of sites reflected a gradient from semi-permanently flooded swamps to seasonally-flooded oak flats to temporarily-flooded natural levees and terraces. However, it was not possible to identify subgroups of Bottomland Oak Flats with this less precise and coarser-scale data set. The first axis of a DCA ordination based of the abundance ranks of

Table 2. Ecological species groups for Caddo Lake wetland communities. Groups are based on a TWINSpan classification of 136 ground flora species from 30 plots along a transect from the Caddo Lake State Park and Wildlife Management Area, except that the TWINSpan did not separate the overcup oak-dominated subtype from the remaining Bottomland Oak Flats. The division is recognized on the basis of DCA. Only 44 species with strong indicator preference are listed. Groups are named after a representative species that has both a high frequency of occurrence in the community types characterized by the group and a high fidelity to those communities. Symbols after the group name (listed in order of importance) refer to the communities in which the species are most often found: MB = Mesic Bottomland Ridges, BFE = Bottomland Oak Flats: *Erianthus* openings, BFW = Bottomland Oak Flats: Willow oak-dominated, BFO = Bottomland Oak Flats: Overcup oak-dominated, CW = Cypress-Water elm Swamps, CS = Closed-canopy Cypress Swamps, and OC = Open Cypress Swamp. Species codes, used in Figure 5, are formed from the scientific name of each species.

Species	Frequency of occurrence (% of sites)							
	Code	MB	BFE	BFW	BFP	CW	CS	OC
1) <i>Chasmanthium</i> group: MB, BFE								
<i>Acalypha virginica</i> L.	ACVI	71	40	13	0	0	0	0
<i>Acer rubrum</i> L.	ACRU	71	0	0	0	0	0	0
<i>Ascyrum hypericoides</i> L.	HYHY	100	60	0	0	0	0	0
<i>Berchemia scandens</i> (Hill) K. Koch	BESC	86	40	0	0	0	0	0
<i>Botrychium biternatum</i> (Savign.) Underw.	BOBI	71	0	0	0	0	0	0
<i>Chasmanthium sessiliflorum</i> (Poir) Yates	CHSE	71	0	0	0	0	0	0
<i>Dichantherium dichotomum</i> (L.) Gould	DIDI	71	60	0	0	0	0	0
<i>Ilex opaca</i> Soland. in Ait.	ILOP	57	0	0	0	0	0	0
<i>Oxalis dillenii</i> Jacq.	OXDI	86	0	0	0	0	0	0
<i>Pinus taeda</i> L.	PITA	100	20	0	0	0	0	0
<i>Quercus nigra</i> L.	QUNI	57	0	0	0	0	0	0
<i>Smilax bona-nox</i> L.	SMBN	100	20	0	0	0	0	0
<i>Smilax rotundifolia</i> L.	SMRO	100	60	13	0	0	0	0
<i>Trachelospermum difforme</i> (Walt.) Gray	TRDI	86	60	38	0	0	0	0
<i>Toxicodendron radicans</i> (L.) O. Ktze.	TORA	86	20	0	0	0	0	0
<i>Ulmus alata</i> Michx.	ULAL	71	0	0	0	0	0	0
2) <i>Carex jorii</i> Group: BFE, BFW, BFO, MB.								
<i>Carex jorii</i> Bailey	CAJO	57	100	100	100	0	0	0
<i>Diospyros virginiana</i> L.	DIVR	57	60	100	100	0	0	0
<i>Erianthus strictus</i> Baldw.	ERST	71	100	62	66	0	0	0
<i>Hibiscus moscheutos</i> L.	HIMO	14	80	62	66	0	0	0
<i>Liquidambar styraciflua</i> L.	LIST	86	80	75	33	0	0	0
<i>Styrax americanus</i> Lam.	STAM	43	100	62	66	0	0	0
3) <i>Brunnichia</i> group: CW, BF, CS, BFO, BFW, BFE.								
<i>Boehmeria cylindrica</i> (L.) Sw.	BOCY	29	60	50	33	100	100	50
<i>Brunnichia ovata</i> (Walt.) Shinnars	BROV	29	100	100	100	100	100	0
<i>Cephalanthus occidentalis</i> L.	CEOC	0	40	25	33	50	100	0
<i>Quercus lyrata</i> Walt.	QULY	29	20	55	66	50	0	0
<i>Planera aquatica</i> (Walt.) J.F. Gmel.	PLAQ	14	100	87	100	100	50	0
<i>Saururus cernuus</i> L.	SACE	0	0	13	66	100	0	0
<i>Taxodium distichum</i> (L.) L. Rich.	TADI	29	80	87	100	100	67	0
<i>Vitis aestivalis</i> Michx.	VIAE	14	100	75	33	100	0	0

Table 2. Ecological Species groups (continued)

4) <i>Nuphar</i> group: OS, CS, CW.		MB	BFE	BFW	BFO	CW	CS	OC
<i>Ludwigia palustris</i> (L.) Ell.	LUPA	0	0	0	33	50	67	100
<i>Ludwigia peploides</i> (Kunth in H.B.K.) Raven	LUPE	0	0	0	0	0	0	100
<i>Myriophyllum aquaticum</i> (Vell.) Verd.	MYAQ	0	0	0	0	50	67	100
<i>Nelumbo lutea</i> (Willd.) Pers.	NELU	0	0	0	0	0	34	100
<i>Nuphar luteum</i> (L.) Sibth. & Sm.	NULU	0	0	0	0	0	34	100
5) <i>Ceratophyllum</i> group: CS, OS, CW								
<i>Azolla caroliniana</i> Willd.	AZCA	0	0	0	0	0	67	50
<i>Cabomba caroliniana</i> Gray	CACA	0	0	0	0	0	67	50
<i>Ceratophyllum demersum</i> L.	CEDE	0	0	0	0	0	67	50
<i>Egeria densa</i> Planch.	EGDE	0	0	0	13	100	100	50
<i>Hydrolea uniflora</i> Raf.	HYUN	0	0	0	33	100	100	100
<i>Limnobium spongia</i> (Bosc.) L.C. Rich ex Steud.	LISP	0	0	0	0	100	100	50
<i>Potamogeton nodosus</i> Poir.	PONO	0	0	0	0	100	67	0
<i>Spirodela punctata</i> (Meyer.) Thomps.	SPPU	0	0	0	0	0	100	100
<i>Wolffia columbiana</i> Karst.	WOCO	0	0	0	0	0	100	100

overstorey trees from the 119 survey sites was highly correlated ( $r^2 = 0.95$ ,  $p < 0.01$ ) with the first axis of the survey ground layer ordination and reflected a gradient similar to that of the ground layer. The second axis of the ground layer ordination was largely related to variation among mesic sites (Figure 4). Sites with high axis 2 scores were associated with steep slopes and narrow ravines near the Caddo Lake State Park campground and headquarters area while terraces and natural levees from higher portions of the Cypress Bayou floodplain had low scores. Ravines and slopes did not occur on the transects which were wholly within the floodplain.

*Ecological species groups.*—DCA orders species simultaneously with samples, displaying near one another in ordination space those species that occur in similar sites and are presumably ecologically similar (Hill & Gauch 1980). Since species ordinations are based on same information that samples ordinations are (Gauch 1982), the ordering of the 136 ground layer species encountered in the 30 transect plots along the first DCA axis was strongly related to water depth as was the first axis of the samples ordination. Species characteristic of well drained mesic sites (*Chasmanthium sessiliflorum* (Poir) Yates) had low first axis scores, species found on seasonally flooded oak flats (*Carex jorii*) had intermediate scores, and swamp species (*Ceratophyllum demersum* L.) had high scores (Figure 5). TWINSpan classified ground layer species into five ecological species groups; groups of species that respond similarly

Table 3. Mean percentage cover of major species (all species encountered with mean cover > 0.75% in at least 1 community type) for species from 30 plots along a transect from the Caddo Lake State Park and Wildlife Management Area. Communities and species are classified on the basis of TWINSPAN except that the overcup oak-dominated subtype is separated from the remaining Bottomland Oak Flats on the basis of DCA. MB = Mesic Bottomland Ridges, BFE = Bottomland Oak Flats: *Erianthus* openings, BFW = Bottomland Oak Flats: Willow oak-dominated, BFO = Bottomland Oak Flats: Overcup oak-dominated, CW = Cypress-Water elm Swamps, CS = Closed-canopy Cypress Swamps, and OC = Open Cypress Swamp.

Species	Mean percentage cover						
	MB	BFE	BFW	BFO	CW	CC	OC
<i>Acer rubrum</i>	0.8	0	0	0	0	0	0
<i>Berchemia scandens</i>	1.0	0	0	0	0	0	0
<i>Botrychium biternatum</i>	1.0	0	0	0	0	0	0
<i>Chasmanthium sessiliflorum</i>	2.1	0	0	0	0	0	0
<i>Dichantherium dichotomum</i>	1.7	0.1	0	0	0	0	0
<i>Smilax bona-nox</i>	0.8	0	0	0	0	0	0
<i>Smilax rotundifolia</i>	2.4	0.3	0.1	0	0	0	0
<i>Ulmus alata</i>	1.7	0	0	0	0	0	0
<i>Carex joorii</i>	7.7	16.0	16.1	0.4	0	0	0
<i>Erianthus strictus</i>	2.9	19.3	0.2	0.1	0	0	0
<i>Ilex decidua</i> Walt.	0.3	1.3	0	0.2	0	0	0
<i>Liquidambar styraciflua</i>	0.5	0.8	0.2	0	0	0	0
<i>Brunnichia ovata</i>	0.5	0.4	1.7	3.7	0.5	0.1	0.1
<i>Tillandsia usneoides</i> (L.) L.	15.1	2.3	2.4	8.5	25.0	25.0	0.3
<i>Cyperus erythrorhizos</i> Muhl.	0	0	0	0	0	5.0	0
<i>Cyperus odoratus</i> L.	0	0	0	0	0	1.0	0.1
<i>Ludwigia palustris</i>	0	0	0	0	0.1	1.1	0.1
<i>Ludwigia peploides</i>	0	0	0	0	0	1.7	0.3
<i>Lindernia dubia</i> (L.) Penn.	0	0	0	0	0.1	6.7	0
<i>Nelumbo lutea</i>	0	0	0	0	0	16.7	37.5
<i>Nuphar lutea</i>	0	0	0	0	0	10.0	30.0
<i>Cabomba caroliniana</i>	0	0	0	0	0	1.0	35.0
<i>Ceratophyllum demersum</i>	0	0	0	0	0	10.0	20.0
<i>Egeria densa</i>	0	0	0	0	0.1	16.7	42.5
<i>Hydrolea uniflora</i>	0	0	0	0	0.5	28.5	0.3
<i>Limnium spongia</i>	0	0	0	0	0.8	21.7	20.5
<i>Spirodela punctata</i>	0	0	0	0	0	14.0	20.5
<i>Wolffia columbiana</i>	0	0	0	0	0	20.0	48.0

to environmental factors and tend to occur together on similar types of sites (Mueller-Dombois & Ellenberg 1974; Barnes et al. 1982). The species groups are listed in Table 2 and their species plotted in ordination space in Figure 5. Only 44 species listed as indicator species or observed to be strong preferential species (present on more than 57% of the samples for a given TWINSPAN division) were included in the species group list. Twenty-eight of the 136 species encountered had a mean coverage of  $\geq 0.75\%$  for at least one community type (Table 3).

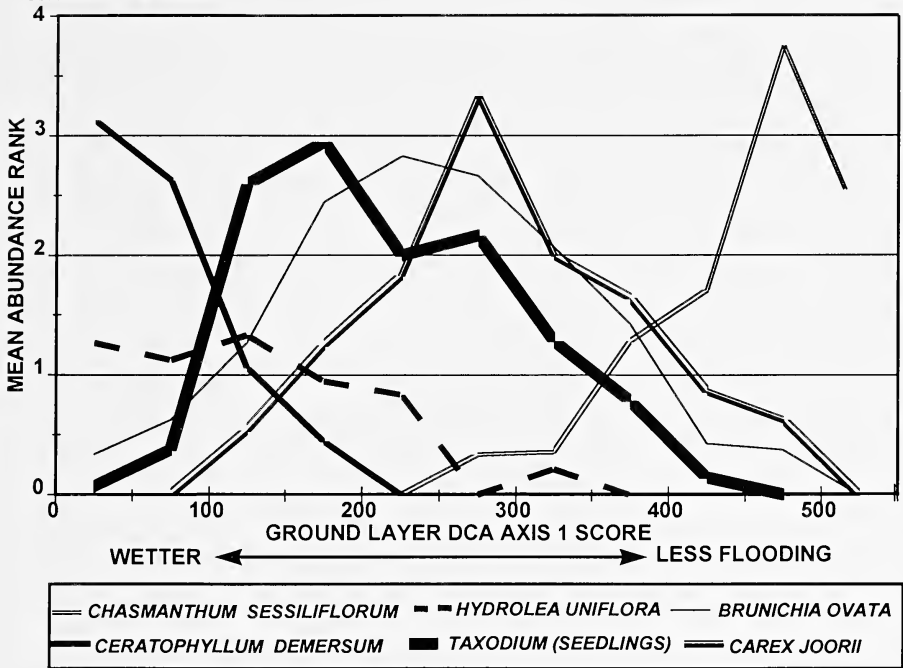


Figure 6. Changes in abundance along a flooding gradient for six ground flora species from the Caddo Lake State Park and wildlife Management Area. Mean abundance ranks for each species are plotted for 11 equal segments of the first axis of a DCA ordination of 119 vegetation survey sites based on ground flora. BROV = *Brunnichia ovata*, CAJO = *Carex jorii*, CEDE = *Ceratophyllum demersum*, CHSE = *Chasmanthium sessiliflorum*, HYUN = *Hydrolea uniflora*, and TADI = *Taxodium distichum*.

Most of these dominant species also had indicator value as a comparison of Tables 2 and 3 reveals. Species groups followed the flooding gradient from the mesic *Chasmanthium* group to the aquatic *Ceratophyllum* group (Table 1, Figure 5). Although 363 species were encountered in the much larger survey data set, the classification and list of indicator species from the 119 survey areas was similar to that from the plots. Overstory species for the transect plots were likewise classified by TWINSPAN (Table 1).

It is also informative to observe changes in abundance for individual species along an environmental gradient (Figure 6). Generally the distributions species along the flooding gradient in this study (expressed by the first DCA axis) are consistent with the bell-shaped Gaussian curves characteristic of the distributions of many species along an environmental gradient (Gauch 1982). For example, cypress (*Taxodium distichum*) seedlings plotted along the first ground layer DCA axis for the survey data reach their peak abundance in shallow cypress-water elm

Table 4. Approximate aerial extent of wetland community types from the Caddo Lake State Park and Wildlife Management Area (estimated from the aerial photograph that forms the basis for the map in Figure 7). RM = Rich-Mesic Slopes and Ravines, MB = Mesic Bottomland Ridges, BO = Bottomland Oak Flats, CW = Cypress-Water elm Swamps, CS = Closed-canopy Cypress Swamps, OC = Open Cypress Swamp, and DI = Disturbed Uplands.

Community	Approx. hectares	Percentage of area
RM	48	1.4
MB	767	21.8
BO	596	17.0
CW	906	25.8
CS	319	9.1
OC	752	21.4
DI	121	3.5
Total	3509	100.0

swamps and decline on drier sites and in deeper water where germination is limited by lack of soil exposure (Figure 6). Likewise, *Cerato-phyllum demersum* is abundant on swamp sites but becomes progressively rarer on drier sites. *Carex jorii* shows peak abundance near the middle of the gradient among the bottomland oak flats, while *Chasmanthium sessiliflorum* is most abundant on mesic sites. Distributions for other species can be inferred from observing rows in Tables 1-3.

*Community types.*—The 119 survey samples were classified into six community types (Rich-Mesic Slopes and Creek Bottoms, Mesic Bottomland Ridges, Bottomland Oak Flats, Cypress Water-elm Swamps, Closed Canopy Cypress Swamps and Deep-Water Open Cypress Swamps) on the basis of multivariate analysis of both overstory and ground flora (Figure 4). This classification forms the basis for a map of the wetland vegetation of Caddo Lake State Park and Wildlife Management Area (Figure 7). Fifty-two percent of the samples (Figure 4) and 56% of the area mapped in Figure 7 consisted of one of the three *Taxodium* dominated swamp types (Table 4); a much higher proportion of cypress swamps than that of any other Texas wetland landscape the authors observed.

**(1) Rich Mesic Slopes and Creek Bottoms:** These hardwood-dominated communities occurred on steep sheltered slopes, creek bottoms, and ravines along the edge of the Caddo Lake basin. The community was mainly observed in the campground and headquarters portion of Caddo Lake State Park, which according to USGS topographic maps, has some of the greatest topographic relief on the Caddo watershed. Ground flora included members of the *Chasmanthium* group



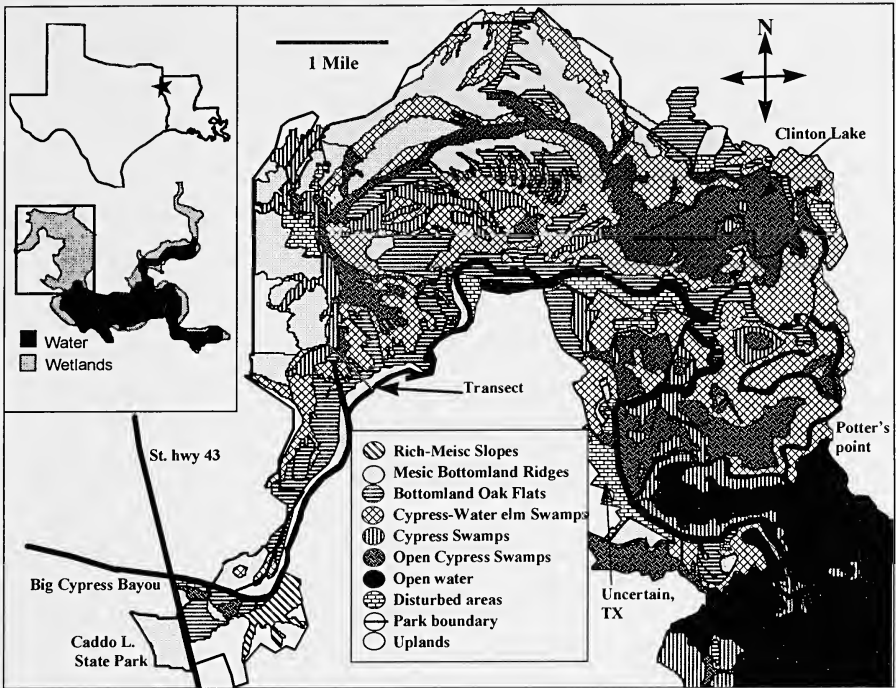


Figure 7. A map of the wetland community types of the Caddo Lake State Park and Wildlife Management Area and adjacent areas. The classification is based on a survey of 119 areas within the State Park and Wildlife Management Area. Other areas outside public lands were classified by interpreting aerial photographs. The southern boundary of the Wildlife Management Area has been omitted in the interest of clarity. Essentially it follows the channel from Clinton Lake to Potter's Point and extends southwest from Potter's Point to the main channel of Cypress Bayou near Uncertain. From there it follows Cypress Bayou north, east, and then southeast to Caddo Lake State Park.

as well as a rich assemblage of mesophytic plants including Florida maple (*Acer barbatum* Michx.), cross vine (*Bignonia capreolata* L.), Christmas fern (*Polystichum acrostichoides* (Michx.) Shott.) and Canada snakeroot (*Sanicula canadensis* L.) that were not found elsewhere. Unlike the remaining five types, these are not wetlands and they did not occur within the Caddo Lake floodplain (<54m elevation). However, these sites have better moisture relations than the adjacent uplands and were possibly better protected from presettlement fires by their topographic location.

