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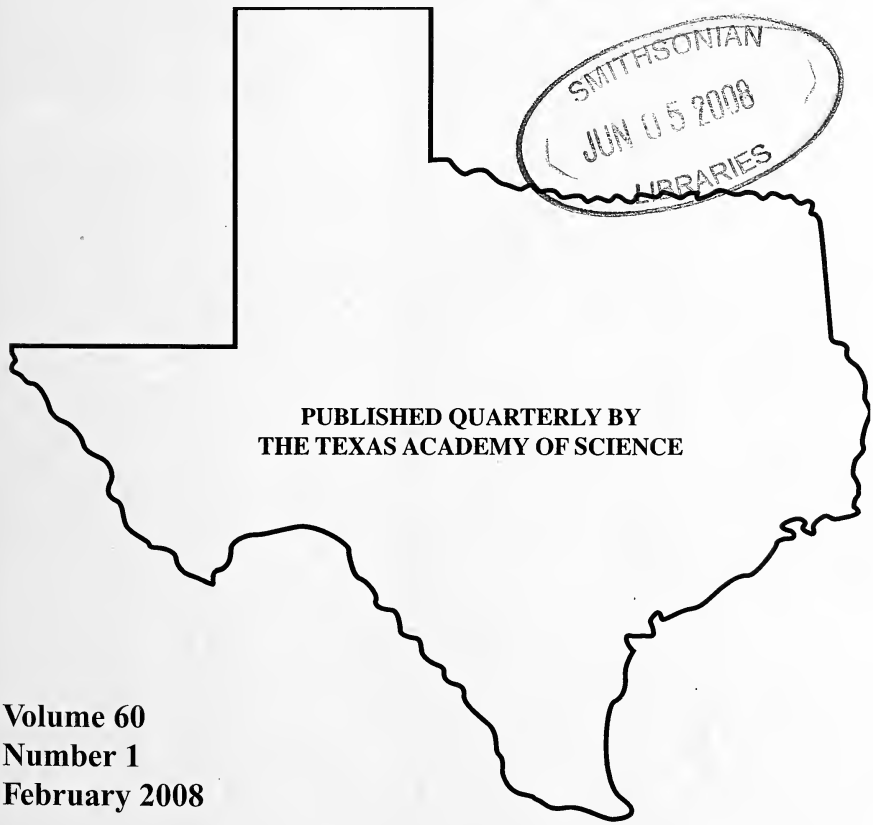

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TROPHIC STATUS RELATED TO PERIPHYTON AND MACROPHYTE DISTRIBUTION AND ABUNDANCE ALONG THE NORTH BOSQUE RIVER IN NORTH CENTRAL TEXAS

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Abstract.–A two-year survey was conducted to estimate periphytic algae and macrophyte diversity and abundance at 11 locations within the North Bosque River watershed. Ash free dry weight by species was used to measure biomass. A total of 30 different species were collected; 24 macrophytes, five periphyton, and one moss. Macrophytes comprised the largest portion of total biomass at most sites, while algae were most frequently collected. The most biomass was contributed by the macrophyte *Justicia americana* (L.) Vahl, while *Cladophora* sp. was the dominant algae. The majority of algae were found in riffles, while macrophyte biomass was fairly evenly distributed between pool and riffle habitats. Nutrient concentrations generally showed saturated conditions for periphyton growth indicating that factors other than nutrients, such as light, may be limiting periphyton and possibly macrophyte biomass along the river. In comparison to proposed trophic state indices for periphyton, mesotrophic or eutrophic conditions were indicated at four of 11 sites. In contrast, measurements of instream chlorophyll-α concentrations of phytoplankton indicated mesotrophic conditions at eight of 11 sites. These differences in tropic status based on periphyton and phytoplankton indicate a need to monitor attached, as well as free-floating algae, when evaluating for water quality impairment in small to intermittent sized creeks and rivers.

In 1998 the North Bosque River was placed on the Texas Clean Water Act section 303(d) list as an impaired water body due to excessive nutrients and plant growth under narrative water quality standards (TNRCC 1998). Besides narrative standards, in Texas, excessive algal growth may be identified through violation of water quality standards for dissolved oxygen (TCEQ 2003). Concern regarding eutrophication may be defined by comparing nitrogen, phosphorus, or sestonic chlorophyll-α (CHLA) concentrations to screening levels. These screening levels are based on an 85-percentile ranking of State water quality data for specific water body types (TCEQ 2003). This ranking allows a relative indication of water quality concerns for nutrients and planktonic algae that is statistically derived but has no direct biological meaning.

For the North Bosque River, phosphorus was identified as the nutrient limiting algal growth (Kiesling et al. 2001). Excessive nutrients, particularly phosphorus (P) in freshwater systems, are major factors in causing excessive algal growth (Gibson 1997). To help control algal growth, two total maximum daily loads (TMDLs) were approved with a reduction goal of about 50 percent in soluble reactive phosphorus along the North Bosque River (TNRCC 2001). Routine monitoring, as part of the post-TMDL implementation effort, includes measurement of sestonic CHLA, which primarily represents the phytoplankton or free-floating algae in the water column. Sestonic CHLA concentrations are often used to measure trophic status as an indicator of eutrophication in lakes and reservoirs (Carlson 1977). Trophic boundaries proposed by EPA (2000) for phytoplankton in rivers and streams are 10 µg/L CHLA for the oligotrophic to mesotrophic boundary and 30 µg/L for the mesotrophic to eutrophic boundary.

Although phytoplankton is a good indicator of eutrophication, in most cases phytoplankton measurements alone underestimate algal biomass in wadeable streams. Periphyton, as attached filamentous algae, is often dominant relative to phytoplankton (Allen 1995). Monitoring periphyton biomass is much more time consuming and labor intensive than measuring sestonic algae, so much less research has been done on defining boundaries for trophic classification of periphytic algae. Despite the limited research, EPA (2000) proposes boundaries of 20 mg/m^2 CHLA for oligotrophic to mesotrophic and 70 mg/m^2 CHLA for mesotrophic to eutrophic for mean periphytic algae. The mesotrophic to eutrophic boundary is relatively close to the 100 mg/m^2 CHLA proposed by Welch et al. (1988) for defining nuisance levels of periphytic algae.

An overabundance of macrophytes is also recognized as a potential water quality problem that is often overlooked in routine water quality assessments (Newton & Jarrell 1999). Macrophytes have clogged intakes for irrigation systems and can interfere with boating and fishing activities (Sosiak 2002). No current trophic

guidelines exist for macrophytes, although management of macrophytes is also important to maintaining healthy aquatic ecosystems (Chambers et al. 1999).

While instream planktonic CHLA concentrations are often monitored with regard to eutrophication, the excessive growth of periphyton and macrophytes are rarely considered with routine water quality monitoring, but may be just as important in defining impairment. The purpose of this study was to estimate the diversity and abundance (as biomass) of macrophytes and periphyton along the North Bosque River and its major tributaries and associate this information with measures of trophic status as indicators of stream eutrophication.

Study Area

The North Bosque River is a fifth-order stream located in north central Texas that begins in Erath County and flows southeast for nearly 179 kilometers through Hamilton, Bosque, and McLennan counties until it enters Lake Waco near Waco, Texas (Figure 1). The mainstem of the North Bosque River for the most part is cut down to bedrock with permanent flow occurring primarily in the lower reaches and intermittent flow in the upper and mid-reaches. A small portion of the upper reach maintains a permanent flow due to discharge from the Stephenville wastewater treatment plant (WWTP). The flow associated with contributions from the Stephenville WWTP can dissipate within a few kilometers, particularly in the summer when rainfall-runoff events are infrequent and generally small and evaporation is at its peak. Lower reaches of the North Bosque River maintain some base flow most notably from seepage through fractured limestone along stream banks in the lower portion of the watershed. A relatively small contribution to base flow is from municipal WWTPs along the river.

The sampling sites represented a broad range of conditions within the watershed (Table 1). Sites were chosen to characterize

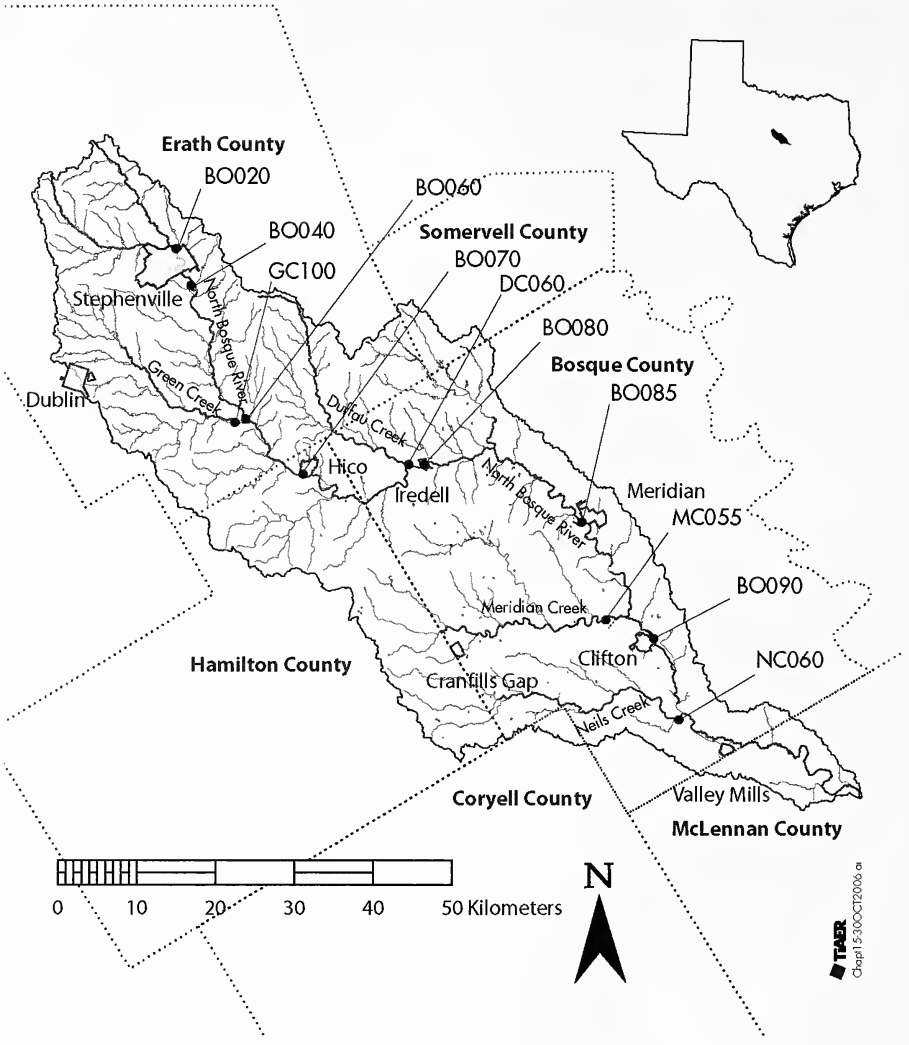

Figure 1. Location of sampling sites used for macrophyte and periphyton survey.

the flora present along the North Bosque River and in the lower reaches of several major tributaries. Included were seven sites along the North Bosque River and four major tributaries (Figure 1). Study reaches at each site were selected that had both riffle and pool habitats, were easily accessible, and were expected to have water present throughout much of the year.

Table 1. Land use and drainage area characteristics above sampling sites.

Site	Wood/ Range (%)	Pasture (%)	Cropland (%)	Dairy Waste Application Fields (%)	Urban (%)	Other (%)	Drainage Area Hectares	Near WWTP Influence
BO020	48.5	29.6	6.6	13.1	1.5	0.7	21,700	No
BO040	47.3	28.4	7.0	12.3	4.4	0.7	25,700	Yes
BO060	57.4	22.8	6.0	9.9	3.3	0.5	48,900	No
BO070	65.3	18.9	6.1	7.3	2.0	0.5	93,100	No
BO080	68.4	16.3	7.0	6.3	1.7	0.3	146,000	No
BO085	71.0	14.7	7.6	4.9	1.6	0.3	190,000	No
BO090	71.5	14.0	9.1	3.7	1.5	0.2	253,000	No
DC060	74.4	7.6	8.7	8.7	0.6	0.0	23,200	No
GC100	67.4	18.1	7.6	5.8	0.6	0.4	25,200	No
MC055	73.6	12.5	13.6	0.0	0.3	0.0	47,200	No
NC060	75.8	10.7	13.2	0.0	0.2	0.0	35,300	No

MATERIALS AND METHODS

Sampling was conducted in March, May, July, August, and October 2004; and January, April, and June 2005 for a total of eight events. Macrophytes were identified to species and periphytic algae to genus in most cases. Diatoms were identified only to Bacillariophyta. A few unknowns were documented that could not be reliably identified. Abundance was measured quanti-tatively by obtaining the ash free dry weight (AFDW) of each species of macrophyte and periphyton collected. Quantitative sampling of macrophytes and periphyton within pool and riffle habitats along each reach was accomplished using a Surber sampler as a 0.093 m^2 plot frame. The lengths of both habitat types ranged from 30 m to 50 m for riffle habitats and 50 m to 60 m for pool habitats. Sampling locations within the stream were determined using a two number coordinate system consisting of points parallel and perpendicular to the length of the stream. A list of random numbers selected prior to sampling was used to reduce the sampler's bias in determining sampling coordinates. A total of 12 sampling points were determined for each site with six sampling points within each of the two habitat types (pool and riffle). Of the six sampling locations within the riffle, a minimum of two were in the margin areas, while the other four could be collected from the margins or

within the channel. Only margin samples were collected within the pool habitat, because greater water depth and turbidity in the channel portions of pools greatly limited the growth of macrophytes and periphyton. An additional reason for sampling only the margin areas of pools was to avoid collecting periphyton that may have been detached through scouring and deposited into the deeper channel portions of the pool.

Macrophytes and periphyton were identified in the field when possible and recorded on the datasheet. Samples were placed in labeled plastic bags and stored on ice for transportation back to the lab. A reference collection of macrophytes collected nearby but outside the study reaches was maintained. Macrophytes used for the reference collection were dried, mounted, and labeled and stored in the Tarleton State University herbarium. Because periphyton are so fragile when dried, a reference collection of periphyton was not maintained, but any unknowns were preserved in water until they could be identified later in the lab. Because many periphyton are very difficult to identify reliably to the level of species (Prescott 1954; Dodds & Gudder 1992), all periphytic algae specimens were identified only to the level of genus, except diatoms, which were identified only to Bacillariophyta. For macrophytes, specimens were identified to the level of species when possible.

All weights were obtained using an analytical balance and recorded to the nearest milligram. Samples were placed into a drying oven for a minimum of 24 hours at 105°C and weighed to obtain a dry weight. Dried samples were placed in a muffle furnace for a minimum of 6 hours at 550°C and weighed to obtain an ash weight (Barber et al. 1999). After obtaining a dry weight and an ash weight, the ash-free dry weight (AFDW), which represents the amount of organic material (biomass) present, was calculated as follows:

$$\text{AFDW} = (\text{Dry Weight} - \text{Tray Weight}) - (\text{Ash Weight} - \text{Tray Weight})$$

Table 2. Suggested stream trophic status boundaries for periphyton and phytoplankton based on chlorophyll (EPA 2000) and estimated boundaries for periphyton based on conversion of AFDW using equations provided by Biggs (2000).

Variable (units)	Oligotrophic-Mesotropic Boundary	Mesotrophic-Eutrophic Boundary
Mean Periphyton Chlorophyll (mg/m^2)	20	70
Maximum Periphyton Chlorophyll (mg/m^2)	60	200
Mean Periphyton AFDW (g/m^2)	7	13
Maximum Periphyton AFDW (g/m^2)	12	24

The total AFDW calculated as the sum AFDW across all eight sampling events by species was used as an indicator of species dominance. The total AFDW by site was calculated separately for the categories of periphyton and macrophyte across riffle and pool habitats and by habitat type to indicate abundance of biomass at a site.

To compare biomass abundance between sites by plant category (periphyton or macrophyte), the analysis of variance and least square means procedures were used on the total AFDW by site and sampling date to determine if significant differences ($\alpha = 0.05$) existed between sites across sampling periods and to indicate which sites were different from one another. The analysis of variance and least square means tests were conducted using the SAS procedure PROC GLM (SAS 2000). A log transformation of the AFDW results was required to obtain a more normally distributed data set for comparisons between sites prior to analysis. The SAS procedure PROC UNIVARIATE was used to confirm the need for a natural log transformation of the data using the Shapiro-Wilks statistic as a test for normality (SAS 2000).

Both CHLA and AFDW are common measures of periphytic algal abundance, but developing relationships between these two measurements to approximate one from the other is a very tedious task. To compare the AFDW measurements to trophic boundaries based on CHLA proposed by EPA (2000) for periphyton, the following equations developed by Biggs (2000) were used:

Table 3. Summary of marcophyte and periphyton species encountered ranked by the number of times collected (N) out of 1068 total sampling points.

Species	Plant Type	Total AFDW (g/m^2)	Mean AFDW (g/m^2)	N
Cladophora sp.	Periphyton	3,390	9.53	356
Justicia Americana (L.) Vahl	Macrophyte	14,900	97.5	153
Spirogyra sp.	Periphyton	347	4.45	78
Najas guadalupensis (Spreng.) Magnus	Macrophyte	83.6	1.37	61
Chara sp.	Periphyton	89.9	1.58	57
Carex sp.	Macrophyte	2,000	43.6	46
Hydrocotyle umbellate L.	Macrophyte	411	10.3	40
Eleocharis sp.	Macrophyte	545	24.8	22
Diatom mat	Periphyton	339	24.2	14
Marsilea vestita Hook. & Grev.	Macrophyte	20.7	2.59	8
Ludwigia peploides (Kunth) Raven	Macrophyte	96.8	16.1	6
Unidentified Macrophyte #3	Macrophyte	93.4	15.6	6
Unidentifed Algae mixed with Diatoms	Periphyton	80.6	13.4	6
Selaginella apoda (L.) Spring	Moss	43.2	7.20	6
Diatom mat with *Spirogyra*	Periphyton	13.8	2.29	6
Diatom/*Cladophora* mix	Periphyton	26.2	5.24	5
Cyperus acuminatus Torr. & Hook. Ex Torr.	Macrophyte	37.9	9.48	4
Ranunculus sceleratus L.	Macrophyte	11.9	2.98	4
Poa sp.	Macrophyte	51.3	17.1	3
Unidentified Macrophyte #4	Macrophyte	29.1	9.69	3
Spirogyra sp., *Chara* sp., *Najas* sp. mix	Periphyton and Macrophyte	17.3	5.77	3
Zizaniopsis miliacea (Michx.) Doell & Aschers.	Macrophyte	274	137	2
Paspalum distichum L.	Macrophyte	90.3	45.2	2
Undientified Macrophyte #1	Macrophyte	73.5	36.8	2
Chaerophyllum tainturieri Hook.	Macrophyte	12.4	6.19	2
Rorippa sessiliflora (Nutt.) A.S. Hitchc.	Macrophyte	6.46	3.23	2
Cynodon dactylon (L.) Pers.	Macrophyte	6.06	3.03	2
Rorippa nasturtium-aquaticum (L.) Hayek	Macrophyte	1.40	0.70	2
Veronica peregrina spp. *Xalapensis* (Kunth) Pennell	Macrophyte	1.08	0.54	2
Steigoclonium sp.	Periphyton	0.32	0.16	2
Panicum dichotomiflorum Michx.	Macrophyte	6.99	6.99	1
Ceratophyllum demersum L.	Macrophyte	0.75	0.75	1
Unidentifed Macrophyte #2	Macrophyte	0.54	0.54	1
Smilax sp.	Macrophyte	0.22	0.22	1

Table 4. Sites ranked by total AFDW summed for all eight monitoring periods.

Site	Total AFDW (g/m^2)	Total Periphyton AFDW (g/m2)	Total Macrophyte AFDW (g/m2)	Number of Periphyton Species	Number of Macrophyte Species
GC100	12,300	975	11,325	4	11
DC060	4,410	945	3,465	4	10
BO070	1,340	811	529	3	7
BO090	1,020	207	817	4	4
BO080	899	440	459	3	8
NC060	865	82	783	3	3
BO040	763	222	541	2	4
BO020	478	66	412	2	2
BO085	227	168	59	3	8
MC055	201	81	120	4	3
BO060	196	21	175	2	4

$$\ln(\text{CHLA, mg/m}^2) = 0.338 + 1.396*\ln(\text{AFDW, g/m}^2) \quad r^2 = 0.79$$

$$\ln(\text{AFDW, g/m}^2) = 0.186 + 0.566*\ln(\text{CHLA, mg/m}^2) \quad r^2 = 0.79$$

These equations provided by Biggs (2000) were based on detailed work conducted on 170 samples collected from a wide range of periphyton communities in streams throughout New Zealand. Trophic boundary values (Table 2) of AFDW as mean and maximum values were then compared to mean AFDW concentrations across sampling events for the North Bosque River sites. Sestonic CHLA collected as part of routine monitoring along the North Bosque River was also compared to trophic boundaries for phytoplankton. Grab samples for sestonic CHLA were analyzed using a spectrophotometer following Standard Method 10200H (APHA 1992).

Results

A total of 30 different species were collected; 24 macrophytes, five periphyton, and one moss (Table 3). Macrophytes comprised the largest portion of the total biomass, while periphyton species were most frequently collected at all sites except GC100. The

majority of biomass obtained, except at sites BO070 and BO085, came from macrophytes rather than periphyton. The species most often encountered was the alga *Cladophora* sp. followed by the macrophyte *Justicia americana* (L.) Vahl (American water-willow).

The amount of biomass collected at each site across habitat types was compared two ways. One by ranking the total biomass collected across all sampling periods by site, and two by statistically comparing mean biomass by site across sampling dates. The largest amount of total biomass collected at the 11 sites came from GC100 as well as the highest number of different species encountered (Table 4). Comparing between sites using analysis of variance (*ANOVA*) and least square means (LSD) procedures, significant differences ($\alpha = 0.05$) in periphytic algal biomass (Figure 2a) and highly significant differences ($\alpha = 0.01$) in macrophyte biomass (Figure 2b) were found between sites. The geometric mean of biomass obtained from periphyton species ranged from 0.03 g/m^2 at site BO060 to 3.32 g/m^2 at site DC060 (Figure 2a). The geometric mean biomass obtained from macrophyte species ranged from 0.01 g/m^2 at site BO085 to 22.1 g/m^2 at site DC060 (Figure 2b). Although the largest amount of total biomass was indicated at GC100, the largest amount of biomass across sampling periods most consistently occurred at DC060.

Macrophytes comprised 74% of the total biomass at DC060 and 92% at GC100 for the nine sampling periods, and the dominant species at both sites was the macrophyte *J. americana*. *Justicia americana* was one of the largest macrophytes collected. The erect stems of *J. americana* can reach up to 100 cm tall, and this plant is known for its extensive adventitious rooting system that can firmly bind to the streambed (Diggs et al. 1999).

The abundance of *J. americana* at DC060 and GC100 may be attributed in part to the physical characteristics of these reaches. *Justicia americana* is sensitive to shading or light limitation (Fritz et al. 2004). Light availability is considered more important than

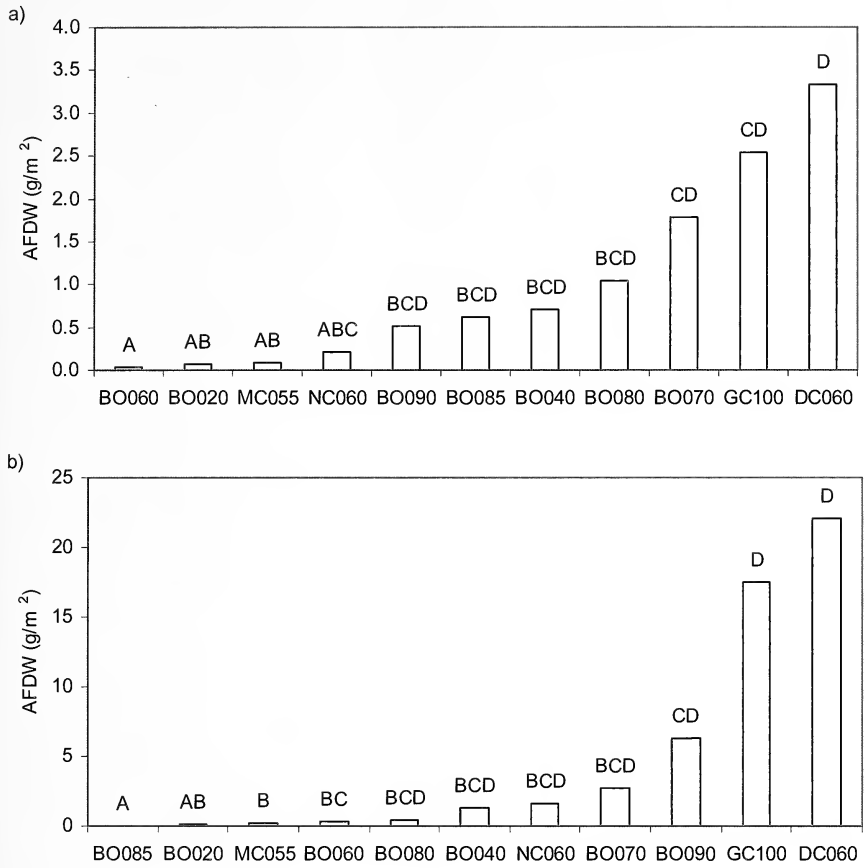

Figure 2. Geometric mean AFDW from (a) periphyton and (b) macrophytes for each site across all sampling events. Means followed by the same letter were not significantly different at a probability level of 0.05 according to a test of least significant differences.

flooding disturbance or parasite abundance as a factor affecting total biomass of *J. americana* as evaluated for six south-central Alabama streams (Fritz et al. 2004). In earlier work by Howell (1975) cited by Fritz et al. (2004), the coverage of *J. americana* in a Kentucky stream was more than twice as much in reaches flowing east to west than in reaches flowing north to south. This greater coverage was attributed to longer daily exposure to direct sunlight of east-west reaches compared to north-south reaches.

The reach at DC060 was open with no notable canopy cover, so sunlight was accessible to the reach regardless of its orientation. The reach at GC100 runs east to west and was the only reach that was oriented in this manner except for sites BO040 and BO070. The east to west orientation of site GC100 coupled with relatively sparse canopy cover allowed aquatic vegetation to be exposed to sunlight longer than the other study sites that were typically oriented north to south. Although the reach sampled at BO040 shared the same orientation characteristics as GC100, the pool habitat of BO040 was heavily shaded by dense canopy cover, which likely inhibited growth of *J. americana* by limiting light. Site BO070 is also oriented east to west, and similar to site GC100 canopy cover was sparse. The orientation and sparse canopy cover of site BO070 likely contributed to the relatively large amount of total biomass associated with *Cladophora* (375 g/m^2) that was second only to site GC100 (927 g/m^2). At BO070, periphyton rather than macrophytes, represented the majority of the biomass (Table 4).

In comparing between habitat types (riffle and pool), the majority of periphyton biomass was obtained from the riffle habitat for all sites except BO060 (Table 5). For sites BO040, DC060, and MC055, the riffle and pool habitats contributed nearly equal amounts of the periphyton biomass. The riffle and pool habitats contributed to nearly equal amounts of the biomass obtained from macrophyte species at sites BO060 and BO085, while the majority of macrophyte biomass for the remaining mainstem sites originated from the pool habitats with the exception of site BO020.

Discussion

A large difference occurred in the estimated trophic status classifications between sites depending on whether periphyton or phytoplankton was used as the indicator (Figure 3). For periphyton, only four sites (BO070, BO080, DC060, and GC100) were classified as mesotrophic or eutrophic using either maximum or mean values. For phytoplankton, all sites but DC060, MC055 and

Table 5. Percentage of total biomass obtained from algal and macrophyte species collected within each habitat type.

Site	Periphytic Algae		Macrophyte	
	Riffle (% biomass)	Pool (% biomass)	Riffle (% biomass)	Pool (% biomass)
BO020	91	9	100	0
BO040	51	49	34	66
BO060	20	80	54	46
BO070	62	38	46	54
BO080	88	12	13	87
BO085	70	30	52	48
BO090	64	36	17	83
DC060	57	43	38	62
GC100	87	13	39	61
MC055	57	43	31	69
NC060	74	26	20	80

NC060 were classified as mesotrophic. This apparent discrepancy may in part be explained by competition between algal types. Streams dominated by periphytic algae may have less phytoplankton due to competition for nutrients, light or other limiting factors and vice versa. Also, the establishment of periphytic algae is dependent on the substrate of the streambed providing adequate attachment sites. Another factor, particularly in comparing the mainstem sites, is the upstream to downstream transport of algae. While periphyton can detach and move downstream, this occurs predominately as a result of scouring associated with elevated flows. Phytoplankton are free-floating in the water column and move with the transport of water. Sestonic CHLA concentrations showed a general decreasing pattern from more upstream sites (BO020, BO040 and BO060) to more downstream sites (BO085 and BO090). For periphyton, some of the lowest biomass estimates were indicated at the most upstream site (BO020).

Nutrient concentrations of total N and total P were also generally higher in the upstream portion of the river than in the more downstream portion (Table 6). Excessive aquatic plant growth is often related to excessive nutrients, but defining target concentrations of N and P to help control algal (or macrophyte) impairment is

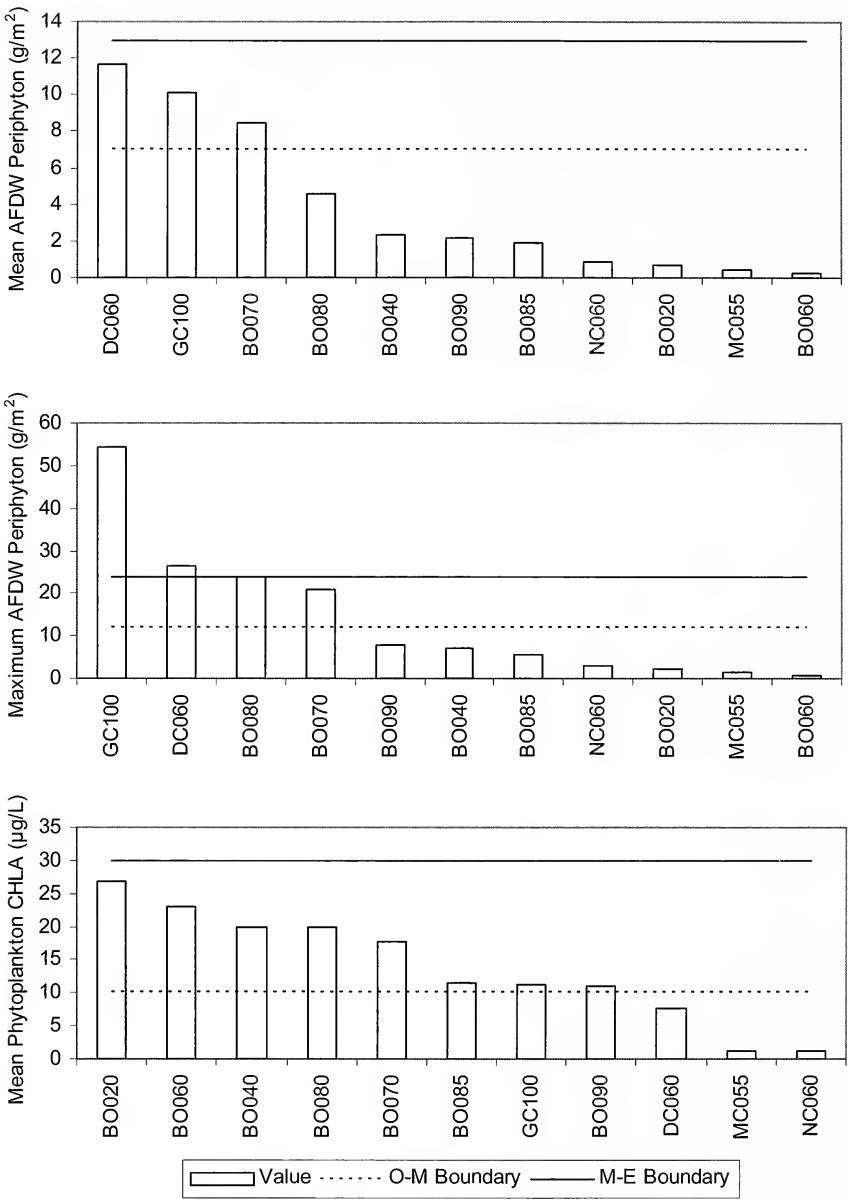

Figure 3. Comparison of potential trophic boundaries for periphyton and phytoplankton for streams based on EPA (2000) and work by Biggs (2000). O-M indicates oligotrophic to mesotrophic boundary and M-E indicate mesotrophic to eutrophic

Table 6. Basic statistics for total N and total P from routine grab samples collected between March 1, 2004 and June 30, 2005. N equals the number of observations.