**(2) Mesic Bottomland Ridges and Flats:** These rarely-flooded communities, found within the Cypress Bayou floodplain (< 54 m elevation), occurred on the crests of natural levees and meander scrolls that developed along stream channels and along the gradually sloping Pleisto-

cene low terraces adjacent to the lower wetlands. Stands were commonly dominated by loblolly pine (*Pinus taeda* L.), sweetgum (*Liquidambar styraciflua* L.), southern red oak (*Quercus falcata* Michx.) and other hardwoods. Important shrubs included *Ilex decidua* Walt and *Vaccinium arboreum* Marsh. Ground vegetation was dominated by members of the *Chasmanthium* and *Carex jorii* groups especially *Chasmanthium sessiliflorum*, sedge (*Carex jorii*) and greenbriar (*Smilax* spp.).

**(3) Bottomland Oak Flats:** These communities occurred on the lower portions of islands and levees that gently slope down into the wetter swamps. Sites were seasonally flooded and soils poorly drained, but were above water for most of the year. Most stands were dominated by willow oak (*Q. phellos*), with lesser amounts of overcup oak (*Q. lyrata*), blackgum (*Nyssa sylvatica* Marsh.) and sweetgum (*Liquidambar styraciflua*). Wetter stands transitional to swamps were generally dominated by *Q. lyrata*. Important shrubs included *Crataegus opaca* Hook & Arn., *Diospyros virginiana* L., *Styrax americana* Lam., *Ilex decidua* and on lower sites, *Foresteria acuminata* (Michx.) Poir. Ground vegetation included *Carex jorii*, and other members of the *C. jorii* group. Members of the *Brunnichia* group were also common, especially on wetter sites. Bottomland Oak Flats can be divided into three community subtypes: (a) willow oak flats, dominated by willow oak and *C. Joorii*, (b) sites on slight mounds with a somewhat open canopy of willow oak and a dense ground cover of *Erianthus strictus* and (c) overcup oak-dominated flats on lower, wetter sites. The environmental and/or historic factors maintaining the canopy openings of the *Erianthus* subtype are not known, but the groundflora appears to be transitional between Mesic Bottomland Ridges and the remaining Bottomland Oak flats (Figure 1, Table 1).

**(4) Cypress Water-elm Swamps:** Cypress-water elm communities were transitional between oak flats and swamps. Occurring slightly below the current normal pool elevation of the lake (Figure 2), they were flooded for much of the year, but had significant periods of exposure. Cypress dominated the overstory but there was an abundant sub-canopy of water elm (*Planera aquatica*). Shrubs were sparse, but included *Foresteria acuminata*, and *Cephalanthus occidentalis* L. The Ground layer was sparse during low water periods, largely represented by the *Brunnichia* group. When flooded, species from the *Ceratophyllum* group were found.

**(5) Closed-Canopy Cypress Swamps:** These swamps, often associated with abandoned stream channels, occurred lower on the landscape

than the cypress water-elm swamps, and were usually under water. The high density of cypress indicated that historically there were periods in which the sites were exposed enabling significant regeneration. The tree layer was pure baldcypress (*T. distichum*). Shrubs were limited to scattered *Cephalanthus occidentalis* growing from stumps and logs. By mid summer, the surface was covered by members of the *Ceratophyllum* group including *Egeria densa*, fanwort (*Cabomba caroliniana*) duckmeat (*Spirodela punctata* (Meyer) Thomps.), water meal (*Wolffia columbiana* Karst) and water fern (*Azolla caroliniana* Willd.).

**(6) Open (Deep Water) Cypress Swamps:** In deeper water, cypress stands became less dense. The deepest swamps were reduced to scattered trees growing in open water covered by floating and submersed aquatic plants. As with the previous community, members of the *Ceratophyllum* group dominated the surface. Dense colonies of *Nuphar luteum* were also common.

#### DISCUSSION

Caddo Lake wetland vegetation corresponds to a flooding and elevation gradient from mesic, rarely-flooded terraces and levees to nearly continuously flooded cypress swamps. While removal of the Great Raft and dam construction may have modified hydrology, modern vegetation appears to be closely adjusted to the current flooding regime as evidenced by the correspondence between the normal pool elevation of 51.36 m and the boundary between semi-permanently flooded swamps and seasonally flooded Bottomland Oak Flats (Figure 2).

*Other bottomland and swamp vegetation studies.*—Other authors have described hydrologic gradients and plant communities comparable to those at Caddo Lake from other locations in the South. Crouch & Golden (1997) surveyed the flora of an Alabama bottomland forest where a seasonally flooded floodplain was dominated by bottomland hardwoods including *Quercus phellos*, *Q. pagoda* Raf., *Q. nigra* L and *L. styraciflua*, with *Q. lyrata* and *Carya aquatica* (Michx f.) Nutt. dominating a somewhat lower slough. An area of slopes and ravines adjacent to the floodplain contained a rich mixture of hardwoods similar to the Rich Mesic Steep Slopes of Caddo Lake.

Thompson (1980) described mesic terrace bottoms, mixed softwood levees, oak hardwood bottoms and shallow *Taxodium distichum* swamps from a floodplain forest in Missouri. The recently-formed mixed softwood levees, characterized by pioneer trees such as *Salix nigra* Marsh. were not observed at Caddo Lake, possibly because the high-energy stream flows responsible for creating such landforms have not been present on Big Cypress Bayou during the last 200 yr as a result of

its partial impoundment. Although many southern bottomland species were present, Thompson (1980) also reported a number of species such as *Acer saccharinum* L. and *Quercus palustris* Muenchh. from this more northerly site that were not found at Caddo Lake.

*Texas and Louisiana studies.*—Marks & Harcombe (1981) identified Floodplain Hardwood Forest and Swamp Cypress-Tupelo Forest from the Big Thicket of southeastern Texas. Their two highest Floodplain Hardwood stands are roughly equivalent to Mesic Bottomland Ridges, except that *Fagus grandifolia*, Ehrh., abundant in the Big Thicket sites, was absent at Caddo. The remaining Big Thicket Floodplain Hardwood stands correspond to Caddo Lakes's Bottomland Oak Flats except that *Quercus lyrata* is less important for the Big Thicket and *Carpinus caroliniana* Walt., abundant in the Big Thicket understory, is absent at Caddo. *Nyssa aquatica* L., dominant in the Big Thicket swamp, was absent from the corresponding swamps at Caddo Lake.

Mathies et al (1983) described a series of wetland communities from Cunningham Brake in Natchitoches Parish, Louisiana. *Taxodium distichum* and *N. aquatica*, were associated with areas flooded for most of the year and lowland hardwoods including *Q. phellos*, *Q. nigra*, *Q. laurifolia* Michx., *Q. falcata* (= *Q. pagoda* ?), and *L. styraciflua* dominated seasonally flooded bottomlands. Open sand bars and stream edges along Kisatchie Bayou, characterized by pioneer species such as *Betula nigra* L. and *Salix nigra*, are equivalent to the softwood levees of Thompson (1980), but with the exception of a few sites observed on the spoils of artificial ditches, were not observed at Caddo Lake.

Delcourt (1976) described cane ridges, levee slope hardwoods (equivalent to Caddo's Bottomland Oak Flats), and cypress/tupelo swamps in her reconstruction of presettlement northern Louisiana vegetation. It is notable that although presettlement accounts described giant cane *Arundinaria gigantea* (Walt.) Muhl. as dominating natural levees, (equivalent to Mesic Bottomland Ridges), cane was uncommon on such sites at Caddo Lake.

*Relation to regional classification systems.*—Van Lear & Jones (1987) developed an ecological site classification system for terraces and floodplains associated with the Savannah River in South Carolina. Resembling the Caddo Lake communities, ecological land units are arranged along a flooding gradient from deep water cypress swamps, shallow cypress and tolerant hardwood swamps, laurel oak-water oak floodplains characterized by prolonged flooding, and loblolly pine-sweetgum-oak communities on terraces above the active floodplain.

Larson et al. (1981) and Christenson (1988) described a "zonal

classification" of southeastern alluvial wetlands which divides them into five zones based on the frequency and duration of flooding. Mesic Bottomland Ridges essentially correspond to zone V (infrequently flooded transition to uplands), Bottomland Oak Flats correspond to zone IV (seasonally flooded forests of backwaters and flats), Overcup oak-dominated lower Bottomland Oak Flats correspond to Zone III (Lower hardwood swamp forest), and the three swamp types described for Caddo lake correspond to zone II (intermittently exposed river swamp forest). Zone I represents the permanent water of river channels and lakes.

The SAF Forest Cover Type classification (SAF 1980), widely used among forestry professionals in the US, places Mesic Bottomland Ridges in the Loblolly Pine-Hardwood cover type (SAF type 82). Higher bottomland Oak Flats (*Erianthus* openings and Willow Oak Flats best fit the Sweetgum-Willow Oak cover type (SAF type 92), but also resemble the Willow Oak- Water Oak- Diamondleaf Oak type (SAF type 88). Overcup oak-dominated Bottomland Oak Flats correspond to the Overcup Oak- Water Hickory cover type (SAF type 96). The three cypress swamp community types of Caddo lake belong to the Baldcypress cover type (SAF type 101), although *Planera aquatica*, common at Caddo Lake, is described as an understory associate for the Baldcypress/Tupelo cover type (SAF type 102), but not for the pure Baldcypress type.

In 1998, The Nature Conservancy released a draft of a comprehensive classification of terrestrial vegetation for the southeastern United States. The system classifies vegetation into Alliances which are in turn made up of one or more Associations (Weakley et al. 1998). While the Caddo Lake communities can generally be placed into an appropriate Alliance, in many cases an Association has yet to be described that closely fits them. Additional quantitative case studies such as this one will be invaluable in refining the classification and in increasing our understanding of regional patterns of vegetation.

Mesic bottomland Ridges appear to best fit in the *Pinus taeda-Quercus (phellos/nigra/laurifolia)* Temporarily Flooded Forest Alliance (I.C.3.N.b.070), but they also have affinity to the *Pinus taeda-Quercus (pagoda/shumardii/michauxii)* Temporarily Flooded Forest Alliance (I.C.3.N.b.060). The willow oak-dominated Bottomland Oak Flats best fit into the *Quercus phellos* Seasonally Flooded Alliance (I.B.2.N.e. 130), but the Alliance usually occurs in upland depressions, rather than bottomlands. Willow Oak Flats also resemble the *Quercus (phellos/nigra/laurifolia)* Temporarily Flooded Forest Alliance (I.B.2.N.d.250) although there are understory differences and Big Cypress Bayou is not a "blackwater or low sediment/nutrient river" as is associated with this

Alliance (Weakley et al. 1998). The lower, overcup oak-dominated Bottomland Oak Flats belong to the *Quercus lyrata* (*Carya aquatica*) Seasonally Flooded Forest Alliance (I.B.2.N.e.100) and possibly to the *Quercus lyrata*/*Liquidambar styraciflua*/*Foresteria acuminata* Forest Association. Cypress-Water elm Swamps best fit the *Taxodium distichum*/*Nyssa* Seasonally Flooded Forest Alliance (I.B.2.N.e.190) although *Nyssa* spp. were absent from Caddo swamps. Weakley et al. (1998) also described a *Planera aquatica* Seasonally Flooded Forest Alliance (I.B.2.N.e.090) with an understory similar to that observed in Cypress-Water elm Swamps at Caddo Lake. However, the emergent cypress canopy at Caddo is too dense to fit the description for this Alliance. Closed Canopy and Open Cypress Swamps belong to the *Taxodium distichum* Semi-permanently Flooded Forest Alliance (I.B.2.N.f.060) and to the *Taxodium distichum*/*Lemna minor*. Association, although at Caddo *Spirodela* spp. and *Wolfia* spp. dominate the surface rather than *Lemna* spp. Some of the larger openings in the Open Cypress Swamps (such as portions of Clinton Lake, Figure 7) belong to the *Nuphar lutea* Permanently Flooded Herbaceous Alliance (V.C.2.N.a.040) where *Nuphar* dominates, or to the southern variant of the *Potamogeton* spp./*Ceratophyllum* spp./*Elodea* spp. Permanently Flooded Herbaceous Alliance (V.C.2.N.a.065).

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IMPORTANCE OF ARBUSCULAR MYCORRHIZAE TO DRYMASS PRODUCTION OF A NATIVE TEXAS C<sub>3</sub> AND C<sub>4</sub> GRASS**O. W. Van Auken and S. C. Brown\***

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**ABSTRACT.**—The importance of mycorrhizal symbiosis for drymass production of the native Texas grasses, *Nassella leucotricha* Trin. and Rupr. (C<sub>3</sub>, Texas Wintergrass, formerly *Stipa leucotricha*) and *Aristida longiseta* Steud. (C<sub>4</sub>, Red Threeawn), were examined. Arbuscular mycorrhizal infection increased total and shoot drymass in *N. leucotricha* and *A. longiseta* in low nutrient soil as well as in fertilized soil when compared to fungicide (benomyl) treated plants. Fertilizer addition reduced the mycorrhizal infection in *A. longiseta* 62% compared to a reduction of 29% in *N. leucotricha*. Fungicide application significantly reduced mycorrhizal infection in both species, but was more effective in reducing mycorrhizal infection in *A. longiseta* than in *N. leucotricha*. Mycorrhizal infection and total drymass of *A. longiseta* grown in heat sterilized soil was reduced 97% and 93%, respectively. Soil inoculum placement was also significant, as percent infection of *A. longiseta* grown in sterile-upper/nonsterile-lower stratified soil was reduced 22% compared to nonsterile-upper/sterile-lower soil, while drymass increased by 17%. Delayed acquisition of mycorrhizae by *A. longiseta* seems to be beneficial to early plant growth. However, prolonged lack of colonization would result in plant mortality because *A. longiseta* is an obligate mycotroph. *Nassella leucotricha* on the other hand, seems to be facultative.

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Focus on the effects of single factors has tended to obscure many complex interactions that occur among species (Clay 1990). Competition is a major force thought to shape ecosystems (Grace & Tilman 1990;), but it is not the only factor (Callaway et al. 1996; Pugnarie et al. 1996), and it is constantly debated (Grace 1993; 1995; Goldberg 1994; Twolan-Strutt & Keddy 1996; Van Auken & Bush 1997). In addition, the results of species interactions may be changed by herbivores (Crawley 1983; Van Auken & Bush 1989; Louda et al. 1990; Van Auken 1994; Bush & Van Auken 1995) and microorganisms (Clay 1990; Clay et al. 1993; Setala 1995; Little & Maun 1996; Watkinson & Freckleton 1997). The role of fungi in mediating the outcome of species interactions in natural communities is complex and not well understood (Clay 1990; Carey et al 1992). However, fungi caused negative effects in many agricultural species are well documented (Burdon 1987)

compared to fungal mutualisms in native plants (Lewis 1987).

Early research on arbuscular mycorrhizal relationships was done with agricultural plants that have well-defined economic value (Peng et al. 1993; Graham & Eissenstat 1994). That trend has shifted to include many nonagricultural species (Allen 1991; Del Vecchio et al. 1993; Gehring & Whitham 1994; Fischer Walter et al. 1996; Weinbaun et al. 1996; Koske & Gemma 1997). But, in spite of considerable research, mycorrhizal-plant interactions are not always easily predicted (Miller & Allen 1992; Nelson & Allen 1993).

In many grasslands, dominant plants appear to be mycorrhizal (Davidson & Christensen 1977; Gibson & Hetrick 1988; Koske & Gemma 1997; Wilson & Hartnett 1997). Nevertheless, different species have different physiological responses to mycorrhizae (Fitter 1977; Allen & Allen 1990). Thus, the role of mycorrhizae is undoubtedly complex. In these grasslands, it is reasonable to assume that native plants and their mutualistic fungi compete for resources and that competitive intensity could change with resource availability, but this idea is widely debated (Campbell & Grime 1992; Turkington et al. 1993; Grace 1993).

Plants that are most often mycorrhizal-dependent usually possess coarse roots and have short root hairs, or lack root hairs completely (Baylis 1975). Grasses, that usually have well developed fibrous roots, were originally thought to be facultatively mycorrhizal. The  $C_3$  grasses generally respond in a negative fashion to mycorrhizae, with plant drymass decreasing as root infection increases (Hetrick et al. 1988, 1990, 1992). Thus, the mycorrhizal symbiosis for some  $C_3$  grasses could be pathogenic (Hetrick et al. 1990) because the energy cost of maintaining the infection is higher than any derived benefits (Hayman 1983). The  $C_4$  grasses, on the other hand, exhibit high mycorrhizal dependency (Hetrick, et al. 1988; Wilson & Hartnett 1997), but some may be facultative under certain conditions and obligative in others (Baylis 1975; Hetrick, et al. 1988; 1989).

The purpose of this study was to examine the importance of arbuscular mycorrhizae in drymass production of two native Texas grasses, *Nassella leucotricha* ( $C_3$ ) and *Aristida longiseta* ( $C_4$ ).

## METHODS AND MATERIALS

Seeds of the C<sub>3</sub> grass *Nassella leucotricha* Trin. & Rupr. (Texas Wintergrass, formerly *Stipa leucotricha*, Barkworth 1990) and the C<sub>4</sub> grass *Aristida longiseta* Steud. (Red Threeawn) were collected from disturbed grasslands in northern Bexar County, Texas (29°37'N, 98°36'W). All experiments utilized the top 15-20 cm of a Patrick-series Mollisol (classified as clayey-over-sandy, carbonatic-thermic, typic-calcicustoll, with the "A" horizon varying in depth from 25-41 cm [Taylor et al. 1966]), collected in northern Bexar County from a disturbed grassland. Surface vegetation and litter were removed, the soil was sieved through a 6.4 mm mesh screen, air dried and mixed. The soil was low in nutrients (12 mg-P/kg, 1 mg-N/kg, 159 mg-K/kg and a pH of 8.4).

For each experiment, 15 cm diameter by 15 cm deep, plastic pots were lined with 3.8 L plastic bags to prevent nutrient loss. Seed dormancy in *N. leucotricha* was broken by soaking the seeds in 18 molar sulfuric acid for 15 minutes (White & Van Auken 1996; Van Auken 1997). *Aristida longiseta* appeared nondormant and was not treated with sulfuric acid. For all experiments, pots were watered thoroughly at planting, and then daily with approximately 150 mL deionized water. Pot locations were rotated weekly to ensure equal light and temperature exposure in the greenhouse. Photosynthetically active photon flux density (PPFD 400-700 nm) in the greenhouse at solar noon, October 10, 1991 was  $1264 \pm 166 \mu\text{mol}/\text{m}^2/\text{s}$ . Outside PPFD was  $1891 \pm 31 \mu\text{mol}/\text{m}^2/\text{s}$ . Light level was measured with a Li-Cor, Li-188 integrating quantum sensor.

At harvest, plant tops were clipped at the soil surface and oven dried at 90°C to a constant mass. Roots were carefully washed to remove the soil. Approximately 0.3 g of fresh root was taken from the upper, middle and lower part of each plant root system. Fresh drymass of the remaining roots was measured prior to oven drying at 90°C to a constant mass. Ash-free root drymass was obtained by ashing the dried roots at 700°C for 3 h (Böhmer 1979). The total ash-free drymass of the root sample was calculated by determining the percent difference in fresh root drymass and ash-free root drymass and multiplying the root sample drymass by the resulting coefficient. In this way, the ash-free drymass of the total root sample could be estimated.

Fresh root samples from all experiments were washed, cut into 1 cm segments, and placed in 10% KOH at 90°C for 20 min. After clearing, the KOH was removed and samples were washed in deionized water. Samples were then soaked in acidified water for 10 min, stained in 0.05% Trypan Blue for 10 min at 90°C, destained in 85% lactic acid at 90°C for 20 min and stored in lactic acid (Phillips & Hayman 1970). Percent root infection was determined using the gridline/intersect method (Newman 1966; Giovannetti & Mosse 1980). If an arbuscule or vesicle was found at an intersect, that intersect was considered infected. One hundred fifty intersects were examined for each sample and the infection presented as a percent.

Data were arcsine transformed prior to making statistical comparisons; however, non-transformed data are presented (Steel & Torrie 1980). Significant differences between treatment means within a species or plant part (aboveground, belowground or total) were evaluated with *t*-tests. Significant differences between soil type (sterile/nonsterile stratified soil or the reverse), root location (upper pot or lower pot) and the interaction between these factors were determined with a two-way analysis of variance (ANOVA) followed by the Scheffé multiple comparison test (Steel & Torrie 1980).

To examine effects of mycorrhizal colonization on plant drymass at low and high levels of soil nutrients, ten pots (1400 g of dry Patrick soil) were planted with *A. longiseta* (20 seeds/pot) and ten pots were planted with *N. leucotricha* on 1 April 1991. Ten additional pots were planted with each species and amended with 0.2 g-N as  $\text{NH}_4\text{NO}_3$ , 0.15 g-P as  $\text{Na}_2\text{HPO}_4$ , 0.1 g-K as KCl and 0.04 g-S as  $\text{MgSO}_4$  per pot on 1 May 1991 (Tiedemann & Klemmedson 1986). Plants were thinned to two per pot two weeks later. Five pots of each species at each nutrient level were randomly chosen for treatment with the fungicide benomyl. Twice weekly, 2.8 mL of a 30 mg/kg aqueous solution of benomyl was applied to the experimental plants through aerial application. All plants were harvested after fourteen weeks. Drymass and percent infection were then determined.

To measure the influence of arbuscular mycorrhizae on *A. longiseta*, plants were grown in sterile soil. 1600 g of soil was used per pot. The soil was autoclaved at 85°C for two hours. Five pots were filled with sterile soil and five with unsterilized soil. All ten pots were sown with twenty *Aristida longiseta* seeds on 1 August 1991 and plant density was

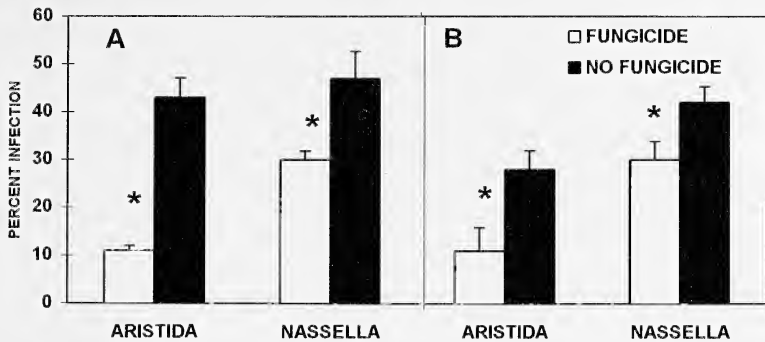


Figure 1. Mean percent arbuscular mycorrhizal infection of *Aristida longisetata* and *Nassella leucotricha* roots in low nutrient soil (A) and in fertilized soil (B). Sample size=5 for each treatment for each species. Benomyl was applied aerially twice weekly for 14 weeks. An \* indicates a significant difference between treatment means within a species ( $P \leq 0.05$ ). The line at the top of a bar is one SD.

adjusted to three plants/pot two weeks later. Harvest occurred after 12 wk. Drymass and percent infection were determined at harvest.

The effect of mycorrhizal inoculum placement on the drymass and colonization of *A. longisetata*, was also examined. Plants were grown in 15 cm diameter by 15 cm deep pots with sterilized soil in the top half and unsterilized soil in the bottom half of the pots. The reciprocal treatment was also established. Eight hundred grams of one soil type was added to the bottom one-half of five pots. On top of this soil layer, 800 g of the second soil type was added. *Aristida longisetata* seeds were sown into the pots on 1 August 1991. Plant density was adjusted to three plants/pot two weeks later. All plants were harvested after 12 wk. Drymass was determined at harvest, and percent infection was measured separately for the roots in the top and bottom halves of each pot.

## RESULTS

In low nutrient soil, percent mycorrhizal infection of *Aristida longisetata* roots was significantly lower in the fungicide treated plants than in the plants receiving no fungicide (Fig. 1a). In no replicate did aerial application of fungicide completely eliminate infection, but it was reduced by 75%. Percent infection was also significantly lower in the fungicide treated *Nassella leucotricha* roots than in the roots of the plants that were not treated with fungicide. Mean infection in the roots of the fungicide treated plants was reduced by 35% compared to the non-fungicide treat plants (Fig. 1a).

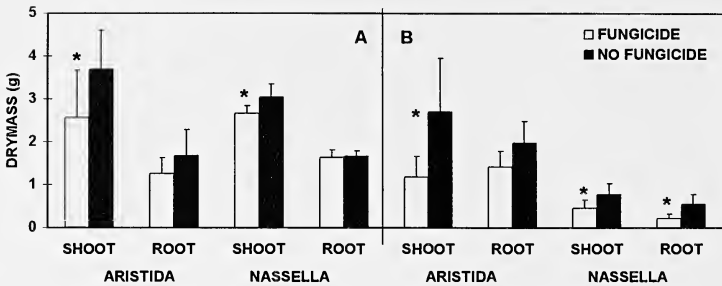


Figure 2. Mean shoot and ash-free root drymass (g) for *Aristida longiseta* and *Nassella leucotricha* in low nutrient soil (A) and in fertilized soil (B). Sample size=5 for each treatment for each species. Benomyl was applied aerially twice weekly for 14 weeks. An \* indicates a significant difference between treatment means within a plant part and species ( $P \leq 0.05$ ). The line at the top of a bar is one SD.

In high nutrient soil, there was a 62% reduction in mycorrhizal infection of the roots of *A. longiseta* plants treated with fungicide compared to plants receiving no fungicide (Fig. 1b). Infection of the roots of fertilized, fungicide treated *N. leucotricha* plants was reduced 29% (Fig. 1b). Percent infection of the roots of *A. longiseta* plants treated with fungicide but with or without added fertilizer were the same at 11% (Fig. 1a&b). However, the percent infection of the roots of *A. longiseta* plants not treated with fungicide but fertilized was 28% compared to 43% in non-fertilized plants (Fig. 1a&b). Percent infection of the roots of *N. leucotricha* plants treated with fungicide but with or without added fertilizer was the same at 30%. However, the percent infection of the roots of *N. leucotricha* plants not treated with fungicide, but fertilized was 42% compared with 47% in non-fertilized plants (Fig. 1a&b).

Ash-free root drymass and total drymass for fungicide treated *A. longiseta* plants was not significantly different from non-treated plants (Fig. 2a). However, shoot drymass in the fungicide treated plants was reduced by 31% from 3.68 g to 2.56 g (Fig. 2a). Total *N. leucotricha* drymass and shoot drymass for plants treated with fungicide was significantly lower than non-treated plants, but the reduction was only 9% (Fig. 2a). Most of the difference was in shoot drymass. There was no significant difference between *N. leucotricha* ash-free root drymass of the fungicide treated plants compared to the roots of those not treated.

Total drymass for fertilized *A. longiseta* plants treated with fungicide was 44% lower than plants not treated with fungicide (Fig. 2b). Shoot

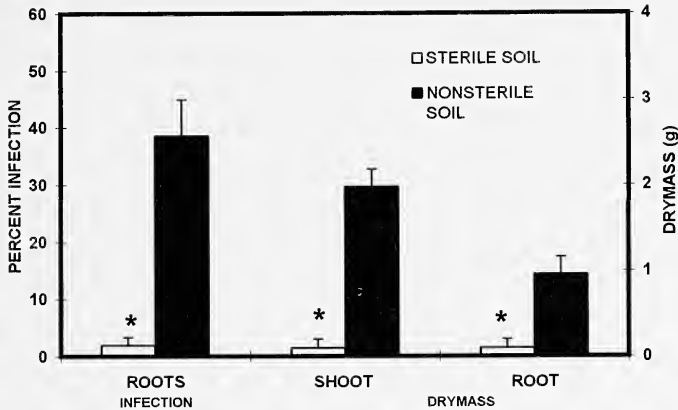


Figure 3. Mean percent arbuscular mycorrhizal infection, shoot and root drymass of *Aristida longiseta* grown in heat-treated, sterile or nonsterile soil for 14 weeks. Sample size = 5 for each treatment. An \* indicates a significant difference between treatment means ( $P \leq 0.05$ ). The line at the top of a bar is one SD.

dry drymass in the fungicide treatment was 56% lower than plants receiving no fungicide and ash-free root drymass was reduced 29% in the fungicide treatment, but this last difference was not significant (Fig. 2b). Fertilized *N. leucotricha* total plant drymass, shoot drymass and ash-free root drymass were reduced by 49, 42 and 40% respectively for the fungicide treated plants (Fig. 2b).

In heat sterilized soil, compared to the nonsterile soil treatment, percent mycorrhizal infection, total plant drymass, shoot drymass and ash-free root drymass of *A. longiseta* were reduced 96, 96, 98 and 91% respectively (Fig. 3). Mycorrhizal infection for the roots of the plants in the heat treated sterile soil was 1% compared to 39% for the nonsterile soil control. Mean total plant drymass for the heat sterilized soil was 0.13 g and it was 2.93 g in the nonsterile soil control.

When the effect of mycorrhizal inoculum placement on percent mycorrhizal infection was examined for *A. longiseta*, ANOVA demonstrated a significant ( $P < 0.05$ ) soil effect (sterile-upper/nonsterile-lower versus nonsterile-upper/sterile-lower), a significant location effect (upper roots versus lower roots), but no significant interaction between the main factors. Percent infection was significantly lower in the roots in both the upper half and the lower half of the soil when the top soil strata was sterile and the bottom soil strata contained the inoculum (Fig. 4). ANOVA of total drymass indicated a significant soil effect ( $P < 0.05$ ), a significant location effect and a significant interaction. Total plant

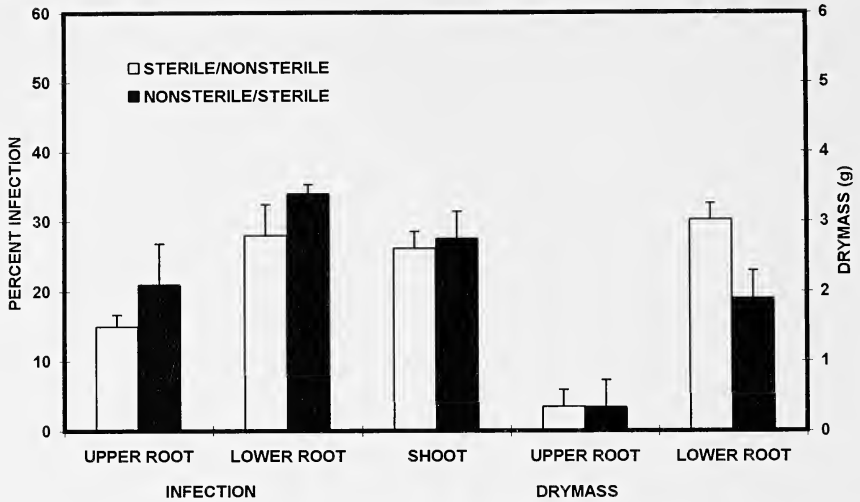


Figure 4. Mean percent arbuscular mycorrhizal infection, shoot, upper and lower root ash free drymass of *Aristida longiseta* as a function of inoculum placement (lower inoculum = sterile soil above/nonsterile below and upper = nonsterile above/sterile below). Sample size = 5 for each treatment. ANOVA of percent infection indicated a significant ( $P \leq 0.05$ ) soil effect (sterile/nonsterile versus nonsterile/sterile), a significant location effect (upper versus lower layer of the soil), but no significant interaction. ANOVA of total drymass indicated a significant soil effect, a significant location effect, and a significant interaction. The line at the top of a bar is one SD.

drymass of *A. longiseta* grown in sterile/nonsterile stratified soil was 17% greater than total plant drymass of plants in nonsterile/sterile soil. The largest difference was in the ash-free drymass of the lower roots (those occurring in the bottom half of the pots) in the sterile/nonsterile, stratified soil, which was 38% higher than in the reverse treatment (Fig. 4).

## DISCUSSION

Mycorrhizal colonization of *Aristida longiseta* roots in low nutrient soil significantly increased plant shoot mass. This is not surprising since it is a commonly held, but not widely tested, belief that most  $C_4$  grasses are obligately mycorrhizal (Hetrick et al. 1988, 1989, 1990; Wilson & Hartnett 1997). Fertilized *A. longiseta* plants showed the same trend as seen in the low nutrient experiment, except total plant as well as shoot drymass was significantly higher for the no fungicide treatment. *Aristida longiseta* plants grown in low nutrient soil had 43% mycorrhizal root infections, compared to 28% in their fertilized counterparts, a 62% reduction. This confirms in another species that increased levels of soil nutrients probably phosphate, increases drymass but inhibits root



colonization by the mycorrhizae, or that low soil nutrients, probably lack of phosphorus, stimulates root infection (Hetrick et al. 1990; Allen 1991; Miller & Allen 1992; Peng et al. 1993; Graham & Eissenstat 1994). A positive nutrient effect overall was not observed, which was probably caused by high summer greenhouse temperatures that had negative effects on both species.

In both the low nutrient and fertilized treatments, *A. longiseta* plants sprayed with the fungicide benomyl maintained mycorrhizal infection levels of 11%. This method of control does not appear to be as effective in prevention of infection as mixing the fungicide with the soil (Hetrick et al. 1989; Wilson & Hartnett 1997). However, the level of plant and soil disturbance is minimal and should allow differential, selective application in the field or in competition experiments. Benomyl apparently does not effect a variety of plants when fungi are lacking (Paul et al. 1989) and has been commonly used to alter mycorrhizal activity (Carey et al. 1992; Hetrick et al. 1994; Wilson & Hartnett 1997).

Mycorrhizal infection also increased *N. leucotricha* drymass in both low and high nutrient soil. However, this increase in drymass was not as dramatic as in *A. longiseta*. This may be due to the fungicide treatments not being as effective in reducing mycorrhizal infection in *N. leucotricha* as in *A. longiseta*. *Nassella leucotricha*'s positive response to mycorrhizal infection is interesting in light of studies showing that some C<sub>3</sub> grasses have a low mycorrhizal dependence (Hayman 1983; Hetrick et al. 1988, 1990, 1992). Apparently mycorrhizal colonization is pathogenic for some C<sub>3</sub> grasses (Hayman 1983; Hetrick et al. 1990).

The range of *N. leucotricha* is strictly south of 35°N latitude (Leithead et al. 1971), which is atypical of C<sub>3</sub> species that are assumed to lose their growth advantages in lower latitudes with higher temperatures and irradiances (Doliner & Joliffe 1979; Hicks et al. 1990). *Nassella leucotricha* is like other C<sub>3</sub> species in terms of cool temperature tolerance because its growth period is during late fall, winter and early spring (Gould 1975). However, its continued growth and survival in C<sub>4</sub> grasslands may be related to its wide temperature tolerance for carbon uptake, efficient water use, double mechanism of seed dormancy, and its tolerance or perhaps requirement for arbuscular mycorrhizae (Hicks et al. 1990; Van Auken 1997).