Constituent	Site	Mean (mg/L)	Median (mg/L)	Standard Deviation (mg/L)	Minimum (mg/L)	Maximum (mg/L)	Number of Obs.
Total N	BO020	1.68	1.61	0.63	0.61	3.90	32
	BO040	3.74	3.47	1.07	2.14	6.15	35
	BO060	1.43	1.38	0.69	0.39	2.75	27
	BO070	1.31	1.21	0.76	0.08	3.08	35
	BO080	1.22	1.05	0.64	0.40	2.91	27
	BO085	0.96	0.82	0.52	0.10	2.22	27
	BO090	0.84	0.78	0.39	0.20	2.07	34
	DC060	0.94	0.62	0.92	0.26	4.68	27
	GC100	2.87	2.23	2.14	0.48	7.53	27
	MC055	0.60	0.54	0.26	0.23	1.19	27
	NC060	0.71	0.64	0.20	0.31	1.10	35
Total P	BO020	0.40	0.41	0.18	0.09	0.85	32
	BO040	1.52	1.11	0.91	0.56	3.66	35
	BO060	0.44	0.42	0.21	0.01	1.06	27
	BO070	0.21	0.21	0.11	0.06	0.49	35
	BO080	0.13	0.10	0.10	0.01	0.40	27
	BO085	0.10	0.08	0.09	0.01	0.38	27
	BO090	0.08	0.07	0.07	0.01	0.32	34
	DC060	0.10	0.06	0.11	0.02	0.44	27
	GC100	0.11	0.06	0.14	0.01	0.60	27
	MC055	0.06	0.06	0.03	0.01	0.15	27
	NC060	0.06	0.06	0.03	0.01	0.13	35

difficult (TNRCC 2001). Chlorophyll-α concentrations of phytoplankton in the water column generally show a relatively close relationship with nutrient concentrations in the water column, particularly in lake systems (e.g., Carlson 1977; Vollenweider et al. 1980). For periphytic algae and macrophytes, a correlation with instream nutrient concentrations generally does not occur (Dodds et al. 2002). Macrophytes, in particular, obtain most of their nutrients from bank or bed sediments rather than the water column.

The growth of macrophytes and periphyton may also be impacted by light availability, as noted earlier with regard to the abundance of *J. americana* at sites DC060 and GC100. The east to

west orientation of these two sites coupled with a sparse canopy cover is thought to have allowed more abundant macrophyte growth at these two sites than at other sites monitored in the watershed. While almost all macrophytes found were emergent or floating, turbidity within the water column can also limit light penetration limiting the growth of submerged macrophytes or periphyton. Although these stream sites can become quite turbid during storm events with total suspended solids (TSS) concentrations of several hundred mg/L, routine grab samples collected on a biweekly basis between March 2004 and June 2006 indicated median concentrations of TSS ranging from <4 to 12 mg/L. Although light availability within the water column is an important factor for aquatic plant growth, variations in TSS were not related to periphyton biomass.

In an attempt to relate instream nutrient to periphytic CHLA concentrations, Dodds et al. (2002; 2006) evaluated almost 300 sampling events using a literature data set representing more than 200 rivers in North America and New Zealand. Dodds et al. (2002; 2006) found very weak ($r \leq 0.32$) but significant ($\alpha = 0.05$) correlations between mean periphytic CHLA, as a measure of algal biomass, and instream nutrient concentrations. In earlier work, Dodds et al. (1997) proposed a saturation effect of nutrients on periphytic algae or a lack of further growth response when nutrient concentrations are high. Using breakpoint regression to define when saturation occurred, Dodds et al. (2002; 2006) determined saturation breakpoints for maximum benthic algal biomass concentrations at about 0.06 mg/L total P and 0.60 mg/L total N. For the North Bosque River sites, median stream concentrations were generally at or well above these breakpoints (Table 6). Because total N and total P concentrations were generally above the breakpoints established by Dodds et al. (2002), nutrients do not appear to be the dominant factor limiting periphyton and macrophyte biomass in the North Bosque River under normal conditions, although at times nutrients may be a limiting factor. Minimum total P concentrations at all but three sites were well

below the breakpoint of 0.06 mg/L total P and only two sites indicated minimum total N concentrations greater than the total N breakpoint of 0.60 mg/L (Table 6).

Earlier work in the North Bosque River focusing on nutrient limitation of periphytic algae using instream periphytometers indicates the potential for nitrogen limitation at some locations in the upper third of the watershed and phosphorus limitation at locations in the lower portion of the watershed (McFarland et al. 2004). In this work (McFarland et al. 2004), dissolved inorganic nitrogen (DIN) concentrations rather than total N concentrations appear to be more strongly related to growth potential when nitrogen limitation was indicated. When nitrogen limitation occurred, DIN concentrations were 0.04 mg/L or less, while total N concentrations averaged 0.45 mg/L with only one observation of 0.74 mg/L greater than the total N breakpoint of 0.60 mg/L determined by Dodds et al. (2006). Minimum DIN concentrations (data not shown) from grab samples between March 2004 and June 2005 for several of the North Bosque River sites were below 0.04 mg/L. Most likely periphytic algae were opportunistic to changing water quality conditions and responded to changes in nutrient concentrations when other factors, such as light, were not limiting.

Besides nutrient and light limitation, periphyton are expected to respond to flow conditions. Low to moderate levels of flow help in supplying a continuous, although often varying, source of nutrients to locations along a stream. Losses of periphyton, and to a lesser degree macrophytes, may result from scouring associated with elevated flows from large rainfall runoff events. These scouring events can reduce stream periphytic algal biomass even when nutrient concentrations are high, causing a disconnect between nutrient concentrations and biomass levels.

Although scouring is known to reduce periphytic algal biomass, macrophytes are generally more resistant to scouring events because their roots are embedded into the streambed substrate

unlike periphytic algae, which are attached to the substrate surface. For sites with continuous flow data, the abundance of macrophytes and periphyton from each sampling event was overlaid to see if an impact from scouring events could be visually observed. While it appeared that there was some impact due to elevated flows between sampling events, a clear pattern could not be construed. The fairly long time periods between sampling events may have allowed regrowth to occur after a potential scouring event prior to the next sampling. Also, macrophyte and periphytic algal biomass indicated some seasonality with the lowest biomass levels most often occurring during the January 2005 sampling events. For periphyton, there was often a noted decrease in the summer months that is probably temperature related. *Cladophora* often dies off in midsummer when water temperatures increase above about 25°C (Dodds & Gudder 1992).

Summary and Conclusions

A survey of macrophytes and periphyton biomass at 11 locations within the North Bosque River system indicated a wide range of spatial variation over the two-year study. Instream nutrient concentrations did not appear to be the primary factor influencing the abundance of periphytic algae and macrophytes, because saturated rather than limiting nutrient conditions generally occurred along the river for both N and P. Other factors, such as light limitation, scouring, and seasonality, were likely more influential than nutrient concentrations in determining the biomass measured at any specific point in time. Trophic status as determined by measurements of phytoplankton differed greatly from trophic status as determined using measurements of periphyton indicating a need to evaluate eutrophication from more than one perspective. Marcrophytes and periphytic algae represent important and dynamic sinks as well as sources of nutrients within the North Bosque River system that need to be considered in evaluating water quality problems associated with aesthetics and eutrophication. Such efforts are taking place as indicated by EPA proposed stream nutrient criteria (EPA 2000), but more work is needed.

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DISTRIBUTION OF THE BANTAM SUNFISH, *LEPOMIS SYMMETRICUS* (PERCIFORMES: CENTRARCHIDAE), IN ARKANSAS

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Abstract.–This study reports 77 new geographic and 15 new county records for the bantam sunfish, *Lepomis symmetricus*, collected between May 1988 and June 2007 in 30 counties of Arkansas. Detailed distributional data are included as well as an updated map showing the overall distribution of this fish in the state. Bantam sunfish were collected from all major drainages within the Gulf Coastal Plain and Mississippi Alluvial Plain physiographic provinces of Arkansas, including the Arkansas, Ouachita, Mississippi, Red, Saline, St. Francis and White rivers. Based on these new collections and historical records of the past, it is believed that populations of *L. symmetricus* in Arkansas are stable and do not warrant a protected legal status.

The bantam sunfish, *Lepomis symmetricus* Forbes is a very small (< 9.0 cm) nongame centrarchid that ranges in the former Mississippi Embayment from southern Illinois to the Gulf Coast, including Gulf Slope drainages from Bay St. Louis, Mississippi, west to the Colorado River, Texas, and McCurtain County, Oklahoma (Smith 1979; Burr 1980; Page & Burr 1991; Miller & Robison 2004). The species inhabits shallow waters of lowland swamps, oxbow lakes and sluggish backwaters of creeks and bayous almost always in association with aquatic vegetation, submerged logs, and stumps over a detritus and muddy bottom (Burr 1977; Robison & Buchanan 1988). In Arkansas, this fish is reported to be more common in the southern part of the state than in the east, restricted to the Coastal Plain lowlands of all major drainages (Robison & Buchanan 1988).

Black (1940) recorded the first occurrence of *L. symmetricus* in Arkansas based on collections from Monroe County (Maple

Slough, 3.2 km east of Brinkley) and St. Francis County (relief off the L'Anguille River, 1.6 km northeast of Palestine). Collections including bantam sunfish in Arkansas were so few that Buchanan (1974) treated the species as having an "undetermined status," although he suggested that the species was "possibly endangered in Arkansas."

Since then, Robison (1975) added three more records including Big Cornie Creek in Columbia County, a site in the Saline River drainage of Bradley County, and another from the Ouachita River backwater in Calhoun County. Loe (1983) provided two records of *L. symmetricus* from Nevada County. Robison & Buchanan (1988) provided 26 collection sites in all major drainages in Arkansas for the period 1960 to 1987, and two additional pre-1960 sites. Additional records for *L. symmetricus* were provided by Buchanan et al. (2003) who reported the species was uncommon in the Red River (upstream and downstream from the mouth of the Little River) of extreme southwestern Arkansas. The most recent records are those of Buchanan (2005) who used rotenone sampling to collect 180 bantam sunfish from six Arkansas reservoirs, including Champagnolle Creek, Dierks, Erling, Merrisach, Millwood, and Pool 2 of the Arkansas River.

Methods and Materials

To update the status of this uncommon sunfish this study sampled streams throughout Arkansas and mapped museum collection data. Between May 1988 and June 2007 bantam sunfish were collected with standard nylon seines (1.8 by 0.5 m and 2.7 by 0.5 m of 3.2 mm mesh) or dipnets. Fishes were preserved in 10% formalin and later transferred to 45% isopropanol. Specimens were field identified, verified in the laboratory, and vouchers deposited in the collections at Southern Arkansas University, Magnolia, Arkansas (SAU), the University of Louisiana-Monroe Museum of Natural History, Monroe, Louisiana (NLU), Henderson State University (HSU), the North Carolina State Museum of Natural Sciences, Raleigh, North Carolina (NCSM), and the University of

Arkansas at Fort Smith, Fort Smith, Arkansas (UA-FS). Detailed data provided on the 77 new sites are as follows: (county, specific locality [township, section, and range when available], date, museum accession number (if known), and number of specimens in parentheses).

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

ARKANSAS COUNTY (n = 14): Cox Cypress Lake, 16.1 km N of Bayou Meto (Sec. 2, T5S, R6W). 4 May 2001. NLU 77315 (2); Arkansas River, Merrisach Lake Park boat dock ramp, River Mile 14.47 (Sec. 6, T8S, R2W). 22 June 2004. NLU 78535 (4); Unnamed creek, 3.2 km N of St. Hwy. 165 (Sec. 25/26, T4S, R3W). 9 October 1996. NLU 74717 (3); Little LaGrue Bayou, 6.4 km NE of Almyra, upstream of weir (Sec. 12, T3S, R4W). 5 September 1996. NLU 74659, 74817 (8).

ASHLEY COUNTY (n = 27)**:** Hanks Creek, 0.3 km W of St. Hwy. 81 on U.S. Hwy. 82 (Sec. 28, T18S, R7W). 24 March 1989 and 9 May 1991. NLU 62310, 62315, 65393 (15); Hanks Creek at U.S. Hwy. 82W, 3.2 km S of Hamburg (Sec. 28, T18S, R7W). 29 April 1990. NLU 64413 (2); Hank's Creek, 1.3 km W of St. Hwy. 81 on U.S. Hwy. 82. 15 June 1993. SAU (4); Thompson Creek, 11.3 km NW of Crossett (Sec. 11, T17S, R9W). 5 July 1996. HSU 1343 (3); Bearhouse Creek at gravel road NE of Snyder (Sec. 22, T16S, R5W). 21 April 2000. SAU (1). Unnamed tributary to Chemin-A-Haut Creek at St. Hwy. 52 (Sec.19, T18S, R7W). 21 April 2000. SAU (2 juveniles).

BRADLEY COUNTY (n = 3)**:** Moro Creek at Moro Bay State Park (Sec. 21, T16S, R12W). 10 June 2005. SAU (1); L'Aigle Creek at county road, 14.5 km S Hermitage (Sec. 18, T16S, R10W). 10 June 2005. SAU (2).

CALHOUN COUNTY (n = 10)**:** Locust Bayou at St. Hwy. 278, 14.5 km E of Camden (Sec. 30, T13S, R15W). 2 May 1990. SAU (1); Locust Bayou at St. Hwy. 278, 14.5 km E of Camden (Sec. 30, T13S, R15W). 1 June 2003. NCSM 36205 (1); Moro Creek at St. Hwy. 160 (Sec. 9, T16S, R12W). 18 June 2007. SAU (5); Champagnolle Creek at co. road (Sec. 27, T15S, R14W). 18 June 2007. SAU (3).

CLARK COUNTY* (n = 8): Tupelo Creek at St. Hwy. 51, 3.2 km E of Arkadelphia. 13 March 1996. UA-FS (6); McNeeley Creek, 6.4 km S Beirne off St. Hwy. 51 (Sec. 31, T10S, R20W). 20 April 1997. HSU 2154 (1); Gurdon Lake. 17 August 2000. UA-FS (1).

CLEVELAND COUNTY (n = 2): Saline River at TAR (Sec. 17, T11S, R9W). 7 July 1991. SAU (1); Big Creek at St. Hwy. 114 (Sec. 10, T9S, R10W). 2 May 1996. SAU (1).

CHICOT COUNTY* (n = 2): Overflow area E of levee on Mississippi River at end of St. Hwy. 144 (Sec. 21, T15S, R1E). 18 June 2002. SAU (2).

COLUMBIA COUNTY (n = 9): Dorcheat Bayou at county road, 4.8 km SW of Philadelphia (Sec. 16, T18S, R22W). 16 September 1989. SAU (7 juveniles); Dorcheat Bayou at St. Hwy. 160, 6.4 km E of Taylor (Sec. 9, T19S, R22W). 19 May 2004. SAU (1); Horsehead Creek at U.S. Hwy. 79, 9.7 km E of Magnolia (Sec. 17, T18S, R20W). 14 May 2005. SAU (1).

DALLAS COUNTY* (n = 7): East Tulip Creek at St. Hwy. 8 at Princeton (Sec. 34, T8S, R15W). 13 March 1996. SAU (5); Unnamed tributary to Freeo Creek at Ramsey (Sec. 5, T10S, R14W). 29 April 2003. SAU (2).

DESHA COUNTY* (n = 1): Canal 19 at St. Hwy. 138, E of Winchester (Sec. 6, T11S, R3W). 27 August 2003. NLU 78310 (1).

DREW COUNTY (*n* = 5): Cut-Off Creek at St. Hwy. 35, 1.1 km E of Collins (Sec. 31, T13S, R4W). 8 April 1994. SAU (2); Bayou Bartholomew at St. Hwy. 35, 6.4 km E Collins (Sec. 28, T13S, R4W). 28 September 1999. SAU (3).

HEMPSTEAD COUNTY (*n* = 5): Millwood Lake at Saratoga Landing, 0.8 km W of Saratoga (Sec. 6, T12S, R27W). 10 August 1990. SAU (5).

HOT SPRING COUNTY (*n* = 1): Saline Bayou, 4.0 km S Friendship (Sec. 23, T6S, R19W). 14 February 1997. HSU 2154 (1).

HOWARD COUNTY* (*n* = 3): Saline River at county road, 3.2 km W of Schaal (Sec. 10, T11S, R28W). 12 June 2000. SAU (1); Mine Creek at county road, 1.6 km W of Tollette (Sec. 6, T11S, R29W). 11 August 1990. SAU (2).

JEFFERSON COUNTY (*n* = 3): Yellow Lake on Pine Bluff Arsenal (Sec. 2, T5S, R10W). 1 July 1999. SAU (2); Bayou Meto WMA, Wrape Plantation, roadside pool (Sec. 6, T6S, R5W). 8 May 2002. NLU 77306 (1).

LAFAYETTE COUNTY (*n* = 8): Bodcau Creek at county road, 1.6 km N Lewisville (Sec. 7, T15S, R23W). 16 March 1990 and 5 July 1992. SAU (2); Lake Erling at St. Hwy. 160 (Sec. 35, T19S, R23W). 24 September 1991. SAU (6).

LINCOLN COUNTY* (*n* = 65): Bayou Bartholomew at Garrett Bridge (Sec. 6, T10S, R5W). 3 June 1989. SAU (3); Oakwood Bayou at St. Hwy. 212, 8.0 km E of government road (Sec. 7, T9S, R7W). 17 April 2003. NLU 77785 (60); Bayou Bartholomew, 6.4 km N of Star City (Sec. 21, T8S, R7W). 7 July 2003. SAU (1); Bayou Bartholomew at St. Hwy. 293 (Sec. 15, T9S, R6W). 7 July 2003. SAU (1).

LITTLE RIVER COUNTY (n = 13): Little River Relief at St. Hwy. 41, 6.6 km SW of Horatio (Sec. 10, T10S, R32W). 25 May 1988. NCSM 37422 (5); Cypress Creek at St. Hwy. 234 in Winthrop (Sec. 7, T11S, R31W). 6 June 1989. SAU (1 juvenile); Flat Creek at St. Hwy. 234, 3.2 km E of Winthrop (Sec. 21, T11S, R31W). 10 July 1990. SAU (1 juvenile); Caney Creek at St. Hwy. 41, 4.0 km SW of Billingsley's Corner (Sec. 28, T10S, R32W). 1 March 1992. SAU (2); Little River backwater at U.S. Hwy. 71, 3.2 km N of Wilton (Sec. 24, T11S, R29W). 5 October 2001. SAU (4).

LONOKE COUNTY* (n = 56): Indian Bayou at Tomberlin (Secs. 21/28, T2S, R8W). 14 July 2000. NLU 76916 (55); Arkansas River at River Mile 104, Willow Bar Cutoff off U. S. Hwy. 165, 0.4 km NW of Pilgrims Rest Church (Sec. 21, T2S, R9W). 13 July 2000. NLU 76772 (1).

MILLER COUNTY (n = 200): Mercer Bayou ramp off County Road 109, above lower structure (Sec. 19, T18S, R27W). 25 May 1988. NLU 75772 (3); Kelly Bayou at county road, 6.4 km SW of Doddridge (Sec. 9, T20S, R27W). 3 September 1990. SAU (1); West Fork Kelly Bayou at county road, 1.6 km S of Brightstar (Sec. 28, T18S, R28W). 3 September 1990. SAU (1). Mercer Bayou at gated structure, 7.6 km W of Beggy (Sec. 2, T18S, R28W). 24 May 1999. NLU 75765 (3); Upper Mercer Bayou, bendway between ramp and gate (Sec. 10, T18S, R28W). 25 May 1999. NLU 75785 (6); Middle Mercer Bayou, upper lateral tributary (Sec. 10, T18S, R28W). 25 May 1999. NLU 75791 (6); Lower reach of Mercer Bayou, ramp at County Road 109 (Sec. 30, T18S, R17W). 4 August 1999. NLU 75825 (4); Mercer Bayou, upper reach at gated structure, 7.6 km west of Beggy (Sec. 2, T18S, R28W). 11 August 1999. NLU 75811 (34); Mercer Bayou, middle reach at lateral trib. (Sec. 2, T18S, R28W). 11 August 1999. NLU 75820 (30); Mercer Bayou, upper reach at bendway between gate structure and ramp (Sec. 10, T18S, R28W). 11 August 1999. NLU 75815 (112).

MISSISSIPPI COUNTY* (*n* = 72): Mississippi River bar pit No. 17, 0.8 km E of Wilson (Sec. 22, T11N, R10E). 4 August 1996 and 4 August 1997. NLU 74322, 74329 (10, 62).

NEVADA COUNTY* (*n* = 27): Middle Creek, 14.5 km N of Prescott on St. Hwy. 19 (Sec. 27, T9S, R23W). 19 February 1983. NLU (1); Caney Creek, 4.8 km N of Bluff City on St. Hwy. 24 (Sec. 22, T11S, R20W). 19 February 1983. NLU (26).

OUACHITA COUNTY (*n* = 5): En Core Fabre Bayou, 1.6 km N of Camden (Sec. 10, T13S, R17W). 22 August 1994. SAU (2); Bragg Lake, 2.0 km SE Bragg City at St. Hwy. 24 (Sec. 33, T12S, R18W). 12 April 1997. HSU 2055 (2); Freeo Creek at St. Hwy. 9, 8.9 km S of Dallas County line (Sec. 36, T11S, R16W). HSU 2184 (1).

PHILLIPS COUNTY* (*n* = 8): Mississippi River borrow pits off St. Hwy. 20, 11.3 km S of Helena (Sec. 35, T3S, R4E). 21 July 1997. NLU 74145 (8).

PIKE COUNTY* (*n* = 1): Unnamed slough, 1.6 km E jct. St. Hwys. 195 and 301 (Sec. 13, T9S, R24W). 6 April 1997. HSU 1557 (1).

PRAIRIE COUNTY* (*n* = 3): Unnamed creek, 4.0 km W of Seidenstricker (Sec. 1 and 2, T1N, R6W). 4 September 1996. NLU 74630 (1); Hooks Canal at St. Hwy. 11, 4.8 km NE of Slovak (Sec. 20, T1N, R5W). 6 September 1996. NLU 74601 (1); South Fork, 2.4 km E of Hazen at U.S. Hwy. 70 (Sec. 21, T2N, R5W). 10 October 1996. NLU 74727 (1).

PULASKI COUNTY (*n* = 3): Backwater area along St. Hwy. 365, 0.8 km N of Woodson (Sec. 8, T2S, R11W). 10 July 2005. SAU (3).

SEVIER COUNTY (*n* = 6): Rolling Fork River at St. Hwy. 24, 4.8 km W of Horatio (Sec. 28, T9S, R32W). 8 October 1989. SAU (3); Millwood Lake, Paraloma Landing at end of St. Hwy. 234 (Sec. 29, T11S, R28W). 18 June 2002. SAU (2); Little River at end of St. Hwy. 317, 4.8 km S of Brownstown (Sec. 25, T11S, R29W). 19 June 2002. SAU (1).

UNION COUNTY* (*n* = 6): Bear Creek at St. Hwy. 160, 8.0 km NE Mount Holly (Sec. 21, T16S, R17W). 17 March 1989. SAU (1); Tributary of Ward Creek at U.S. Hwy. 82, E of El Dorado (Sec. 15, T18S, R14W). 29 October 2000. NLU 77006 (2); unnamed tributary to Big Creek at U.S. Hwy. 82, 6.4 km W El Dorado (Sec. 30, T17S, R16W). 26 February 2002. SAU (1); Big Cornie Creek at St. Hwy. 15 (Sec. 35, T19S, R18W). 3 March 2002. SAU (1); Lapoile Creek at U.S. Hwy. 82, 16.1 km NE Strong (Sec. 18, T18S, R10W). 7 April 2003. SAU (1).

WOODRUFF COUNTY* (*n* = 3): Bayou DeView at St. Hwy. 38, 1.6 km E of Cotton Plant (Sec. 33, T5N, R2W). 29 October 2001. NLU 78209 (3).

Results And Discussion

This study documents the collection of 576 (10 juvenile, 566 adult) *L. symmetricus* from 30 of 75 counties (40%) of Arkansas (Fig. 1). Of those, 176 specimens (31%) were taken on 11 August 1999 from Mercer Bayou in Miller County. The second largest collection, 62 *L. symmetricus* or 11% of the total were collected on 4 August 1997 from a Mississippi River borrow pit in Mississippi County. Loe (1983), in his unpublished thesis, also collected 27 specimens of *L. symmetricus* from Nevada County not included in Robison and Buchanan (1988).

Bantam sunfish were collected from all major drainages within the Gulf Coastal Plain (GCP) and Mississippi Alluvial Plain (MAP) physiographic provinces of Arkansas, including the Arkansas, Ouachita, Mississippi, Red, Saline, St. Francis, and White rivers.

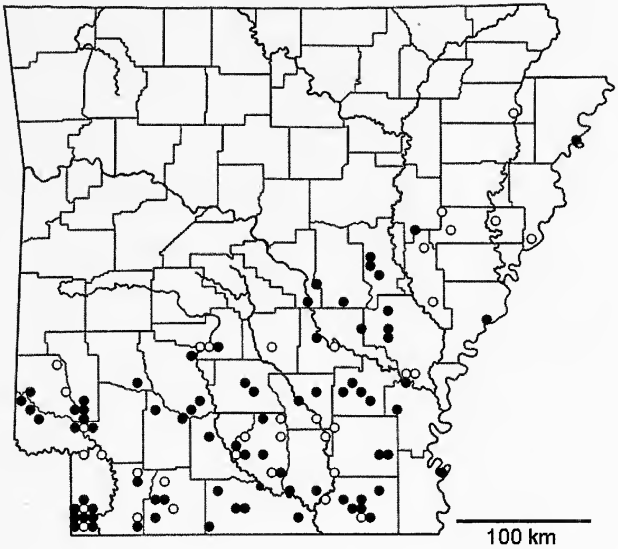

Figure 1. Records of the bantam sunfish (*L. symmetricus*) in Arkansas. Open circles (○) are older records referenced herein; closed circles (●) are current records.

Compared with the records in Robison & Buchanan (1988), this study adds 15 new county records of *L. symmetricus*, including the northeasternmost, southeasternmost, and southwesternmost range extensions in the state for Mississippi, Chicot, and Little River counties, respectively. Further, numerous other collections of the species are noted from new localities in counties with historical records. All specimens originated from habitats within the GCP and MAP, although several collections were taken near the interface of the GCP and the Ouachita Mountains uplift. Because collections presently indicate that *L. symmetricus* is more widespread and common than was previously known, the authors believe that its populations in Arkansas are stable and do not warrant a protected legal status.

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A POSITIVE CONTROL FOR DETECTING HETERODUPLEXES IN DGGE FOR MICROBIAL COMMUNITY FINGERPRINTING

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Abstract.–Denaturing gradient gel electrophoresis in combination with PCR has found a wide application for analysis of genetic variants in many disciplines of bioscience, especially evaluation of microbial diversity in environmental samples. However, amplification of an environmental sample containing multiple DNA species can lead to formation of heteroduplexes (HDs), a PCR artifact. Appearance of HDs on the denaturing gradient gel can lead to a skewed interpretation of results and inaccurate conclusions. The present study is designed to develop a simple methodology to verify presence or absence of heteroduplexes in PCR samples which in conjunction with 'reconditioned' PCR can be used as an effective control to indicate presence and positions of HDs during DGGE analysis. Identification of HDs can allow effective design of reconditioned PCR by varying template and primer amounts and PCR cycle number.

Denaturing gradient gel electrophoresis (DGGE) is a powerful technique for separation of double-stranded DNA molecules of nearly identical size according to their nucleotide composition. This technique has a wide range of applications, from detection of point mutations to evaluation of microbial community diversity. DGGE exploits the phenomenon of discrete melting behavior of double stranded DNA. PCR-amplified fragments are separated during electrophoresis according to their melting behavior on a polyacrylamide gel with a linearly increasing denaturant gradient. The resulting DGGE band patterns reflect sequence diversity in a given sample. However, during the final PCR cycles when the concentration of primers is low, heteroduplexes (HDs) can form. HDs result in "phantom bands" on the denaturing gradient gel and often are erroneously interpreted as additional DNA species or sequence variants (Acinas et al. 2005). This can lead to overestimation of sequence population diversity. Cloning of PCR products from a mixed DNA template sample can exacerbate the

problem. The non-directed mismatch repair system of *E. coli* generates a number of novel sequences from a single HD clone (Ruano 1992; Qiu et al. 2001). Other screening methodologies which utilize PCR products such as RFLP and Thermal Gradient Gel Electrophoresis (TGGE) are prone to the same problem (Osborn & Moore 2000).

In the past there were a number of reports of PCR artifacts pertaining to DGGE. With the use of constant denaturing capillary electrophoresis, formation of HDs was demonstrated and a simple "reconditioning PCR" suggested (Jensen 1993; Thompson & Marcelino 2002) to reduce the number of HDs produced. In another study to eliminate HDs, samples were treated by T7 endonuclease (Qiu et al. 2001). Surprisingly, a recent literature search revealed neither evidence of research being conducted according to suggested improvements nor a general awareness of the problem within the DGGE community. This is probably due to a fact that in spite of recommended methods, researchers lack control measures to judge the absence or presence of HDs. Therefore, this study attempts to identify the presence and positions of HDs on DGGE gels and suggest a simple methodology which can serve as a positive control to indicate the presence of HDs on DGGE gels.

Material and Methods

DNA extraction and amplification.–Three groups of samples were prepared from 119bp fragments of 18S rDNA from the protistian parasites *Eimeria tenella* (A), *Eimeria maxima* (B) and *Eimeria acervulina* (C). Group I contained seven mixes: A only, B only, C only, AB, AC, BC and ABC. Group II contained group I mixes which were subjected to heating to 92°C and then allowed to cool to room temperature. The heating treatment was done in order to mimic one denaturing step of the PCR cycle in absence of primers. Group III was prepared by PCR (50 µl final volume) using group I mixes as templates (0.5 µl) with *Eimeria* species-specific primers (forward primer containing GC-clamp 5'-

GCCCGCCGCGCCCGCGCCCGTCCGCCGCCCCCGCCCGGATT AGATACAAAACCAACCC-3', and reverse primer 5'-GCTGATA GGTCAGAAACTTG-3', 0.8 ng/µl final concentration for both primers). The amplification process was carried out using 25 µl of JumpStart™ REDTaq® ReadyMix™ PCR Reaction Mix (Sigma Chemical Co., St. Louis, MO) in a Mastercycler (Eppendorf, Scientific Inc., Westbury, NY) according to the following program: initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 92°C for 45 s, annealing at 60°C for 35 s, and an extension at 72°C for 2 min. The final extension was performed at 72°C for 7 min. To demonstrate formation of HDs as a function of PCR cycles, a sample containing all three fragments (ABC), was amplified using the same set of primers and amplification conditions as described above but with a reduced number of cycles (20, 16, 15, 14, 13 and 12 cycles). All PCR products were purified with a Wizard PCR purification kit (Promega, Inc., Madison, WI) and run on a 1% agarose gel to check for quality of amplification.