Both *A. longiseta* and *N. leucotricha* typically grow in soil with high calcium levels and moderately alkaline pH (Taylor et al. 1966), thus acquisition of phosphate could be difficult because it is bound as  $\text{CaPO}_4$  (Lapeyrie & Chilvers 1985; Gahoonia et al. 1992). In such situations, the ability of the mycorrhizae to exploit bound nutrients such as phosphate could be beneficial and negate the energy cost to support the symbiosis (Peng et al. 1993; Graham & Eissenstat 1994). Lapeyrie & Chilvers (1985) showed that ectomycorrhizae allowed *Eucalyptus dumosa* seedlings to grow in a calcareous soil that would not normally support their growth.

The 97% reduction of *A. longiseta* total drymass in heat sterilized soil compared to nonsterile soil provide insight into the mycorrhizal dependency of this species. Stribley (1987) criticized the use of heat sterilized soil for mycorrhizae experiments since heat sterilization alters more than just the presence of mycorrhizal propagules. Heat treatment would remove all pathogenic fungi as would general fungicide treatments (Carey et al. 1992; Wilson & Hartnett 1997). Still, *A. longiseta* plants grown in heat sterilized soil produced only 0.129 g drymass with low infection. The potential for other factors to cause the same reductions seem remote.

Previously it was thought that seeds exposed to mycorrhizae propagules at germination and early growth would have a greater drymass at inflorescence, even if the developing roots grew into lower soil layers containing no mycorrhizae (Schwab & Reeves 1981). If that premise were true, then it would have strong management implications, particularly in reclamation sites where soils are heavily disturbed. It appears the reverse may be true for some species, because the drymass of *A. longiseta* grown in the sterile-upper/nonsterile-lower soil stratification treatment was 17% higher than the reverse treatment. It seems that *A. longiseta* prefers early growth at lower levels of mycorrhizal infection.

*Aristida longiseta* appears to be an early successional species or one that exploits disturbances (Gould 1975). It appears to increase in grasslands after heavy grazing (Gould 1975). Thus, it may be adapted to low nutrient, low resource gaps in grasslands where other species cannot easily establish, where soil surface temperature may be very high and the surface layer biota may be reduced. *Aristida longiseta* seems to be an obligate mycotroph based on its poor growth in heat sterilized

and/or fungicide treated soil. However, *N. leucotricha* is probably a facultative mycotroph.

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TWO POPULATIONS OF  
*SOLANUM ELAEAGNIFOLIUM* CAV. F. *ALBIFLORUM* COCKLL.  
(SOLANACEAE) ARE BLOCKED AT DISTINCT SITES IN THE  
ANTHOCYANIN BIOSYNTHETIC PATHWAY

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**Abstract.**—Floral anthocyanidin pigments from *Solanum elaeagnifolium* Cav. were identified as delphinidin and petunidin. The glycosidic portion of the anthocyanins was a 3' O-linked glucose monomer. White corollas from two distinct populations were unpigmented and differed in the anthocyanin precursors that they accumulated. Corollas from one population accumulated kaempferol and produced colored pigment when fed dihydromyricetin or dihydroquercetin but not dihydrokaempferol. Corollas from the second population failed to accumulate kaempferol and produced colored pigment when fed dihydrokaempferol, dihydromyricetin or dihydroquercetin. The two white-flowered populations apparently arose from different progenitors with distinct lesions. One population lacks the activity of flavonoid 3',5'-hydroxylase. The other population is deficient in the activity of flavanone 3-hydroxylase. The phylogenetic identity of the two populations implied by the uniform designation of *Solanum elaeagnifolium* Cav. f. *albiflorum* Cockll. is, therefore, misleading.

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*Solanum elaeagnifolium* Cav. (silverleaf nightshade, horsenettle) is a rhizomatous perennial occurring in dry, sterile soils, open woods, and prairies from March to October. The natural range of *S. elaeagnifolium* extends from Missouri and Kansas south to Louisiana, Texas, Arizona and adjacent Mexico. The species has been introduced and become a troublesome weed of crop lands in many subarid regions of the world by distribution of propagules mixed with agricultural exports (Boyd et al. 1984).

The corollas of *S. elaeagnifolium* range from pale to deep violet in color presumably due to anthocyanin pigments as is true for other members of the Solanaceae (Harborne 1967). White-flowered populations of the species, designated as *S. elaeagnifolium* Cav. f. *albiflorum* Cockll. by Correll & Johnston (1970), Stanford (1976) and others, occur rarely. Biosynthesis of corolla pigments has not previously been examined in this species. However, anthocyanin biosynthetic pathways have been characterized in maize (Poaceae), snapdragon (Scrophulariaceae) and petunia (Solanaceae). A composite biosynthetic pathway is presented in Figure 1 (Holton & Cornish 1995). The branch points and consequent biosynthetic product(s) of the pathway are species-specific. White

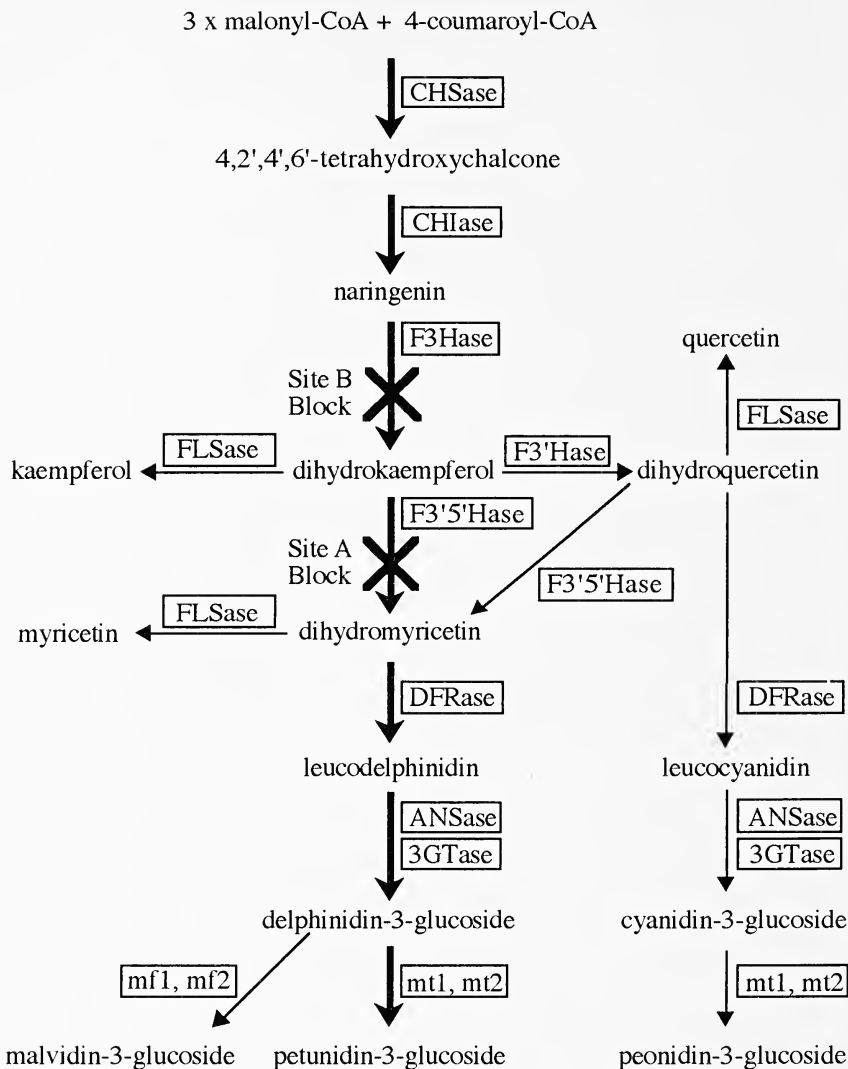


Figure 1. The anthocyanin biosynthetic pathway. The enzymatic pathway proposed for *Solanum elaeagnifolium* is highlighted in bold lines. The block in the pathway for each population of *S. elaeagnifolium* f. *albiflorum* is indicated by an "X". (Adapted from Holton & Cornish 1995).

(colorless) phenotypes result from blocks in the biosynthetic pathway prior to the production of colored products (Durbin et al. 1995; Levin & Black 1994).

Thin-layer chromatography (TLC) was employed to identify the



anthocyanin pigments in colored corollas of *S. elaeagnifolium* and to locate the sites of biosynthetic blocks resulting in white corollas in two distinct populations of *S. elaeagnifolium* f. *albiflorum*. Precursor feeding experiments confirmed the TLC results.

#### MATERIALS AND METHODS

*Collection sites.*—Colored corollas of *Solanum elaeagnifolium* were collected from plants growing adjacent to Sikes Lake (Wichita Falls, Wichita County, Texas). White corollas were collected from two distinct populations growing in the same locality. Specimen plants from each collection site were transplanted to pots and grown in the greenhouse where subsequent corolla sampling occurred.

*Chemicals.*—Pelargonidin, taxifolin (+/-dihydroquercetin), kaempferol, myricetin, quercetin, D-(+)-glucose, D-(+)-galactose, D-(-)-arabinose, D-(+)-xylose, L-(+)-rhamnose and N-methyldioctylamine, purchased from Sigma Chemical Company, were of reagent grade. Cyanidin was extracted from corollas of *Centaurea cyanus*. Delphinidin was extracted from *Delphinium hybrida* (Harborne 1967). All other chemicals were of reagent grade.

*Extraction and purification of pigments and precursors.*—Corollas were excised, weighed and extracted in 10 mL acidified methanol [1% (w/v) HCl in methanol] per gram tissue. Extractions were performed overnight in 15 mL polypropylene tubes at room temperature in the dark. Plant tissues were removed by extraction with 2.4 volumes chloroform:water (5:1). The pigmented aqueous phase was placed in a 1.5 mL microcentrifuge tube and dried under vacuum overnight.

*Deglycosylation of anthocyanins.*—The dried extract from one corolla was dissolved in 1 mL methanol, combined with 1 mL 4 N HCl in a 15 mL conical polypropylene tube and placed in a boiling water bath for 40 minutes (Strack & Wray 1989). Cooled hydrolyzate was extracted with 0.5 mL *Iso*-amyl alcohol. The pigmented organic layer was dried under vacuum overnight.

*Anthocyanidin chromatography.*—The dried pigment extract was reconstituted in 40 mL 0.1% HCl/methanol (w/v) and spotted onto cellulose plates. Plates were developed in the dark with Forestal (glacial

Table 1. Composition of acid-methanol solutions containing corolla extracts or standards with constituents identified by  $R_f$  ranges in Forestal mobile phase.

Sample	$R_f$ Ranges of Compounds Resolved by TLC		
	$R_f = 67-75$	$R_f = 51-57$	$R_f = 32-36$
<i>Solanum elaeagnifolium</i>			
wild type corollas	+	+	+
site A white corollas	+	—	—
site B white corollas	—	trace	trace
upper leaves	—	—	+
Cyanidin	—	+	—
Delphinidin	—	—	+
Kampferol	+	—	—

acetic acid:concentrated HCl:deionized water, 30:3:10) (Francis 1982), or *Iso*-PrOH (isopropanol:5% aqueous HCl, 55:45) (Mullick & Brink 1967) mobile phases. Developed plates were dried at room temperature and observed under visible and ultraviolet illumination. Spots were marked, their colors were noted and  $R_f$  values were calculated.

*Sugar analysis.*—Following deglycosylation, the aqueous phase was washed three times with 10% (v/v) N-methyldioctylamine in chloroform to remove acid residue. Samples were washed once with chloroform and dried overnight under vacuum. Dried sugars were dissolved in 40 mL water and spotted on cellulose plates with glucose, galactose, and rhamnose as reference solutions. TLC was developed in BBPW (*n*-butanol-benzene-pyridine-water, 5:1:3:3, upper layer) or Phenol (phenol:water, 4:1) and dried before spraying with aniline-hydrogen phthalate sugar reagent (1.6 g sodium hydrogen phthalate, 9.1 mL aniline, 48 mL *n*-butanol, 48 mL ethyl ether, 4.0 mL water) (Francis 1982). The sprayed plates were heated at 93°C for 10 minutes to visualize sugars. Spots were marked and  $R_f$  values were calculated.

*Feeding corollas anthocyanin precursors.*—Corollas were excised and placed in 1.5 mL microcentrifuge tubes filled with feeding solution. Feeding solutions consisted of 2 mg of commercially prepared dihydroquercetin (DHQ), quercetin, myricetin, or kaempferol dissolved in 10 mL water or synthesized dihydrokaempferol (DHK) or dihydromyricetin (DHM) (Pew 1948) dissolved in about one mL of water. Control corollas received water. Feeding occurred under ambient light at room temperature (Forkmann 1977).

Table 2. Composition of acid-methanol solutions containing corollas extracts or standards with constituents identified by  $R_f$  ranges in *Iso*-PrOH mobile phase.

Sample	$R_f$ Ranges of Compounds Resolved by TLC	
	$R_f = 67-74$	$R_f = 56-60$
<i>Solanum elaeagnifolium</i>		
wild type corollas	—	+
site A - DHQ fed corollas	+	—
site B - DHQ fed corollas	—	+
Cyanidin	+	—
Delphinidin	—	+

## RESULTS

*Anthocyanidin identification.*—Deglycosylation of anthocyanin extracts yielded two violet-colored bands with different mobilities following thin-layer chromatography (TLC) with Forestal (Table 1). The  $R_f$  value of the lower mobility band corresponded with delphinidin. The  $R_f$  of the higher mobility band was comparable to that of cyanidin, but its violet color differed from the magenta color of cyanidin. TLC with *Iso*-PrOH yielded distinct  $R_f$  values for cyanidin and the *S. elaeagnifolium* pigment (Table 2). Furthermore, the absorbance maximum of the unknown pigment was 556 nm while that of cyanidin was 546 nm. Based on these data, the higher mobility aglycone of *S. elaeagnifolium* was identified as petunidin. The intensity of pigmentation of *S. elaeagnifolium* corollas varies dramatically from population to population, ranging from pale to deeply colored. Extracts of corollas from several different populations contained pigments of identical color and TLC mobilities.

*Sugar analysis.*—The sugar component of *S. elaeagnifolium* anthocyanin was analyzed by TLC of extracts following acid hydrolysis. TLC with glucose, galactose and rhamnose standards and BBPW as mobile phase identified the moiety as glucose (Table 3). This result was confirmed by TLC with galactose and glucose standards and Phenol mobile phase for increased resolution (Table 4).

*TLC analysis of white corollas.*—Extracts of white corollas were compared by TLC with flavonol standards and Forestal mobile phase. White corollas from site A contained the same non-pigment compounds

Table 3. Composition of aqueous solutions containing hydrolyzate or sugar standards with constituents identified by  $R_f$  ranges in BBPW mobile phase.

Sample	$R_f$ Ranges of Compounds Resolved by TLC		
	$R_f = 22-25$	$R_f = 17.2-18.5$	$R_f = 16-17$
<i>Solanum elaeagnifolium</i>			
wild type corollas	—	+	—
site A white corollas	—	+	—
Glucose	—	+	—
Galactose	—	—	+
Rhamnose	+	—	—

as colored corollas. Kaempferol accumulated in site A corollas, although the quantity was not significantly increased compared with colored corollas. In contrast, white corollas from site B did not accumulate kaempferol or any other compounds that corresponded to flavonol standards on TLC. Based on the presence of kaempferol in site A corollas, a biochemical block subsequent to the conversion of DHK to DHM was hypothesized. The absence of flavonol precursors in site B corollas suggested that a block occurs prior to the conversion of naringenin to DHK.

*Precursor feeding of white corollas.*—Cut white corollas were fed kaempferol, quercetin and myricetin as well as the corresponding dihydroflavonols. Corollas fed flavonols failed to produce colored pigments. Corollas from site A did not produce colored product when fed DHK, but converted both DHQ and DHM to colored pigments. The color of the product was determined by the precursor that was fed to the corollas. DHQ was converted to a magenta pigment whose aglycone exhibited mobility in Forestal and *Iso*-PrOH mobile phase similar to cyanidin. Corollas from the same site converted DHM to a violet product resembling delphinidin. Site B corollas produced colored pigmentation when fed any of the three dihydroflavonol precursors. In each case, the pigment exhibited a violet color. Extracts of DHQ-fed site B corollas exhibited mobility similar to delphinidin in *Iso*-PrOH (Table 2). Naringenin-fed corollas from each site failed to accumulate pigmentation.

#### DISCUSSION

Timberlake & Bridle (1982) identify petunidin as a component pigment of nearly all members of the genus *Solanum*. The pigments of

Table 4. Composition of aqueous solutions containing hydrolyzate or sugar standards with constituents identified by  $R_f$  ranges in Phenol mobile phase.

Sample	$R_f$ Ranges of Compounds Resolved by TLC	
	$R_f = 37.5-39$	$R_f = 31.2-32.1$
<i>Solanum elaeagnifolium</i>		
wild type corollas	—	+
site A white corollas	—	+
Glucose	—	+
Galactose	+	—

*S. elaeagnifolium*, which have not previously been examined, are based on the aglycones delphinidin and petunidin (3'-methyl delphinidin). Rare white-flowered populations of *S. elaeagnifolium* fail to synthesize the colored pigments, presumably as a consequence of the loss of a specific enzymatic activity.

White corollas from site A accumulated kaempferol but not quercetin or myricetin. This suggested that a block occurred after the conversion of naringenin to DHK but before the synthesis of either DHQ or DHM (Figure 1). Two enzymes are potentially involved in the conversion of DHK to either DHQ or DHM: flavonoid 3'-hydroxylase (F3'Hase) and flavonoid 3',5'-hydroxylase (F3'5'Hase), respectively. Feeding experiments indicated that the corollas could produce cyanidin when fed DHQ, delphinidin when fed DHM, but no colored product when fed DHK. The failure to produce colored product when fed DHK confirms the absence of F3'Hase and F3'5'Hase activities in the corollas. The production of only cyanidin when fed DHQ in this population indicates that F3'5'Hase is not present to convert DHQ to DHM. However, the enzymes necessary to convert DHQ or DHM into the corresponding colored products are present. The natural violet pigment composition of *S. elaeagnifolium* colored corollas coupled with the production of cyanidin when white corollas were fed DHQ indicates that these organs do not naturally exhibit F3'Hase activity. If this activity were present, DHK would presumably be partitioned between pools of DHQ and DHM and corolla pigment composition should include a mixture of cyanidin and delphinidin or their derivatives. The absence of cyanidin from colored corollas indicates that DHQ is not naturally formed. Therefore, site A white corollas are colorless due to a failure to produce DHM resulting from a deficiency in F3'5'Hase activity.

White corollas from site B accumulated no kaemferol, suggesting that DHK is not produced in the corollas. Feeding experiments showed that the corollas were capable of producing colored pigments utilizing DHK, DHQ or DHM. It is likely that chalcone synthase (CHSase) and chalcone isomerase (CHIase) activities are present in white corollas from site B and that flavanone 3-hydroxylase (F3Hase) activity is absent or reduced to trace levels for the following reasons. In all plants which have been studied, CHSase is encoded by a multigene family consisting of as many as six members (Holton & Cornish 1995), although all copies may not be expressed in corollas. Isomerization of 4,2',4',6'-tetrahydroxychalcone to naringenin is reported to occur spontaneously at a low rate (Holton & Cornish 1995). Even if CHIase activity were absent, natural pigments would presumably be produced at a low rate and might accumulate. Site B corollas produce a very low level of pigment which was detected only upon extraction of corollas. This would be consistent with the hypothesis that site B corollas are white due to the absence of CHIase activity. However, it is the opinion of the authors that these corollas actually lack F3Hase activity. TLC analysis of site B corollas on Forestal produces a very high mobility compound that is also present in colored corollas. The compound exhibits several characteristics of naringenin. Naringenin accumulation would be consistent with the absence of F3Hase activity rather than CHIase activity. Additionally, feeding of naringenin to corollas of this population failed to produce pigment. The accumulation of a small amount of pigment in the corollas may be due to an incomplete block in the conversion of naringenin to DHK. Regardless of the specific site of blockage in the anthocyanin pathway of site B corollas, it is not the same as the block in site A corollas.

The potential for molecular verification of these results is simplified by the fact that genes encoding both F3Hase and F3'5'Hase have been isolated and their nucleotide sequences are known (Britsch et al. 1992; Britsch et al. 1993; Charrier et al. 1995; Deboo et al. 1995; Holton et al. 1993; Pelletier & Shirley 1995; Tanaka et al. 1996; Weiss et al. 1993). Northern blot analysis of poly (A)+ RNA from colored and white corollas will indicate whether the genes are transcribed at normal levels in the white corollas. The identification of two distinct populations of white flowered *S. elaeagnifolium* raises the question of whether other white-flowered populations may have arisen from genetic lesions which block other steps in anthocyanin synthesis, such as CHIase or dihydroflavonol reductase (DFRase).

Strict interpretation of the nomenclatural designation of these populations infers descent from a common progenitor and, consequently, derivation from a common molecular event. The results obtained from two distinct white-flowered populations refute this inference.

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## DIFFERENTIATION OF MISTLETOES (SANTALES) ON THE BASIS OF GEOGRAPHICAL ORIGIN

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**Abstract.**—Multivariate statistical analysis of gas chromatographic data has been applied to the differentiation of species of mistletoe based on their geographic origin. Mistletoe plants were collected from 26 locations in Texas and 13 locations in Tennessee, Alabama, Georgia and Florida. Hexane extracts were analyzed by gas chromatography. When the normalized chromatographic data were subjected to hierarchical cluster analysis, three clusters were seen. Two contained only Texas samples, and the other contained southeastern United States samples plus several Texas samples, possibly due to overlapping ranges of the species. This method appears to have value as a chemotaxonomic technique for differentiation of mistletoes, but further study is warranted. Compounds that are independent of parasitic host, season, gender and method of collection must be carefully selected for use in speciation of mistletoe.

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Mistletoes are found in one of four biological families within the order Santales; Misodendraceae, Loranthaceae, Viscaceae and Eremolepidaceae. The members of this family are either hemiparasitic (with chlorophyll) or parasitic (without chlorophyll) and attach to the roots or stems of other plants. The four family classification consists of over a hundred genera and thousands of species (Cronquist 1968; Kuijt 1968).

Of the four families of mistletoe that exist, the two most common are: Loranthaceae and Viscaceae. The differences between these two families center around floral structure and chromosomal count (Barlow 1971; Wiens & Barlow 1971). The Loranthaceae have large, conspicuous brightly colored flowers, and the Viscaceae have small, simply constructed inconspicuous flowers. The chromosomal count for Loranthaceae varies from 8 to 12, whereas in the Viscaceae the number is constant at 14.

Mistletoes are found throughout the entire world, but are concentrated in paratropical regions. The Loranthaceae are found in the southern hemisphere, and the Viscaceae are found in the northern hemisphere including the United States (Barlow 1983).

The family Viscaceae consists of seven genera and about 400 species.

The genus, *Phoredendron*, is found in the western hemisphere and consists of 170 species. Seven species are found in the United States (Barlow 1983). This genus is divided regionally into species; *P. serotinum*, found in the eastern United States and *P. tomentosum*, found in the central to western United States (Wiens 1964). *Phoredendron tomentosum* is further divided into two subspecies, *P. tomentosum tomentosum* growing in central Texas from Mexico to Oklahoma and *P. tomentosum macrophyllum* growing from West Texas to California (Wiens 1964). The range of *P. serotinum* is from eastern Texas through the southeastern United States (May 1971) up to Ohio through New Jersey (Spooner 1983). The two mistletoe species that are the subject of this study are *P. tomentosum* and *P. serotinum*.

In order to classify biological species, the taxonomist observes taxonomic characters. A taxonomic character is "any attribute referring to form, structure, physiology, or behavior which is considered separately from the whole organism for a particular purpose such as comparison, identification, or interpretation (Haywood 1970)." Defined in this way, chemical data could be used in the same way as traditional morphological features.

Chemotaxonomy is a very powerful tool that has been used for many years to distinguish species of biological samples. Chemotaxonomy is a hybrid subject encompassing chemistry, a highly specific and exact science, and systematics, which is more of an art. Chemical methods in taxonomy are used to record the presence or the absence of various compounds, and to some extent, concentrations of these compounds. The taxonomist usually decides which are important characters for classification or interpretation and which are not. Large classes of compounds that are used for taxonomic purposes are high molecular weight molecules, such as proteins, (Fitch 1970; Hall et al. 1971) and relatively low molecular weight compounds, such as alkaloids (Hegnauer 1963) and terpenes (Irving 1969). Chromatographic methods are usually used, since they are relatively fast, accurate and inexpensive. Gas chromatography can be applied to taxonomic studies since almost every major plant group can be identified using volatile compounds (Flake & Turner 1973). Gas chromatography is the analytical method that was used in the current project.

The current taxonomy of mistletoe leaves much to be desired. At the family and genus level the classifications are clear, but the subgenera

level it is an entirely different matter. The following observations have been made on the difficulty of studying the taxonomy of *Phoredendron* by Kellogg (1991):

1. any classification of herbaria samples is almost impossible due to their deterioration over time,
2. there are between 100 and 150 classified species,
3. leaf shape varies within a particular species.

These facts lead to the observation that even highly trained taxonomists have a difficult task of differentiating species of mistletoe; thus chemotaxonomy may be an important technique.

Mistletoe has received very little attention in the area of taxonomy or chemotaxonomy over the years. Paper chromatography has been used as an aid in differentiating ecotypes of *P. tomentosum* (cf. May 1972), to differentiate species of Loranthaceae (Tilney & Lubke 1974), and in the study of species of dwarf mistletoe (Crawford & Hawksworth 1979). Gas chromatographic profiles of polysaccharides have been used to differentiate three genera of mistletoes (Gedalovich-Shedletzky et al. 1989).

The chromatographic data were analyzed using a pattern recognition computer program (Ein\*Sight) to run hierarchical cluster analysis (HCA) to determine whether the two species of mistletoe can be distinguished from each other. HCA is a method by which distances between points in N-dimensional space are calculated. The samples that are closer together are more similar than those that are farther apart. A dendrogram, a tree-shaped map, is developed from distance calculations by linking samples and clusters of samples as a function of distance. Many linkage strategies are used which are mathematical formulas for calculating the distance matrices in various ways. Basically, each sample is considered a cluster of one. Then the two most similar samples are linked. Once this new cluster has been linked, it is linked to another cluster and this combination defines a third cluster. The distances between all existing clusters are computed, and the smallest distance is again searched and another cluster is created. Continuing this process links all the samples at some level of similarity. A dendrogram is produced from this linking procedure. The branches of the dendrogram have lengths that are proportional to the distances between the connected clusters. Similarity units are used to represent the distances in the

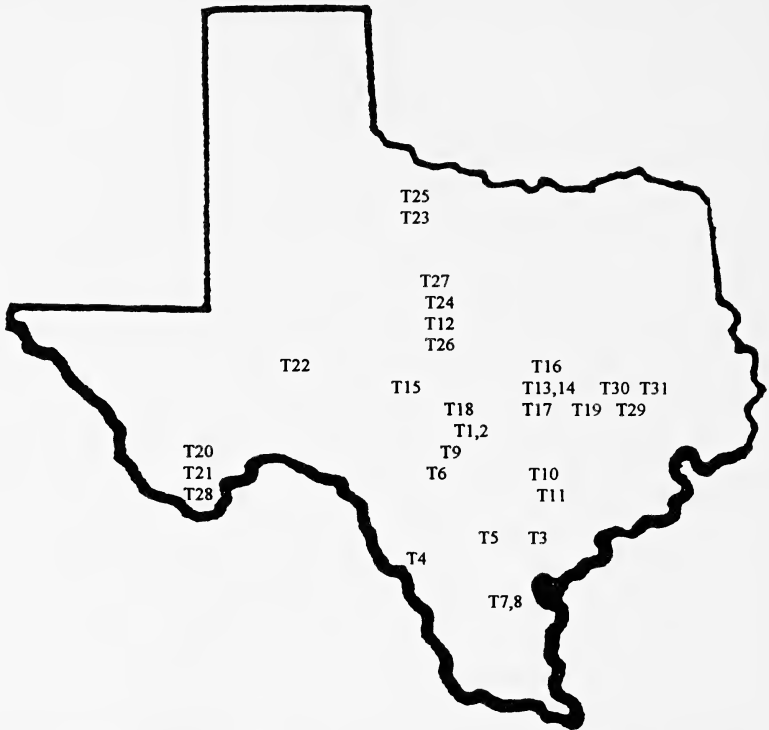


Figure 1. Mistletoe collection sites in Texas T1,2 = San Antonio, T3 = Beeville, T4 = Laredo, T5 = George West, T6 = Devine, T7,8 = Falfurrias, T9 = Hondo, T10 = Stockdale, T11 = Kenedy, T12 = Burnet, T13,14 = Austin, T15 = Kerrville, T16 = Georgetown, T17 = Fischer, T18 = Boerne, T19 = San Marcos, T20 = Terlinqua, T21 = Lajitas, T22 = Sonora, T23 = Mineral Wells, T24 = Lampasas, T25 = Iowa Park, T26 = Marble Falls, T27 = Hamilton, T28 = Marathon, T29 = Bastrop, T30 = Giddings, T31 = Brenham.

dendrogram with the most similar samples assigned a value of one and the most dissimilar samples are assigned a value of zero.

#### METHODS AND MATERIALS

*Sample collection.*—Mistletoe samples (*Viscaceae* - *Phoradendron tomentosum*) were collected from 28 locations in south, central, near east, north and west Texas (Figure 1). In addition, 18 samples (*Viscaceae* - *Phoradendron serotinum*) were collected from 13 locations in Alabama, Georgia, Florida and Tennessee (Figure 2). The samples collected in Texas should be a different species than the samples collected in the Southeast (Wiens 1964).

*Extraction method.*—Undamaged leaves collected from individual

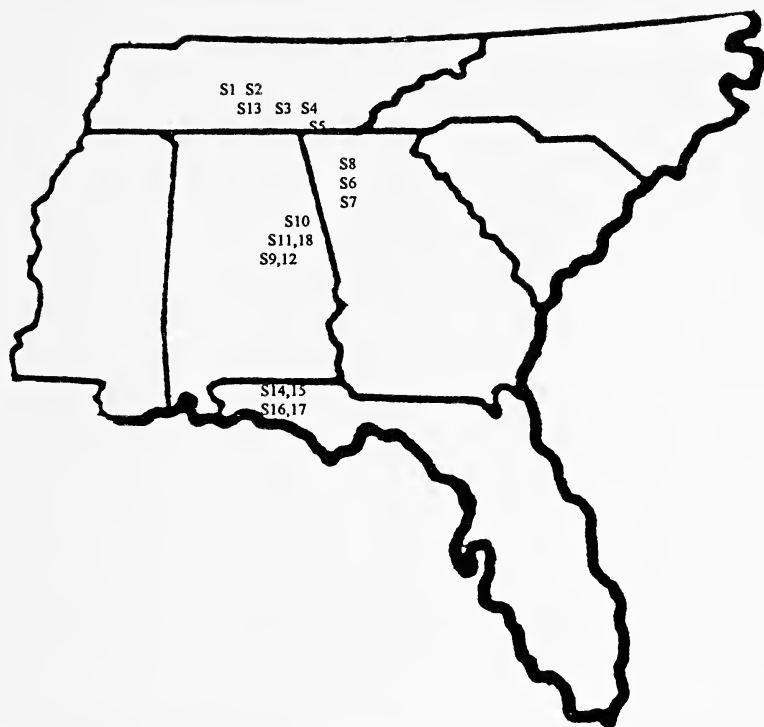


Figure 2. Mistletoe collection sites in the Southeast S1 = Tennessee, I-24E, Mile 124; S2 = Tennessee, I-24E, Mile 125; S3 = Tennessee, Monteagle; S4 = Tennessee, I-24E, Mile 143; S5 = Tennessee, I-24E, Mile 144; S13 = Tennessee, I-24E, Mile 112; S6 = Georgia, I-59S, Mile 10; S7 = I-59S, Mile 11; S8 = I-59S, Mile 9; S9,12 = Alabama, I-59S, Mile 215; S10 = Alabama, Collensville; S11,18 = Alabama, I-59N, Mile 176; S14,15,16,17 = Florida, Ponce de Leon

plants were allowed to dry, at room temperature, in open containers for a minimum of 14 days. After drying, approximately three grams of leaves were crumpled into small pieces, placed in labeled 50-milliliter Erlenmeyer flasks and extracted in 20 milliliters of chromatography grade hexane (OmniSolve HX0297-1 EM Science) for 96 hours. The extracts were removed from the leaves and allowed to evaporate for 24 hours. The dry samples were dissolved in three milliliters of hexane and filtered using a one milliliter syringe and 25 mm diameter PTFE membrane with 0.2  $\mu\text{m}$  pore size microfilter disks (Supelco ISO-DISC P-252). The samples were stored at 8°C until analyzed.

*Sample analysis.*—The samples were analyzed using a Hewlett Packard (HP5890II) capillary gas chromatograph which was fitted with a "split/splitless" capillary injector operated in split mode, a flame ionization detector and an automatic sampler (HP7673). The column of

choice was a Hewlett Packard capillary column Ultra 1, 25 meters by 0.2 millimeter I.D. and 0.11 micrometer film thickness. Helium was used as the carrier gas with a head pressure of 76 pounds per square inch (psi) and a flow rate of 0.4 milliliter/minute (mL/min). The detector make-up gas was nitrogen with a head pressure of 40 psi and a flow rate of 30 mL/min through the detector. The flame gases were air and hydrogen with head pressures of 40 psi and 18 psi respectively and flow rates of 400 mL/min and 30 mL/min respectively. The initial oven temperature was set at 100°C which was increased to 315°C at a rate of 7.5°C per minute. The oven remained at 315°C for 30 minutes for a total run time of 58.7 minutes. The injector temperature was 330°C, and the detector temperature was 325°C.

Instrument control, data collection, and analyses were performed using Hewlett Packard Series II Chemstation (HP3365) software loaded on a Hewlett Packard Vectra 486/33U computer where the data was stored. The chromatograms were printed on a Hewlett Packard Model LaserJet 4 printer.

*Computer analysis of data.*—The data obtained were analyzed using a computerized pattern recognition program (Ein\*Sight, version 3.0) (Infometrix, Seattle, Washington). This program was loaded onto a Packard-Bell Statesman 486 microcomputer. The results were printed on a Hewlett Packard DeskJet 600C printer. The data were furthered analyzed by Microsoft Excel spreadsheet software loaded on the same computer system. The data analysis performed was hierarchical cluster analysis (HCA).

## RESULTS AND DISCUSSION

Visual comparison of chromatograms of mistletoes growing in Texas and those growing in the southeastern United States show some differences. The chromatograms of the southeastern samples are very similar while those of the Texas samples showed more variation. The peak area data were entered in a spreadsheet, and a mean and a standard deviation was calculated for each peak. Those peaks showing the greatest difference in mean value and the least difference in standard deviation when comparing each group were chosen for further analysis. This resulted in seven chromatogram peaks with the following relative retention times 7.63, 8.34, 8.45, 11.90, 12.81, 12.88 and 13.28 minutes. These seven peaks were well resolved and consistent across samples. A peak at 17.9 minutes, which appeared in all chromatograms, was used as an internal

Table 1. Relative retention times and normalized peak areas of Texas samples.