DNA extraction and amplification from a field sample.–Fecal field samples from a poultry farm located in east Texas were collected and used for DNA extraction. The birds were infected with several species of *Eimeria*. The extractions were carried out using the QIAamp DNA Stool Mini Kit QIAGEN (QIAGEN, Valencia, CA). The extracted DNA (0.5ul) was used as a template for PCR reactions with the same set of primers and PCR conditions as described above. The product of the PCR reaction (0.5ul) was used as a template to carry out three cycles of reconditioned PCR. One field sample was used to carry out a series of PCR reactions with an increasing number of cycles from 11 to 23.

DGGE analysis.–Polyacrylamide gels (8%, 0.75mm thick) were prepared using a 35-45% gradient of urea-formamide mix (Myers & Maniatis 1987). Electrophoresis was conducted in 1×TAE buffer at 60°C using a DGGE-2001 apparatus (CBS Scientific Co., Del Mar, CA). After electrophoresis, the gels were stained with SYBR Green I and subsequently photographed using a BioRad Imager

System equipped with a Gel Doc XR camera and Quantity-One software (BioRad Inc., Hercules, CA).

RESULTS

Formation of HDs.–We demonstrated the formation of HDs using a simple system on denaturing gradient (DG) gel. The model system contained the PCR products from the single A, B, and C fragments and their mixture ABC which we loaded on the DG gel (Group I) to show that no heteroduplexes were present in the original single template PCR products or in their mixtures. The heat-treated samples (Group II) along with the PCR products (Group III) were also loaded on the gel (Figure 1). As revealed by DGGE analysis, the PCR products from mixtures BC, AC, and AB contained in addition to the expected two original bands, two HD bands located higher on the gel than their parental bands (Group III, Figure 1). In the same group, the PCR product of the ABC mixture template showed five bands in addition to the three parental bands (Six HD bands were expected, however due to co-migration only five bands were visible). The HD bands represent HDs derived from all possible matches of the parental single strands. By matching the band patterns from the dual mixture samples (Group III) the origin of all the HD bands can be traced back to their corresponding parental bands. Group II samples which included the heat treated mixtures (ABC, BC, AC, and AB) showed striking similarity to their mixed template PCR counterparts from Group III. Moreover, by comparing bands from Group I and Group II or III it is possible to clearly identify HDs by a simple elimination of common bands.

Elimination of HDs.–The presence of identical HDs in Group II and Group III as shown on Figure 1 leads to a conclusion that a single denaturation step in absence of primers is sufficient for detection of HDs. After the denaturation step during the last cycles of PCR when the primer:template ratio is low, single strands can rehybridize and form all possible combinations of hetero- and homoduplexes (Ruano 1992; Jensen 1993). To determine the first

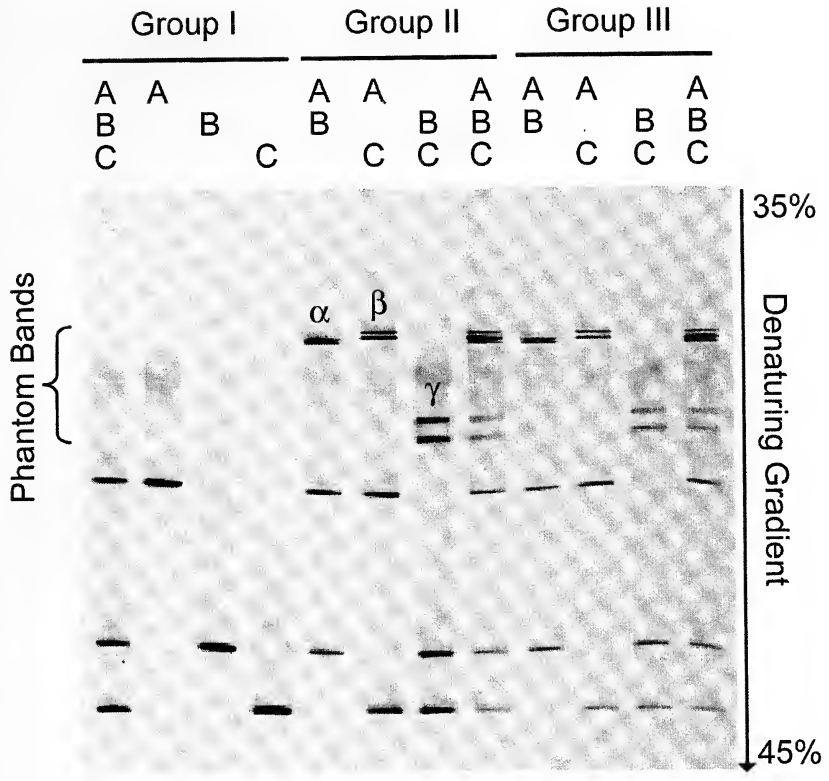

Figure 1. Formation of HD bands by PCR and heat treatment. Group I contained amplicons from single template PCR products and their mixture. Group II represents Group I samples which were subjected to heat treatment at 92°C and then cooled to room temperature. Group III samples contained PCR products obtained from mixed templates. Patterns of HD migration: α - co-migration (HDs migrate as a single band), β - double-band migration (HDs migrate close to each other), γ - separate migration (HDs migrate as two separate bands).

PCR cycle when HDs can be detected, PCR products of the ABC mixture obtained from six amplification reactions with decreasing number of cycles were analyzed by DGGE (Figure 2). Loading Set I contained equal volumes of PCR products from every reaction. Loading Set II contained adjusted volumes of the same set of PCR products as in Loading Set I. The volumes were adjusted to emphasize that the disappearance of the HD bands was due to reduction of PCR cycles and not due to a lower amount of PCR product. Therefore, volumes of samples with less PCR product

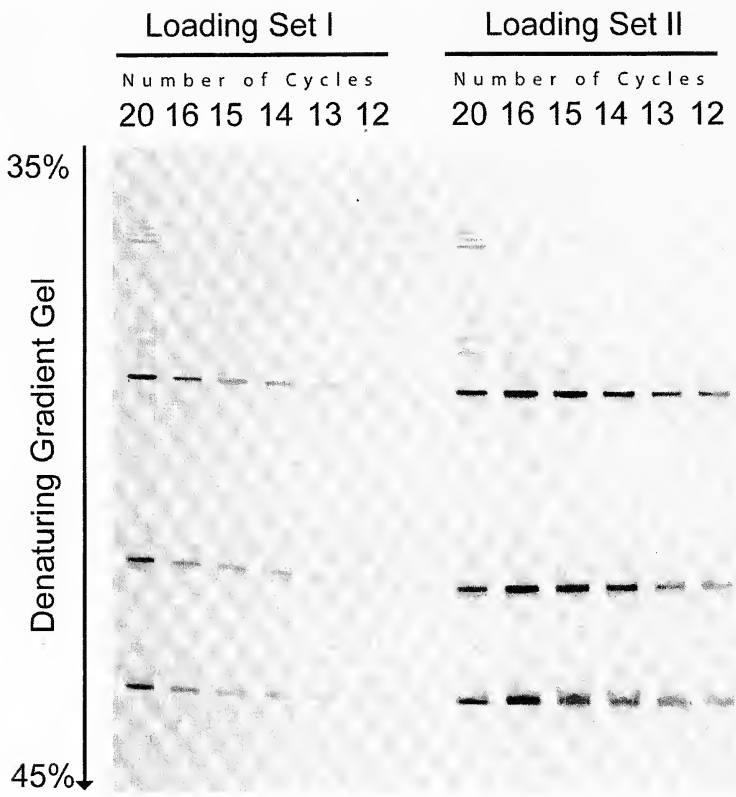

Figure 2. Elimination of HD bands by reduction of PCR cycles. PCR product from a mixture of ABC loaded on a DG gel in two groups. Group I contained equal volumes of all the PCR products (2.5 µl). To confirm the absence of HD bands in some samples, the volumes of samples in Group II were adjusted to maximize the volume of the loaded sample and to be inversely proportional to the PCR cycle number.

were increased to confirm the absence of HDs. Detection of HDs at different PCR cycles is complicated by different parameters such as PCR efficiency and initial copy number of template DNA therefore the proposed control measure is required to judge the presence, absence and position of HDs.

Environmental sample.–Elimination of HDs by reduction of PCR cycles was tested using a field sample of unknown template composition. A series of PCR reactions was performed with reduced numbers of cycles from 22 to 11 (Figure 3). Sixteen cycles

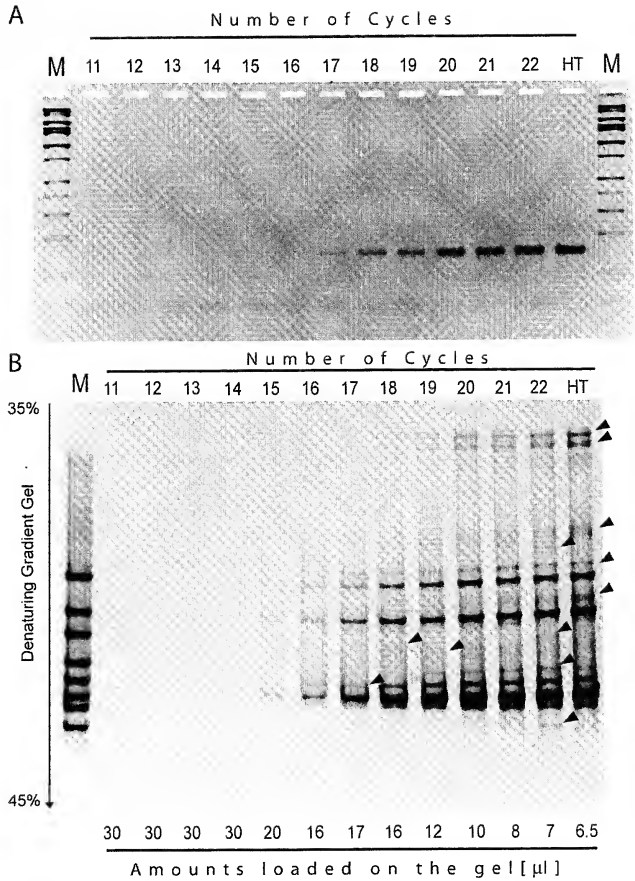

Figure 3. Elimination of HD bands in a field sample by cycle reduction: (a) Two percent agarose gel of PCR products obtained with different cycle numbers using field sample DNA extract. The gel was stained in ethidium bromide. Each well contains 2 µl of PCR product. (b) Parallel denaturing gradient gel of the same samples as in Figure 3a. Volumes of samples resulting from fewer PCR cycles were increased to demonstrate absence of heteroduplexes. The last lane (HT) contained a heat-treated PCR product. Heteroduplexes are marked with arrows.

were required to reach the detection limit on agarose gel (Figure 3, A). The DGGE analysis of the corresponding reactions demonstrated the gradual appearance of HDs in the upper part of the gel (Figure 3, B). No HD bands were detected before the 18th cycle. Thus, a controlled reduction in the number of PCR cycles

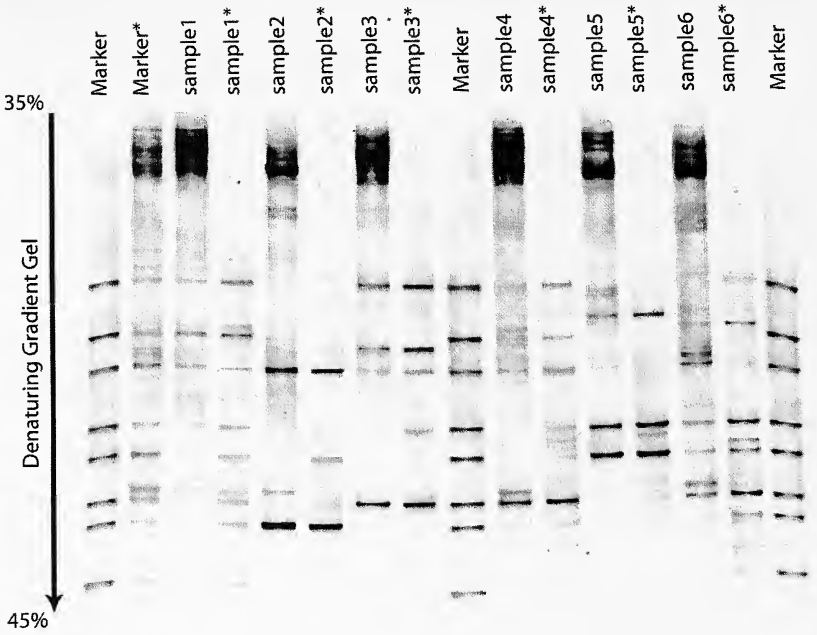

Figure 4. Elimination of HD bands in field samples by reconditioned PCR: Parallel denaturing gradient gel was loaded with six heat treated samples and their respective PCR reconditioned samples. Reconditioned samples are marked with an asterisk. HDs can be identified by comparing the heat treated samples with the adjacent reconditioned sample lanes. Reconditioned PCR was performed by running another three cycles of PCR using μl of original PCR product as template.

demonstrates formation of HDs as a function of PCR cycles. However, elimination of HDs by the PCR cycle reduction strategy is not practical for a large number of samples.

Elimination of HDs by PCR reconditioning carried out with six field samples (Figure 4) showed a marked decrease in number of bands in reconditioned samples. Most of the HDs as expected were found in the upper part of the gel, however several were found in the lower part. In addition, we noted several bands in the lower part of the gel which were present in the reconditioned samples and absent in the heat treated samples pointing out that any given DNA species can disappear by forming HDs.

Discussion

The ability of denatured nucleic acid molecules to renature or hybridize forms the basis for numerous methods and applications including southern blot, northern blot and PCR. This same property, if not accounted for, can lead to the misinterpretation of results (Thompson & Marcelino 2002). In the present study using DGGE, clear evidence was obtained of quantitative and qualitative parameters that effect HD formation. These are not the artifactual bands which can form during the late PCR cycles in a single template containing samples due to secondary structure formations (Janse et al. 2004).

This study demonstrates by using a simple model system where mixtures of double stranded homologous molecules were subjected to heating and cooling, that any two homoduplexes can form heteroduplexes in a single step. For instance A^-A^+, where A^- designates the antisense and A^+ the sense strand of DNA respectively, and B^-B^+ require a single step of denaturation-renaturation to produce two HDs A^-B^+ and A^+B^- (Group II, Figure 1). Quantitatively, the number of possible hybrids will depend on the number of original homoduplex molecules present in the mixture. The maximum number of possible HDs can be calculated according to the formula: $N_{ht}=N_{hm}(N_{hm}-1)$ where N_{ht} is the number of HDs and N_{hm} is the number of homo-duplexes. Thus, two homoduplex species during rehybridization will generate two additional heteromolecules; three will produce six, four - twelve and so on. Qualitatively, the structure of these hybrids as compared to the structure of homoduplexes has shape-distorting mis-match(es); thus, they melt under milder denaturing conditions and have different rates of migration on the denaturing gradient gel. It is therefore safe to say that HD bands on the denaturing gradient gel will always occur above the parental homoduplex bands. However, in a mixed template sample it is possible to have some homoduplex bands with lower melting temperature than some of the HDs. So, bands appearing on the upper part of the gel cannot always be

discarded as HD bands. Therefore, to identify HDs on the gel use of a positive control is required.

Stability of the new hybrid molecules depends on the GC content of parental sequences and on their similarity. HDs can exhibit three different patterns of migration (Figure 1): (1) Co-migration (HDs migrate as a single band), (2) Double-band migration (HDs migrate close to each other), (3) Separate migration (HDs migrate as two separate bands). Due to the unstable structure of HDs, some may denature without forming a distinct band or may disappear after some time during the electrophoresis and leave smears. However, the number and amount of HDs can be significant and, if co-migrating, they can provide a strong fluorescent signal on a gradient gel and lead to false conclusions regarding total number of sequences and DNA species diversity. Using the A, B and C fragments in all possible combinations as a template for four PCR reactions this study has demonstrated that HD formation occurs in absence of primers during a single denaturation step (Group II, Figure 1).

During the first cycle of PCR, when different but closely related templates with identical priming sites are present, double the number of original homoduplexes is generated. As PCR progresses, the number of primers decreases exponentially at the same rate as the new strands are synthesized. Therefore, each consecutive PCR cycle creates an equilibrium shift from primer-single strand complex formation towards the formation of homo- and heteroduplexes. At a low concentration of primers or in their absence, there is an equal probability of formation of homo or heteroduplexes upon renaturation. The amount of heteromolecules depends entirely on the amount of primers, which are better competitors for hybridization sites. The presence of a GC clamp on one or both primers is required for DGGE resolution and provides favorable conditions for HD formation. As demonstrated in Figure 1 (Group II), a single denaturation-renaturation step is sufficient to form HDs. Such a condition repeatedly occurs during late cycles of PCR and it results in an even redistribution of homo and HDs.

Formation of HDs in multi-template PCR is inevitable and it contributes to the 'C_0t effect' allowing amplification of less dominant DNA species during late cycles of PCR (Mathieu-Daudé et al. 1996).

Several methods have been proposed to reduce or eliminate HDs to obtain a representative picture of DNA species diversity by DGGE analysis. Some of the unstable HDs could be eliminated by increasing denaturant concentration in the gel although this may not always remove all the hybrids (Qiu et al. 2001). Elimination of HDs by the T7 endonuclease I (Lowell 2000) demands strict experimental conditions with respect to enzyme concentration and incubation time which differ from sample to sample and can not be predicted (Qiu et al. 2001). PCR reconditioning (Thompson & Marcelino 2002) can be helpful, but with the limitation that a ten fold increase in primer concentration will only provide enough primers for three additional PCR cycles and a hundred-fold increase would allow for only five additional PCR rounds before depletion of primers. In addition, PCR amplification depends on many factors such as primer efficiency, concentration of inhibitors and initial concentration of the template. Therefore, conditions for reconditioning PCR must be determined empirically. This study proposes a simple positive control which can be used to control for the presence of HDs in a PCR sample containing multiple templates. Heating an aliquot of the individual PCR sample to 96°C followed by an on-bench cooling will generate all possible HDs. By comparing the DGGE band profiles of the original PCR product and the heat treated sample, a researcher can reach a grounded conclusion about the presence, absence and location of HD bands on the gel (Figure 4). Hopefully this finding will help the DGGE community to generate reliable data and make interpretation of DGGE results easier.

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SURVIVORSHIP OF RADIO-TRANSMITTERED URBAN WHITE-WINGED DOVES ON THE EDWARDS PLATEAU, TEXAS

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Abstract.–This study evaluates survivorship of White-winged Doves (*Zenaida asiatica*) with subcutaneously implanted radio-transmitters in Mason, Texas from January to March of 2006. Attempts were made to compare survivorship of transmittered doves (0.70 ± 0.135) with untransmittered doves using mark-recapture methodology in program MARK. No mark-recapture survivorship models had adequate fit for comparisons. Consequently, annual survivorship was estimated from period survivorship data for transmittered doves. This study concluded that the use of radio-transmitters had a detrimental effect on White-winged Dove survival for this particular study.

George et al. (2000) reported highly variable survival rates for White-winged Doves (*Zenaida asiatica*) in Texas and New Mexico from 1960 to 1978 using banding data. However, no recent reliable estimates of survival exist for White-winged Doves, and no comparisons of survival estimation methods have been conducted for this species.

Radio-telemetry has become a frequently used tool for studying doves in the U.S. in recent years (Schulz et al. 1998; 2001; Schaefer et al. 2004; Small et al. 2005; Berdeen & Otis 2006). Captive studies of both Mourning Doves (*Zenaida macroura*) and White-winged Doves indicated subcutaneous implantation of transmitters were the preferred method of attachment (Schulz et al. 2001; Small et al. 2004b). A study of free-ranging Mourning Doves in South Carolina indicated implanted radio-transmitters did not detrimentally affect survival from hunter harvest (Berdeen & Otis 2006). However, doves in that study showed higher survival rates when held 24 h post-implantation prior to release.

This study estimates annual survival for free-ranging White-winged Doves with subcutaneously implanted radio-transmitters, and tests the concept put forth by Guthrie & Lusk (2004) that using radio-transmitters for estimating survivorship may negatively bias estimates. This study provides an evaluation of the efficacy of this transmitter attachment technique in wild White-winged Doves.

All activities were conducted in accordance with Texas State University-San Marcos IACUC approval #06-05CC59736D, state permit #SPR-0890-234, and federal permit #06827.

Methods

Study area.–This study was conducted in Mason, Texas in the Edwards Plateau ecoregion (Gould et al. 1960). Mason encompasses 958.3 ha with a population of about 2,211 (City-data.com 2005). Dominant tree species in Mason include oaks (*Quercus* sp.) and pecan (*Carya illinoinensis*) mixed with ornamentals and an understory of ornamental shrubs and turf grass. Mason is inhabited by a variety of mid-size predators including domestic cats (*Felis domesticus*), raccoons (*Procyon lotor*), opossum (*Dasypus virginianus*), domestic dogs (*Canis familiaris*), and hawks (*Accipiter* sp.). White-winged Doves in Mason occur in significantly higher densities in discreet core areas of town (Schwertner & Johnson 2006). Also, backyard bird feeders are abundant in Mason and are frequently used by White-winged Doves.

Areas of White-winged Dove habitat in Mason were delineated using ArcGIS 9.1 (Environmental Systems Research Institute, Inc., Redlands, CA, USA). The 1992 National Land Cover Data (U.S. Geological Survey 1999) were used to identify areas of urban land cover. These areas were then buffered at 500 m and the resulting polygons defined as White-winged Dove habitat (Schwertner & Johnson 2006), thus designating the study area.

Radio-transmittered doves.–Forty-four White-winged Doves were implanted with subcutaneous radio-transmitters (Advance Telemetry Systems, Isanti, Minnesota) between 13 January and 11 March 2006 following the field implantation procedure described by Small et al. (2004a). Transmitters were 41.83 (±0.05) by 26.83 (±0.05) by 7.62 (±0.05) mm with 15.24 (±0.01) cm antennas and had a mean weight of 3.41 g. (*SE* = 0.01) (<3.0% dove body weight). Transmitters had a range of 1.14 km (±0.02) and an expected battery life of 334 d. Doves were released upon regaining a lucid state.

Transmittered doves were monitored 5 days/week from time of implantation until 15 May 2006 (123 days) over the entire study area. White-winged Doves in Mason are urban obligate nesters and roosters (Schwertner & Johnson 2006). Although searches for doves were conducted during all periods of the day, because the study area was small in relation to transmitter range 2-3 days/week included monitoring radio-transmitter signals in early morning before daylight when the doves were assumed to be stationary. This enabled full coverage of the study area and consequently the conclusion that an individual had left the study area when a signal was not received for ≥5 days. On these occasions it was not possible to determine where the individual had relocated (Small et al. 2006b). There was no reason to believe that any transmitters failed during the course of this study. In two previous radio-telemetry studies with monitoring lasting 120 days (Small et al. 2005) and 96 days (Small et al. 2006a), all transmitters that failed gave off a distinctive whine signal for several days pre-failure, which did not occur during this study. In this study, all transmitters from known predations were found because they continued to transmit a signal (including one transmitter that had passed through the digestive tract of a cat and was embedded in the scat). Consequently, there was no evidence that any transmitters were destroyed by predators.

In the event of mortality, cause of death was determined by observation of predators with the dove or by the condition of the carcass at the transmitter recovery site and the presence of predator scat, fur, or feathers.

Estimated pooled survivorship for transmittered White-winged Doves was determined using Kaplan-Meier product limit estimator (Kaplan & Meier 1958; Pollock et al. 1989; White & Garrott 1990). White-winged Doves were categorized as dead on the day of transmitter recovery. Because the fate of missing doves was unknown they were right censured on the last day a signal was received. The Kaplan-Meier method bases survivorship estimates on survival days only. Consequently, all individuals which die or leave the study (regardless of their fate) are treated the same and right-censored. Analyzing the data without including those individuals of unknown fate (i.e., those believed to have left the study area) would be a misuse of the technique and provide erroneous results.

Survivorship estimates were obtained by extrapolating period survival to annual survival. While this requires the typically unreasonable assumption of equal survivorship across periods, there is sufficient natural history information available on White-winged Doves to validate the method (see discussion). Consequently, mean daily survival rate (S_d) was estimated by multiplying estimated survivorship for the period being monitored (S_{Pr}) by the number of days (t) in the sample period (in this case 123 days). Thus the mean daily survival estimate was:

$$S_d = (S_{Pr})(t).$$

Annual survival rate (S_a) was then estimated using the formula

$$S_a = (S_d)/(365),$$

the probability of surviving one year (365 days).

These data were assessed for demographic plausibility by calculating the demographic age ratio (R) for juvenile to adults required for population stability (finite growth multiplier = 1.0) (Guthrey 2002; Guthery & Rusk 2004). In other words, given the estimated annual survivorship, how many young would have to be produced in order to maintain a constant population size? This can be determined from the following formula,

$$R = (1/S_a)\text{-}1,$$

where R refers to the number of young needed to be produced to maintain the population size, $(1/S_a)$ represents the proportion which survive one year. By subtracting the proportion expected to survive one year from 1.00, the number of young needed to be recruited into the population can be estimated.

An $R = 2$ (2 young produced/adult) value was considered conservatively plausible and $2 < R \leq 4$ was considered possible but unlikely for telemetry derived survival rates (Small et al. 2005; 2006b). Values > 4 were considered physiologically unrealistic.

Mark-recapture.–In addition to monitoring transmittered White-winged Doves, untransmittered doves were trapped and banded and mark-recapture methodology employed to estimate survivorship of untransmittered doves to those with radio-transmitters. White-winged Doves were trapped using modified wire funnel traps (Reeves et al. 1968) on 78 days during the period in which transmittered doves were monitored. Time between the first trap and last trap days was 118 days. During this period 476 individual doves were captured and recaptured and each dove's capture history recorded.

Program MARK (White & Burnham 1999) was used to evaluate survivorship models. Models were constructed and run using designated trap occasions ranging from 1 to 24 days. Program MARK models indicated the most parsimonious models were obtained using a trap occasion consisting of 12 days. Thus, 123

days/12 days yielded 10.25 trap occasions. Pooled survivorship for untransmittered doves was estimated for the period 18 January through 15 May 2006 using recaptures only models with a sin link function and 2nd part variance estimation in Program MARK. Four models were constructed using parameters for survival, Φ, and probability of capture, p, with and without time dependency. Akaike Information Criterion corrected for small sample size (AIC_c) was used to select the most parsimonious model, $\Phi(.)$ $p(t)$ (Burnham & Anderson 2002). Parameter index matrices (PIMs) of the selected model were adjusted and all values in the Φ PIM set to 1 and values in the p PIM set to 2 through 11 to reflect constant survivorship with time-dependent probability of capture. The $\Phi(.)$ $p(t)$ model was then rerun using these PIMs to improve model fit.

To evaluate apparent overdispersion of the data ($\hat{c} = 4.405$), $\hat{c}$ was estimated using the median $\hat{c}$ approach and logistically regressed for $c = 1$ to 4.5 (4.5 represents a slightly higher value than the observed $\hat{c}$ of 4.405) at 5 intermediate points for each of 100 simulations (Cooch & White 2007).

Results

Radio-transmittered doves.–Thirteen of 44 radio-transmittered White-winged Doves were known to have died during the monitoring period. All mortality was attributed to predation. Of these, eight were killed by domestic cats, two by raccoons, one by a hawk (*Accipiter* sp.), one by a domestic dog, and one in which the predator could not be determined. By the end of the monitoring period, 21 transmittered White-winged Doves had left the study area, the status of six doves was undetermined (a signal was received but the dove could not be visually located), and four were known to be alive. Estimated period survivorship for transmittered White-winged Doves over the 123 day period was 0.43 (95% CI: 0.22-0.65) (Fig. 1).

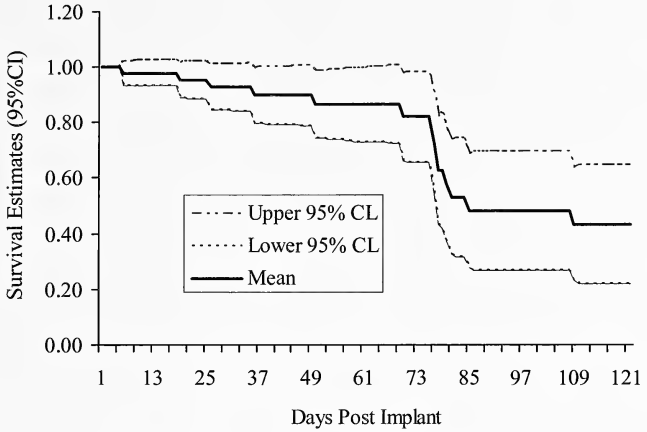

Figure 1. Kaplan-Meier survival curve for transmittered white-winged doves in Mason, Texas in 2006 with 95% confidence intervals.

Following the protocol assuming constant survival across sample periods a daily survival can be determined as [(0.43)(123)]/365 = 0.145 and from this $R = 6.12$. The implication being that for the population to maintain a constant size, each adult would have to produce 6.12 young per year.

Mark-recapture.–Sixty-nine untransmittered doves were recaptured during the trapping period (49 once, 10 twice). Overall recapture rate was 14.5% and individual recapture rate was 12.4% (to date the highest recapture rate achieved for this species). The model for constant survival and time dependent probability of capture, with 12 days designated a trap occasion, was selected as the most appropriate model because it had the smallest AIC_c and its Akaike weight suggested it was 7 times more likely to fit the data than the other models. We used the median $\hat{c}$ approach to obtain an estimated $\hat{c} = 1.01$ ($SE = 0.018$).

Estimated pooled survivorship for untransmittered White-winged Doves was 0.16 for a 118 day period, an intuitively unrealistic value. However, none of the models fit the data sufficiently to provide results considered reliable. Goodness-of-fit tests consistently showed lack of model fit to the data from either

failure to reach numerical convergence or P-values < 0.0001. Consequently, mark-recapture calculations of survivorship were excluded from the analysis.

Discussion

Fundamental to the use of radio-transmitters is the assumption that transmitters do not adversely affect activity patterns and related parameters of individuals being monitored (White & Garrott 1990). The use of radio-transmitters in the study of avian species has been more problematic in meeting these assumptions than in other vertebrate taxa because of the added constraint of flight, which must be considered in such studies. Other than transmitter weight, method of attachment of radio-transmitters has been of particular interest (Gaunt et al. 1997), primarily as related to limiting movement and behavior (Hooge 1991).

In recent years, studies of Mourning Doves and White-winged Doves have indicated that subcutaneously implanted transmitters with external antennas are a feasible, if not preferred, method for attachment of radio-transmitters (Schulz et al. 2001; Small et al. 2004b; Berdeen & Otis 2006).

Previous studies evaluating radio-transmitter attachment methods for White-winged Doves in flight pens have been conducted. No differences were found in subcutaneously implanted doves versus controls for physiological parameters (Small et al. 2004b), hematological parameters (Small et al. 2005b) or time budget behavior analysis (Rosales 2000). In field studies with White-winged Doves, subcutaneously implanted radio-transmitters were used with a high degree of success (period mortality rates $\leq$ 5%) over 120 days (Small et al. 2005a) and 96 days (Small et al. 2006b) in two other urban habitats. In Mason, Texas, recaptured White-winged Doves with subcutaneously implanted radio-transmitters showed no obvious signs of adverse reaction to the attachment technique (i.e., surgical procedure) (Small et al. 2007).

Berdeen & Otis (2006) verified that subcutaneously implanted radio-transmitters had no substantial effect on Mourning Dove survival and met the assumption that radio-tagging did not affect survival. However, their study focused on hunter harvest as the primary source of mortality. Additionally, they had a higher survivorship for implanted individuals held 24 h prior to release compared to those released immediately.

It is not realistic to assume constant annual survival from period survival rates. Because White-winged Doves are a game bird in Texas, mortality increases substantially during the 2-month dove hunting season each autumn. Therefore, annual survival estimates for this species, derived from a 123 d period during the spring and summer, most likely represent an overestimate of the true survival. However, this does not necessarily render the analysis meaningless. White-winged Doves typically lay two eggs per clutch. Based on estimates derived from this study, each pair would have to produce >6 successful 2-egg clutches per year to offset the high mortality rates of radio-transmittered birds in our study. The greatest documented number of clutch attempts by an individual White-winged Dove is four, two of which were successful (Schaefer et al. 2004). Based on previous studies, nest success, defined as at least one successful fledgling, is about 50%. Therefore, the mortality rates associated with transmittered birds appear to be unsustainable, suggesting that these rates are substantially higher than for normal (i.e., untransmittered) birds.