RRT:	7.63	8.34	8.45	11.90	12.81	12.88	13.28
Sample							
T1	17.6	5.3	0.0	50.2	1.7	9.3	15.9
T2	23.8	5.7	0.0	41.2	1.4	7.3	20.4
T3	38.8	6.0	0.0	21.5	1.9	11.8	20.0
T4	11.8	5.9	0.9	50.4	1.5	8.3	21.2
T5	11.8	2.2	0.2	69.2	1.1	4.8	10.7
T6	21.9	5.6	0.0	54.7	1.6	7.9	8.2
T7	19.4	5.6	1.3	57.0	1.0	7.2	8.6
T8	11.2	6.3	0.0	61.2	1.9	9.0	10.4
T9	28.8	5.6	0.0	38.4	2.1	8.8	16.3
T10	26.4	3.3	0.0	49.1	2.0	5.9	13.4
T11	53.4	14.6	0.0	0.0	2.8	14.8	14.3
T12	6.5	2.5	5.0	52.4	0.9	5.6	27.0
T13	11.4	4.1	0.0	65.2	1.0	5.7	12.6
T14	12.6	4.5	0.4	66.4	1.0	5.4	9.6
T15	6.0	4.7	0.5	65.2	1.6	5.9	16.1
T16	1.7	2.4	0.1	62.2	1.9	5.8	25.9
T17	1.7	6.6	2.5	25.6	4.6	12.2	46.8
T18	9.0	11.7	0.0	35.2	2.0	13.1	29.1
T19	0.7	3.1	0.0	44.9	3.7	4.0	43.5
T20	11.1	27.0	0.0	5.1	3.3	24.6	28.9
T21	3.6	41.6	0.0	0.0	6.4	35.7	12.7
T22	3.4	5.6	1.0	0.0	1.6	11.6	76.9
T23	10.3	3.5	0.4	50.1	1.9	5.5	28.2
T24	2.8	7.4	1.1	62.2	7.5	19.0	0.0
T25	2.7	11.1	4.5	57.2	6.0	18.6	0.0
T26	12.0	5.9	1.0	38.1	1.6	11.3	30.1
T27	3.2	4.0	6.4	73.9	3.4	9.1	0.0
T28	6.5	8.0	0.0	1.3	4.4	15.6	64.2
T29	2.7	16.4	0.0	2.4	3.7	18.9	55.8
T30	11.0	10.7	1.1	4.6	3.1	15.0	54.5
T31	7.2	16.9	0.0	5.2	0.0	18.6	52.1

standard to calculate these relative retention times (RRT) to ensure the identity of each peak. A RRT of 10.00 minutes was assigned for the peak at 17.9 minutes. The data were further treated by normalizing each peak area to the total area of all peaks chosen for analysis. This was done in order to eliminate any differences in sample concentration or in size of sample injected. Table 1 contains the RRT and normalized peak area for the Texas samples and the same data for the southeastern samples are contained in Table 2. Figure 3 illustrates two typical chromatograms, one from Texas and one from the southeastern United States. Some distinct differences do exist, but they are not consistent across all chromatograms. The subtle differences in peak area are difficult to compare when one is comparing several chromatograms; therefore the data were subjected to cluster analysis.

Table 2. Relative retention times and normalized peak areas of southeastern samples.

RRT:	7.63	8.34	8.45	11.90	12.81	12.88	13.28
Sample							
S1	23.9	15.5	2.7	1.3	5.6	27.4	23.6
S2	0.9	18.2	23.7	2.7	5.9	27.1	21.4
S3	10.1	14.4	28.2	2.1	3.7	18.5	23.0
S4	12.4	9.7	25.5	0.0	3.3	14.3	34.8
S5	14.2	11.5	20.3	2.4	3.3	17.9	30.3
S6	8.0	19.1	0.0	2.6	3.5	27.3	39.5
S7	6.5	16.7	44.6	0.7	3.4	20.7	7.4
S8	6.3	8.2	40.1	2.5	1.7	10.8	30.5
S9	19.8	18.8	17.5	0.0	3.9	22.2	17.6
S10	5.5	16.4	25.1	5.4	3.6	24.6	19.4
S11	14.8	14.5	0.0	8.6	3.3	25.2	33.6
S12	12.4	15.1	28.8	3.2	3.4	17.9	19.2
S13	11.0	18.4	0.0	3.3	3.4	25.5	38.4
S14	5.2	13.4	18.1	3.7	5.7	18.5	35.3
S15	3.8	33.5	0.0	0.0	7.2	35.2	20.2
S16	18.3	15.4	6.5	3.5	3.8	25.4	27.1
S17	17.9	11.5	12.5	5.7	2.7	18.6	31.2
S18	3.1	16.7	0.0	6.6	5.0	25.0	43.6

Data for 49 mistletoe samples (31 collected in Texas and 18 collected in the southeast) were subjected to HCA. The preprocessing technique chosen for this data set was mean centering. The clustering technique chosen was incremental sum of squares. A dendrogram (Figure 4) was constructed using this data. The top cluster consists of 22 samples, all 18 southeastern samples appear in this cluster along with four Texas samples. The middle cluster consists of five samples, all from Texas. The third cluster consists of 22 samples, again all from Texas.

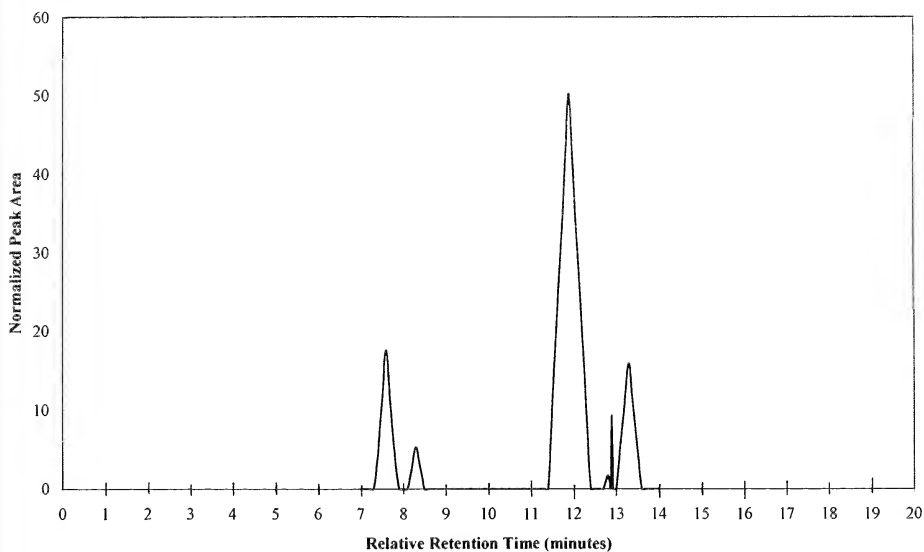
The cluster of five Texas samples consists of samples T22, T28, T29, T30 and T31. Three of these samples T29, T30 and T31 are the easternmost samples collected in Texas and could be *P. serotinum*. Samples T22 and T28 are two of the westernmost samples collected. Of the four Texas samples that were classified among the southeastern samples, T20 and T21 were the other western Texas samples. The remaining Texas samples that were clustered with the southeastern samples, T3 and T11, were the most southeastern of the Texas samples.

## CONCLUSIONS

Mistletoe is a very complex biological specimen to study. The growth ranges of mistletoes are very broad, and ecotypes have been shown to exist in complex populations of species with the possibility of



**Typical Chromatogram of a Texas Sample**



**Typical Chromatogram of a Southeast Sample**

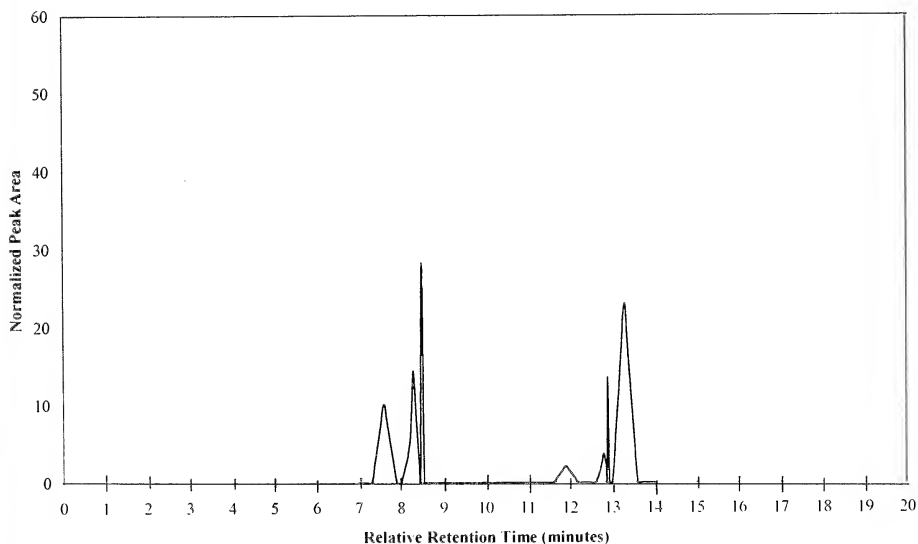


Figure 3. A comparison of chromatograms of samples from Texas and from the southeast. Note the differences at relative retention times of 7.63, 8.34, 8.45, 11.90, 12.81, 12.88, and 13.28 minutes.

hybridization occurring; thus detailed taxonomy should be considered in any future study with mistletoe.

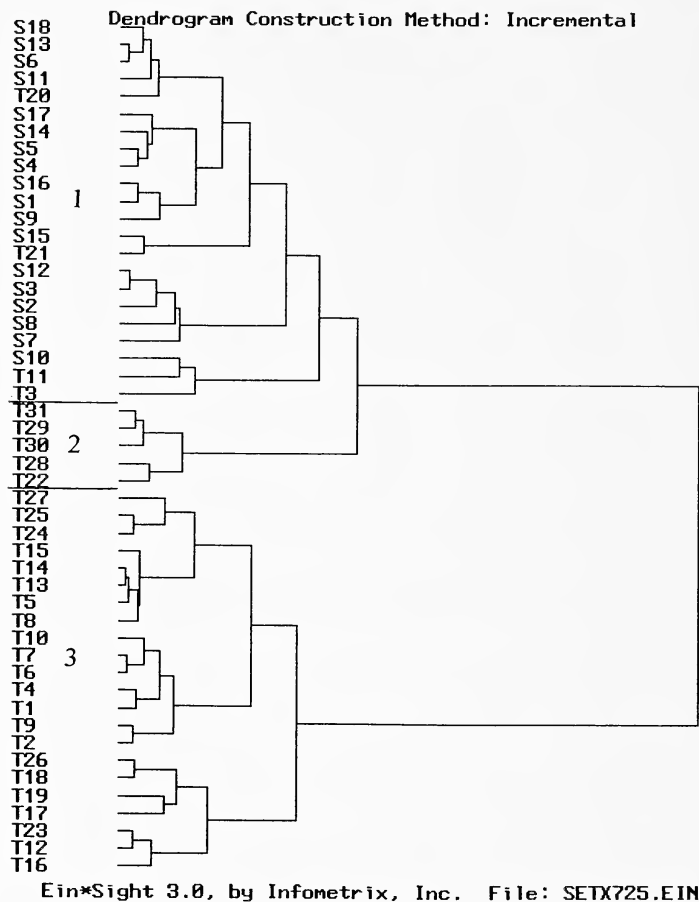


Figure 4. A hierarchical cluster analysis dendrogram produced by Ein\*Sight for the data set of Texas and southeast mistletoe samples using seven peaks. The preprocessing technique was mean centering and the clustering technique was incremental sum of squares. Note the presence of three clusters. Cluster one consists of 22 samples, all 18 southeastern samples and four from Texas. Cluster two consists of five Texas samples. Cluster three consists of 22 samples, all from Texas.

In the current study, capillary gas chromatography was the method of choice for the analysis of samples. This method coupled with profile analysis and multivariate statistics helped to differentiate the mistletoe samples. There have been many studies using these techniques on biological samples, but no direct references to mistletoe have been found in the current literature.

The southeastern United States sample chromatograms showed the

most consistency probably due to the fact that one species of mistletoe grows in this geographic area. The Texas samples showed more variation in their chromatograms possible due in part to three species growing in Texas. Samples of all three species were probably collected. On the basis of HCA, the Texas samples and southeastern samples could be differentiated from one another, separating into two major clusters and one minor cluster. All of the southeastern samples were clustered together along with four samples from Texas. A cluster of five Texas samples was evident and the remaining 71% of the Texas samples were clustered.

This method appears to have value as a chemotaxonomic technique for the differentiation of mistletoes but, further study is warranted. Compounds that are independent of host, seasonal variation, gender and method of collection must be carefully selected for analysis.

#### ACKNOWLEDGMENTS

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## PRODUCTION OF PECTOLYTIC ENZYMES BY *CURVULARIA SENEGALENSIS*, A PHYTOPATHOGENIC FUNGUS

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**Abstract.**—The effect of different carbon sources on the production of extracellular pectinases of *Curvularia senegalensis* was investigated. Production of polygalacturonase and pectinesterase was induced by sodium polypectate, pectin and lactose. Production of these enzymes was repressed or completely inhibited in the control cultures containing maltose or glucose as carbon sources and enzyme inducers. Maximum production of polygalacturonase and pectinesterase was induced when sodium polypectate was used as carbon source. The results of this work indicate that the pectic enzymes of *C. senegalensis* may be produced in sequence, i.e., the production of polygalacturonase is followed by the secretion of pectinesterase.

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Direct penetration of susceptible hosts by the infective hyphae of phytopathogenic fungi is facilitated by the production of cutinases (Agrios 1988). Penetration of the host is followed by disintegration of the pectic substances of the middle lamella and the primary cell wall by pectolytic enzymes (Kenaga 1974; Agrios 1988). Production of pectolytic enzymes is induced in plant pathogenic fungi when these organisms are grown on media containing various carbon sources and enzyme inducers. These carbon sources include sugar polymers (Cooper & Wood 1973), sodium polypectate and pectin (Pegg 1981; Alana et al. 1989) and sugar beet cell walls (Bugbee 1990).

*Curvularia senegalensis* (Speg.) Subram., is primarily a pathogen of cereal crops and grasses including maize and sugar cane (Cook 1981; Nyvall 1989). The main objectives of this work were to study the components of extracellular cell wall degrading enzymes of *C. senegalensis* and to determine the effects of the carbon source on the production of these enzymes by the test fungus.

### MATERIALS AND METHODS

**Organism and culture conditions.**—Stock cultures of *C. senegalensis* were maintained on potato-dextrose-agar slants (PDA, Difco, B13). The fungus was previously grown in 250 mL flasks with 125 mL of a medium containing: 0.02% MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.01% Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, 2.0% glucose in Na-citrate buffer at pH 4.8. After four days growth at

26°C, 5 mL of mycelium inoculum was washed twice in distilled water and transferred to the growing medium. The medium for the production of pectinases contained: 0.15%  $\text{NH}_4\text{NO}_3$ , 0.24%  $\text{K}_2\text{PO}_4$ , 0.08%  $\text{MgSO}_4$ , 0.08%  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 0.72 ppm  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ , 0.44 ppm  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 2.0 ppm  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 0.40 ppm  $\text{ZnCl}_2$  and 1.0% carbohydrate. The carbohydrates used as carbon sources and enzyme inducers included: sodium polypectate, apple pectin, xylan (Sigma Chemical Company), lactose (Mallinckrodt Chemical Works) maltose and sucrose (Fisher Scientific). The control cultures had glucose (Fisher Scientific) as the sole carbon source. The pH of the growing medium was adjusted to 5.0 with 0.1 N KOH. Incubation of the cultures was carried out for seven days in covered 250 mL flasks on an orbital shaker at 80 rpm at 24°C.

*Enzyme preparation and assays.*—Culture fluids were collected after seven days of growth in the liquid medium. The culture fluids were centrifuged (4,500 rpm, 30 minutes, 10°C) to obtain a clear supernatant. The supernatant was subsequently used for the determination of extracellular enzyme activity. For simplification, the collected supernatant is hereafter referred to as the enzyme.

*Polygalacturonase* (Pectinase, EC 3.2.1.15).—Polygalacturonase activity was measured by combining 1 mL of enzyme with 10 milligrams of sodium polypectate in 1 mL of 0.05 M sodium citrate buffer (pH 5.0) and incubating the reaction mixture for 120 minutes at 40°C. The tubes were stirred once during incubation. After centrifugation, the concentration of galacturonic acid or its reducing sugar equivalent in the supernatant was determined by the dinitrosalicylic acid reagent of Miller (1959).

*Pectinesterase* (Pectin methylesterase, EC 3.1.1.11).—Pectinesterase activity was measured by combining 1 mL of enzyme with 10 milligrams of pectin in 1 mL of 0.05 M sodium citrate buffer, (pH 5.0). The reaction mixture was incubated at 40°C for 120 minutes. After centrifugation, the concentration of galacturonic acid or its reducing sugar equivalent in the reaction mixture was measured by the dinitrosalicylic acid reagent of Miller (1959).

*Protein determination.*—Extracellular total protein in the crude supernatant was determined with the BCA reagent (Pierce Chemical Company) using bovine serum albumin as standard.

Table 1. Specific activities<sup>1</sup> of two pectolytic enzymes produced by *Curvularia senegalensis* grown in liquid medium containing different carbon sources.

Carbon source	Enzymes		
	Polygalacturonase	Pectinesterase	Total protein <sup>2</sup>
Sodium polypectate	21.38±1.01*	12.43±5.30*	0.92±0.32
Pectin	1.39±0.25	0.27±0.10	16.10±0.65
Xylan	0.0	2.11±0.28	1.99±0.15
Lactose	1.40±0.66	0.49±0.37	12.60±0.07
Maltose	0.0	0.0	7.40±0.26
Sucrose	0.60±0.11	0.0	38.60±1.20*
Glucose	0.0	0.0	15.18±0.75

<sup>1</sup> Usp/min/mg of protein. Mean ± SD of four replications.

<sup>2</sup> mg/mL.

\* Using one-way ANOVA and Duncan's MRT, significantly different from other values in the same group.

*Data analysis.*—All enzyme activity tests and protein determinations were replicated four times. Enzyme activities were expressed as units of specific enzyme activity (Usp) and represent means plus or minus the standard deviation of four replications. Each unit of enzyme specific activity was calculated as the amount of enzyme that liberated one micromole of galacturonic acid (or its reducing sugar equivalent) per minute per milligram of protein under the assay's conditions. Statistical analyses of experimental data were made with one-way analysis of variance (ANOVA) and Duncan's multiple range test (MRT).