Although previous studies using subcutaneously implanted radio-transmitters showed low mortality rates (≤5%) (Small et al. 2005: 2006b), this study definitively had a minimum predation rate of 29.5% over the observational period. This suggests that in some circumstances implanted radio-transmitters may be detrimental to individual survivorship. Consequently, it is suggested that care be taken when designing radio-telemetry studies with this or similar species.

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GENERAL NOTES

SOME OBSERVATIONS ON THE MATING BEHAVIOR OF THE GIANT WALKING STICK, *MEGAPHASMA DENTRICUS* (ORTHOPTERA: PHASMIDAE)

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Sexual dimorphism is a prominent feature throughout the animal kingdom, from birds to ungulates to arthropods (Blanckenhorn et al. 2007). In many stick insects, sexual dimorphism is two-fold. First, the males may bare enlarged femoral spines, perhaps used as weapons in male-male competitions (Sivinski 1978; Brock 1999). Second, the males can be smaller than females, which may be a benefit to both males and females, as during mating, the male hangs on to the female, and a smaller male may allow the female to be more mobile for feeding and/or have more energy to invest in reproduction (Sivinski 1978). However, the idea that relatively small males have a higher fitness than large males is contradictory to a body of sexual selection theory that suggests that larger males, specifically in species that have weapons and engage in male-male competitions, often obtain *more* matings (Andersson 1994). The preliminary observations on the male-male and male-female interactions in the giant walking stick *Megaphasma denticrus* (Stål; also referred to as *Megaphasma denticrum*), while too few for statistical analyses, suggest that future investigations of the following hypotheses could be profitably pursued: (1) males compete with each other over access to females or resources, (2) large males are more likely to mate than small males, (3) males missing legs (autotomy) experience any mating disadvantages, (4) males prefer large females, and (5) females prefer small males.

The giant walking stick is one of the largest phasmid species in North America and is found in the south central United States (Figure 1; Hebard 1943; Wilkings & Breland 1951). Both sexes

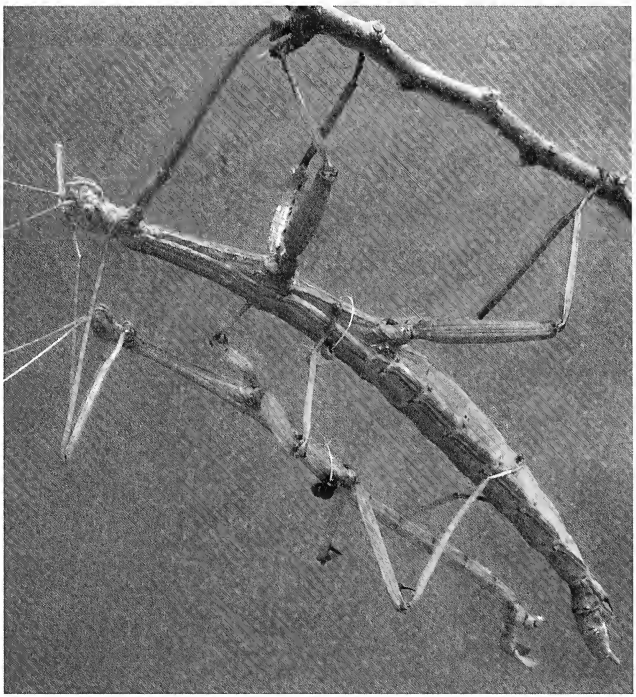

Figure 1. A female (top) and male *Megaphasma dentricus*.

have femoral spines on their mid and hind legs, but males have a single large spine and females a row of small spines. Both sexes are green and brown in color, and females are generally larger than males (females in this study ranged from ≈105-135 mm and males ranged from ≈90-125 mm). Females drop their eggs to the ground, and can lay up to three eggs per hour and thirteen per day for several months. Like some other phasmids, males have a clasping organ - modified cerci used to wrap around a female's abdomen during copulation and/or mate guarding (Figure 2; Wilkins & Breland 1951; Sivinski 1978).

In May 2007, 29 females and 30 males were collected in Blanco County, Texas (mostly found on mesquite, *Prosopis glandulosa* Torr.). Individuals were brought to the lab, measured for body size (anterior of head to tip of abdomen), inspected for missing legs, and

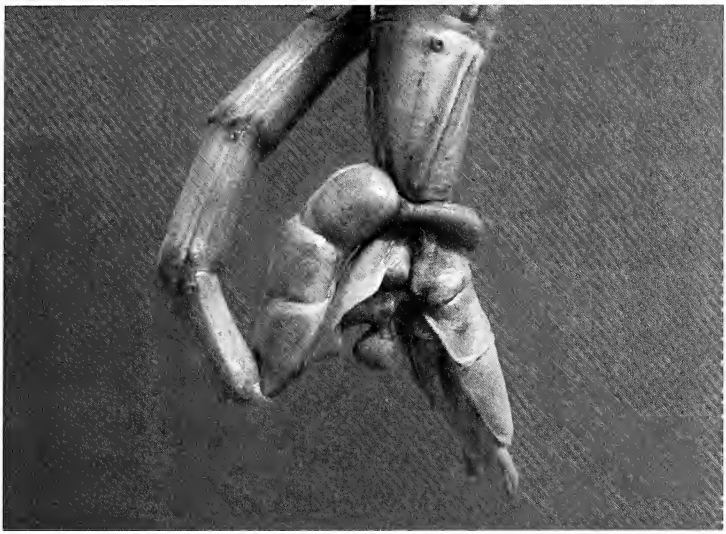

Figure 2. Clasping organ of a male (left) around a female.

given a unique color band for identification (Figure 1). All animals were fed hackberry leaves *ad libitum* (*Celtis laevigata* Willdenow) and kept in cages in a large greenhouse. Groups of individuals (see Table 1) were placed in a 37.85 L fish tank and recorded for 12 h with a red light/ lamp and Sony Handycam DCR-SR42 (≈7:00 p.m. to ≈7:00 a.m.; like many insects, phasmids are thought to be unable to see red light). No individuals were used in observations more than once, and once used, individuals were put together in a large holding cage where their mating behaviors were recorded approximately every twelve hours. During filming, six categories of behaviors were noted: mating effort, mating, grappling, feeding, walking, and motionless. 'Mating effort' was defined as either a male simply hanging on a female (Figure 1) or a male attempting to wrap his clasping organ around the female. 'Mating' was defined as a male having his clasping organ wrapped around the female (Figure 2; actual copulation was not determined in this study because it was difficult to tell whether the genitalia were in physical contact or whether the male simply has his claspers around the female). 'Grappling' was defined as two individuals who engaged

Table 1. Mating behavior in *Megaphasma dentricus*. Small males were shorter than 101.3 mm, and large males were longer than 115.7 mm. Similarly, small females were shorter than 110.2 mm, and large females were longer than 127.7 mm. All small and large pairs were at least 15 mm difference in size. Average males were ≈105 mm and average females were ≈117 mm.

Individuals	Trial	Successful Mating Pair	≈ Minutes until claspers attached	Time spent on mating effort by 'loser' male (out of ≈720min)	
Small ♂	1	♀ + Small ♂	1	70.1%	(505 min)
Large ♂					
Average ♀	2	♀ + Large ♂	<1	97.2%	(700 min)
	3	♀ + Small♂	4	3.5%	(25 min)
	4	♀ + Large ♂	2	0.0%	(0 min)
	5	♀ + Large ♂	2	29.2%	(210 min)
♂ *	1	♀ + ♂ (-) 1 leg	<1	47.9%	(345 min)
♂ (-) 1 leg					
♀	2	♀ + ♂	2	3.5%	(25 min)
	3	♀ + ♂	2	91.0%	(655 min)
♂ *	1	♀ + ♂ (-) 2 legs	<2	39.6%	(285 min)
♂ (-) 2 legs					
♀	2	♀ + ♂ (-) 2 legs	1	41.7%	(300 min)
Small ♀	1	♂ + Large ♀	2	N/A	
Large ♀					
Average ♂	2	♂ + Small ♀	<1	N/A	
	3	♂ + Small ♀	3	N/A	

* Body size in males was controlled for in each of these experiments/trials (e.g., the male with all six legs and the male missing legs were within two mm of each other).

in extensive touching of their legs and is considered to be mildly aggressive (Sivinski 1978).

Males do not appear to compete with each other over food or females, as grappling behavior occurred less than 2% of the time ($\bar{x}$ = 10.9/720 min, *SD* ± 6.3). This was true for all trials involving females (listed in Table 1) and trials with only two males (e.g., small vs. small, large vs. large, small vs. large, and average vs. average - one trial each, not listed in Table 1, same filming methods as above). With or without a female present, males often came in contact without grappling, and simply walked over or around each other with no signs

of aggression. In addition, they spent many minutes feeding in close proximity, and on several occasions, one male hung off the other.

These preliminary observations suggest that mating is opportunistic and on a 'first-find, first-mate' basis (Table 1). Large males were no more likely to find and successfully attach to females first, and males missing appendages appeared to be at no mating disadvantage. Females may show little preference for small males, and males no preference for large females; a male clasped to the first female he encountered, and remained attached for at least the next twelve hours, even if the other individual approached the mating pair. In each of the observations, a male had his claspers around a female in four minutes or less (most within two minutes), and he remained there for at least the next twelve hours. That is, there was no instance where a male successfully clasped around a female and then abandoned that female, even with no other male present. On many occasions within each of the 12h observations, the 'loser' male also got his claspers around the mating female, in which scenarios he attached anterior to the other male, further away from the female's genitalia. This did not appear to be very stable, as this second male would repeatedly slip off the female. However, 'second' males spent up to 90% of their time attempting to mate with a paired male and female ($\bar{x}$ = 305/720 min, *SD* ± 253.7), often hanging or following the mating pair around the cage and/or swinging their abdomen around in an attempt to attach. On average, both males and females spent ≈15% of their time feeding ($\bar{x}$ = 105.2/720 min, *SD* ± 71.7), and ≈85% of their time walking or motionless ($\bar{x}$ = 614.8/720 min, *SD* ± 71.7). The exceptions to this were single females; in the three observations with a male, a large female, and a small female, the unmated female spent up to 95% of her time feeding ($\bar{x}$ = 490/720 min, *SD* ± 190.8).

Other interesting observations arose after the experimental animals were placed together in one large cage. First, two mating pairs were together for over 60 hours (but <72), presumably extensive mate guarding (Sivinski 1983; Brock 1999). Second, five males changed partners at least three times in a 36-hour period, and most males changed partners once every 24-36 hours. Moreover, their mate switching did not follow any pattern of preferring smaller or larger

individuals. Third, available males and females did not always form a mating pair (e.g., just because there was an available mate did not mean a mating pair formed). These observations provide more support that both males and females do not show strong preferences for body size or overall morphology of their mate.

In conclusion, mating in *Megaphasma dentricus* appears to be opportunistic. Although there are strong adaptive hypotheses for both sexes related to their sexual dimorphism, our limited observations illuminated no apparent patterns of behavior involved in male-male competition or female choice. Other North American phasmids, such as *Diapheromera veliei* and *D. covilleae*, are different; larger males win contests over access to females and larger males mate longer than small females (Sivinski 1978). Perhaps the femoral spines function for crypsis and/or predator defense (Bedford 1978), and/or their sexual dimorphism is an evolutionary relic of other closely related species. Future work will reveal more details about the mating behavior seen in *M. dentricus*, and if it is typical of other phasmids.

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FIRST REPORT OF *OCHETOSOMA ANIARUM* (DIGENEA: OCHETOSOMATIDAE) FROM THE BRAZOS WATER SNAKE, *NERODIA HARTERI* (SERPENTES: COLUBRIDAE), IN TEXAS, WITH A SUMMARY OF DEFINITIVE HOSTS OF THIS PARASITE

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The Brazos water snake, *Nerodia harteri*, is a medium-sized colubrid endemic to the Brazos River system of 11 counties of northcentral Texas (Dorcas & Mendelson 1991; Conant & Collins 1998; Dixon 2000; Werler & Dixon 2000; Ernst & Ernst 2003). Discovered in 1936 in Palo Pinto County in the Brazos River, the species was later described by Trapido (1941). This snake fills a unique niche within this riverine habitat typified by fast-flowing rocky stream riffles of the upper Brazos River and two of its tributaries. Although not currently listed as federally threatened or endangered by the U.S. Fish and Wildlife Service, the species is considered threatened by the Texas Parks and Wildlife Department (TPWD 2007). In addition, *N. harteri* is globally listed G2 (NatureServe 2007) due to its restricted range and habitat requirements.

Detailed information is available on various aspects of the natural history of this snake (Mecham 1983; Scott et al. 1989); however, very little is known about its parasites. McAllister & Upton (1989) reported three eimerians and a *Cryptosporidium* sp. from Brazos water snakes from Somervell and Palo Pinto counties, and Upton et al. (1989) further described a *Cryptosporidium* sp. in a single *N. harteri* from the latter county. The only previous report on helminth parasites was by Rossi & Rossi (2000) who reported *Strongyloides* sp. and unidentified filiarial worms from captive *N. harteri.*

On 18 July 1987 and again between 6 May 1988 and 29 July 1988, 10 juvenile and adult *N. harteri* (four males, six females; SVL range = 340-710 mm, mean ± 1*SD* = 547.5 ± 113.4 mm) were collected by hand or tong from two sites on the Brazos River, one below Possum Kingdom Dam, 11.3 km SW Graford off FM 4, Palo Pinto County (*n* = 3, 32° 52.02'N, 98° 25.03'W), and the other 8.0 km S Glen Rose off FM 200, Somervell County (*n* = 7, 32° 16.16'N, 97° 39.51'W). Several of these snakes were the same specimens reported in McAllister & Upton (1989). Specimens were placed in individual collecting bags on ice and within 24 hr anesthetized with sodium pentobarbital (Nembutal®) overdose, and their gastrointestinal tract from their mouth to cloaca examined for helminth parasites. Trematodes were fixed in 10% neutral buffered formalin, transferred to 70% ethanol, stained with Semichon's acetocarmine, and mounted in Canada Balsam. Voucher specimens of parasites are deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, USA, as USNPC 84293. Snake voucher specimens are deposited in the Arkansas State University Museum, Herpetological Collection, State University, Arkansas, as ASUMZ 8420, 11221, 11762-11766, and the Cedar Valley College Collection (CVC), Lancaster, Texas, as 880520-18, 880708-6, and 880729-1.

One of 10 (10%) *N. harteri* (655 mm SVL female, CVC 880708-6) collected from the Somervell County site on 8 July 1988 was found to be infected in its mouth with four specimens of the digenean trematode *Ochetosoma aniarum*. This was the only helminth found in this small sample of snakes. The parasite was originally described by Leidy (1890) as *Distomum aniarum* from the northern water snake, *Nerodia sipedon,* from Pennsylvania. In addition, Dubois & Mahon (1959) listed *Renifer acetabularis, R. orula, R. natricis, R. texanus,* and *R. wardi* as synonyms of *O. aniarum*. It would appear that Dubois & Mahon (1959) were unaware that Skrjabin & Antipin (1957) had previously transferred the above species of *Renifer* to *Ochetosoma*. The current arthors are in agreement with the latter synonymy although some disagreement currently exists (see Ernst & Ernst 2006).

Table 1. North American definitive hosts of *Ochetosoma aniarum*. Variously reported as synonyms *Neorenifer aniarum*, *Neorenifer acetabularis*, *Renifer acetabularis*, *R. orula*, *R. natricis*, *R. texanus*, and *R. wardi*. Host scientific names follow Crother et al. (2000) and Crother et al. (2003).

Family/Host	Locale	Reference
Colubridae		
Coluber constrictor constrictor	unknown	Wallander (1968)
C. constrictor flaviventris	Nebraska	Brooks (1979)
C. constrictor foxii	unknown	Wallander (1968)
Farancia abacura abacura	unknown	MacCallum (1921)
Heterodon platirhinos	Texas	Harwood (1932)
	unknown	Wallander (1968)
Heterodon simus	unknown	Wallander (1968)
Lampropeltis getula floridana	Florida	Parker (1941)
	unknown	Wallander (1968)
L. getula holbrooki	Louisiana	Rabalais (1969)
	Texas	Dronen & Guidry (1977)
Nerodia cyclopion	Florida	Parker (1941)
	Illinois	Dyer & Ballard (1991)
	Louisiana	Rabalais (1969); Brooks (1979); Fontenot & Font (1996)
	Mississippi	Byrd (1935)
N. erythrogaster erythrogaster	North Carolina	Collins (1969)
	Tennessee	Parker (1941)
N. erythrogaster flavigaster	Alabama	Detterline et al. (1984)
	Arkansas (Lonoke County)	previously unpublished
	Illinois	Dyer (1999)
	Louisiana	Rabalais (1969); Brooks (1979)
	Mississippi	Byrd (1935)
N. erythrogaster transversa	Mexico (Nuevo Leon)	Jimnez & Caballero (1975)
	Texas	Curfman & Davidson (1966); previously unpublished
N. fasciata confluens	Louisiana	Rabalais (1969); Brooks (1979); Fontenot & Font (1996)
	Texas	Harwood (1932)
N. fasciata fasciata	Louisiana	Rabalais (1969)
N. fasciata pictiventris	Florida	Parker (1941)
N. harteri	Texas	This study (new host record)
N. rhombifer rhombifer	Illinois	Dyer (1999)
	Kansas	Crow (1913)
	Louisiana	Rabalais (1969); Fontenot & Font (1996)
	Mississippi	Byrd (1935); (1937)
	Tennessee	Parker (1941)
N. sipedon pleuralis	Alabama	Detterline et al. (1984)
	North Carolina	Collins (1969)
	Texas	Harwood (1932)
N. sipedon sipedon	Michigan	Talbot (1934)
	Pennsylvania	Leidy (1890)
N. taxispilota	Georgia	Camp (1980)
	North Carolina	Collins (1969)
Seminatrix pygaea	Florida	Parker (1941)
Viperidae		
Agkistrodon piscivorus leucostoma	Louisiana	Rabalais (1969)
A. piscivorus piscivorus	North Carolina	Collins (1969)

This parasite has previously been reported in two viperids and several colubrid snakes from various North American localities, including Texas (Table 1). From this summation it is obvious that most of these hosts are semiaquatic or aquatic species (primarily *Nerodia* spp.) that occasionally feed on frogs.

In the life cycle, eggs containing miracidia hatch after being eaten by pulmonate snails (*Physa* spp.) and develop into daughter sporocysts. After leaving snails, cercariae penetrate and encyst in tadpoles of the genera *Lithobates, Hyla* and *Pseudacris* , which, when fed to snakes of the genus *Nerodia*, adult flukes occurred in the mouth and esophagus of definitive hosts 35 days later (Byrd 1935; Walker 1939; Schell 1985). Although *N. harteri* most often feeds on small fishes, primarily minnows (Tennant 1984; Werler & Dixon 2000) related neonate Concho water snakes, *N. paucimaculata*, has been reported to eat cricket frogs, *Acris crepitans*, by Greene et al. (1994). This may help explain the low prevalence of *O. aniarum* in *N. harteri.*

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INTRODUCTION OF THE BLUEFIN KILLIFISH (*LUCANIA GOODEI*) IN TEXAS

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In October 1997, E.I. Dupont de Nemours and Company, Inc. constructed a 22.9-ha artificial wetland at a plant near Victoria, Victoria County, Texas, as part of a new biotreatment process of effluent water prior to its discharge into the Guadalupe River. As recently as 2001, the wetland had 10.6 ha of open water, bordered by extensive growths of emergent vegetation that had been planted on shallow shelves bordering several deep-water zones and on shallow flats separating the deep-water zones. Vegetation consisted of 24 species of marsh and water plants. Most were obtained from an onsite nursery, but three species (pickerelweed, *Pontederia cordata*; softstem bulrush, *Scirpus validus*; and giant cutgrass, *Zizaniopois miliacea*) were imported from a Florida nursery (Duckworth-Cole, Inc. 2001).

Following completion of the planting of the aquatic vegetation, the Texas Parks and Wildlife Department (TPWD) seined a collection of fish from the Guadalupe River and released some of them into the wetland in late 1997 (Duckworth-Cole 1999). All were native species considered compatible with the wetland habitat. Following acquisition of the property in 2004 by INVISTA, S.à.r.l., the wetland became known as the INVISTA Wetland.

Beginning in 1998, the wetland was sampled by seine and hoop net annually through 2002 and again in 2004 to determine the fate of the introduced fish stocks over time. When the wetland was first sampled in May 1998, collections included not only native fishes but also 20 small (26 to 40 mm in total length) killifish, tentatively

identified as the bluefin killifish (*Lucania goodei*) by Howells (2001). Prior to these collections, the introduced range of the bluefin killifish was believed restricted to Florida, southeastern Georgia, and southeastern Alabama, with introduced populations in North and South Carolina (Gilbert & Burgess 1980; Page & Burr 1991; Mettee et al. 1996; Fuller et al. 1999). This species is represented in the aquarium trade (e.g., Axelrod & Schultz 1971) and, as such, it is a candidate for introductions outside its range. In the wild, it inhabits vegetated ponds, lakes, and other still waters such as sloughs, pools and backwaters of streams. During spawning, adhesive eggs are deposited on vegetation or algae (Mettee et al. 1996). Giant cutgrass dominates the perimeter shores of the open-water cell where bluefin killifish were collected, and the species may have been introduced into the wetland as eggs attached to plants imported from Florida.

By 2000, it became clear that the bluefin killifish was reproducing in the Invista Wetland. Fish increased in total length (TL) from 26-40 mm in spring 1998, to 30-52 mm in spring 1999. The appearance of numerous small fish 22-36 mm TL in spring 2000 indicated that successful spawning had occurred. Subsequent sampling periods (Table 1) demonstrates that the species is established and is successfully reproducing in the Invista Wetland.

A representative male and female bluefin killifish were sent to the TPWD Heart of the Hills Fisheries Science Center (HOH), Ingram, Texas, for confirmation of the identification. Voucher specimens were placed in the Texas Cooperative Wildlife Collection (Catalogue number 10492.01), Texas A&M University, College Station, Texas. Additional specimens remain with LGL Ecological Research Associates, Inc., Bryan, Texas, and with the HOH.

Water discharged from the Invista Wetland overflows a weir and drops vertically about 1 m into a large underground pipe. This pipe empties into an open channel that flows into holding ponds that also

Table 1. Number of bluefin killifish (*Lucania goodei*) collected by 2-mm size intervals (total length) from the Invista Wetland, Victoria County, Texas.

Length Interval (mm)	1998	1999	2000	2001	2004
22			1		3
24			2		2
26	3		6		3
28	1		15		8
30	2	1	23	1	6
32	4		14	1	5
34	5	1	12	1	10
36	3	8	13	2	7
38	1	23	10	1	15
40	1	36	11	2	18
42		58	7	1	16
44		68	11	1	2
46		75	11	1	3
48		56	8	1	1
50		23	6	1	
52		6	2		1
54			1		
56			1		
Total	20	355	154	13	100

collect plant site runoff water and water discharged from an onsite cooling basin. The Invista Wetland is not accessible to fish from the holding ponds, but fish from the wetland can be transported into the holding ponds. In May 2002, 526 bluefin killifish were collected from the holding ponds. Water collected here is discharged through an underground pipe into a backwater slough of the Guadalupe River. Seining surveys in this slough conducted in May 2002 yielded 19 bluefin killifish. Bluefin killifish have not only established a reproducing population in the wetland, but, apparently, they have also dispersed from this site through the holding ponds into the Guadalupe River.

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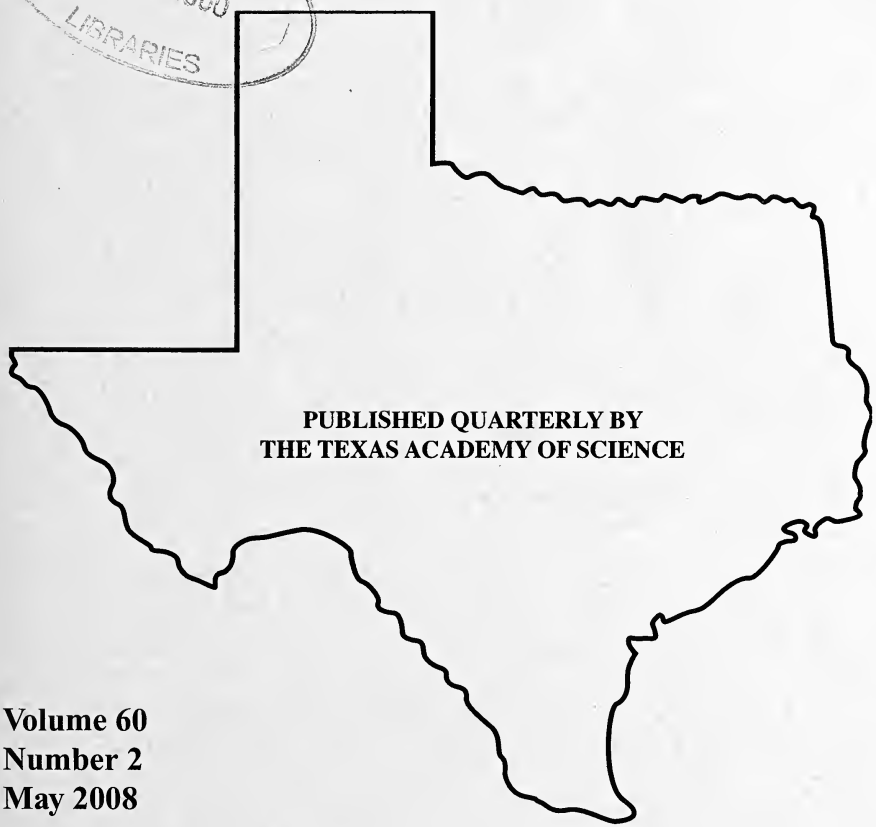

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TEXAS J. OF SCI. 60(2):83-96 MAY, 2008

A REEXAMINATION OF *SYNGNATHUS AFFINIS* GÜNTER 1870, WITH COMPARISONS TO *SYNGNATHUS SCOVELLI* (EVERMANN AND KENDALL 1896) (TELEOSTEI: SYNGNATHIDAE)

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Abstract.–The Texas pipefish (*Syngnathus affinis*) is currently known from only a small number of specimens collected from geographically isolated locations in the vicinity of Corpus Christi Bay, Texas. The general rarity of this species, coupled with the fact that no new collections have been recorded in over 30 years, has led numerous researchers to postulate that this taxon represents a marine species that has become extinct. To determine the status of *S. affinis*, a reexamination of all known collections was undertaken and these were compared to recent collections of *S. scovelli*, a common syngnathid ranging throughout the Gulf of Mexico. Multivariate statistical techniques were used to compare the morphometric and meristic characters currently used to differentiate syngnathids. Analysis of Similarity revealed that a low degree of separation exists between meristic characters of the two species, and an even lower degree of separation was found with the morphological values. Based on the results of this study, the taxonomic validity of the nominal designation *S. affinis* is questioned. Previous records of *S. affinis* most likely represent misidentified specimens of *S. scovelli*.

Considerable confusion surrounds the status of the Texas pipefish, *Syngnathus affinis* Günther 1870. This nominal species is based on a single specimen (Lot 233) purchased at Steven's sales room (London) and registered simply as "Louisiana" and the questionable nature of the type-locality was indicated by Günther's failure to discuss the source of the holotype (Dawson 1982). Nearly all occurrences of this species have come from the Corpus Christi Bay area of Texas, with only one other subsequent collection from Louisiana (five specimens from Prien Lake; Fowler 1933). A total of 36 specimens have reported since 1926, with 20 of the 30 Corpus Christi Bay specimens taken with *S. louisianae* and *S. scovelli* in two seine collections at Fish Pass (Dawson 1982). A single specimen collected in 1970 (but subsequently lost) constitutes the collection from the Laguna de Terminos area (Dawson 1982).

Early accounts of short-snouted pipefishes (snout length-to-head length ratios of 0.400 to 0.588) of the genus *Syngnathus* from the western Gulf of Mexico originally included only *S. fuscus* and *S. scovelli* (see Evermann & Kendall 1894:109 as *Siphostoma fuscus*; Breder 1929; Jordan et al. 1930; Herald 1942; Hoese 1958). These two species were separated primarily by the total number of trunk rings (19-21, rarely 18 in *S. fuscus*; 15-18, rarely 19 in *S. scovelli*) and dorsal fin rays (35-43 in *S. fuscus*; 27-35 in *S. scovelli*). Based on a reexamination of the holotype, Herald (1965) was the first to adopt the subspecies designation of *S. fuscus affinis* (Relict Northern Pipefish), although it was originally postulated that the holotype was a specimen of *S. fuscus* from an erroneous locality. Later, Hoese & Moore (1977) recognized *S. fuscus affinis* as a subspecies of *S. fuscus* known only from Corpus Christi Bay. *Syngnathus fuscus fuscus* is distributed along the Atlantic coast from Gulf of St. Lawrence in Canada southwards to northern Florida (Lazzari & Able 1990). Dawson (1982) dropped the subspecies designation, using the combination of trunk rings (modally 18), tail rings (33-34), and dorsal fin ray counts (37-41) to separate *S. affinis* from *S. fuscus*. Other characters presented by Dawson (1982) for distinguishing *S. affinis* from other western Atlantic congeners include moderate snout length (averages 0.42 snout-to-head length ratio) and depth (averages 0.33 snout depth-to-length ratio) in addition to a narrower preorbital bone. By contrast, Hubbs et al. (1994) incorporated all previous Gulf of Mexico specimens of *S. fuscus* as *S. affinis*. This practice was followed by McEachran & Fechhelm (1998), thereby eliminating the Gulf of Mexico from the distributional range of *S. fuscus*.

In order to resolve some of the confusion surrounding the identity and status of *S. affinis*, a reexamination of all known collections of this species was conducted and these were compared to recent collections of *S. scovelli* from the Corpus Christi Bay area. In this paper, the species status of *S. affinis* is questioned based on a multivariate analysis of the meristic and morphological characters currently used to identify pipefishes.

Materials and Methods

Collections of *Syngnathus affinis* were obtained from museums (Academy of Natural Sciences [ANSP], British Museum of Natural History [BMNH], California Academy of Science [CAS], Field Museum of Natural History [FMNH], Gulf Coast Research Laboratory [GCRL], Texas A&I University [TAIC], Texas A&M University [TCWC], Texas Memorial Museum [TNHC], National Museum of Natural History [USNM], and Yale University [YPM]). Collections of *S. scovelli* were made by the author from every location in the Corpus Christi Bay area where *S. affinis* were previously recorded (Fig. 1). Each specimen was examined using the characteristics outlined in Dawson (1982), with the following measurements taken with dial calipers under a stereo-microscope (10X): head length, snout length, snout depth, trunk depth, anal depth, pectoral fin length, and dorsal fin base length. Measurements were taken to the nearest 0.1 mm and expressed as a percentage of head length. Meristic counts included: trunk rings, tail rings, doral fin rays, dorsal fin rings (subdorsal rings) over the trunk, subdorsal rings over the tail, and total dorsal fin rings.

Multivariate statistical techniques were used to compare the similarity of the meristic and morphological characters among the samples (i.e., individual pipefish specimens). Analyses were performed using Primer-E (Version 6.0) software (Clarke & Warwick 2001). Samples were standardized to account for scale differences in measurement units. A matrix of Euclidean distance similarities between samples was created. Significant differences in rank similarities between groups of samples were tested by Analysis of Similarity (ANOSIM). In the ANOSIM procedure, the probability of *a priori* groupings of samples is estimated by repeated permutations of the data (i.e., repeated random relabelling of samples in the matrix). Values of the *R* statistic can range from -1 to 1, although *R* will usually fall between 0 and 1 with *R* values > 0.4 indicating higher degrees of discrimination between groups. Similarities between the samples are graphically represented with non-metric multidimensional scaling (MDS) ordinations (Kruskal

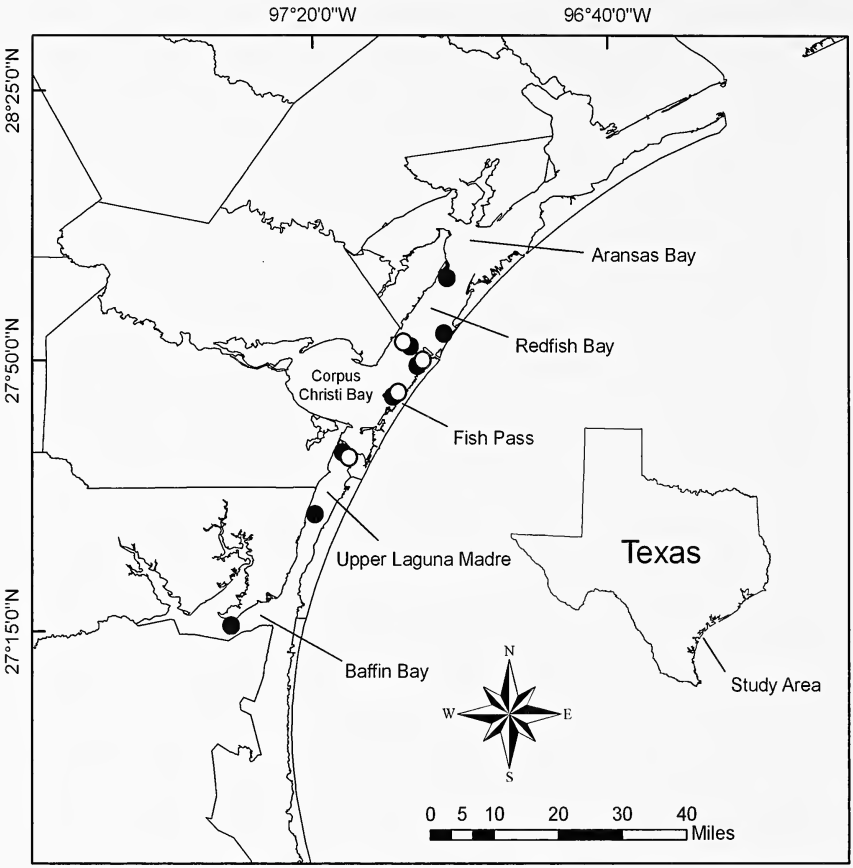

Fig. 1. Sampling locations in the vicinity of Corpus Christi Bay of *Sygnathus affinis* (◦, open circles) ans *S. sovelli* (•, solid circles) collected for this study.

1964). Although outcomes of the ANOSIM are not dependant on MDS ordinations, the ordinations are presented here as they are a helpful way of visualizing patterns in the data. Stress values indicate how well the two-dimensional plot represents relationships among samples in the multidimensional space. Stress values < 0.15 indicate a good fit. MDS ordinations may be arbitrarily rotated so axes are not labeled.

Material examined.–Syngnathus affinis (N = 106). Numbers in parenthesis are number of specimens followed by size range; measurements are in mm SL. "Louisiana", BMNH 1854.7.3.2

(holotype)(1, 144); Texas, Corpus Christi Bay and vicinity: CAS 39674 (1, 80); FMNH 40309 (2, 206, 218); GCRL 15252 (10, 55-123); TAIC 3807 (as *S. fuscus* 1, 123); TAIC 5307 (as *S. fuscus* 72, 65-148); TNHC 27813 (9, 78-93); TNHC 28102 (as *S. fuscus* 1, 91); USNM 132675 (2, 117-174); Galveston Bay, TCWC 11633.01 (1, 109); Florida, Egmont Key, YPM 8724 (1, 89); Louisiana, Prien Lake, ANSP 55455 (5, 95-102).

Syngnathus scovelli (N =170), uncataloged. Texas, Corpus Christi Bay and vicinity: Corpus Christi Bay (16, 74-118); Fish Pass (18, 72-120); Upper Laguna Madre (9, 75-105), Baffin Bay (17, 60-86); Aransas Bay (2, 61-85); Redfish Bay (3, 57-113).

Results

A total of 170 pipefish were collected by the author between June 2005 and November 2005 from the Corpus Christi Bay area. Field identifications resulted in the collection of 148 *S. scovelli* and 22 *S. louisianae*. Other collections provided by the Texas Parks and Wildlife Department (TPWD) Coastal Fisheries' systematic bag seine sampling efforts in Upper Laguna Madre, Corpus Christi, and Aransas bays during this same time period resulted in an additional 19 *S. scovelli* and 3 *S. louisianae* specimens. No new specimens of *S. affinis* were collected during this study. Individuals of *S. scovelli* (n = 65) were randomly selected from this collection and used in the multivariate comparisons.

ANOSIM results of the meristic characters (Table 1) revealed a relatively low degree of separation of the *a priori* designations (R = 0.363, $P < 0.001$). MDS configuration of the meristic characters is shown in Fig. 2. The most cohesive group of *S. affinis* samples fell on the left side of the MDS, with the majority of these samples constituting the Fish Pass (GCRL 15252) and the Upper Laguna Madre (TAIA 5307) collections. The remainder of the *S. affinis* collections from other Texas bays, as well as the Louisiana (ANSP 55455) and Florida (YPM 8724) specimens were scattered throughout the ordination among the *S. scovelli* collections. Based on the meristic information, the holotype was located in the middle

Table 1. Counts and proportional measurements of the holotype and non-type specimens of *Syngnathus affinis* Günter 1870 with comparisons to *S. scovelli*. Morphometric characters expressed in percent of head length, except for SL in mm and snout depth-to-length ratio.

Character	Holotype (BMNH 1854.7.3.2)	Non-type Specimens *S. affinis* (n = 50)			Corpus Christi Bay Area *S. scovelli* (n = 65)		
		Range	Mean	*SD*	Range	Mean	*SD*
Morphometric							
Standard Length	144	64-218	–	–	57-120	–	–
Head length	17.8	8.4-23.2	–	–	7.2-15.0	–	–
Snout length	0.42	0.37-0.52	0.42	0.04	0.37-0.47	0.42	0.02
Snout depth	0.18	0.13-0.24	0.19	0.03	0.13-0.22	0.18	0.02
Snout depth-to-length	0.43	0.28-0.61	0.46	0.08	0.32-0.56	0.42	0.06
Trunk depth	0.28	0.23-0.38	0.31	0.03	0.22-0.37	0.31	0.03
Anal depth	0.24	0.19-0.38	0.27	0.04	0.20-0.35	0.27	0.03
Pectoral length	0.14	0.14-0.30	0.21	0.03	0.15-0.26	0.20	0.03
Dorsal base length	1.05	0.86-1.44	1.11	0.12	0.89-1.18	1.04	0.07
Meristic							
Trunk rings	18	16-19	17.5	0.97	15-17	16.0	0.43
Tail rings	33	31-36	33.7	1.45	31-34	32.2	0.81
Total rings	51	47-55	51.1	2.28	46-50	48.2	0.94
Subdorsal trunk rings	5.75	3.00-5.50	4.30	0.60	2.25-4.50	3.72	0.55
Subdorsal tail rings	3.50	4.00-5.75	4.81	0.49	3.50-5.75	4.54	0.55
Total subdorsal rings	9.25	7.50-10.5	9.11	0.83	7.00-9.25	8.27	0.45
Dorsal fin rays	35	31-40	36.0	2.63	30-36	32.5	1.59

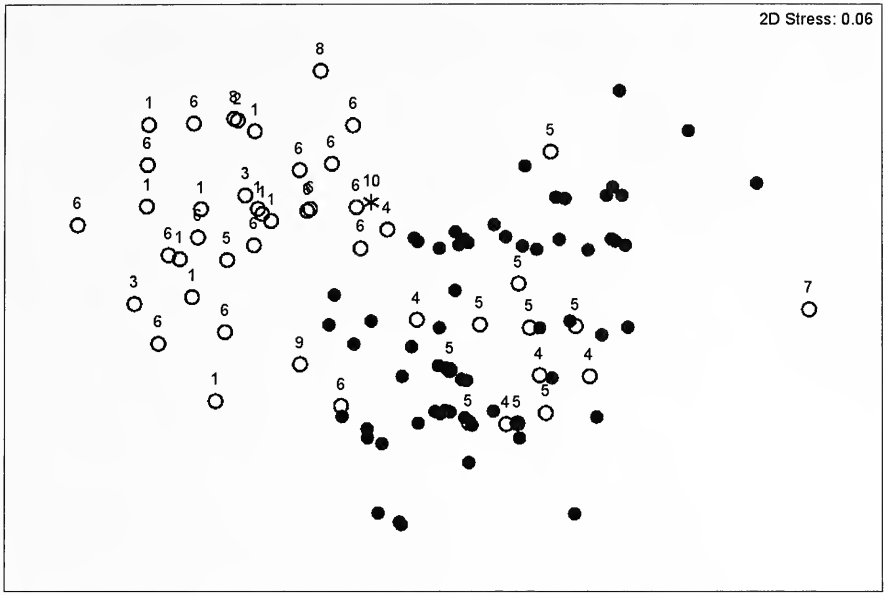

Fig. 2. Non-metric multidimensional scaling ordination of the multivariate analysis (Euclidean distance measure) performed on the meristic features of *Syngnathus affinis* (°, open circles) and *S. scovelli* (•, solid circles) collections. *S. affinis* holotype identified by the star (*) symbol. Sample superscripts correspond to the following museum collections: 1-GCRL 15252; 2-CAS 39674; 3-USNM 132675; 4-ANSP 55455; 5-TNHC 27813; 6-TAIA 5307, TAIA 3807; 7-YPM 8724, 8-FMNH 40309; 9-TCWC-11633.01; 10-BMNH 1854.7.3.2.

of the variability exhibited by the *S. affinis* collections. Proportional morphological measurements between the groups were less distinct, as shown in Fig. 3. While statistically significant (R = 0.123; $P < 0.001$), the morphologically-based R value between *S. affinis* and *S. scovelli* is negligibly small. The test reveals that the two 'species' probably do not have exactly the same proportional characteristics (the null hypothesis $R = 0$ can be rejected) and that these measurements are strongly overlapping and differ somewhat (R is closer to zero). Unlike the meristic characters, ordination of the morphological information failed to detect any cohesive groupings of the *S. affinis* samples. The holotype was again located within the range of variability encompassed by *S. affinis*.

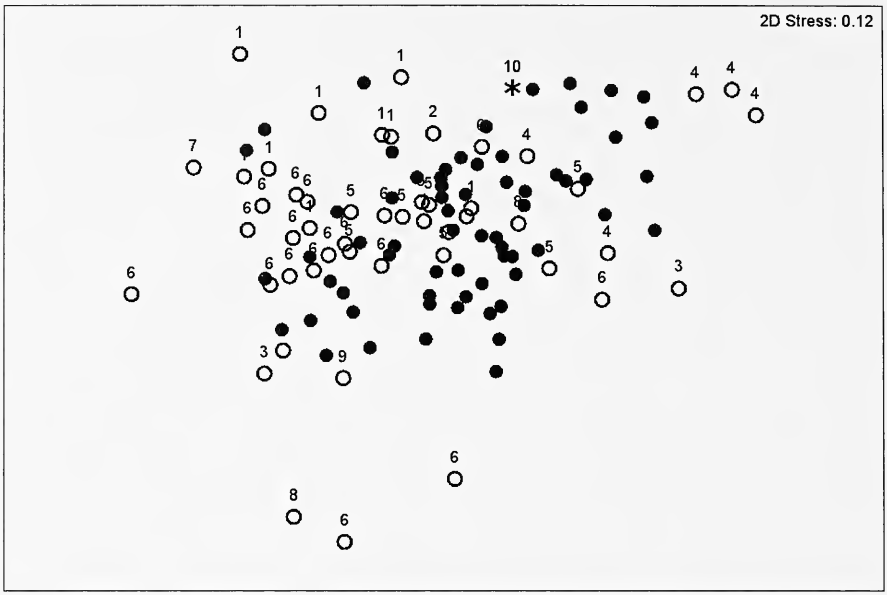

Fig 3. Non-metric multidimensional scaling ordination of the multivariate analysis (Euclidean distance measure) performed on the morphological features of *Syngnathus affinis* and *S. scovelli* collections. Sample superscripts correspond to the following museum collections: 1-GCRL 15252; 2-CAS 39674; 3-USNM 132675; 4-ANSP 55455; 5-TNHC 27813; 6-TAIA 5307, TAIA 3807; 7-YPM 8724, 8-FMNH 40309; 9-TCWC-11633.01; 10-BMNH 1854.7.3.2.

Discussion

Little or no literature is available describing the specific characteristics, physiology, or habitat requirements of *S. affinis*. Presumably, *S. affinis* shares similar habitat (shallow, nearshore submerged aquatic vegetation) and food requirements as *S. scovelli* (Joseph 1957; Huh & Kitting 1985; Bolland & Boettcher 2005). Because few specimens have been reported since 1977, this species is currently described as among the rarest in the Gulf of Mexico and it is presumed to be extinct (Roberts & Hawkins 1999). Reasons for disappearance include habitat decline and degradation, caused by human-influenced fishing, shrimping, and dredging activities (Roberts & Hawkins 1999; Musick et al. 2000). While seagrass decline is cited as a specific reason for this demise, seagrass beds in the vicinity of Corpus Christi Fish Pass (the area of highest concentrations of *S. affinis* to date) have increased dramatically

between 1974 and 1994 (Pulich & White 1997). Similar declines in populations of *S. scovelli* from comparable locations around the Corpus Christi Bay area have not been noted (bag seine collections 1982-2005; TPWD Coastal Fisheries database, Austin, Texas). While not currently listed as threatened or endangered by the National Marine Fisheries Service, the American Fisheries Society recommends listing the Distinct Population Segments of *S. affinis* as 'endangered' because of their "general overall rarity, endemic nature, and restricted geographic range" (Musick et al. 2000).

Extensive sampling of the known localities around the Corpus Christi Bay area where *S. affinis* had previously been reported was unsuccessful. In the limited time frame used for this study, a total of 192 pipefishes were collected from nearshore habitats. *Syngnathus scovelli* made up the majority of these collections, revealing that this species is indeed the dominant syngnathid found in coastal regions of the Gulf of Mexico (Bolland & Boettcher 2005). Admittedly, the field sampling used in this study was limited in scope, concentrating primarily on the immediate vicinities of known locals of *S. affinis* populations.

A spatially larger scope of sampling within the shallow, nearshore habitats is performed by TPWD, which conducts systematic bag seine sampling in each bay system along the Texas coast as a fishery-independent measure used to track the relative abundance and sizes of finfish (Martinez-Andrade et al. 2005). Since 1975, approximately 37,500 bag seine samples in shallow, nearshore habitats along the Texas coast have produced over 3,600 *S. scovelli* (TPWD Coastal Fisheries database, Austin, Texas). By stark contrast, no *S. affinis* have been recorded during these 30 years of sampling. This systematic monitoring of nearshore habitats has revealed that the highest densities of *S. scovelli* are found in Coastal Bend region of Texas (San Antonio, Aransas, Corpus Christi, and Upper Laguna Madre bay systems; see Fig. 1), and these areas of abundant populations of *S. scovelli* are generally the same areas that were sampled extensively for this study. Given

the general overlap in the meristic characters between *S. affinis* and *S. scovelli* as outlined in this paper, it is quite possible that field identifications of *S. scovelli* by TWPD staff could have erroneously included at least some *S. affinis* in the thousands of individuals reported. Previous taxonomic keys (Parker et al. 1972; Hoese & Moore 1977; Murdy 1983) recognized *S. fuscus* as the only other short-snouted pipefish in the genus *Syngnathus* from Texas waters, yet *S. fuscus* was similarly absent from TPWD surveys during the 1975-2005 period. Again, misidentifications could account for this apparent absence of *S. fuscus* from TPWD surveys of the Texas coast.

With no recent collections of *S. affinis* available for investigation, investigations of its specific status rest on existing museum specimens. This study did locate additional museum specimens (n = 74) of *S. affinis* not listed in Dawson (1982), and these were included in the present analysis. These new records call into question the limited distributional range of *S. affinis*, with this 'species' now recorded from around the northern Gulf of Mexico (1 from near Galveston Bay in Texas; 72 from the Upper Laguna Madre in Texas, and 1 from Egmont Key in Florida). This new range is fully encompassed by the known range of *S. scovelli* (Dawson 1982).

The ANOSIM tests used for this study revealed that for the meristic information, a relatively low degree of separation was found between *S. affinis* and *S. scovelli* (Table 1). Early works describing *S. affinis* were primarily based on meristic differences found in the short-snouted pipefishes from the western Gulf of Mexico (Herald 1965; Parker et al. 1972), yet those works recognized that only a few individuals had ever been collected. The present analysis also found differences in the mean numbers of dorsal fin rays, trunk rings and tail rings, as well as trunk and tail subdorsal rings, although the ranges of these counts always overlapped, in some cases greatly, between the two nominal taxa. While the meristic-based ANOSIM was significant, this low of an R

value is indicative of a high degree of overlap in the *a priori* groupings. This can happen when the number of replicates is large for the groups, thus giving a very large number of possible permutations. Biologically trivial differences can still be statistically significant when power is large. More importantly, the ANOSIM procedure based on the morphometric values failed to detect any consistent pattern of differences between the two forms. While the stress value calculated for the morphometric data was larger than the meristic configuration (0.12 vs. 0.06, see Fig. 2), the ordination of the morphological information still provides a potentially useful 2-dimensional picture (below the 0.15 level, see Clarke & Warwick 2001). Scaled as a function of head length, average ratios of snout depth, pectoral fin length, and dorsal fin base length were higher in *S. affinis*, whereas snout length, trunk depth, and anal depth were equivalent with *S. scovelli*. Each of these characters used in this analysis varied between the two forms by no more than, at most, the standard deviation of the average ratios. This lack of divergence can clearly be seen in the MDS ordination (Fig. 3).

Given the plasticity of meristic characters within western Atlantic species of *Syngnathus* (e.g., tail rings ranging from 28 - 34 in *S. scovelli* and 32-35 in *S. affinis*; dorsal fin rays ranging from 25 - 37 in *S. scovelli* and 35 - 41 in *S. affinis*; see Tables XL, XLI, XLII, and XLIII in Dawson 1982), the variability and gradients of these traits suggests that all the material examined for this study represent different phenotypes of *S. scovelli*. Individuals currently identified as *S. affinis*, on the basis of higher counts of trunk rings, tail rings, and dorsal fins rays, most likely represent individuals at the upper limits of these features currently seen in western Gulf of Mexico populations of *S. scovelli*. Based on the multivariate techniques used for this study, there appears to be little justification for recognizing *S. affinis* and *S. scovelli* as distinct species as the former is shown herein to be indistinct from the latter.

Before invalidating *S. affinis* as a nominal taxon, extensive field work must be conducted in the western Gulf of Mexico to document that there is indeed only a single specimen of short-snouted *Syngnathus* within the area. These collections should undoubtedly encompass a much greater temporal scale than the six months used for this study. Given the uncertainty in the type locality for *S. affinis*, "Louisiana" is certainly within the geographic range of *S. scovelli*. Morphometric and meristic characters of the holotype of *S. affinis* all lay within the range of variation found in *S. scovelli*. If invalidation of *S. affinis* ultimately proves to be the proper action, the Principle of Priority (International Commission of Zoological Nomenclature (ICZN) 2000) states that the valid name of any taxa be the oldest available name applied to it (in this case, *S. affinis* is senior to *S. scovelli*). While *S. affinis* is senior, the Principle of Priority further states that it is not intended to be used to upset a long-accepted name in its accustomed meaning by the introduction of a name that is its senior synonym or homonym (ICZN 2000: Article 23.2). Therefore, if further research corroborates the present hypothesis of invalidating *S. affinis*, all short-snouted pipefishes from the western Gulf of Mexico should be referred to as *S. scovelli*.

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ISOLATION AND CHARACTERIZATION OF NITROGEN-FIXING BACTERIA NODULATING *PHASEOLUS VULGARIS* IN THE LOWER RIO GRANDE VALLEY OF TEXAS

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Abstract.–To establish a highly effective symbiosis between a legume plant and its bacterial symbiont it is essential to develop a successful program for the selection of superior strains to maximize production through N_2 fixation. Nitrogen-fixing bacteria from representative soil series of the Lower Rio Grande Valley region of southern Texas were examined for their symbiotic association with common 'Pinto' bean (*Phaseolus vulgaris* L.). PCR-RFLP analyses of genes associated with nitrogen fixation indicated that the bacterial isolates belong to the recently described *Rhizobium etli* and none were identified as *R. tropici*. Examination of symbiotic responses of the rhizobial isolates under controlled, aseptic conditions, indicated that 20% produced effective nodula-tion, 35% partially effective, and the remaining 45% produced parasitic or ineffective nodulation on the host legume. Strains UTPA-P 66, P82 and P80, each isolated from a different soil series, accounted for maximum plant response and nitrogen fixation activity. Although nodule morphology and size markedly varied among isolates, those with the highest tissue mass were associated with the highest production of shoot biomass. Electron micrographs of cross sections of 2-week old nodules indicated that effective bacterial cells were rod shaped, and more abundant than those enclosed in the peribacteroid membranes of ineffective nodules. To ensure adequate nodulation and N_2-fixation, it is necessary to inoculate beans with locally isolated nitrogen-fixing bacteria, regardless of any previous history of bean cultivation.

The inoculation of legume seeds with soil nitrogen-fixing bacteria (referred generically as 'rhizobia' or 'rhizobial cells') is a well-established technology of major economic and environmental importance. Natural populations of rhizobia can establish symbiotic associations with legumes. The success of the symbiosis, which is reflected by enhanced yield, depends largely on the performance of the rhizobial strains (Materon & Zibilske 2001). When needed, the symbiotic association could be optimized through seed inoculation (Materon & Ryan 1996). A well-nodulated common bean (*Phaseolus vulgaris*) crop can fix up to 100 kg of N_2 per ha annually, which is equivalent to 208.3 kg of urea fertilizer (Somasegaran & Hoben 1994). This legume is cultivated in areas

totaling 20,550 ha in the state of Texas (Smith & Anciso 2005). Most of this acreage is dedicated for commercial or processing purposes and approximately 60% of it is located in the Texas Winter Garden and in the High Plains region. The remaining production is obtained from small farms located in the southern part of the state (Smith & Anciso 2005).

Unfortunately, according to many researchers (Graham 1981; Graham et al. 2003), inoculation with introduced inoculant rhizobial strains does not always lead to an abundant effective nodulation to secure high rates of nitrogen fixation and seed production (Graham 1981; Graham et al. 2002; Materon 1991; Materon & Zibilske 2001; Mrabet et al. 2005). This fact could be related to the promiscuity observed in *P. vulgaris* (cf. Michiels 1998) or to other limiting factors, like the high rate of nitrogen fertilizer used in intensive agriculture, which is particularly detrimental for this legume species (Materon & Ryan 1996).

In addition, this grain legume species also has a reputation for weakness in its ability to nodulate and efficiently fix nitrogen in symbiosis (Graham 1981; Graham & Halliday 1987; Graham et al. 2003). The diversity of rhizobial species that are able to nodulate *P. vulgaris* in relation to symbiotic effectiveness has been recently reported by various researchers (Amarger et al. 1997; Bal et al. 1982; Mostasso et al. 2002; Mrabet et al. 2005).

A clearer knowledge of indigenous nitrogen-fixing bacteria is thus essential for the understanding of the ecological consequences of native biodiversity for legume production (Tamimi 2002). Information is virtually lacking on the symbiotic properties of indigenous populations of rhizobia and their capacity to nodulate and fix nitrogen when in symbiosis with locally-adapted cultivars of *Phaseolus* and other legume species grown in southern Texas. Other important characteristics of selected elite strains include their ability to compete and survive in the inoculant carrier and in soil, to colonize the rhizosphere, and to migrate in soil (Materon 1991;

Materon & Zibilske 2001; Tamini 2002). It is expected that further research on rhizobial ecology will illustrate the relationship between population size in the rhizosphere and nodulation. Local farmers should avoid the application of nitrogen fertilizers due to the inefficient nitrogen fixation capacity of the indigenous and introduced inoculant strains. In the present study, a wide variety of agricultural soils were sampled in southern Texas to identify and evaluate the presence of any of five rhizobial species known to nodulate *P. vulgaris*.

Materials and Methods

Soil sampling procedures.–Soil samples were collected from 83 agricultural sites representing the different soil types of the region. The sites were selected using soil survey maps of four counties (Hidalgo, Cameron, Willacy and Starr) located in the lower Rio Grande Valley of Texas. The Hidalgo soil series is classified as fine-loamy, mixed, active, hyperthermic typic Calciustoll; the Willacy as fine-loamy, mixed, super-active, hyperthermic Udic Argiustolls; the Cameron as Sodic Haplusterts; and, the Starr soil series as fine-loamy, mixed, semi-active, thermic Fluventic Dystrudepts (USDA, 2007). These soils are mildly to moderately alkaline with pH values ranging from 7 to 8, and very low soil organic matter contents ranging from 0.1 to 0.8 % organic carbon. The total area covered by these counties is 8,859 km^2.

At each sampling site, three soil samples were taken at 0, 5 and 10 m within a linear transect. Approximately 120 g of soil was collected from each point from a depth of 5-20 cm using a hand shovel. Samples were mixed and placed inside a plastic container and kept in a box away from sunlight. To prevent cross contamination, the shovel was sprayed in between sampling with a wash bottle containing a 50% Clorox™ (commercial hypochlorite bleach) solution, and rinsed with water three times (Beck et al. 1993). Elevation and air temperature were recorded as well as the longitude/latitude coordinates of the sampling site corresponding to the center of the transect using a manual Magellan Map 410 Global

Positioning System (GPS) receiver. Samples were kept refrigerated at 3°C before analyses. The most probable number technique (MPN) procedure was applied for the calculation of number of nitrogen-fixing bacteria per gram using *Phaseoulus vulgaris* L. as a trap species (Vincent 1970).

Inoculation and cultivation of bean seedlings.–Trays containing layers of Sunshine™ soil mix potting medium made of composted bark, peanut hulls, Canadian sphagnum peat moss, perlite, and a wetting agent, were sterilized at 120°C, 0.1 MPa for 1 h. Plastic pots with a diameter of 10 cm were then filled to 70% capacity with the sterile potting medium. Seeds of *P. vulgaris* L. cv. 'Pinto' were surface sterilized by exposing them to 95% ethanol for 1 min followed by immersion in a 5.25% (vol/vol) sodium hypochlorite (NaOCl) solution for 1 min and then repeatedly rinsed in deionized sterile water (Beck et al. 1993; Vincent 1970). Two seeds were aseptically planted in each pot within a hole containing two spoonfuls of soil sample that acted as the inoculant with the intention to induce colonization and infection of the seedling root hairs. The inoculated seeds were then covered with more sterile potting soil. Pots were covered with saran wrap and distributed on tables inside a greenhouse, located at the USDA Kika de la Garza Center for Subtropical Research in Weslaco (Texas), equipped for ambient temperature control and deionized water for irrigation purposes.

The average temperature throughout the experiments was 21°C with a maximum of 23.6°C and a minimum of 15.6°C. Uninoculated controls included sixteen pots each with seeds and no soil inoculum. Aseptic handling of pots and instruments were meticulously practiced to avoid any cross contamination. Plants were watered once a day by an automatic sprinkler system using deionized water. Additional pots were set aside for early harvesting of effective and ineffective nodule samples for electron microscope observation. Plants were harvested after a 30-day period of growth

for observation of patterns of nodulation and isolation of rhizobia from nodules.

Isolation of rhizobia from trap legume host nodules.–Roots were gently removed from pots and rinsed with running tap water to remove soil and debris. Three nodules were carefully detached from the crown area of the primary root. Nodules were immersed into a beaker with 95% ethanol for 10 sec, and then transferred into another beaker with a 2.5% (vol/vol) sodium hypochlorite (NaOCl) solution for 30 sec. Nodules were then rinsed five times in sterile deionized water to remove any excess of chlorine (Beck et al. 1993; Vincent 1970). Each nodule was crushed with a tip of a flamed broad tip forceps and its contents deposited onto the surface of a yeast-extract mannitol (YM) agar plate. Inoculated plates were incubated overnight at 37°C. Once bacterial growth was detected, a loopful of the confluent growth was streaked onto the surface of another YM agar plate to obtain single colonies. Samples of each isolate were examined microscopically using the Gram's stain technique. Nitrogen-fixing bacteria specific to *Phaseolus* are rod shaped and Gram negative (Vincent 1970).

All the presumptive isolates were grown aerobically on YM agar slants and stored at 4°C for further examination. Once tested for their symbiotic ability to produce N_2-fixing nodules, isolates were assigned a collection name with the prefix UTPA-P followed by its respective catalog number as a suffix. To prevent genetic variation and loss of effectiveness traits, the bacterial cultures were lyophilized and kept in small vials at 4°C (Beck et al 1993; Somasegaran & Hoben 1994).

Symbiotic evaluation of rhizobial cultures previously isolated from nodules.–A total of 690 pots of 10.2 cm diameter were filled with coarse grade vermiculite that had been previously rinsed, drained with deionized water and sterilized using an autoclave set at 121°C and 0.1 mPa for 60 min. Two *P. vulgaris* L. cv. 'Pinto' bean seeds were surface sterilized (see procedure above) and planted in

each pot. The solutions of each of the bacterial cultures, grown at 27°C, had been adjusted by turbidity and plate count to provide a uniform density of approximately 10^8 bacteria per ml (Beck et al. 1993; Somasegaran & Hoben 1994). One ml of the bacterial suspension was added to inoculate each of the seeds. Pots were then covered with a polyvinyl chloride plastic wrap to prevent any cross contamination during the early growth stage of the seedlings. After five days of growth an extra cover of plastic wrap was used to reseal around the stem of the plant. Pots were arranged in 23 groups of 30 replicated plants per isolate. Each group was split into three groups and randomly assigned a permanent position in the greenhouse. The inoculated treatments included an isolate from a commercial peat-based inoculant obtained from Becker Underwood (Ames, Iowa), labeled as strain 132, for comparison and contrast with the isolates under study. Uninoculated controls were not supplemented with nitrogen fertilizer, received no rhizobia, and were hydrated only with deionized water. Uninoculated nitrogen-fertilized controls were irrigated with N-free Hoagland solution supplemented with 5 mM KNO_3 (Beck et al. 1993). All pots were regularly weighed and adjusted to a weight of 480 g by carefully adding N-free Hoagland solution. Plants were harvested after 30 days of growth. The rooting medium was carefully removed under a gentle stream of water so that not to detach nodules. For each plant, numbers and nodule distribution, as well as total nodule mass, were graded and recorded. Other plant parameters such as number of leaves and flowers were recorded. Means of dry shoot mass were used to measure plant response to inoculation for comparison with the nitrogen-deficient dry shoot mass mean of the uninoculated plants and referred to as the 'symbiotic index' (Beck et al. 1993; Vincent 1970).

Genotypic characterization of rhizobial cultures.–Advanced molecular techniques allows a more accurate and precise determination of phylogenetic relationships among species. For this reason, PCR-RFLP analyses of symbiotic and genomic genes were used to determine the taxonomic position of twenty-one of the

rhizobial strains able to nodulate *P. vulgaris*. Three restriction endonucleases, *Hin*fI, *Rsa*I, and *Alu*I, were used in this study to digest the PCR amplification products for *nod*A, *nif*H, and *GSII* genes from all isolates representing the different locations sampled in the Lower Rio Grande Valley. Oligonucleotides used as primers included GSII-1&4 (Turner & Young 2000) for sequencing, nodA-1&2 (Zhang et al. 2000) for the amplification of *NodA*, and nifH-1 (Haukka et al. 1998), and nifH-2 (Early et al. 1992) for the amplification of the *nifH* gene. RFLP products were run on 1.5% agarose gels, stained with ethidium bromide, and patterns were scored manually. Representatives of each of the PCR-RFLP types were sequenced using GSII PCR products. Purification using QIA quick PCR Purification Kit (Qiagen Hamburg GmbH) was performed following the manufacturer's instructions. Sequencing was done for each product in both directions, and analyzed using Clustal-X (Thompson et al 1997) and BLAST analysis. The Basic Local Alignment Search Tool (BLAST) was used to identify regions of local similarity between sequences.