## RESULTS

*Extracellular protein.*—The total extracellular protein that was measured in the fluids of the liquid cultures of this study was used to calculate the enzyme specific activities of the fungus grown in liquid media containing different carbon sources and enzyme inducers. Maximum concentration of extracellular protein (38.60 milligrams per mL) was measured in fluids collected from cultures containing sucrose as the carbon source (Table 1). The accumulation of protein in the fluids of cultures containing sucrose was significantly higher ( $P = 0.05$ ) than the concentration of total protein measured in fluids containing other carbon sources. Total protein accumulated in the fluids of cultures containing pectin, lactose or glucose was significantly higher ( $P = 0.05$ ) than the protein accumulated in cultures containing sodium polypectate, xylan or maltose (Table 1). The lowest amount of total protein (0.92 milligrams per mL) was measured in fluids collected from cultures containing sodium polypectate.

*Polygalacturonase*.—Production of polygalacturonase was induced in cultures containing sodium polypectate, pectin, lactose and sucrose as carbon sources and enzyme inducers. No polygalacturonase activity was detected in the fluids obtained from cultures containing xylan, maltose or glucose as carbon sources (Table 1). Maximum polygalacturonase activity (21.38 Usp) was measured in fluids collected from cultures containing sodium polypectate (Table 1). This activity was significantly higher ( $P = 0.05$ ) than the activities assessed in fluids obtained from cultures with pectin, lactose or sucrose (1.39, 1.40 and 0.60 Usp, respectively).

Polygalacturonase activities measured in fluids from cultures with pectin or lactose as carbon sources were significantly higher than the activity determined in fluids from cultures containing sucrose (Table 1). No significant difference was determined in the polygalacturonase activities measured in fluids of cultures containing pectin or lactose. The lowest polygalacturonase activities were measured in fluids containing sucrose.

*Pectinesterase*.—Production of pectinesterase was induced in cultures containing sodium polypectate, pectin, xylan and lactose as carbon sources. Maximum pectinesterase specific activity (12.43 Usp) was measured in fluids harvested from cultures containing sodium polypectate (Table 1). This activity was significantly higher ( $P = 0.05$ ) than the activities assessed in fluids obtained from cultures with pectin, xylan or lactose (0.27, 2.11 and 0.49 Usp, respectively). The pectinesterase activity measured in fluids obtained from cultures containing xylan (2.11 Usp) was higher than the activity measured in fluids collected from cultures with pectin or lactose (0.27 and 0.49 Usp, respectively) as carbon sources. However, the differences between these activities (1.84 and 1.62 Usp, respectively) were not significant. No pectinesterase activities were detected in fluids harvested from cultures containing maltose, sucrose or glucose as carbon sources and enzyme inducers.

## DISCUSSION

Although the highest amount of extracellular protein determined in this study was found in fluids collected from cultures containing sucrose as the carbon source, it seems that only a small part of it was polygalacturonase. Whereas the production of polygalacturonase was induced in cultures of *C. senegalensis* containing sodium polypectate, pectin, lactose and sucrose as carbon source, sodium polypectate was the



most effective inducer of this enzyme. Xylan, maltose or glucose did not induce the secretion of polygalacturonase by *C. senegalensis* under the conditions of this study.

It has been shown in similar studies that plant pathogenic fungi can be induced to secrete polygalacturonase and pectinesterase when grown in liquid media with the appropriate carbon source (Cleveland & McCormick 1987; Cooper & Wood 1973; Crawford & Kolattukudy 1987; De Lorenzo et al. 1987).

Pectinesterase activities were measured in fluids collected from cultures containing sodium polypectate, pectin, xylan and lactose. Production of both enzymes by the test fungus was repressed or completely inhibited when the growing medium contained maltose or glucose as the sole carbon source. Repression of the induction of polygalacturonase and other pectic enzymes by glucose or galacturonic acid used as carbon sources has been shown in other studies of phytopathogenic fungi (De Lorenzo et al. 1987; Leone & Van Den Heuvel 1987). The polygalacturonase accumulated in fluids obtained from cultures containing sodium polypectate was nearly two times higher than the amount of pectinesterase determined in the same fluids. In fluids containing pectin, the difference in enzyme activity was five times higher and in fluids with lactose as the carbon source, the activity of polygalacturonase was nearly three times higher than the activity of pectinesterase.

It is probable that the concentration of these enzymes in relation to each other is an indication of the sequence in which the enzymes were produced, i.e., the production of polygalacturonase is followed by the secretion of pectinesterase. The production in sequence of pectic enzymes has been demonstrated in similar studies of plant pathogenic fungi (Bahkali 1987; Leone & Van Den Heuvel 1987). In these studies, polygalacturonase was detected first in the culture filtrates. Pectinesterase appeared later.

#### SUMMARY

Secretion of polygalacturonase and pectinesterase was induced in liquid cultures of *C. senegalensis* when sodium polypectate, pectin or lactose were used as sole sources of carbon and enzyme inducers. Sodium polypectate was the most effective inducer in the production of both enzymes.

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NEARSHORE *PERUVIELLA DOLIUM* (ROEMER 1849)  
PALEOCOMMUNITY, CRETACEOUS (ALBIAN)  
WALNUT FORMATION, CORYELL COUNTY, TEXAS

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**Abstract.**—The large gastropod species, *Peruviella dolium* (Roemer 1849) occurs in the Walnut Formation in the city of Gatesville, Texas. Stratigraphic relations show that the beds containing *P. dolium* are limestones overlying the clastic sandstones of the Paluxy Formation, and underlying *Texigryphaea* oyster banks of the Walnut Formation. The paleocommunity dominated by *P. dolium* defines a specific nearshore environment that existed on both sides of the Cretaceous Tethyan seaway.

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*Peruviella dolium* (Roemer 1849) is a large gastropod species that is abundant at several Cretaceous localities in central Texas (Adkins 1928; Moore 1964; Moore & Martin 1966), and is the dominant taxon of a unique paleocommunity that defines a very precise paleoenvironmental location. Commonly misidentified in stratigraphic literature as *Actaeonella*, Kollmann & Sohl (1979) reevaluated the taxonomy of the genus *Peruviella* and transferred it from the Actaeonellacea to the Nerinaecea. Working only with curated museum specimens they failed, however, to discuss the very interesting paleoecological aspects of these molluscs. The *P. dolium* paleocommunity defines a low diversity, nearshore environment for the Cretaceous.

#### STRATIGRAPHY

A location within the city limits of Gatesville, in central Coryell County, Texas exposes the *Peruviella dolium* paleocommunity in a roadcut section on the south and north sides of U. S. Highway 84 as it descends eastward into the Leon River valley.

**Locality information.**—University of California Museum of Paleontology (UCMP) 11087 may be found on the Gatesville, Texas 7.5 minute US Geological Survey quadrangle, 1957 version, photo-revised 1979. The UTM coordinate is 14RPK17647826. Geographic coordinates are 31°26'05"N, 97°45'44"W.

The stratigraphic section exposes the boundary of the Paluxy Formation and the overlying Walnut Formation. This is the same as "locality 19" described in Atlee (1962), and "Stop 13" in Hendricks et al. (1969). The section south of the highway is better exposed and was collected for this report. Collected fossil specimens are deposited in the University of California Museum of Paleontology (UCMP).

Table 1. Paleoecological characters of the *Peruviella dolium* paleocommunity at UCMP 11087, Gatesville, Texas.

Taxon	# Collected / % Of Total	% On Slabs	Feeding Type	Life Habit
<i>Peruviella dolium</i>	76 / 97%	45%	detritus	infaunal
<i>Pinna comancheana</i>	1 / 1.5%	—	suspension	infaunal
<i>Scabrotrigonia emoryi</i>	1 / 1.5%	—	suspension	infaunal
Nereineid gastropods	— / —	34%	detritus	infaunal
<i>Texigryphaea</i> fragments	— / —	14%	suspension	epifaunal
Bivalvia indet.	— / —	7%	?detritus	epifaunal

The Paluxy/Walnut contact is conformable and inter-tonguing. Unfossiliferous, medium to fine grained sandstones of the Paluxy are replaced by limestones of the Walnut containing abundant *Peruviella dolium*. These are not discontinuous carbonate mounds within the Paluxy as reported by Moore & Martin (1966) for Burnet County, Texas to the south, but the basal beds of the Walnut Formation at this location and throughout north-central Texas (Lozo 1959; Hendricks 1967). These basal beds are replaced 1.5 meters upsection by the *Texigryphaea* oyster packstone beds that typify the Walnut Formation (Adkins 1928; Flatt 1976).

The Gatesville stratigraphic section is here interpreted as one of transgression from the nonmarine sand facies of the Paluxy Formation, through the shallow marine, oyster bank facies of the Walnut Formation. There are no detectable unconformities in this section and so Walther's Law is applicable (for discussion, see Middleton 1973). The *Peruviella dolium* paleocommunity occurs between the two facies and thus occupied the extreme nearshore position of the rising Fredericksburg sea during the Cretaceous.

#### PALEOECOLOGY

The *Peruviella dolium* paleocommunity at UCMP 11087 (Table 1) is a low-richness assemblage dominated by this large (up to 95.5 mm length, 79.25 mm width) gastropod (Figure 1). The surrounding sediments are packstones, with some grainstones - evidence for high-energy environments. The non-carbonate fraction of fine- to medium-grained sand does not exceed 5% by weight. Minor biologic components of the paleocommunity include the bivalves *Pinna comancheana* (Cragin 1894) and *Scabrotrigonia emoryi* (Conrad 1857). High-spined, nereineid gastropods are abundant in polished slabs, but do not occur loose on outcrop. Differences in collected, loose fossils on an outcrop, and those observed in polished rock slabs is a common phenomenon in paleoecological studies (Watkins 1996), and usually reflects the difficulty in breaking out specimens from hard rock surfaces.

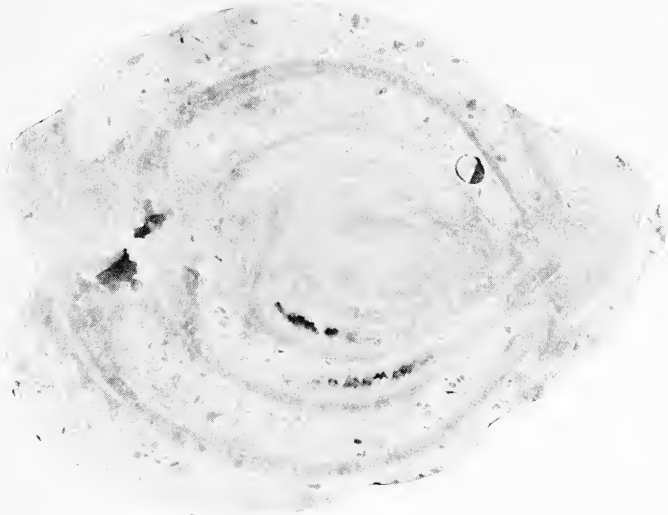


Figure 1. Cross section of *Peruviella dolium* (Roemer 1849) X1, from UCMP 11087. Length of specimen is 74.25 mm.

Paleoecologic comparison may be made with several other Mesozoic faunas. Scott (1990) described a nerineid gastropod-dominated paleocommunity of low richness for coeval rocks of the Rodessa Formation from cores recovered in Houston County, Texas. Scott (1990) notes the occurrence of 5% nerineids, plus trace occurrences of foraminifera, bivalves, echinoids, serpulids and red algae. Stratigraphic evidence suggests that this nerineid paleocommunity occupied a back-reef area updip from rudist reefs. Scott (1990) listed the environmental factors necessary for dominance of detritus-feeding nereidids as muddy substrates, plus stable, low-energy environments. In contrast, offshore dominance of nerineids in coarse substrate, high-energy environments is documented by Barker (1990) for Jurassic rocks of Europe. The nearshore, high-energy environment described by Lescinsky et al. (1991) from the Cretaceous of Mexico is dominated by encrusting, boring and nestling fauna on hard substrates, and bears no resemblance to the *P. dolium* paleocommunity.

*Peruviella* dominance has widespread paleoenvironmental significance. Kollmann & Sohl (1979) showed that the genus *Peruviella* had a "southern submarginal or marginal Tethyan" distribution in Peru, Brazil and western Africa that parallels other marginal marine or brackish-freshwater transition gastropods of the Thiariidae. Kollman & Sohl (1979) incorrectly recognized the existence of *Peruviella* in the Albian of Texas as "different" because of the association with the rudist framework facies.

Stratigraphic relations at Gatesville, Texas and elsewhere (Moore 1964;

Moore & Martin 1966) show that *Peruviella dolium* is not part of the rudist framework facies, and in fact, confirm the nearshore interpretation implied by the South American and African occurrences. The *P. dolium* paleocommunity is the northern hemisphere counterpart of a finely delimited paleo-environment occurring on both sides of the Cretaceous Tethyan seaway.

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## EVALUATION OF CYCLIC DAMAGE IN CARBON FIBER REINFORCED PLASTICS

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**Abstract.**—The progressive nature of fatigue damage due to cyclic loading on carbon (graphite) fiber reinforced plastics (CFRP) was analyzed. After cycling approximately to the  $10^7$  number of cycles, the specimens were released from the material testing system (MTS) machine to prevent any additional damage. The residual strength of the specimens subjected to fluctuating tension with a given number of cycles was then measured. This was achieved by a three point bending test on specimens. The results suggested that the process of cyclic damage in this composite could be classified into three stages. In the first stage, there was no significant decrease in static strength in spite of various cracks in the test specimen. The second stage consisted of a decrease in static and residual strength of CFRP with an increase in cyclic ratio. Finally, in the third stage, the static strength was reduced with an increase in cycle ratio until the endurance limit was reached, thereafter, the specimen failed suddenly.

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Fatigue damage in composite materials usually results in various modes of failure (Harris 1961). A failure mode is defined as an event or response of events which cause significant changes in mechanical response characteristics of scientific importance.

Numerous studies have shown that cyclic damage in reinforced plastics is progressive in nature (e.g. Boller 1964; Pipes 1974). Despite these studies however, phenomenon of cyclic damage is still poorly understood. The present investigation makes an attempt to study the cyclic damage in carbon fiber reinforced-plastics. By examining the interlaminar-shear strength of the specimen subjected to changing tension for various number of cycles.

### MATERIALS AND METHODS

The specimens used for this study were quasi-rectangular consisting of carbon fiber-epoxy. All cyclic loading was carried in variable tension (zero to a tension value) at a frequency of 25 Hz. Test specimens were subjected to different number of loading cycles at a particular stress level. After fatigue loading to a certain number of cycles the specimens were released from the MTS machine. The tensile and shear (interlaminar) strengths were then measured to examine the change experienced by the materials at a particular fraction of cyclic life. Flexural methods

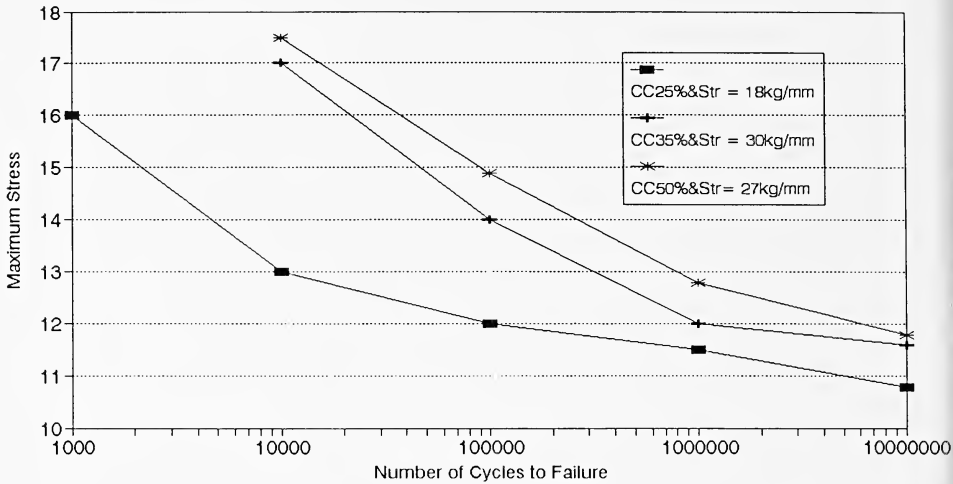


Figure 1. S-N curve for carbon content of 25%, 35% and 50%.

were used to measure the interlaminar-shear strength of the pieces under a span/depth ratio of four.

### EXPERIMENTAL RESULTS AND DISCUSSION

The standard S-N (stress vs. # of cycles) curve for carbon content of 25%, 35%, and 50% are shown in Figure 1. The CC25% and Str, CC35% and Str, and CC50% and Str represents carbon content of 25%, 35% and 50% respectively with applied stress (Figure 1).

The residual strength in interlaminar-shear test after cyclic loading for the specimens with carbon content of 25%, 35% and 50% at a variety of stress levels is shown in Figures 2, 3 and 4. In the graphs the ratio  $\tau/\tau_{ult}$  (ultimate strength to original ultimate strength) and the cycle ratio (various fraction of fatigue life) are plotted against each other. The word "Ult" represents ultimate applied stress during the test.

The interlaminar-shear test results shown in Figs. 2 - 4 were subjected to some uncertainty due to material variability. Correlation coefficients were taken into account for the carbon content of 25%, 35% and 50% in interlaminar-shear test. The coefficients were 0.041, 0.053 and 0.061 for the carbon content of 25%, 35% and 50% respectively. The differ-



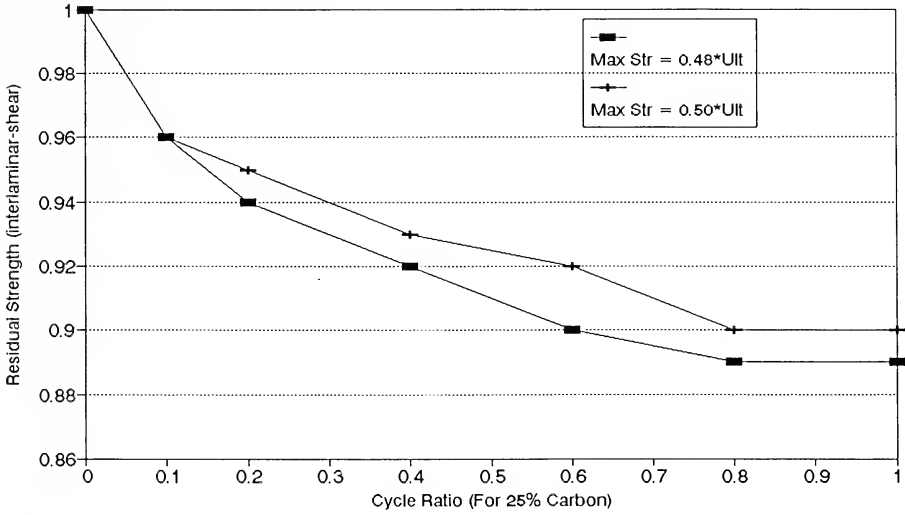


Figure 2. Residual strength in interlaminar-shear vs. cycle ratio for 25% carbon content.