Preparation of nodule tissues for transmission electron microscopy.–Clean 14-day old nodules were washed in 70% ethanol and rinsed in distilled water three times. Nodules were then fixed in formalin-alcohol (70%)-acetic acid (FAA) overnight and dehydrated in 70, 80, 90 and 100% ethanol for 30 min at each dilution. The nodules were then placed in a Samdri critical point dryer (Bozzola & Russell 1999). Nodule samples were immersed in liquid CO_2 at -10°C and exposed to a critical point pressure of 7.4 mPa. After the drying process, treated samples were mounted on stubs and sputter coated with gold-platinum for 60 sec. Images were observed on a Leo 453 scanning electron microscope operated at low beam voltage value (EHT) of 15 kv, and digitally recorded for further use. Ten replications of each leghemoglobin-containing nodules and ineffective nodules were prepared for observation.

Statistical analysis.–Plants were arranged in a randomized block design in the two greenhouse studies. Data were evaluated by

analysis of variance using the general linear models procedure of the Statistical Analysis Software (SAS Institute, Cary, NC). Significant differences ($P < 0.05$) between mean values of main effects were determined using the Ryan-Einot-Gabriel-Welsh Multiple Range Test separation procedure for all analyses.

Results and Discussion

Genetic characterization.–There are five described bean rhizobial species to this date: *Rhizobium leguminosarum* bv. *phaseoli* (Jordan), *R. tropici* (Martinez-Romero et al.), *R. etli* (Segovia et al. 1993), *R. gallicum* and *R. giardinii* (Amarger et al.). In addition, other isolates able to nodulate bean show distinct phylogenetic positions and may well represent other species (Eardly 1992). To establish the rhizobial species designation for the region under study, twenty-one isolates were genetically characterized on the basis of the polymorphism of 16S rRNA genes. The procedure differentiates between *R. etli* and *R. tropici* that are known to nodulate *P. vulgaris* L. in this continent (Segovia et al. 1993). Results indicated that the PCR-RFLP profiles of the 16S rRNA gene region obtained with nodule-producing strains detained during this study matched those of *R. etli.* In addition, the digestion patterns were not similar to those produced by *R. tropici.* Thus, the predominant populations of nitrogen fixing bacteria from this collection were phylogenetically assigned to the species *R. etli.* These results are in accordance to those of Segovia et al. (1993) who proposed that the species *R. etli* predominates in Mesoamerica, a region considered one of the main centers of origin of *P. vulgaris*.

Rhizobial strain selection.–None of the soil collection sites had a recent history of inoculation with N_2-fixing bacteria with most soils being slightly alkaline. The predominant bean-nodulating population of *R. etli* in soils of the Lower Rio Grande Valley could be attributed to (a) introduction of the cultivation of beans by past agriculturalists who could have obtained seed from central or southern Mexico (Perez-Ramirez 1998), and (b) edaphic properties and favorable environmental conditions (c). Determinations of

Table 1. Physical and biological characteristics where soil samples containing rhizobia able to nodulate *Phaseolus vulgaris* L. were collected from agricultural soils of the Lower Rio Grande Valley during the spring season of 2004.

Isolate	Soil series	Coordinates	Altitude (m)	pH	MPN rhizobia/ g soil
UTPA-P5	Zapata loamy	26.31.22N 98.41.26W	146.86	7.53	2.1×10^4
UTPA-P6	Reynosa silty clay	26.22.11N 98.47.44W	43.89	7.61	4.2×10^3
UTPA-P0	Ramadero loam	26.31.37N 98.41.12W	13.41	7.65	8.3×10^4
UTPA-P17	Hidalgo sandy loam clay	26.28.57N 97.43.11W	3.05	7.84	4.0×10^4
UTPA-P18	Hargill sandy loam	26.26.43N 97.57.21W	13.10	8.01	1.1×10^3
UTPA-P19	Wallacy fine sandy loam	26.28.55N 97.44.47W	14.93	7.75	8.3×10^3
UTPA-P21	Wallacy fine sandy loam	26.28.56N 97.44.24W	13.41	7.72	4.1×10^4
UTPA-P34	Randado Cuevitas loamy	26.32.34N 98.10.21W	22.55	7.81	5.4×10^4
UTPA-P35	Evant silty clay	26.26.57N 98.34.05W	9.75	7.69	7.3×10^4
UTPA-P39	Wallacy fine sandy loam	26.26.56N 98.07.07W	21.94	7.87	6.9×10^3
UTPA-P44	Delfina fine loamy	26.26.54N 98.03.05W	21.33	7.84	8.7×10^3
UTPA-P46	Delfina fine loamy	26.21.46N 98.12.46W	25.91	7.73	5.5×10^4
UTPA-P48	Delmita fine loamy	26.32.23N 98.09.13W	13.71	8.02	4.4×10^4
UTPA-P58	Delfina fine loamy	26.32.10N 98.07.47W	17.98	7.81	5.1×10^4
UTPA-P62	Harlingen clay	26.07.35N 97.49.24W	10.67	7.56	6.7×10^4
UTPA-P66	Camargo silty loam	26.04.11N 97.48.54W	12.19	7.63	3.3×10^3
UTPA-P69	Matamoros silty clay	24.04.20N 97.48.55W	8.23	7.60	2.1×10^3
UTPA-P72	Raymonville clay loam	26.20.17N 97.49.13W	27.43	7.45	6.4×10^4
UTPA-P80	Hidalgo fine sandy loam	26.12.27N 97.30.29W	12.19	7.63	5.9×10^4
UTPA-P82	Toscano clay	26.06.31N 97.48.36W	16.76	7.41	8.2×10^3

bacterial numbers by the MPN method indicated that in most of the soils of the region the indigenous rhizobial population of bean rhizobia ranged between 1.1×10^3 to 8.3×10^4 rhizobia per gram of soil (Table 1). This number is low but could be higher if stimulated by the presence of the host legume.

Infection and structure of nodules.–When preparations of nodule tissue were observed under the scanning electron microscope, it was evident that bacteroids from effective nodules were more visible and abundant inside peribacteroid membranes than those found in tissue preparations from ineffective nodules. Functional nitrogen-fixing bacteria were rod shaped and displayed a non-marked pleomorphism (Fig. 1). Conversely, membrane tissues of ineffective nodules had a low amount of bacteroids. These observations are in accordance to those of Holl & LaRue 1976, Bal

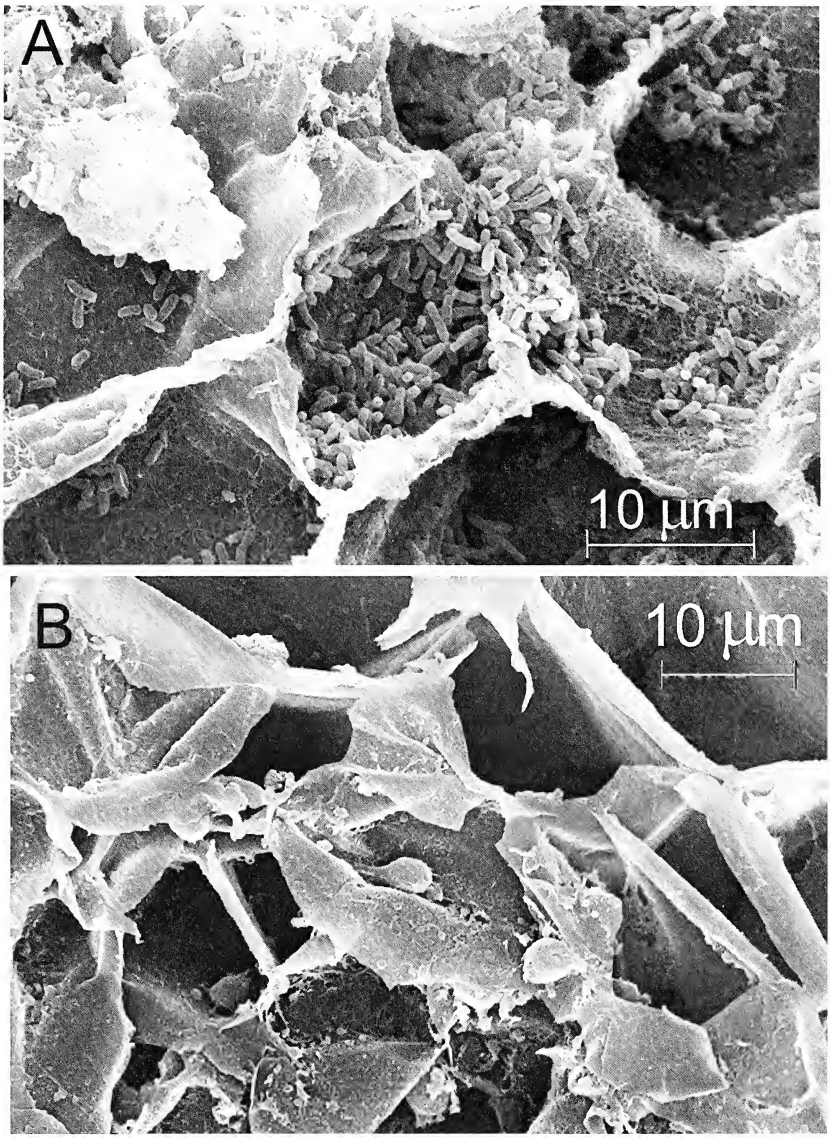

Fig. 1. Examination of sections of nodule tissues of *Phaseolus vulgaris* L. by transmission electron microscopy. Image (a) shows an abundant number of bacteroids inside peribacteroid membranes of an effective nodule corresponding to strain UTPA-P66 at × 2,180. In contrast, emage (b) indicates a lack of bacteroids inside bacteroid membranes from a 10-day old ineffective nodule of strain UTPA-P69 at 1,840×.

et al. (1982) and Sprent & De Faria (1988). Infection threads were seen only in young cells of nodules. Bacteria were released from these cells soon after the end of the infection process. Infection threads appeared to have a defined sheath with a low number of bacteria visible inside. It was also evident that populations of the effective isolates were enclosed in large peribacteroid membranes (Fig. 1). Differences in the degree of adhesion between perio-bacteroid membranes and the envelope outer membranes may explain variations in the number of bacteriods enclosed by peribacteroid membranes in nodules of this host plant.

Nodulation and N_2-fixing potential.–Some researchers have reported that this legume species has a weakness in the ability to nodulate and fix nitrogen in symbiosis (Graham 1981; Graham & Halliday 1977; Graham et al. 2003). Contributing factors may include poor soil conditions under which the crop is grown (*e.g.*, low soil organic matter, phosphorus content, intermittent or terminal drought – which are considered typical situations in the Rio Grande Valley region of Texas). Other factors that merit consideration are the short growing season of many cultivars, the limited energy supply to the nodule, and, in some areas, the effects of mineral nitrogen fertilization commonly applied to inoculated *Phaseolus* plants in the field (Graham & Halliday 1977; Michiels et al. 1998).

Of the 83 sites examined for soil containing indigenous nitrogen-fixing bacteria for *Phaseolus*, only 20 of those sites contained rhizobia that were able to nodulate the host plant (Table 1). The rhizobial isolates varied markedly in their degree of nitrogen fixation as well as in size and shape of nodules produced. Several of the isolates formed nodules with an average dry mass tissue ranging from 160 to 620 mg per plant and were ranked as effective (E) or functional in fixing nitrogen (Table 2). Another cluster of strains were ranked as partially-effective (PE), and those producing lower nodule tissue with no visible traces of leghemoglobin were classified as ineffective (I) in fixing nitrogen.

Table 2. *Phaseolus vulgaris* response to inoculation with isolates of *R. etli* under controlled growth conditions. Means with the same letter within a column are not significantly different at 5% probability level. Symbiotic effectiveness index calculated as shoot biomass of the mean of the inoculated plant divided by the mean of the shoot biomass of the non-inoculated control. E=effective, PE=partially-effective and I=ineffective nodulation (Beck et al. 1993). Strain 132 is a commercial strain from Becker Underwood (Ames, Iowa).

Rhizobial isolate	Shoot biomass (g)	Total nodule mass (g)	Number of crown nodule	Number of lateral mass	Symbiotic effectiveness index
UTPA-P5	2.76^{cde}	0.26^{de}	7.04^{ef}	17.80^{def}	7.26 (PE)
UTPA-P6	1.96^{ef}	0.18^{de}	8.17^{e}	12.09^{ef}	5.16 (I)
UTPA-P10	3.25^{bcd}	0.38^{bcd}	17.52^{cd}	22.52^{c}	8.55 (E)
UTPA-P17	2.24^{def}	0.17^{fg}	2.02^{g}	13.00^{efg}	5.89 (I)
UTPA-P18	3.03^{cde}	0.41^{bc}	25.93^{b}	17.33^{de}	7.97 (PE)
UTPA-P19	2.80^{cde}	0.46^{bc}	11.00^{cd}	39.26^{a}	7.36 (PE)
UTPA-P21	2.29^{def}	0.28^{de}	9.90^{cde}	14.52^{de}	6.02 (I)
UTPA-P34	2.73^{cde}	0.22^{de}	3.48^{efg}	18.74^{de}	7.18 (PE)
UTPA-P35	2.47^{def}	0.19^{de}	0.83^{gh}	18.57^{de}	6.50 (I)
UTPA-P39	2.15^{def}	0.20^{fgh}	1.38^{gh}	11.23^{fg}	5.65 (I)
UTPA-P44	2.78^{cde}	0.28^{de}	13.87^{cd}	8.77^{fg}	7.31 (PE)
UTPA-P46	2.23^{def}	0.16^{fg}	0.88^{gh}	15.00^{def}	5.86 (I)
UTPA-P48	3.10^{cde}	0.32^{cd}	8.28^{e}	30.14^{b}	8.15 (PE)
UTPA-P58	2.97^{cde}	0.26^{ef}	3.07^{efg}	15.15^{def}	7.81 (PE)
UTPA-P62	2.83^{de}	0.20^{fg}	4.17^{ef}	13.04^{ef}	7.44 (I)
UTPA-P66	4.19^{ab}	0.55^{ab}	13.24^{cd}	41.76^{a}	11.02 (E)
UTPA-P69	1.95^{ef}	0.23^{de}	1.10^{gh}	22.30^{c}	5.13 (I)
UTPA-P72	1.64^{fg}	0.18^{fgh}	0.89^{gh}	12.93^{ef}	4.31 (I)
UTPA-P80	4.29^{a}	0.55^{ab}	40.30^{a}	18.11^{def}	11.28 (E)
UTPA-P82	3.78^{abc}	0.62^{a}	43.08^{a}	27.50^{bc}	9.95 (E)
Strain 132	2.53^{def}	0.27^{de}	6.63^{ef}	21.5^{c}	6.65 (I)
UC+N	5.31^{a}	0^{i}	0^{h}	0^{h}	--
UC-N	0.38^{g}	0^{i}	0^{h}	0^{h}	1.00

Isolates producing ineffective nodulation were not associated with plants having higher amounts of shoot biomass and thus had the lowest symbiotic effectiveness indices (Table 2). Strains P82, P80 and P66 produced an average of 55.8 mg of nodule tissue, a significantly higher ($P < 0.05$) amount of tissue biomass than any of the other rhizobial strains tested.

It has been known that crown nodulation is an indication of competitive nodulation success in the early events of root hair infection by rhizobia colonizing the rhizosphere (Graham & Halliday 1977; Graham 1981). All rhizobial isolates formed nodules in both the primary and secondary root system. In only 4 cases (strains P18, P44, P80 and P82), the highest proportion of nodules was located within the crown or upper section of the primary root. Strain P80 formed 40 effective functional nodules in the crown and 18 on the lateral root system. On the other hand, strains P19 and P66 produced a higher ($P < 0.05$) number of nodules in the lateral root system as compared to those produced by other rhizobial isolates (Table 2). Nodule distribution due to strains P80 and P82 was more concentrated at the crown section of the primary roots than in the secondary root system. Although P19 was not a good nitrogen fixer, it had 78% of its nodules positioned in the lateral root system. Despite a uniform cell density of 10^8 cells applied to the emerging root, the response of strain P19 may suggest that early root hair infection events for nodule formation at the crown area could involve a complex series of physiological events.

The commercial inoculant strain, Becker Underwood No. 132, currently used to inoculate crops of *P. vulgaris* in the region, proved to be inferior to most of the indigenous rhizobial isolates in their ability to fix nitrogen. Shoot and nodule tissue mass produced using this inoculant was significantly lower ($P < 0.05$) as compared to strains P66, P80 and P82 (Table 2). Furthermore, strains P80 and P66 produced a comparable biomass ($P > 0.05$) as that produced by plants that received no inoculation but were supplemented with

mineral nitrogen. The ability of these indigenous rhizobia to produce nodules with visible interior red pigmentation due to leghemoglobin, indicated that they were effective with a potential to establish successful symbiotic associations. The elite nitrogen-fixing strains identified in this study, were able to produce up to eleven times more biomass than plants with nitrogen deficiency symptoms. Conversely, the shoot biomass of plants that received no inoculation and no mineral nitrogen was lower ($P < 0.05$) than any shoot mass value of any rhizobial strain under study. No nodules were observed in plants of the uninoculated or mineral nitrogen control treatments.

The potential of using strains P66, P80, and P82 as local inoculants for bean plants depends, however, on their growth, competitiveness and persistence under the soil conditions of the Lower Rio Grande Valley. Among these conditions, pH, drought, and microbial competitors should be evaluated. More information is required about the performance of these elite rhizobial isolates under field experimentation to further establish their value as commercial inoculants. These results point out the benefits which could be achieved by selecting efficient and competitive strains from populations of indigenous nitrogen-fixing bacteria from agricultural soils of the region. It was evident that these elite isolates are better adapted to local environmental conditions than the introduced commercial inoculant product. The latter resulted in low symbiotic effectiveness indices comparable to plants with ineffective nodulation (Table 2).

Selecting highly effective rhizobia for inoculants should not neglect the ability of the introduced bacteria to compete with the indigenous populations of nitrogen-fixing bacteria. Introduction to agricultural fields, in different geographical areas, remains a decisive step when testing selected strains. These results point out the benefits which could be achieved by selecting efficient and competitive strains among natural populations of *Rhizobium etli*.

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NOTABLE COUNTY RECORDS FOR ROLLING PLAINS ANGIOSPERMS OF NORTH CENTRAL TEXAS

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Abstract.–Based on data from fieldwork in Wichita County on a privately owned ranch located in the Rolling Plains region of Texas, thirty-nine species of flowering plants in 23 families are reported as new distribution and occurrence records for Wichita County, Texas. Twenty-one of these species represent major range extensions for the species in Texas. These new distribution and occurrence records provide baseline floristic data from a region that is poorly known compared to other regions of the state. Most of the new distributions are native but 31% are introduced species. Distribution data is critical for understanding native plant ranges and the spread of introduced species in the state.

The flora of the Rolling Plains region of Texas is poorly known compared to other regions of the state (Turner et al. 2003a; 2003b). In fact, no comprehensive flora has been published for this region. Wichita County is located within the Rolling Plains region of Texas and little floristic work has been conducted in the county. The Rolling Plains form a partial boundary with the southeastern edge of the Panhandle region of Texas and extend southward to the northern Edwards Plateau and eastward to the West Cross Timbers regions of Texas. The Rolling Plains is considered a subsection of the Great Plains region of the central United States (Correll & Johnson 1970; Diggs et al. 1999). Rainfall ranges from approximately 56cm (22 in.) in the west to almost 76cm (30 in.) in the eastern region with a summer dry period and corresponding high temperatures (Correll & Johnston 1970). Soils vary from coarse sands to heavy clays, or red-bed clays and shales with much of the area in crop or range land (Correll & Johnston 1970). The original prairie vegetation included tall and mid-grasses with short-grass species increasing under grazing. Mesquite (*Prosopis glandulosa*) is a common invader of all soil types, and heavy grazing by livestock (among other factors) has resulted in invasion of various

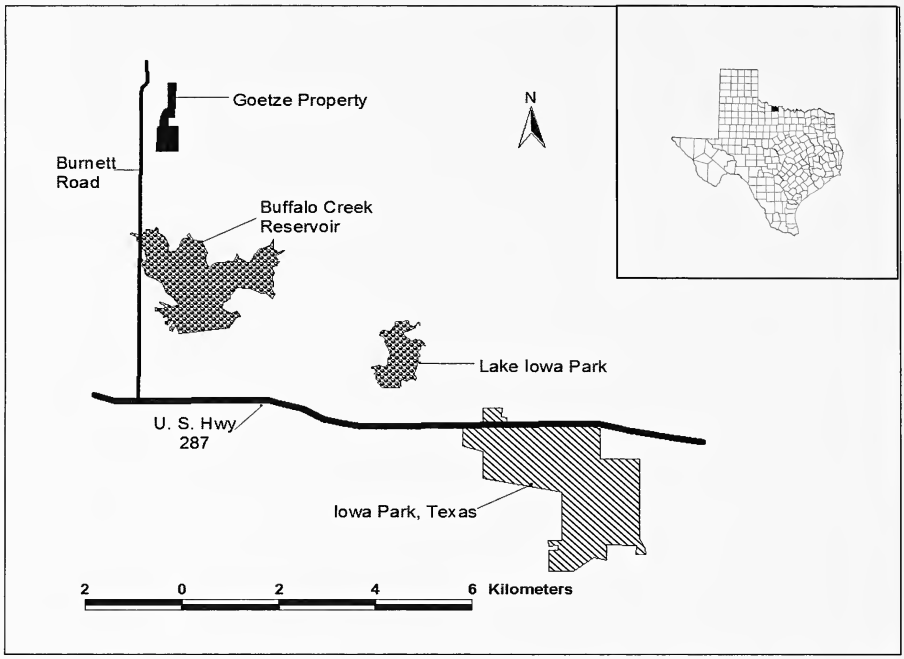

Figure 1. Map showing the general location of floristic surveys within Wichita County. Inset shows location of Wichita County.

species of grasses and forbs (Correll & Johnston 1970; Diggs et al. 1999).

Study Area and Methods

Plants were collected in Wichita County, Texas, mostly from a site 3 km N of Buffalo Creek Reservoir previously described by Stangl et al. (1992) as study site 2 - Goetze property or at Buffalo Creek Reservoir (Figure 1). Soils at the collecting sites were primarily clay loams. Much of the area is utilized for crop production and pasture lands. Plants were collected along roads, on the margins of wheat fields, in heavily grazed pasture lands, and around the margins of Buffalo Creek Reservoir.

Collecting activities occurred in July 2003, April and May 2004, as well as May and September 2005. Plants were identified using *Shinners & Mahler's Illustrated Flora of North Central Texas* (Diggs et al. 1999) and the *Manual of the Vascular Plants of Texas*

(Correll & Johnston 1970). Nomenclature of native or naturalized plants was standardized and county records as well as range extensions are based upon the *Atlas of the Vascular Plants of Texas* (Turner et al. 2003a & b). Taxa are discussed alphabetically according to family. Voucher specimens are deposited in the Tarleton State University Herbarium (TAC).

Results and Discussion

Range extensions and reports of county records are critical in providing baseline floristic data with which to evaluate introduced species and better understand distribution of native species (Turner et al. 2003a). This study resulted in new distribution and occurrence records for 39 species from Wichita County in relation to information currently available in the *Atlas of the Vascular Plants of Texas* (Turner et al. 2003a; 2003b). Sixty-nine percent of the plants collected were native species. Eighteen species represent new county records for Wichita County but also have been reported (Turner et al. 2003a; 2003b) to occur in counties bordering Wichita County (Table 1).

Twenty-one species represent major range extensions for plant species in Texas. While most of the flora collected in this investigation was native (69%), 31% of the major range extensions are introduced species. Each of these new species records is arranged by family and discussed individually within the accounts below.

Family Apiaceae

Torilis arvensis (Huds.) Link (Hedge-parsley; TAC 4007), an introduced species from the Mediterranean region known from disturbed habitats (Diggs et al. 1999), is chiefly found in the Blackland Praires and Edwards Plateau regions of Texas (Correll & Johnston 1970). The closest collections to the Rolling Plains locality in Wichita County are Grayson County to the east, Palo Pinto County to the southeast, and Garza County to the southwest (Turner et al. 2003a).

Table 1. Floral records for Wichita County that have also been reported from bordering counties (Turner et al. 2003a & b) including Archer (A), Baylor (B), Clay (C), and Wilbarger (W). For species that have common names, they are included in parentheses at the end of the scientific name. Plants that are native (N) to north central Texas and introduced (I) are indicated. Accession numbers for the Tarleton State University Herbarium (TAC) are included.

Family	Species	Bordering Counties	N/I	TAC
Agavaceae	*Yucca arkansana* Trel. (Arkansas yucca)	A, C	N	3992
Asteraceae	*Aphanostephus ramosissimus* DC. var. *ramosissimus* (Plains lazy daisy)	A, W	N	4005
	Helianthus maximiliani Schrad. (Maximilian sunflower)	C	N	4018
	Senecio tampicanus DC. (Yellowtop)	A	N	4027
	Sonchus asper (L.) Hill (Prickly sow-thistle)	C	I	4015
Boraginaceae	*Buglossoides arvensis* (L.) I.M. Johnst.	C	I	4021
Brassicaceae	*Lepidium oblongum* Small (Veiny pepperweed)	B	N	4020
	L. virginicum L. (Virginia pepper-grass)	A	N	4019
Cuscutaceae	*Cuscuta cuspidata* Juss. *ex* Choisy (Cusp dodder)	W	N	4010
Nyctaginaceae	*Mirabilis linearis* (Pursh) Heimerl (Linear-leaf four-o'clock)	A, C	N	4002
Onagraceae	*Oenothera laciniata* Hill (Cut-leaf evening-primrose)	C, W	N	4026
Papaveraceae	*Argemone polyanthemos* (Fedde) G.B. Ownbey	C, W	N	3991
Pedaliaceae	*Proboscidea louisianica* (Mill.) Thell. (Common devil's-claw)	A	N	4004
Poaceae	*Cynodon dactylon* (L.) Pers. (Bermuda grass)	C	I	3996
	Poa arachnifera Torr. (Texas blue grass)	A	N	4001
Scrophulariaceae	*Linaria texana* Scheele (Texas toad-flax)	A, C, W	N	4023
Solanaceae	*Solanum rostratum* Dunal (Buffalo-bur)	A	N	3989
Ulmaceae	*Celtis reticulata* Torr. (Net-leaf hackberry)	C	N	4011

Family Asteraceae

Tragopogon dubius Scop. (Goat's-beard; TAC 3997), introduced from Eurasia, is common in the west Cross Timbers and is migrating from the Panhandle to at least Grayson County in the east (Diggs et al. 1999). The collection in Wichita County helps affirm its migration from the Panhandle, where it is known from most of the counties of that region (Turner et al. 2003a).

Family Brassicaceae

Chorispora tenella (Pall.) DC. (Blue-mustard; TAC 4012), introduced from Asia, occurs in disturbed areas. This species has only recently been found in Texas (Diggs et al. 1999). The collection in Wichita County is the first report of this species from the Rolling Plains region of the state (Turner et al. 2003a).

Family Cactaceae

Echinocactus texensis Hoffer (Horsecrippler; TAC 3993) is native to Texas and usually found in sandy or limey soils in north central Texas, south and west to the South Texas Plains and Trans Pecos regions (Diggs et al. 1999). The closest collections to the locality in Wichita County are Foard County to the west and Young County to the south (Turner et al. 2003a).

Mammillaria heyderi Muehlenpf. (Nipple cactus; TAC 4016) is native to Texas and the closest collections to the locality in Wichita County are Randall County to the northwest and Brown County to the south (Turner et al. 2003a). The collection in Wichita County is the first report of this species from the Rolling Plains region of the state.

Family Geraniaceae

Geranium carolinianum L. (Carolina crane's-bill; TAC 4006) is a native species occurring in woods, fields, and waste places in

various kinds of soils throughout most of Texas (Diggs et al. 1999). The collection from Wichita County extends its known range from Throckmorton County to the south and Montague County to the east into the northern Rolling Plains (Turner et al. 2003a).

Family Moraceae

Maclura pomifera (Raf.) CK Schneid. (Osage-orange; TAC 4024) is found in stream bottoms, lower slopes, and waste places mainly in northeastern to north central Texas and southward into Central Texas and was originally native to only about 12 counties in this region (Diggs et al. 1999). *Maclura pomifera* is most common in eastern Texas (Correll & Johnston 1970) but has been reported from localities in the Texas Panhandle (Turner et al. 2003a). The collection represents the first record of this species from the Rolling Plains.

Family Phytolaccaceae

Phytolacca americana L. (Pokeweed; TAC 3999) is a native species found in stream bottom woods and thickets or occasionally in disturbed areas throughout much of Texas (Diggs et al. 1999). The collection represents the first report of this species from the Rolling Plains (Turner et al. 2003a).

Family Poaceae

Andropogon glomeratus (Walter) Britton, Sterns, & Poggenb. (Bushy bluestem; TAC 3998) is a native species known from roadsides and low moist areas throughout Texas (Diggs et al. 1999), but is more frequent in the eastern one-half of Texas than westward (Correll & Johnston 1970). The closest collections to the locality in Wichita County are Motley County to the west, Throckmorton County to the south, and Collin County to the southeast (Turner et al. 2003b).

Arundo donax L. (Giant reed; TAC 4009), introduced from the Mediterranean region, is known from nearly thoughout Texas (Diggs et al. 1999). The collection represents the first record of this species from the Rolling Plains outside Garza County to the southwest (Turner et al. 2003b).

Avena fatua L. (Wild oats; TAC 4014), introduced from Mediterranean region, is scattered in Texas along roadsides and in other disturbed habitats (Diggs et al. 1999). The closest collections to the locality in Wichita County are Childress County to the west and Jack County to the southeast (Turner et al. 2003b).

Bromus tectorum L. (Cheat grass brome; TAC 4003), introduced from Europe, is found in disturbed sites from north central Texas south and west to the Panhandle region of Texas (Diggs et al. 1999). This collection represents the first location of this species from the Rolling Plains of Texas (Turner et al. 2003b).

Poa annua L. (Annual bluegrass; TAC 3990), introduced from Europe, is found in disturbed sites throughout Texas (Diggs et al. 1999). This collection represents the first location of this species from the Rolling Plains region of Texas (Turner et al. 2003b).

Triticum aestivum L. (Wheat; TAC 3994) is a commonly cultivated species that was introduced from Asia. It frequently escapes into disturbed areas throughout Texas (Diggs et al. 1999). The closest collections to the locality in Wichita County are Randall County to the west and Young County to the south (Turner et al. 2003b).

Family Polygonaceae

Polygonum punctatum Elliott (Water smartweed; TAC 3995) is a native species occurring in wet areas throughout Texas, but it is more common in the eastern one-half of the state (Diggs et al. 1999). This collection represents the first location of this species from the Rolling Plains region of Texas. The nearest records are from Hemphill County in the Texas Panhandle and Tarrant County to the southeast (Turner et al. 2003a).

Rumex crispus L. (Curly dock; TAC 4013), an introduced species from Europe occurring in disturbed, moist areas, is widespread throughout Texas (Diggs et al. 1999). The closest collections to the locality in Wichita County are Childress County to the west, Throckmorton County to the south, and Cooke County to the east (Turner et al. 2003a).

Family Ranunculaceae

Ranunculus sceleratus L. (Blister buttercup; TAC 4008) is a native species found in moist, often sandy soils in north central Texas, South Texas, the Edwards Plateau, and Rolling Plains (Diggs et al. 1999). This collection represents the first location of this species from the Rolling Plains (Turner et al. 2003a).

Family Rosaceae

Crataegus viridis L. (Greenhaw; TAC 4022) is a native species occurring in stream bottoms, fields, and slopes in east and southeast Texas, west to the Edwards Plateau and Rolling Plains (Diggs et al. 1999). This specimen represents the first collection of this species from the Rolling Plains region of Texas (Turner et al. 2003a).

Family Sapindaceae

Sapindus saponaria L. (Western soapberry; TAC 4017) is a native species scattered throughout Texas. *Sapindus saponaria* occurs in stream bottoms, forest margins, and disturbed sites (Diggs et al. 1999). The closest collections to the locality in Wichita County are Childress and Cottle counties to the west, Young County to the south, and Grayson County to the east (Turner et al. 2003a).

Family Scrophulariaceae

Castilleja indivisa Engelm. (Entire-leaf paintbrush; TAC 4025) is a native species favoring sandy or silty soils within open woods, prairies, and disturbed areas in southeast and east Texas, west to the East Cross Timbers, but this species has been widely planted by the Texas Highway Department (Diggs et. al. 1999). This collection in Wichita County extends its range northward from Callahan County in the Rolling Plains (Turner et al. 2003a).

Family Typhaceae

Typha domingensis Pers. (Narrow-leaf cat-tail; TAC 4000) is a native species found in shallow water or wet ground throughout most of Texas (Diggs et al. 1999). The closest collections to this locality in Wichita County are Childress and Cottle counties to the west, Throckmorton County to the south, and Grayson County to the east (Turner et al. 2003b).

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HERPETOFAUNAL INVENTORY OF CAMP MABRY, AUSTIN, TEXAS: COMMUNITY COMPOSITION IN AN URBAN LANDSCAPE

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Abstract.–Amphibians and reptiles were surveyed using standard sampling techniques at Camp Mabry, Austin, Texas in 2004 and 2005 as part of the Texas National Guard's biological inventory of the property. A total of 555 individuals representing 20 species were documented including six anurans, five turtles, four lizards, and five snakes. Amphibians compromised 55% of all captures followed by turtles with 36%. Of the 555 specimens of reptiles and amphibians captured, 77.4% consisted of the following five species: *Bufo nebulifer, Syrrhophus marnocki, Trachemys scripta, Chelydra serpentina,* and *Rana catesbeiana.* Results suggest that despite Camp Mabry's potential habitat and its location within an ecoregion known to support a diverse assemblage of reptiles and amphibians, the herpetofaunal community is characterized by a lack of certain groups (e.g., large colubrid snakes and lizards) known from the surrounding area.

Camp Mabry, in north central Austin, Travis County, Texas, is the headquarters for the Texas Military Forces. Despite its urban location, this 152-ha training site retains some native habitat, in contrast to the surrounding urban environment. It is this undeveloped acreage that makes Camp Mabry a unique site for reptiles and amphibians within the City of Austin. At this location, populations of native animals remain within a dense urban context (Ferguson et al. 2005; Matthews & Abbott 2005; McDonough et al. 2005). Camp Mabry is geographically within the border of the Edwards Plateau ecoregion (The Nature Conservancy 2004). The Edwards Plateau is well known for its unique flora and fauna, and supports a relatively high level of faunal endemics (Goetze 1998). Several endangered species, threatened species, and species of special concern have been reported from this region (TPWD 2003), including the Golden-cheeked Warbler (*Dendroica chrysoparia*), Barton Springs salamander (*Eurycea sosurum*), and Tobusch fishhook cactus (*Ancistrocactus tobuschii*). It is important that the

managers of Camp Mabry remain informed regarding the current composition, relative abundance, and any subsequent changes in their faunal assemblages. To address this concern, Camp Mabry has authorized inventories to assess floral and faunal diversity on the base. Avian (Matthews & Abbott 2005), mammalian (McDonough et al. 2005) and insect (Abbott & Broglie 2006) inventories have been performed, however, until now a systematic survey of the herpetofauna had not been completed.

This paper reports results for the baseline herpetofaunal survey of Camp Mabry, Austin, Texas. The design of this study allows for ease of replication and subsequent long-term monitoring of the herpetological assemblage on the facility. The main objectives were to (1) inventory the herpetofaunal community; (2) provide abundance, relative density, and distributional data on the species found; and (3) suggest any management options affecting these taxa on the facility that were seen as appropriate.

Study Site

Camp Mabry is located within the city limits of Austin, Texas. The site is bound to the north by 45th Street, the east by Loop 1 expressway, to the south by 35th Street, and to the west by residential properties (Figure 1). Eighty-one hectares are available for training activities and include numerous buildings, such as houses, offices, maintenance facilities, storage, and indoor training facilities (Wermund & Avakian 1994). However, a significant portion of the site (40%) retains a variety of native vegetation that has been detailed by Wermund & Avakian (1994).

In 1996, The Nature Conservancy (TNC) defined four vegetation communities on Camp Mabry (Wolfe et al. 1996). These were meant to be descriptive designations within the site and do not strictly define divergent floristic community boundaries. The four designations provided by TNC were: Ashe Juniper-Oak Woodland (AJOW), Live Oak Woodland (LOW), Sugarberry-Elm

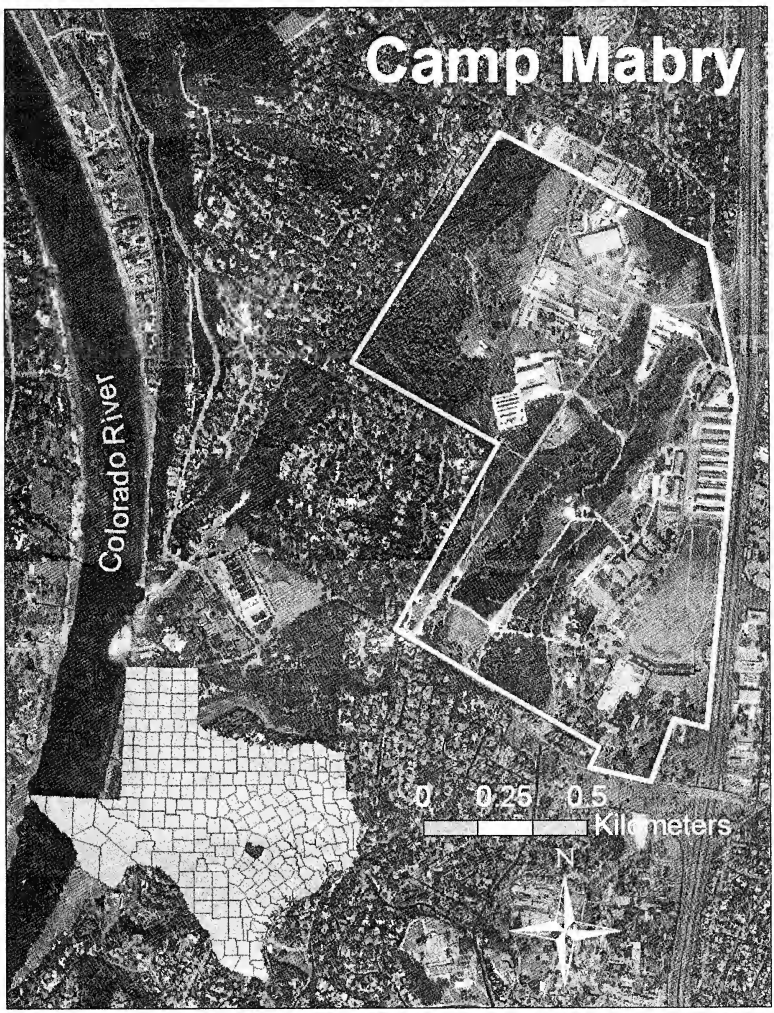

Figure 1. Map of Texas identifying Travis County and an aerial photograph of Camp Mabry illustrating its relation to the city of Austin and the Colorado River.

Woodland (SEW), and Live Oak Savanna (LOS). In addition to the native plants, several invasive plant species were found to occur on the site (Reinecke et al. 2002). The most abundant of those include chinaberry (*Melia azedarach)*, tree-of-heaven (*Ailanthus altissima*), and Japanese ligustrom (*Ligustrum japonicum*). The locations of these plants were typically in or adjacent to disturbed sites

including concrete waste piles, construction disposal sites, and other development sites, which were scattered throughout Camp Mabry fragmenting vegetation communities (Wermund & Avakian 1994; Wolfe et al. 1996). Water resources, including both ephemeral and permanent wetland areas are particularly important areas for reptile and amphibian fauna. There are several drainages on the northwest portion of the property that flow during rain events. The approximate bankful sizes of the two permanent ponds are 2.0 ha and 0.5 ha with depths of 3.7 meters and 2.1 meters, respectively (Farquhar et al. 1996).

Materials and Methods

Vegetation communities (Wolfe et al. 1996) were subdivided into eleven distinct zones. AJOW was divided into WA1, WA2, and WA3. LOW was divided into W1, W2, W3, W4, and W5. SEW was divided into RIP1 and RIP2. Category eleven, manicured area (MA), encompassed all areas of the facility managed as manicured lawns or parkland and represented the LOS category of vegetation (Ferguson et al. 2005). These zonal descriptions allowed a systematic inventory of the property conducive to future monitoring designs, while emphasizing the current vegetative structure of the facility.

Several survey techniques were used within each of the ten regions in an attempt to detect all possible reptiles and amphibians on the facility. Different species as well as ecological groups of reptiles and amphibians are often sampled more successfully using survey techniques specific to particular taxa (Parris et al. 1999; Sutton et al. 1999; Christiansen & Vandewalle 2000; Jenkins et al. 2003; Ford & Hampton 2005). Therefore, targeted species survey techniques were employed to maximize chances of documenting all reptile and amphibian taxa. For example, snakes and lizards were targeted by drift fences with funnels, but also detected using visual encounter searches and time constrained searching (Christiansen & Vandewalle 2000). Funnel placement was concentrated along RIP 1 and RIP 2 where erosion control fences had been previously

installed, although opportunistic placement of other funnel traps did occur. Due to high levels of human activity and constant training activity on the base, pitfall traps with drift fence arrays were not used, although with proper planning and placement this technique could be applied in the future.

Anurans were also monitored using standard call surveys (Heyer et al. 1994; Ford & Hampton 2005) at four designated sites (Ferguson et al. 2005). Call surveys were performed for 20-minute intervals after a five-minute acclimatization period and standard weather variables including relative humidity, temperature, and wind speed were monitored using a Kestrel hand-held weather meter. Abundances of anurans were estimated for large choruses (Heyer et al. 1994) by locating individuals calling along the shore with artificial lights to obtain relatively accurate numbers. To minimize double counting, anuran individuals were included in the results only if they were counted in different aggregates or locations during surveys. This helped to reduce over-estimating individuals at the four call sites and provided locations of other known anuran breeding sites on the property.

Aquatic sampling with eel pots in the creek and springs found in W1 sought to document karst dwelling salamanders (e.g., *Eurycea spp.*). Turtles were trapped using hoop nets (Steen & Gibbs 2004) and basking traps as well as by visual surveys of basking individuals. All terrestrial and aquatic species encountered during visual surveys were recorded. The site was visited four times a month at all times of the day and night, totaling 60 visits averaging 9.2 person hours per visit, for a total of more than 700 person hours.

Nocturnal road searches were conducted along a pre-determined 7.7-km stretch of roads that encompassed all of the habitat types, including the savannah (McDonough et al. 2005). A minimum of two persons performed road searches, driving no faster than 8.0 km/h, with one individual operating a spotlight and the driver scanning the roadways for reptiles and amphibians. Road cruising was conducted at various times of the night across all months surveyed.

All species encountered were opportunistically collected by hand capture, rubber bands, lizard nooses, and nets (Anderson 1965).

Taxonomic identification followed Dixon (2000). Each individual animal had standard measurements recorded, was marked, and released at their point of capture. Marking systems employed were approved by Texas State University's International Animal Care and Use Committee (IACUC #03FF16A316_03) and the Society for the Study of Amphibians and Reptiles and included toe clippings for lizards and anurans, drilling of carapace marginal scutes on turtles, and ventral pre-anal scale clips for snakes (Ferner 2007). Anurans were collectively marked based on capture year (e.g., toe four for 2004 and toe five for 2005) and all other individuals were assigned unique numbers via a combination of toe clippings. Most of the species encountered are considered common throughout their distribution and are well represented in vertebrate collections from surrounding areas. This fact, in conjunction with the low abundances of each species observed at the site resulted in the decision that the taking of traditional voucher specimens was not warranted. However, tissue samples were collected from each taxa and were accompanied by photographic vouchers when possible. All tissue samples were deposited in Dr. Michael R. J. Forstner's frozen tissue collection housed in the Biology Department of Texas State University-San Marcos. Exact locations of captures were recorded using a Garmin GPS II Plus unit.

Results

During the 14-month study period (21 April 2004 – 7 May 2005), 555 animals were documented representing 20 species, including six anurans, five turtles, four lizards, and five snakes (Table 1). An additional species, a juvenile Texas rat snake (*Elaphe obsoleta)* was salvaged in October of 2005 during a bat survey of Camp Mabry and is deposited in the Angelo State Natural History Collection, Angelo State University, San Angelo, Texas (ASNHC #14243). Amphibians compromised 55% of all captures followed by turtles with 36%. Of the amphibians, Coastal Plain toad (*Bufo nebulifer*)

Table 1. Amphibian and reptile species captured by method for Camp Mabry, Austin, Texas 21 April 2004 – 7 May 2005.

Species	Time Searches	Road Searches	Basking Trap	Hoop Net	Minnow Trap	Calls	Incidental	Total	%
Amphibia									
Anura									
Bufo nebulifer	9	3			5	144	4	165	29.7
Rana catesbeiana	4					42		46	8.3
Syrrhophus marnocki	6				1	83		90	16.2
Gastrophryne spp.						1		1	0.2
Acris crepitans						1		1	0.2
Pseudacris clarki						2		2	0.4
Reptilia									
Testudines									
Chelydra serpentina	3	1	2	43				49	8.8
Pseudemys texana	3			23			38	64	11.5
Sternothorus odoratus			1	2				3	0.5
Trachemys scripta	2		2	66			10	80	14.4
Trionyx spp.							4	4	0.7
Trionyx spiniferus				1				1	0.2
Squamata									
Scincella lateralis	6				1		11	18	3.2
Sceloporus undulatus	2							2	0.4
Sceloporus olivaceus	1				1		2	4	0.9
Hemidactylus turcicus	4						3	7	1.3
*Elaphe obsoleta**							1*		
Leptotyphlops dulcis	3							3	0.5
Nerodia erythrogaster	4							4	0.7
Tantilla gracilis	3							3	0.5
Thamnophis marcianus	2							2	0.4
Virginia striatula	4						1	5	0.9
Total	56	4	5	135	8	273	74	555	
%	10.1	0.7	0.9	24.3	1.4	49.2	13.3		

*Captured after the survey period

was the most abundant (ca. 165) while the red-eared slider (*Trachemys scripta*) was the most abundant reptile (ca. 80). Among the four species of lizards captured, the ground skink (*Scincella lateralis*) was the most frequently encountered with 18 individuals (56% of lizards captured) followed by the introduced Mediterranean gecko (*Hemidactylus turcicus*) with seven individuals or 22%. Among the five species of snakes captured, rough earth snake (*Virginia striatula*) was the most frequently captured with five individuals representing 29% of snake captures (Table 1).

Turtle trapping resulted in the capture of 132 unique individuals including 69 *T. scripta*, 31 common snapping turtle (*Chelydra serpentina*), 24 Texas river cooters (*Pseudemys texana*), and two stinkpots (*Sternothorus odoratus*), all of which received unique marginal scute marks.

Of the 555 specimens of reptiles and amphibians captured, 77.4% were five species (Table 1), *Bufo nebulifer,* cliff chirping frog (*Syrrhophus marnocki*), *Trachemys scripta, Chelydra serpentina,* and bullfrog (*Rana catesbeiana*). Additionally, 13 of 20 species were represented by five individuals or less including the cricket frog (*Acris crepitans*) and narrowmouth toad (*Gastrophryne spp.*), both heard only once.

Of the survey methods, time-constrained searches and incidental encounters yielded the greatest species richness, with 15 and 11 species respectively. All five snake species and two lizards (*Hemidactylus turcicus* and *Sceloporus undulatus*) were captured during either time-constrained searches or incidental encounters. For both reptiles and amphibians, time-constrained searches yielded the highest capture rate among active collecting techniques (one capture per 3.2 hr). Nocturnal searches lead to the capture of only four individuals composed of two species, *Bufo nebulifer* and *Chelydra serpentina*, with a capture rate of one capture per 4.9 hr. Among indirect methods, hoop net trapping provided the highest capture success for turtles (one capture per 0.13 trap night) whereas

anuran call surveys provided the highest detection rates for anurans (one capture per 4.5 min).

Discussion

This survey provides strong evidence that Camp Mabry's herpetofaunal community is characterized by a subset of reptiles and amphibians known to occur in this region of Texas (Dixon 2000). The natural histories of the five dominate species, *Bufo nebulifer, Syrrhophus marnocki, Trachemys scripta, Chelydra serpentina*, and *Rana catesbeiana* best explains the presence, and high relative abundance of these species on the site. With the exception of *Syrrhophus marnocki*, whose range is restricted to the Edwards Plateau ecoregion of Texas, all of these species are found across a broad geographic range (Conant & Collins 1998). Such broad scale distributions inherently imply a level of tolerance in both habitat and food resources in order to fill regionalized niches and may help explain why this suite of five species continues to thrive in Camp Mabry's urban context.

Based on these survey efforts, it appears that the current herpetofaunal diversity of this Texas National Guard site is characterized by a subset of reptiles and amphibians and a lack of particular species (e.g., large colubrid snakes) when compared to herpetofaunal surveys of other Travis County sites and potential species known to occur in the county. After removing species unlikely to occur based on the availability of habitat, food resources, and other environmental factors, the estimated number of potential species occurring on Camp Mabry is seventy-eight (Dixon 2000). This study found only 25%, or 20, of these potential species during the surveys on Camp Mabry.

Even after refining the potential species list there are a number of notable species that were not encountered during the survey. Although published works on herpetofaunal communities exist for Travis County and surrounding areas (e.g., Strecker & Williams 1927; Strecker 1930; Smith & Buechner 1947), relatively few inventories of proximal properties have been conducted in recent

years. O'Connor (2003) conducted a herpetofaunal survey on the Lady Bird Johnson (LBJ) Wildflower Center in west-central Travis County and found 13 species of reptiles and amphibians. Although the total number of species documented was less than the 20 species recorded for Camp Mabry, the author did not survey for aquatic turtles. Turtles account for 25% of the species diversity at Camp Mabry. O'Connor (2003) documented a much higher diversity of snakes with seven total species including large species such as the bullsnake (*Pituophis catenifer*), coachwhip (*Masticophis flagellum*), and western diamondback rattlesnake (*Crotalus atrox*). Two noteworthy snakes that were located on the LBJ Wildflower Property but absent from Camp Mabry were the rough green snake (*Opheodrys aestivus*) and the black-necked garter snake (*Thamnophis cyrtopsis*), both of which are known to occur near Austin (Milstead 1953).

A survey of The Nature Conservancy's Barton Creek Habitat Preserve (TMM/TNHC 2008) highlights other typical species of the Edwards Plateau not encountered at Camp Mabry. To date, the survey of BCHP has documented 27 reptiles and amphibians including eight species of snakes and five species of lizards not found at Camp Mabry. Some common species detected at BHCP but not located at Camp Mabry include green anole (*Anolis carolinensis*), Texas spotted whiptail (*Aspidoscelis gularis*), and ribbon snake (*Thamnophis proximus*). Other species of reptiles and amphibians expected yet undetected on Camp Mabry include three species of caudates (*Ambystoma texanum, Eurycea neotenes,* and *Plethodon albagula*), five species of anurans (*Bufo woodhousii, Hyla cinerea , Rana sphenocephala, R. berlandieri,* and *Scaphiopus couchi*), and four turtle species (*Kinosternon flavescens, K. subrubrum, Terrapene carolinensis, T. ornata*). However, only one of these species (*Rana sphenocephala*) was detected during two surveys of other Travis County sites (O'Connor 2003; TMM/TNHC 2008).

The reduced diversity of Camp Mabry's herpetological community is also evident when comparing capture rates to other

inventories with similar intensity. A vertebrate inventory of Richland Creek Wildlife Management Area, Freestone and Navarro counties, Texas (Ryberg et al. 2004) found nocturnal road searches provided the highest capture rate (one capture per 3.5 min) totaling 141 individuals of 11 species, which differed from the results of this study, where only 4 individuals of two species were captured (one capture per 4.9 hr). Although Richland Creek WMA is much larger (ca. 5,583 ha) than Camp Mabry, efforts were similar among sites $n = 17$ for 18.8 hr and $n = 15$ for 19.5 hr, respectively. Similarly, survey results of Camp Maxey, Lamar County, Texas, detected 44 species of herpetofauna representing 5009 individuals, or 54% of the species expected from known geographic ranges (Ford & Hampton 2005). Again, Camp Maxey is larger (ca. 2570 ha), and not bound by urban surroundings.

The low number of individuals captured (ca. 555), low species richness, and dominance of a particular suite of reptiles and amphibians could possibly be the result of three factors: nonnative plants and animals, unsuitable habitat, and the urban context. Each of these factors plays a role in limiting diversity of reptiles and amphibians at Camp Mabry.

One of the most substantial threats to Camp Mabry's herpetofaunal diversity is the large populations of nonnative animals such as roof and Norwegian rats (*Rattus spp.*) and red imported fire ant (*Solenopsis invicta*) which are known to prey upon a variety of vertebrates (Clark 1982; Wojcik et al. 2001). The presence of the nonnative rats at Camp Mabry, especially at the high densities found by McDonough et al. (2005) could negatively affect the herpetofaunal community found on Camp Mabry. These nonnative rodents compete for space and often reside in habitats highly suited for native reptiles and amphibians including old logs, debris piles, under large rocks, and even in trees. The imported red fire ant provides nearly the same conflicts in several of these habitat situations as well. In addition to these non-native species, several house cats (*Felis catus*) were documented across Camp Mabry,

which have also been shown to impact native reptile and amphibian assemblages (Henderson 1992).

Finally, habitat degradation, which results from both nonnative vegetation and anthropogenic disturbance, is a likely factor influencing diversity. Nonnative vegetation, which most often originates from anthropogenic sources, can cause serious alterations of a habitat and has been documented to cause declines in reptile and amphibian communities (Minton 1968; Gibbons et al. 2000). Camp Mabry's tracts of chinaberry, tree of heaven, and Japanese ligustrum create relatively large areas of unsuitable habitat for native herpetofauna (Reinecke et al. 2002).

Conclusions

These survey results indicate that Camp Mabry's herpetofaunal community is diminished in terms of potential diversity. Over 74% of total specimens captured were made up of five species: *Bufo nebulifer, Syrrhophus marnocki, Trachemys scripta, Chelydra serpentina,* and *Rana catesbeiana.* Of additional concern, is the lack of certain species groups, most notably large colubrid snakes and lizard species, which have been documented on neighboring properties in Travis County. The prevalence of both nonnative plants and animals, including *Rattus spp.* and fire ants, may be influencing the herpetofaunal community on Camp Mabry and adding to the limited diversity documented during this survey. This survey provides the first systematic inventory of Camp Mabry's herpetofaunal community, establishing baseline data needed for monitoring the responses of Mabry's reptiles and amphibians to active management practices already underway at Camp Mabry.

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NOVEL AUTOCAD METHOD FOR PERFORMING SURFACE ENERGETICS ANALYSES.

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Abstract–This study reports the development of an economical apparatus and method for measuring contact angles of liquids on solid surfaces using a digital microscope and AutoCAD© 2005 drafting software. Digital images (JPG) of 1.0µl droplets for a probe liquid triad (ultrapure water, diiodomethane, ethylene glycol) resting on the substrates of interest are produced. Using the AutoCAD© design window, images of droplets were overlaid with lines and shapes matching all profiles of the images. Once these reference lines were applied, contact angles (Θ) of the probe liquids at the three phase boundary were determined using AutoCAD© software and geometric principals. Multiple surfaces were analyzed using this method, including N-type silicon wafers, glass microscope slides, 130Å thickness gold layers electrosprayed onto both N-type silicon wafers and glass slides, and a carboxyl-terminated self-assembled monolayer (SAM) deposited onto the gold layers. Ten replicates of each surface and treatments were tested independently to determine repeatability of the procedure. Contact angles determined for probe liquids were consistent and comparable to literature values. Surface free energy calculations for solids were calculated with the van Oss-Chaudhury-Good equation and were likewise consistent with literature values. This method of determining the contact angles of probe liquids on solid surfaces has proven reliable in confirming surface energy predictions based on the chemical and physical properties of those surfaces tested. Results support the premise that expensive optical apparatus and associated software are not required to perform accurate comparisons of surface wetting characteristics.

Numerous fields of science and engineering require detailed understanding of material surface characteristics in order to select or properly prepare surfaces. A common and familiar example is that paints are many times formulated for very specific applications. Spreading and adhesion of paint onto a target surface strongly influences the appropriateness of that paint for particular surfaces. For these reasons, paints intended for coverage of wood surfaces are often unsuitable for application to plastic or metal surfaces. Additionally, considerations must also be made concerning the surface characteristics of dried paints: Dried paints formulated for applications such as metal surfaces exposed to outdoor conditions

should have low surface energies in order to repel precipitation and thus protect the underlying materials from oxidation. In another entirely different and strongly contrasting utilization, surface wetting characteristics of plant and tree leaves have also been studied by environmental toxicologist to demonstrate exposures to automobile exhaust (Pal et al. 2002) and exposure to sulfur and deposition of heavy metals (Turunen et al. 1997).

Under certain circumstances, observing changes in wetting properties of solid surfaces may be the only rapid and reliable method for confirming the presence or absence of modifications made to those surfaces. The application of self assembled monolayers (SAMs) to gold-coated silica or glass is one such situation. A readily accessible and inexpensive technology capable of imaging these chemical monolayers is currently unavailable. Yet, by observing the contact angles of probe liquids of known polarities on these surfaces pre- and post-application of a SAM, researchers can compare changes in surface energies and determine the degree of success of the procedure. Hydrophobic/hydrophilic interactions between the solid surfaces and the applied probe liquids rapidly allow inferences to be made regarding any observed changes.

Since surface energetics analyses and tensiometry are essential to many fields and have been in use for decades, many methods of acquiring and analyzing contact angles have been developed, and much debate has gone into determining which methods produce the most accurate and precise results. In fact, So many methods exist that to list and describe each here would not give fair treatment to any, and would be beyond the scope of any single manuscript. A review of literature comparisons reveals the Wilhelmy Balance method to be the benchmark, followed by the tilted plate and the sessile drop methods (Lander et al. 1993; Chibowski et al. 2002; Krishnan et al. 2005).

While this need for testing the wetting properties of solid surfaces has widespread application, the associated costs may be

prohibitive to small laboratories where research grants may not be prevalent: Low-end commercial manual goniometers can cost $5,000-$6,000 new, and frequently require additional proprietary software and dedicated computer equipment in order to be fully functional. This report represents the use of inexpensive apparatus adapted for goniometric contact angle (Θ) analyses and an innovative use of AutoCAD© 2005 drafting software for determining Θ values.

Materials and Methods

Probe liquids and surface preparation.–Ninety-nine percent pure diiodomethane (Alfa Aesar, Pelham, NH, USA), 99.0% ethylene glycol (VWR International), and ultrapure water were utilized as probe liquids. Selection of this triad was based on the simplification of surface free energy (γ_S^{TOT}) calculations via their utilization, and due to the preponderance of use in literature.

Droplets of probe liquids (1.0µl) were applied to test surfaces via hand delivery with 10.0µl glass syringes (Hewlett Packard pn5181-1267). Syringes were washed and autoclaved prior to use. Separate syringes were used for each probe liquid. The syringe for diiodomethane was wrapped in Al foil during the procedure to prevent photodegradation of its contents. Droplet images were acquired within 3-5sec of deposition in order to minimize evaporative loss.

Glass and silica wafers were immersed in chromic acid for 24 h, thoroughly rinsed with ultrapure water, and dried in a Precision Model 70D Laboratory Oven for 12h at 100°C. Gold layers applied to glass slides and silica wafers were tested within one week of the electrospray gold deposition and atomic force microscopy. Self-assembled monolayers were likewise tested immediately upon completion (1-2h) of their deposition and drying.

Self-assembled monolayer.–SPT-0014 ($C_{29}H_{50}O_9S_2$; SensoPath Technologies, Bozeman, MT, USA) a dithiol-tethered SAM precursor molecule, was dissolved in an organic solvent and

deposited onto gold coated glass slides from solution. Upon completion, films were rinsed with anhydrous ethanol (Fisher Scientific, Pittsburg, PA 15219, USA) and dried under nitrogen stream.

Apparatus.–An adjustable instrument base with bubble leveler (Clay Adams, Parsippany, NJ 07054, USA) provided the foundation of this apparatus (Figure 1). Aluminum optical mounting dovetails (40 by 50mm) with ¼"-20 counter bores (Thorlabs, Inc., 435 Route 206 North Newton, NJ 07860) were mounted onto the metal base as attachment points for the digital microscope and stage. A Digital Blue™ QX5™ Computer Microscope (e-bay, USA) was mounted by using two, ½" posts and 3" post holders (Thorlabs, Inc.) which were mounted to optical mounting dovetails. These mounting dovetails were joined to a single base-mounted dovetail via 40mm double dovetail clamps (Thorlabs, Inc.). This arrangement allowed horizontal and vertical adjustments of the microscope. To the second base-mounted dovetail, an in-house machined three-planar goniometer was similarly affixed. A 49.0cm^2 aluminum plate was attached to the goniometer to serve as the stage. A 30.5cm section of 40mm optical dovetail rail was mounted to the metal base for attaching the diffuse light source.

Analysis.–Fifty kilobyte, 512 x 384 digital images (JPG) of 1.0µl droplets liquids resting on solid surfaces were acquired with a Digital Blue™ QX5™ Computer Microscope with the focus of the image on the profile of the droplet at its greatest height and diameter (Figure 2). Images were then imported into the AutoCAD© 2005 design window where tools were employed to overlay simple geometric shapes onto the images. First, a circle was overlaid onto the image and manipulated to match the profile of the droplet surface. This was accomplished by altering the diameter of the circle and moving the location of the mathematical center. Once this circle was best fit onto the image, a single straight line (Line 1) was applied to match the edge of the solid surface onto which the droplet is resting. The combination of this circle and Line 1 become the basis for which Θ calculations are performed.

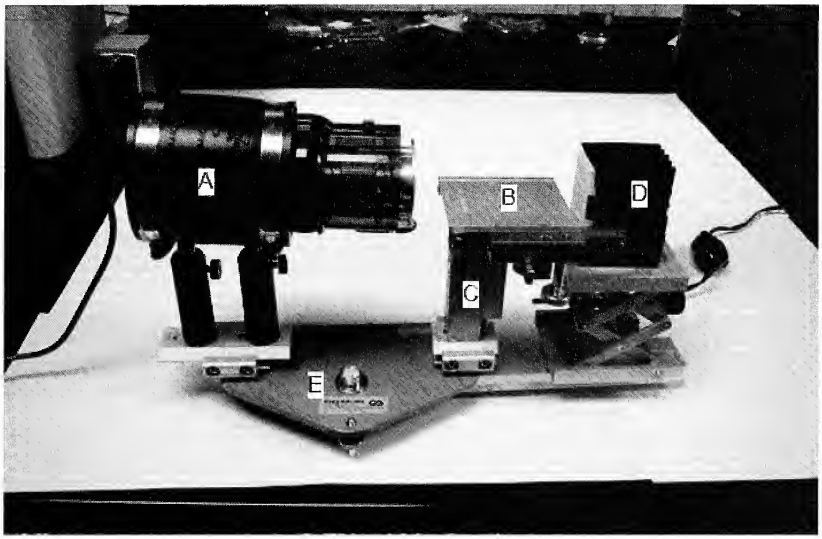

Figure 1. Image of the apparatus developed for acquiring macro images of liquid droplets resting on solid surfaces (major components labeled). Digital Blue™ QX5™ Computer Microscope (A), stage (B), goniometer (C), diffuse light source (D), adjustable instrument base (E).