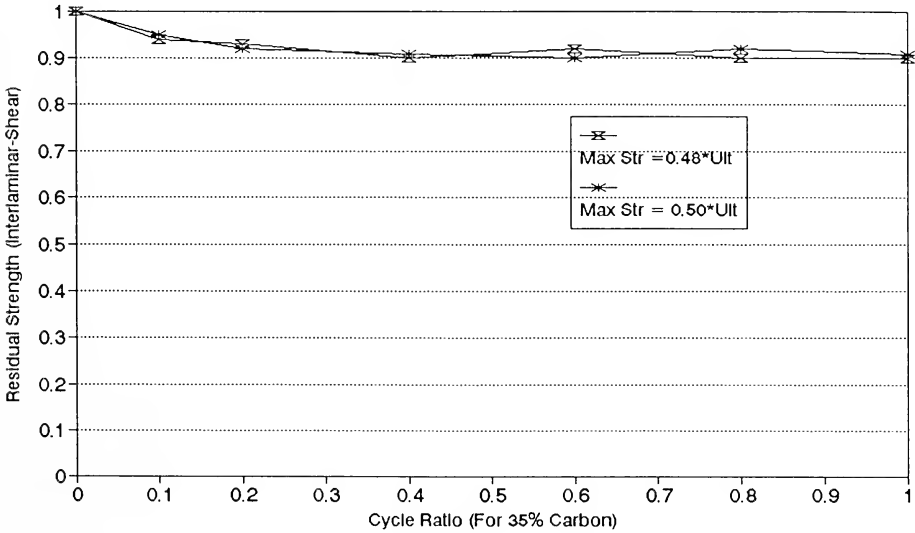


Figure 3. Residual strength in interlaminar-shear vs. cycle ratio for 35% carbon content.

ence in residual strength before and after stress cycling below the cycle ratio of 0.01 was not significant for each carbon content (correlation coefficients are not significantly large). The residual static strength of carbon reinforced plastics subjected to fatigue loading to moderate

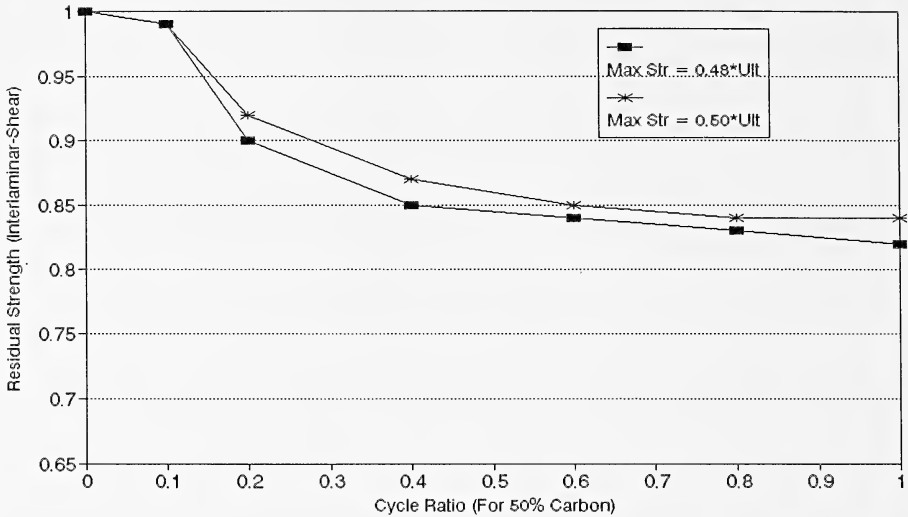


Figure 4. Residual strength in interlaminar-shear vs. cycle ratio for 50% carbon content.

number of cycles was greater than that of virgin material. For cycle ratios greater than 0.01, the residual strength started to decrease due to fatigue for all carbon contents. From the plots of residual strength for various carbon content, it was obvious that the decrease was independent of carbon content and cyclic stress level. The decrease in the residual static strength was very rapid until a cycle of about 0.45 was reached. Thereafter, rate is decreased slowly until the fatigue life was reached at which the specimen failed.

Residual strength in tension vs. cycle ratio of the specimen for the carbon content of 25% is shown in Figure 5. The residual strength showed the same trend as observed in interlaminar-shear tests.

From various observations, the science of cyclic damage in carbon reinforced plastics can be divided into three stages as follows:

1. The first stage,  $N/N_f$  (cycle ratio) = 0 to 0.01. There was not much decrease in residual strength at this stage in spite of many cracks in the specimen.
2. The second stage, cycle ratio = 0.01 to 0.45. Many cracks were produced in the specimen and the residual strength decreased continuously with an increase in cycle ratio.

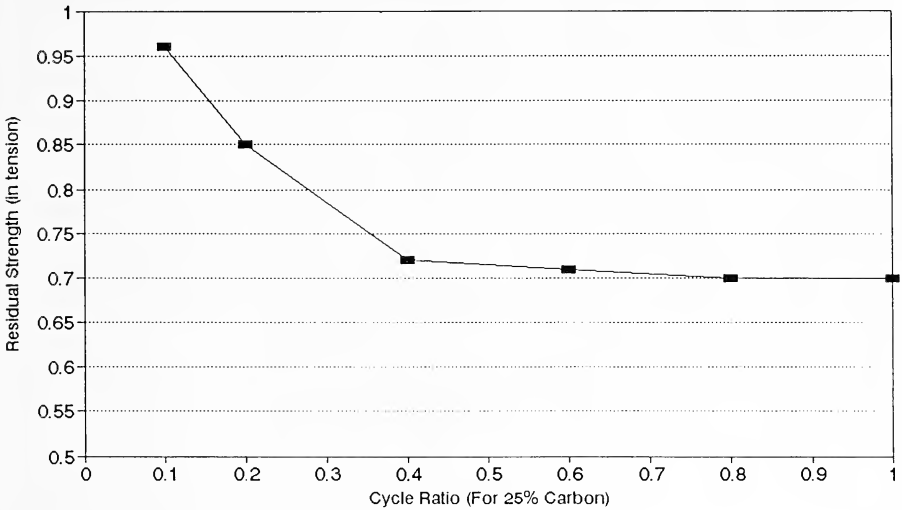


Figure 5. Residual strength in tension vs. cycle ratio for 25% carbon content.

3. The third stage, cycle ratio = 0.45 to 1. Residual static strength continues to reduce until the fatigue life was reached and thereafter, the specimen failed suddenly.

The cyclic behavior of carbon reinforced plastics may be compared with metallic materials. In metals, most of the life is spent before the cracks appear, once a crack appears it propagates rapidly and the metal fails. In carbon reinforced plastics, on the other hand, cracks form after a few cycles even at a lower stress levels (20 to 30% of ultimate strength).

### CONCLUSIONS

The following conclusions can be drawn from the experimental results

1. The residual static strength of carbon fiber reinforced plastics subjected to a cyclic range of 0 to 0.1 is equal or greater than that of composite material that has not been subjected to any loading.
2. The decrease in the residual strength was independent of carbon content and cyclic stress. Therefore, the cyclic damage process in carbon fiber reinforced plastics was divided into three stages.

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## GENERAL NOTES

LARGE SPRING GAMBUSIA (*GAMBUSIA GEISERI*)  
PRODUCES YOUNG IN ALL SEASONS**Clark Hubbs***Department of Zoology, University of Texas  
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Many spring-adapted fishes have reproductive seasons that extend throughout the year (Schenck & Whiteside 1971; Hubbs 1985); however, others have long, but not year-round reproduction (Hubbs 1971), and do not produce young during winter. Stevens (1977) reported female large spring *Gambusia* (*Gambusia geiseri*) in the San Marcos River to have advanced embryos during all months. Stevens report treated only the San Marcos populations; numerous other populations in Texas were studied to determine whether the year-round reproduction occurred elsewhere in the species, because *Gambusia* life history traits have been shown to vary among populations (Hubbs 1996; 1997).

Female *G. geiseri* brought into the laboratory previously have produced young in spring, summer and fall, but winter data were not yet available. Laboratory temperatures were 21°C, near that of the aquifers inhabited by *G. geiseri* (Table 1). Adult *G. geiseri* were obtained in December from five Texas populations, then 12 females of each population were isolated from males, and brood production was monitored for one month. The five populations were collected at: (1) San Marcos head springs (just upstream from Stevens' Station 1), (2) San Marcos River at Interstate 35 (the same as Stevens' Station 2.5), (3) Phantom Cave just north of Toyahvale, (4) Diamond-Y Spring just north of Fort Stockton, Texas, and (5) Comal Springs in New Braunfels, Texas. The Phantom Cave and Diamond-Y populations are introduced, most likely from the San Marcos River (Hubbs & Springer 1957). The San Marcos and Comal populations were in clear, stenothermal, cool, low-salinity waters. The Phantom Cave population differs by living in waters that are about 4°C warmer than three of the other populations (Table 1). The Diamond-Y population lives in much saltier waters than do the other populations. Thus, the tests involve two source populations and three different physical/chemical backgrounds.

Table 1. Number of broods from *Gambusia geiseri* females isolated from males in winter.

<i>Gambusia geiseri</i>	Number of Reproductive Females (out of 12)	Number of Broods	Temperature* (°C)	Specific Conductivity ( $\mu$ S)
San Marcos head spring	5	6	20.3	0.60
Interstate 35 in San Marcos	7	9	21.0	0.50
Diamond-Y Spring	7	8	18.9	6.50
Phantom Cave Spring	7	7	24.9	2.80
Comal Spring	7	9	23.4	0.58

\* Measurement of the water from which each population was collected.

Forty-two to fifty-eight percent of females from all five populations produced offspring (Table 1). Oddly, the low percentage is for the San Marcos head spring population near Stevens' Station 1 where Stevens (1977) found winter reproduction in approximately 75%. The data indicate that *G. geiseri* in stenothermal waters breeds throughout the year regardless of other environmental factors.

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\* \* \* \* \*

NOTEWORTHY RECORDS OF MAMMALS FROM  
SCURRY COUNTY, TEXAS

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There are few reports of mammals from Scurry County, Texas, and these are county records-of-occurrence mapped by Davis & Schmidly (1984). The county lies in the southwestern corner of the Rolling Plains. It is one of many regions in the state that historically has been neglected by mammalogists, and exists outside the coverage of nearby regional treatments of north-central Texas (Dalquest & Horner 1984) and the Llano Estacado (Choate 1997). The nearest geographic treatment is a research note on the mammals of Stonewall County to the immediate northeast (Ruhl & Stangl 1997).

Native vegetation primarily is mesquite (*Prosopis glandulosa*) savannah, although juniper (*Juniperus* sp.) prevails along the rugged slopes of the northern and western parts of the county, and expanses of sand sage dominate much of the sandy soils and river terraces of the Colorado River in the southwestern corner of the county.

A survey of the Midwestern State University (MWSU) Collection of Recent Mammals produced specimens of six species collected in the 1960s by Walter W. Dalquest and MWSU students during the course of fossil excavations. A more recent field trip to the county in the spring of 1998 resulted in six additional species for which there were no previous records from Scurry County. Two of the 12 county records reported herein (*Perognathus flavescens* and *Reithrodontomys fulvescens*) constitute range extensions, and the others help fill in distributional gaps.

*Sylvilagus floridanus*.—A single specimen of an adult male eastern cottontail (MWSU 21417) was salvaged from a road-killed carcass 3 mi SW of Union. The species probably occurs throughout the county wherever sufficient vegetation exists to provide protective cover.

*Perognathus flavescens*.—This is the rarest of pocket mice in Texas, although in places it is abundant on sandy soils dominated by sand sage. A series of four adult plains pocket mice (MWSU 1917, 1919-1921) was trapped 4 mi SW of Snyder. This record fills a broad hiatus in the range as mapped by Davis & Schmidly (1994) and extends the known

range limits of the species southeastward.

*Chaetodipus hispidus*.—The hispid pocket mouse prefers loose soils. Two subadult animals (MWSU 1915, 1916) were taken from sandy soils 4 mi SW of Snyder, in association with the plains pocket mouse and hispid cotton rat.

*Dipodomys ordii*.—This species is locally restricted to the deep sandy soils of river terraces. Ten specimens of Ord's kangaroo rat (MWSU 5131, 5138, 5140, 5744, 5823, 5913, 6456-6459) were taken 3 mi SW of Ira, and another five (MWSU 1827, 1829, 1830, 1831, 1905) were trapped 4 mi SW of Snyder. One of the specimens from near Ira (MWSU 5744) was marked by a large white patch on the left rear flank and a white-tipped tail.

*Reithrodontomys fulvescens*.—Two specimens of the fulvous harvest mouse (MWSU 21412, 21413) represent an extension of the known range of the species farther west into the southern Rolling Plains. The animals were taken 2.4 mi S of Camp Springs, in a grassy association also populated by cotton rats.

*Reithrodontomys montanus*.—The county is well within the known range of the plains pocket mouse. A single specimen (MWSU 21419) was taken 4.8 mi E of Fluvanna, along the sparsely vegetated base of a rocky, juniper-dominated slope.

*Peromyscus attwateri*.—The Texas mouse is a common resident of rugged slopes cloaked in juniper. One animal (MWSU 9684) was taken from 4 mi SW of Snyder, and two (MWSU 21410, 21411) were collected from 1 mi E of Camp Springs and 2.4 mi SW of Camp Springs, respectively.

*Baiomys taylori*.—The pygmy mouse is presently distributed over most of the western two-thirds of the state. A single specimen (MWSU 21420) was trapped from 4.8 mi E of Fluvanna, in the same trapline that yielded the plains harvest mouse.

*Sigmodon hispidus*.—The hispid cotton rat occurs throughout the state in all but the most xeric of plant communities. It was taken at most trap localities in Scurry County, but only four specimens (MWSU 3857, 3858, 3868, 5411) from 3 mi SW of Ira were saved.

*Neotoma albigula*.—The white-throated woodrat shares its preferred rugged slopes with the Texas mouse. Two specimens (MWSU 1553, 1554) were taken from 4 mi SW of Snyder, one (MWSU 21414) from



2.4 mi SW of Camp Springs, and another (MWSU 21418) from 4.8 mi E of Fluvanna.

*Procyon lotor*.—The raccoon is a locally common carnivore. A single specimen (MWSU 21416) is represented by the skull from a road-killed carcass collected 2.4 mi E of Snyder.

*Odocoileus virginianus*.—The white-tailed deer occurs throughout the county. The skull of a young adult doe (MWSU 21415) was salvaged from along a roadway 4.8 mi W of Union.

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\* \* \* \* \*

### NOTEWORTHY RECORD OF THE YELLOW-NOSED COTTON RAT (*SIGMODON OCHROGNATHUS*) FROM TRANS-PECOS TEXAS

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The yellow-nosed cotton rat (*Sigmodon ochrognathus* V. Bailey, 1902) usually is associated with montane grassland, at high elevation, in the southern Trans-Pecos of Texas (Jones & Jones 1992). It is known from the Chisos Mountains in Big Bend National Park, Brewster County, the Sierra Vieja, Presidio County, and the Davis Mountains, Jeff Davis County (Davis & Schmidly 1994). Yancey & Jones (1996) reported a specimen from "non-montane" habitat, adjacent to a stream

bed in desert scrub, on the Big Bend Ranch State Park, in Presidio County.

While conducting field surveys at Elephant Mountain Wildlife Management Area, approximately 23 miles South and 7 miles East of Alpine, Brewster County, Texas on 12 October 1997, a single female yellow-nosed cotton rat was captured in rocky upland habitat. Standard measurements of the female include: total length, 235 mm; length of tail vertebrae, 103 mm; length of hind foot, 26 mm; length of ear, 18 mm; and mass, 52.5 g. This rat gave birth to two babies in the trap. Measurements of one of the neonates follows: total length, 74 mm; length of tail vertebrae, 27 mm; length of hind foot, 11 mm; length of ear, 7 mm; and mass, 4.8 g. Dominant vegetation at the capture site included cholla (*Opuntia* sp.), sotol (*Dasyilirion* sp.), saltbush (*Atriplex* sp.), persimmon (*Diospyros* sp.), yucca (*Yucca* sp.) and sparse short grasses. We saw no evidence of cotton rat "runways" in this sparsely vegetated desert habitat. Other small mammals taken on the same trap-line include; Nelson's pocket mouse (*Chaetodipus nelsoni*), white-ankled mice (*Peromyscus pectoralis*) and white-footed mice (*Peromyscus leucopus*). The locality of capture is approximately 60 miles North and 15 miles West of the Chisos Mountains, in desert scrub, a non-montane habitat. This is the only Brewster County record outside the Chisos Mountains and it represents the northeasternmost known record of occurrence in Texas. The adult cotton rat was prepared as a voucher specimen (TTU # 76315, skin and skull) and deposited in the collection of Recent mammals at the Natural Science Research Laboratory, the Museum of Texas Tech University, Lubbock, Texas.

The status of this species, at Elephant Mountain Wildlife Management Area, is unknown. Based on number of captures per trap night effort (580 trap nights in March, 1996; 260 trap nights in March, 1997; and 108 trap nights in October, 1997), it appears to be uncommon, as only one specimen was collected (in October).

#### ACKNOWLEDGMENTS

Small mammals were collected at Elephant Mountain Wildlife Management Area, under the aegis of a scientific collecting permit issued by The Texas Parks and Wildlife Department (permit number, SPR-1192-569, and park entry permit #97-5). We thank Clay Brewer and Scott Lerich for allowing us access to the area and thank them for

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INDEX TO VOLUME 50 (1998)  
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This index has separate subject and author sections. Words, phrases, locations, proper names and the scientific names of organisms are followed by the initial page number of the articles in which they appeared. The author index includes the names of all authors followed by the initial page number of their respective article(s).

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