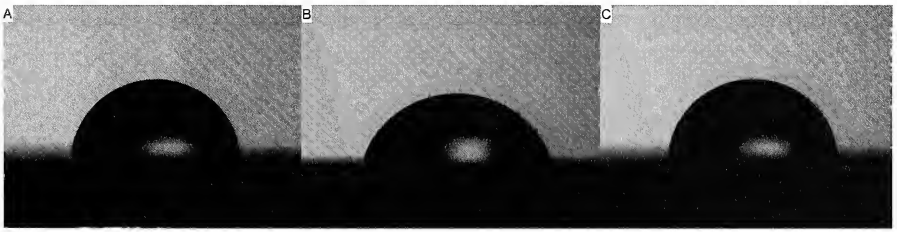

Figure 2. Representative images of 1.0µl droplets of test liquids resting on glass acquired with the apparatus developed during this study. (a) Diiodomethane, (b) Ethylene glycol, (c) MiliQ water.

Contact angle was determined by the application of two additional straight lines and a rectangle to the image. Line 2 was formed by connecting the center of the circle and the point of intersection of the circle and Line 1, and was facilitated by the AutoCAD© design software: Points of intersection and center

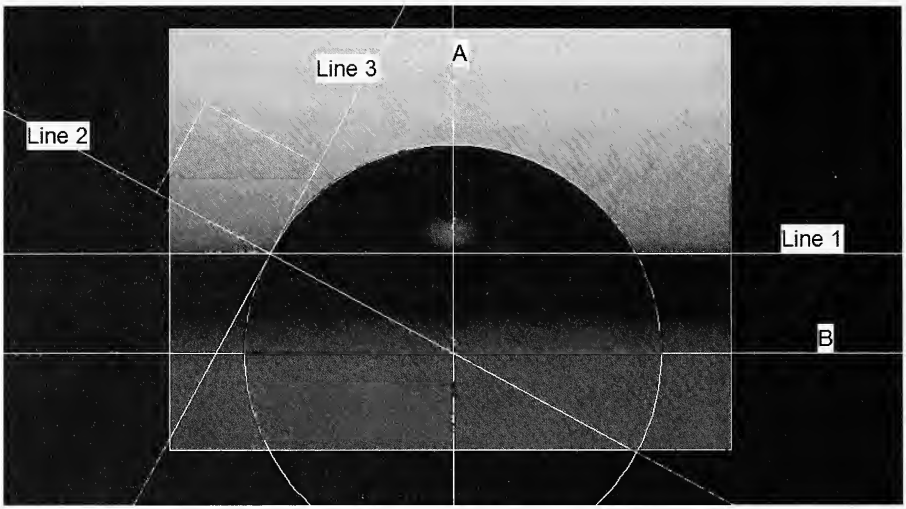

Figure 3. Droplet image demonstrating key elements of the AutoCAD analysis for determining theta. Lines A and B added to show true center and quadrants of the circle.

points of circles are highlighted during modification of these files and are readily accessed by the user. The point where Line 2 intersects the circle forms one vertex of a rectangle that has Line 2 as the bottom side. A rectangle was then inserted into the design window where the program auto-positions this shape so that two sides will parallel Line 1. The rectangle was rotated to a position where one side is coincident with Line 2. A third line (Line 3) was then applied to the image using the side of the rectangle which lies closest to the circle and is perpendicular to Line 2. At this juncture, all elements are in position for determining Θ for the droplet and solid surface (Figure 3).

<TOOLS> <INQUIRY> <DISTANCE> are chosen from the AutoCAD© toolbar at the top of the design window. Two points within the design window are selected in order to receive output from this function. Two points along Line 3 are selected to generate Θ. Theta is reported in degrees by the AutoCAD© software in the Command dialog box at the bottom of the screen as "Angle in XY Plane =…".

Table 1. Total surface tension and components (in mJ/m^2) for water (W), diiodomethane (DIM), and ethylene glycol (EG) used for surface energy estimations.

Liquid	γ_L	γ_L^{LW}	γ_L^{AB}	γ_L^-	γ_L^+
Water	72.8	21.8	51	25.5	25.5
DIM	50.8	50.8	0	0	0
EG	48	29	19	47	1.92

Surface free energy estimation.–Surface free energy estimations were calculated by inserting experimentally determined mean Θ values into the van Oss-Chaudhury-Good (vOCG) equation

$$(1+\cos\Theta)\gamma_L = 2(\sqrt{\gamma_S^{LW}}\sqrt{\gamma_L^{LW}} + \sqrt{\gamma_S^+}\sqrt{\gamma_L^-} + \sqrt{\gamma_S^-}\sqrt{\gamma_L^+})$$

(Van Oss 2004). For the vOCG computational method, known reference values of the polar and dispersion components of a triad of pure liquids (Table 1) are utilized, along with experimentally determined Θ values for each. Three equations with three unknowns are then solved to obtain the values of γ_S^{LW}, γ_S^+, and γ_S^-. The total surface free energy is then calculated as $\gamma_S^{TOT} = \gamma_S^{LW} + 2(\sqrt{\gamma_S^+}\sqrt{\gamma_S^-})$. Microsoft® Excel© (XP, 2003) was used to generate surface free energy estimations.

Single factor analyses of variances (*ANOVA*s) were performed on contact angles for each probe liquid between surface treatments (none, gold, SAM). Statistical comparisons were not made between data for glass and silica, nor were they made between the separate probe liquids.

RESULTS

Mean contact angles for droplets of probe liquids resting on foundations of silica wafers and glass slides and the surface treatments were consistent, with standard errors generally increasing as the γ_S^{TOT} values increased (Table 2, Figures 4 & 5). Standard deviations for contact angles of droplets of all three probe liquids resting on each surface as determined by this method ranged from 1.07 to 9.59 (Table 2, Figures 4 & 5), with the poorer performance occurring for water on gold (glass foundation).

Table 2. Sessile drop contact angles (in degrees) of probe liquids (Water, DiIodoMethane, and Ethylene Glycol) on foundations of glass microscope slides and silica wafers with various surface treatments and total surface free energies (mJ/m^2) calculated with the vOCG formula.

Foundation	Surface treatment	θ W	θ DIM	θ EG	γ_S^{TOT}
Glass	None	32.4±1.07	50.9±2.96	19.0±2.94	46.00
Glass	20Å gold	78.7±9.59	45.5±1.72	55.2±4.52	41.20
Glass	SAM	37.9±9.21	22.5±3.31	21.2±6.83	62.84
Silica	None	62.0±3.43	54.7±2.00	50.5±3.27	39.62
Silica	20Å gold	60.1±4.36	37.6±3.10	42.3±3.56	50.91
Silica	SAM	53.1±9.24	31.1±8.85	30.0±6.48	55.54

Surface free energy estimations for solid foundations and treatments, as expected, were highly variable depending on uppermost exposed layer contacting the probe liquids, and ranged from 31.81 mJ/m^2 (silica, no surface treatment) to 62.84mJ/m^2 (Au-coated glass with SAM) (Table 2). One way *ANOVA* p-values (n=10, α=0.05) within probe liquids between surface treatments indicated extremely significant differences ($p \leq 1.04E^{-9}$) existed between all surface treatments for both glass slides and silica wafers.

Discussion

The treated and untreated surfaces were expected to interact uniquely when brought into contact with the probe liquids. This was experimentally verified by comparing the statistical mean of the respective contact angles. Statistically significant and consistent probe liquid contact angle results between these surface treatments confirm the successful deposition of gold and the subsequent deposition of the SAMs.

The applicability of this method to research is demonstrated most fully by the contact angle results shown after the SAM deposition onto the gold surfaces. While the application of the gold was confirmed visually and through atomic force microscopy prior

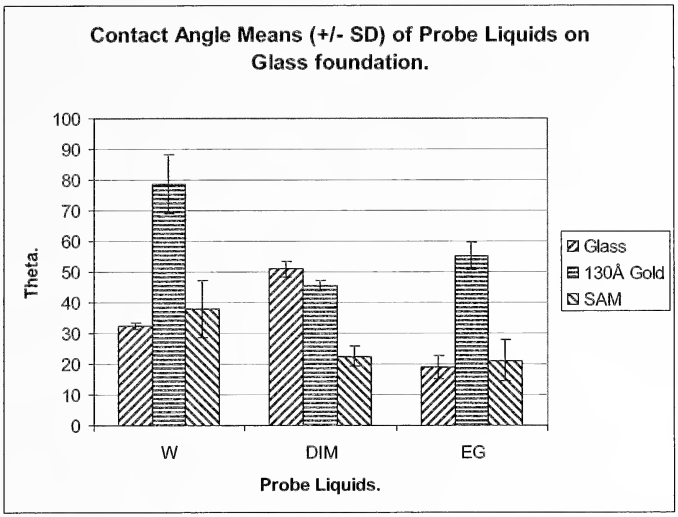

Figure 4. Contact angle means and standard errors for test liquids measured on glass microscope slides as determined with AutoCAD software. n=10 for all six combinations of test liquids on solid surfaces. DIM=diiodomethane, EG=ethylene glycol, W=MiliQ water.

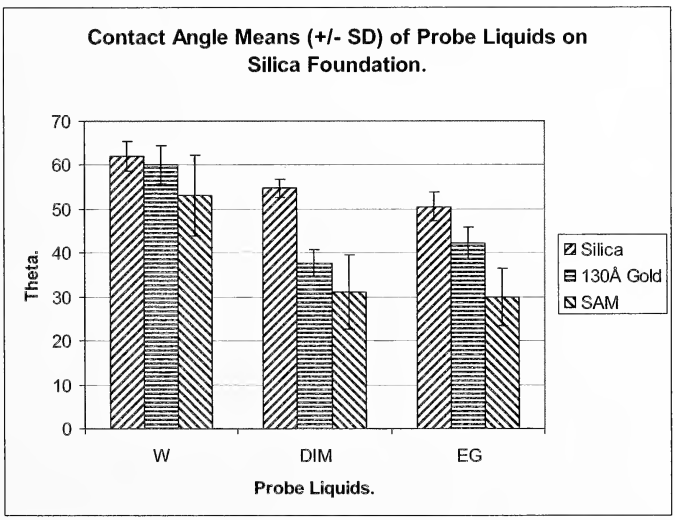

Figure 5. Contact angle means and standard errors for test liquids measured on N-type silica wafers as determined with AutoCAD software. n=10 for all six combinations of test liquids on solid surfaces. DIM=diiodomethane, EG=ethylene glycol, W=MiliQ water.

to these analyses, no analytical method is capable of confirming the successful deposition of a chemical monolayer as rapidly and as contact angle determination. Although the standard deviations from the means of the Θ values were elevated above desirable levels for probe liquids applied to SAMs on both foundations, the statistical analyses demonstrate that relatively inexpensive equipment and software can be coupled in a manner which is capable of producing reliable results.

The standard deviations were consistently elevated for contact angles of probe liquids on SAM's and were randomly elevated for several other surfaces (Table 2). While no surface energetics analyses studies for a SAM identical or similar to the one used herein could be located, Lu et al. (1998) reported Θ values for the same triad of liquids on a hexadecyltrichlorosilane (HTS) SAM, similarly formed on both glass and silicon. Due to the molecular differences between these SAM's, the contact angle means are not comparable, but the standard deviations for those measurements are useful and ranged from 0.8 to 5.0, whereas standard deviations in this study ranged from 3.31 to 9.24. Incomplete deposition of SAM's onto the gold substrates (e.g., islands of exposed gold) is a likely explanation for elevating the standard deviations of observed means. Additionally, 1.0μl droplets of probe liquids were approaching the lower limit of volume that can be accurately measured and hand delivered. This phenomenon was addressed when Lander et al. (1993) determined that the sessile drop method gave the lowest contact angles and was the least reproducible when compared to the Wilhelmy balance and tilted plate methods. In spite of these limitations, the sessile drop method continues to be a valuable tool while receiving ongoing scientific validation (Tadmor & Yadav 2007). Under ideal circumstances, probe liquid droplets should be mechanically measured and delivered, but the results show that hand delivery of droplets was a suitably reproducible.

Of all methods of contact angle determination currently in use, the nearest equivalent to the Θ values generated herein is the advancing contact angle (Θ_a), regardless of which method is used,

although it has been concluded that needle presence in droplets affects the three phase boundary line (Lander et al. 1993). Since sessile droplets are at static hydraulic pressure, while advancing and receding contact angles (Θ_r) are at increasing and decreasing hydraulic pressures, respectively, the sessile Θ for any given liquid will reside between Θ_a and Θ_r, but is more closely associated with Θ_a. Chibowski et al. (2002) reported that the Θ_a for water, DIM, and EG on glass microscope slides using the tilted plate method were 33.07 (±2.06), 46.12 (±0.74), and 24.95 (±2.36), which were in good agreement with corresponding experimentally determined Θ values of 32.4 (±1.07), 50.9 (±2.96), and 19.0 (±2.94) (Table 2). Chibowski et al. (2002) also found that Θ_a values for DIM and water on glass microscope slides generated with the syringe method were 47.75 (±1.24) and 29.91 (±1.69), respectively, which also strongly agree with corresponding experimental Θ values of 50.9 (±2.96) and 32.4 (± 1.07) (Table 2). Radelczuk et al. (2002) reported similar findings for water and EG on glass slides with Θ_a values of 33.6 (±1.1) and 48.8 (±0.8), respectively, which compared well with corresponding Θ values of 32.4 (±1.07) and 50.9 (±2.96) (Table 2).

Contact angles determined on cleaned glass microscope slides and the subsequently calculated surface energies have become the predominant standard literature values for method and calculation comparisons. Observed γ_S^{TOT} estimate for cleaned glass slides (46.0mJ/m^2) was in good agreement with literature values using the Θ_a values of the same triad of probe liquids (Radelczuk et al. 2002; γ_S^{TOT}= 41.5mJ/m^2, Chibowski et al. 2002; γ_S^{TOT}= 43.5 ± 0.7mJ/m^2). Surface free energy estimates for cleaned glass slides were also in strong agreement with the mean of γ_S^{TOT} calculations determined with Θ hysteresis for seven probe liquids (Radelczuk et al. 2002; γ_S^{TOT}= 50.6 ±9.7mJ/m^2) and through the Θ_a and Θ_r measurements of eight combinations of six probe liquid triads by the tilted plate method (Chibowski et al. 2002; γ_S^{TOT}= 50.6 ±8.8mJ/m^2). Since the γ_S^{TOT} estimates for cleaned glass slides were calculated with a commonly used triad of probe liquids and method, the agreement

seen between three findings and literature values demonstrate the suitability of this method of calculating Θ.

Volpe et al. (2003) stated that there is no "handbook-level" collection of contact angles of common liquids on common solids, nor measured or calculated surface free energies of common solids, and that 21st century scientists are not in agreement, even on the values of surface free energies of common solids. As such, it becomes apparent that variability between results remains inherent to the study of surface energetics, and that even large differences between data are to be expected. However, for the data presented herein, no such accommodations are required: Observed experimentally determined contact angles easily fall within range of frequently encountered data, while the calculated value for surface free energy of glass agrees strongly with values seen in current literature.

Several steps of this procedure rely heavily on the patience and consistency of the analyst. The first and most important step is acquiring an analysis-appropriate image of the droplet on a solid surface. As mentioned earlier, it is essential that the focal point of the image be the profile of the droplet at its greatest height (true midpoint, Figure 3). This is to ensure that the points of contact between the droplet and solid surface are also clearly in focus. Macro photographs can be focused along a continuum of the droplet from leading edge to beyond. If the contact points at maximum droplet height are not properly in focus, application of the circle and Line 1 in AutoCAD© cannot be accurately achieved. It is essential to the success of the analysis that the droplet be in sharp focus thereby blurring the edge of the solid surface: Figures 2 and 3 show the blurring of the solid surface while the droplet and its reflection at point of contact remain clearly visible. Gaclawski & Urbaniak-Domagala (2007) emphasize the significance of capturing the reflection point clearly for the sessile drop method. For the analysis described, images should be of the highest quality resolution so pixilization is avoided: As images can be enlarged in the AutoCAD© design window during Θ determination to better fit

the components, increased resolution ensures accurately fitting the components necessary for analysis.

An additional point is that the profile of a droplet resting on a surface is rarely the perfect arc of a circle bisected by a plane. This is especially true for droplets of low energy liquids laid onto low energy surfaces (eg., EG on glass, Figure 2b). While excessive droplet spreading may prevent the user from accurately applying a circle to such an image, the persistence of the user in fitting the circle to the best capabilities of the software increases the reliability and reproducibility of the results. Under instances where application of an ellipsoid to droplet profile is more appropriate, spheres in AutoCAD© can be elongated along the horizontal axis accordingly without negatively effecting results.

Surveys of existing literature for universal agreement on method of contact angle determination, acceptable probe liquids, and standard free energy values for common surfaces demonstrates that variability between these elemental components remains an open problem. Currently, investigators seem to have narrowed these problems and indications are that surface free energies are constant only under ideal conditions and, in reality, are best taken as the result of averaged values (Volpe et al. 2003, Chibowski et al. 2002).

In spite of the limitations to the method described herein, developing an in-house apparatus and method for contact angle analysis is beneficial in many aspects. As demonstrated, the usefulness of such a method in confirming successful surface treatments can be readily accomplished by novice operators. If these analyses are performed by using commonly accepted probe liquids, contact angle data can be inserted into surface free energy equations which result in estimations which concur strongly with literature values. In laboratories where surface energy studies play a small but significant role, such economical and reliable equipment and methods have great potential for applicability.

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GENERAL NOTES

REPRODUCTIVE CYCLE OF THE COMMON BARKING GECKO, *PTENOPUS GARRULUS* (SQUAMATA: GEKKONIDAE) FROM SOUTHERN AFRICA

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The common barking gecko, *Ptenopus garrulus* occurs in western arid regions of southern Africa and ranges from little Karoo to Kruger National Park (Branch 1998). Hibbitts et al. (2005) studied the reproductive ecology of *P. garrulus* and Girard (1997) described mating behavior in captive specimens. Clutch sizes are in Haacke (1975), Pianka (1986), Pianka & Huey (1978). The purpose of this paper is to provide additional information on the reproductive biology of *P. garrulus* from a histological examination of museum specimens as part of an ongoing effort to characterize the reproductive patterns of geckos from southern Africa.

Materials and Methods

A total of 171 *P. garrulus* including 91 females (mean snout-vent length, SVL = 46.7 mm ± 3.0 *SD,* range = 39-53 mm), 61 male (mean SVL = 47.0 mm ± 3.6 *SD,* range = 38-58 mm), 10 subadults (mean SVL = 34.1 mm ± 1.6 *SD,* range = 33-37 mm) and 9 presumed neonates (mean SVL = 24.0 mm ± 1.5 *SD*, range = 21-25 mm) *P. garrulus* were examined from the herpetology collection of the Natural History Museum of Los Angeles County (LACM), Los Angeles, CA. Lizards were collected 1969, 1970, 1972 and 1976.

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

performed using Instat (vers. 3.0b, Graphpad Software, San Diego, CA). An unpaired *t*-test was used to compare *P. garrulus* male and female mean body sizes (SVL).

Ptenopus garrulus from southern Africa examined from the herpetology collection of the Natural History Museum of Los Angeles County, (LACM), Los Angeles, California.

BOTSWANA

Kgalagadi District, 11 km S Tsabong (26°08'S, 22°28'E) LACM 83270, 83275, 83281, 83288, 83292, 83293, 83295, 83296, 83299, 83313, 83316, 83321, 83324, 83325, 83330, 83334, 83335, 83342, 83346, 83350, 83351, 83353, 83354, 83358, 83362, 83366, 83371-83373, 83383, 83384, 83388, 83393, 83395, 83396, 83405, 83408, 83409, 83413, 83415, 83416, 83421, 83423, 83425, 83429, 83436, 83492, 83503, 83506, 83507, 83509, 83510, 83514, 83515, 83517, 83518, 83525; 9 km N, 11 km E Twee Rivieren (26°23'S, 20°43'E) LACM 83261, 83263, 83266.

NAMIBIA

Erongo Region, Walvis Bay (22°95'S, 14°50'E) LACM 127468, 127469; Karas Region, 89 km ENE Koes (26°00'S, 19°15'E) LACM 77292, 77293, 77299, 77300, 77302- 77307, 77310-77312, 77315, 77318-77323; Karas Region, 25 km WNW Helmering-hausen (25°88'S, 16°81'E) LACM 77047, 77050, 77052; Karas Region, 46 km N, 17 km E Aroab (26°22'S, 19°49'E) LACM 83206, 83207, 83210, 83211, 83213, 83215.

REPUBLIC OF SOUTH AFRICA

Northern Cape Province, 31 km N,100 km E Upington (28°13'S, 22°16'E) LACM 83138, 83146, 83148, 83150-83152; 24 km N, 83 km E Upington (28°17'S, 22°05'E) LACM 83223, 120 km N, 54 km W Upington (27°22'S, 20°43'E) LACM 83038, 83041, 83044-83051; 121 km N, 16 km E Upington (27°22'S, 21°25'E) LACM 83238, 83243, 83247; 129 km N, 65 km W Upington (27°17'S, 21°54'E) LACM 83055, 83059, 83064, 83066, 83072, 83074, 83076, 83077, 83080-83087, 83093, 83095, 83097, 83102, 83103, 83105-83111, 83113- 83115, 83117, 83120-83122, 83125-83129, 83131, 83132, 83134, 83136; Kalahari-Gemsbok National Park, 1 km W. Kameelsleep (25°45'S, 20°44'E) LACM 83156, 83160, 83163,

83165, 83167, 83177, 83193, 83195, 83201-83204; 18 km S, 22 km E Witkoms (27°58'S, 21°32'E) LACM 83251, 83254; 50 km W, 7 km S of Vanzylsrus (27°04'S, 21°48'E) LACM 83256, 83257.

Results

Males followed a seasonal testicular cycle (Table 1) in which three stages were represented: (1) Regression (non-reproductive) in which the germinal epithelium is exhausted and the predominant cells are Sertoli cells and spermatogonia; (2) Recrudescence (recovery) characterized by renewal of the germinal epithelium for the next period of sperm formation; primary and secondary spermatocytes are the predominant cells; (3) Spermiogenesis (sperm production) in which the seminiferous tubules are lined by clusters of spermatozoa and metamorphosing spermatids; the epididymides are packed with sperm. The peak period of sperm production occurred in austral spring when 100% of 31 males were undergoing spermiogenesis (Table 1). Recovery for the next period of sperm formation occurred during summer-autumn when all three stages (= regression, recrudescence, spermiogenesis) were present in the population (Table 1). Recovery is completed by the next spring. The smallest reproductively active male (spermiogenesis in progress) measured 38 mm SVL (LACM 83125) and was collected in October.

There was no significant size difference (mean SVL) between males and females (unpaired *t* test). Monthly changes in the ovarian cycle are in Table 2. Egg production was limited to the right ovary. Females with enlarged ovarian follicles (> 4 mm), oviductal eggs or corpora lutea were recorded from October, December and January. Two females from April were undergoing early yolk deposition (marked by the presence of vitellogenic granules). There was no evidence (corpora lutea and early yolk deposition) in the same female to suggest more than one egg clutch is produced in the same reproductive season. Mean clutch size (n = 21) was 1.0 ± 0.0. The smallest reproductively active females measured 43 mm, follicles > 4 mm, (LACM 77323 from October and LACM 83223 from January) or, oviductal eggs, (LACM 77299 and LACM 77332 from October).

Table 1. Monthly changes in the testicular cycle of *Ptenopus garrulus* from southern Africa.

Month	*n*	Regression	Recrudescence	Spermiogenesis
September	9	0	0	9
October	20	0	0	20
November	2	0	0	2
January	6	4	0	2
February	6	3	2	1
April	15	2	6	7
May	3	1	1	1

Table 2. Monthly changes in the ovarian cycle of *Ptenopus garrulus* from southern Africa.

Month	*n*	No yolk deposition	Early yolk deposition	Enlarged follicles > 4 mm	Oviductal eggs	Corpora lutea only
Oct.	21	2	3	9	6	1
Dec.	5	4	0	0	0	1
Jan.	23	21	0	2	0	0
Feb.	25	25	0	0	0	0
Mar.	4	4	0	0	0	0
Apr.	12	10	2	0	0	0
May	1	1	0	0	0	0

Discussion

In a previous study on the reproductive ecology of *P. garrulus* Hibbitts *et al.* (2005) reported peak reproductive activity for both sexes occurred in September and October. Data from this study as well as values in Pianka (1986) and Hibbitts et al. (2005) indicate that clutch size in *P. garrulus* consists of one egg. Hibbitts et al. (2005) reported that based on the presence of two eggs in different stages of development in 4 (8%) females, two egg clutches in the same year were possible; only the right ovary produced eggs. However, in contrast, no females were observed that would likely have produced more than one egg clutch in the same year (females with oviductal eggs and concomitant yolk deposition and/or females with corpora lutea and concomitant yolk deposition). This finding may be due to the small sample sizes.

Hibbitts et al. (2005) reported reproductive activity commenced at smaller SVLs (male = 36 mm; female = 31 mm) than observed in this study. Gonads from ten *P. garrulus* from October in this study, (mean SVL = 34.1 mm ± *SD*, range = 33-37 mm were "extremely small" and

consequently classified these lizards as sub-adults. These differences in onset of reproductive activity may suggest regional differences in the sizes that *P. garrulus* populations commence reproduction.

It is not known if the two April *P. garrulus* that had initiated vitellogenesis (Table 2) would have deposited eggs or undergone follicular atresia with the yolk being reabsorbed (see Moodley & van Wyk 2007). Follicular atresia is common late in the reproductive season when follicles that do not complete vitellogenesis deteriorate (Goldberg 1973, Moodley & van Wyk 2007).

Presumed neonates were collected in January (n = 6), February (n = 1) and May (n = 2). This is consistent with the observation of Haacke (1975) that young of *P. garrulus* hatch from early summer into autumn and Auerbach (1987) that eggs of *P. garrulus* probably hatch in late summer to autumn and possibly as early as October.

To date reproductive cycles of African gekkonid lizards appear to fall into two types: (1) a seasonal cycle in which reproduction occurs mainly in spring-summer as found in *P. garrulus* described herein and in Hibbitts et al. (2005), *Chondrodactylus angulifer,* (Goldberg 2006a), *Pachydactylus capensis,* (Goldberg 2006b), *Colopus wahlbergii,* (Goldberg 2006c; Whiting et al. 2007), *Pachydactylus bibronii,* (Flemming & Bates 1995), *Hemidactylus mabouia* (Moodley & van Wyk 2007) and; (2) extended reproduction occurring through much of the year as appears to be typical for *Lygodactylus,* (Simbotwe 1983, Vitt 1986, Goldberg 2007, Goldberg 2008, Vences et al. 2004). Subsquent studies on additional species of geckos are needed before the variations in the timing of gekkonid reproductive cycles from southern Africa can be known.

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SITE FACTORS AFFECTING PINYON-JUNIPER OCCURRENCE IN THE CAPULIN VOLCANO FIELD OF NORTHEASTERN NEW MEXICO

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The Capulin Volcano Field covers approximately 207,192 km² in Union and Colfax counties in northeastern New Mexico and southernmost Las Animas county in Colorado. The geology of the area is characterized by Pleistocene and Holocene extrusive volcanic activity through sandstone, shale and limestone of the Late Cretaceous Period (Trauger & Kelly 1987). Average annual precipitation and temperature for the area is 45.2 cm. and 9.6°C respectively (McCurdy 1994). Although, much work has been published on pinyon (*Pinus* spp.) – juniper (*Juniperus* spp.), little data have been reported on pinyon-juniper stands from the Capulin Volcano Field. Species studied in this investigation include pinyon (*Pinus edulis* Voss.), one-seed juniper (*Juniperus monosperma* Sarg.) and Rocky Mountain juniper (*J. scopulorum* Lemmon.) [SAF Cover Type 239 (Larson 1980)]. Although not timber producing, these stands supply posts, fuelwood, and pinyon nuts. They provide nesting platforms for birds and edge and cover for wildlife and livestock.

The objectives of this study were to locate pinyon-juniper stands in the Capulin Volcano Field and determine what environmental factors are affecting their occurrence.

Pinyon-juniper stands were located and sampled on privately owned rangeland. Individual stands tended to be small (<3 ha) and surrounded by typical western Great Plains short grass dominated by blue grama (*Bouteloua gracilis* [Lag.]) and western wheatgrass (*Agropyron smithii* [Rybd.]). Data were obtained by approximating

the center of each stand and randomly selecting the direction of a transect. Randomness was assured by following the direction of a second hand on a watch. Azimuth of the facing slope was recorded as was Universal Transverse Mercator (UTM) coordinates of the initiating point. Soil data were collected along transects at zero meters (the initiation point) and every five meters thereafter until the transect extended at least 10 meters beyond the edge of the stand. Each sample point was monumented by pin flags and numbered. Soil depth was measured with a soil probe. Soil depth was recorded by transect and flag number. Depth was measure to the nearest centimeter (cm) to 25 cm. Depths that exceeded 25 cm were recorded as >25 cm. Soil samples were collected at each sample point, recorded and returned to the laboratory. Mechanical analysis of soil samples was conducted using the method prescribed by Bouyoucous (1951; 1962). Soil pH was characterized using a glass electrode pH meter. Aspect was determined with the aid of a hand compass and azimuth was recorded. Percent slope was measured with a clinometer. Summaries of these data are shown in Table 1.

Environmental variables were evaluated by means of stepwise regression with the presence of pinyon-juniper stands as the dependent variable. Environmental factors used as independent variables included: soil depth to 25 cm, percent clay, percent silt, percent sand, soil pH and percent slope. Data were standardized before statistical analysis was performed (SPSS 2003).

Soil depth had the greatest impact on pinyon-juniper growth. Soil depth accounted for 31.2 percent of the variation of whether pinyon-juniper stands occurred and was negatively correlated. Soil pH accounted for an additional 7.8 percent of variation and was also negatively correlated with pinyon-juniper occurrence. Azimuth explained 4.2 percent of variation and was positively correlated. The resulting equation accounted for 43.2 percent of variation in seedling growth ($P = 0.00$).

Table 1. Summaries of environmental data

Variable	Mean	Standard Deviation	Range
Soil Depth (cm)	13.61	1.02	1 - 25
Soil pH	7.95	0.06	6.65 – 8.81
% Sand	39.09	10.83	12.00 – 72.72
% Silt	44.21	0.81	30.16 – 66.00
% Clay	17.18	8.51	0.72 – 41.28
Azimuth (degrees)	241	64	100-312
Slope (%)	25.4	3.19	6.00-37.00

$$\Gamma = 0.4996 - 0.739(\text{DEPTH}) - 0.281(\text{pH}) + 0.222(\text{AZIMUTH})$$
$$(r^2 = 0.432, P = 0.00)$$

where:

Γ = presence of pinyon-juniper stands [1].

Soil depth was negatively correlated to favorable stand conditions. This may appear counter-intuitive, however other investigators found that when grass cover was heavy, seedlings probably could not compete for limited soil resources (Woodbury 1947; Aro 1971). These shallow, rocky sites provided conditions that result in little competition from herbaceous vegetation, leaving pinyon-juniper able to survive. Soil pH was also negatively correlated, but that is not to say the more favorable sites were acid in nature; pH ranged from 6.7 to 8.8. Thus, a low pH for this study was one that approaches neutral. The data indicate that north to northwest faces were the most favorable exposures for pinyon-juniper stands which is consistent with similar results from the southern part of the state where pinyon exhibited higher cover values on northern exposures (Pieper & Lymbery 1987). The data support, that in the Capulin Volcano Field of northeastern New Mexico, sites with shallow, rocky soils, moderate pH and northern exposures provide suitable conditions for pinyon-juniper to occur.

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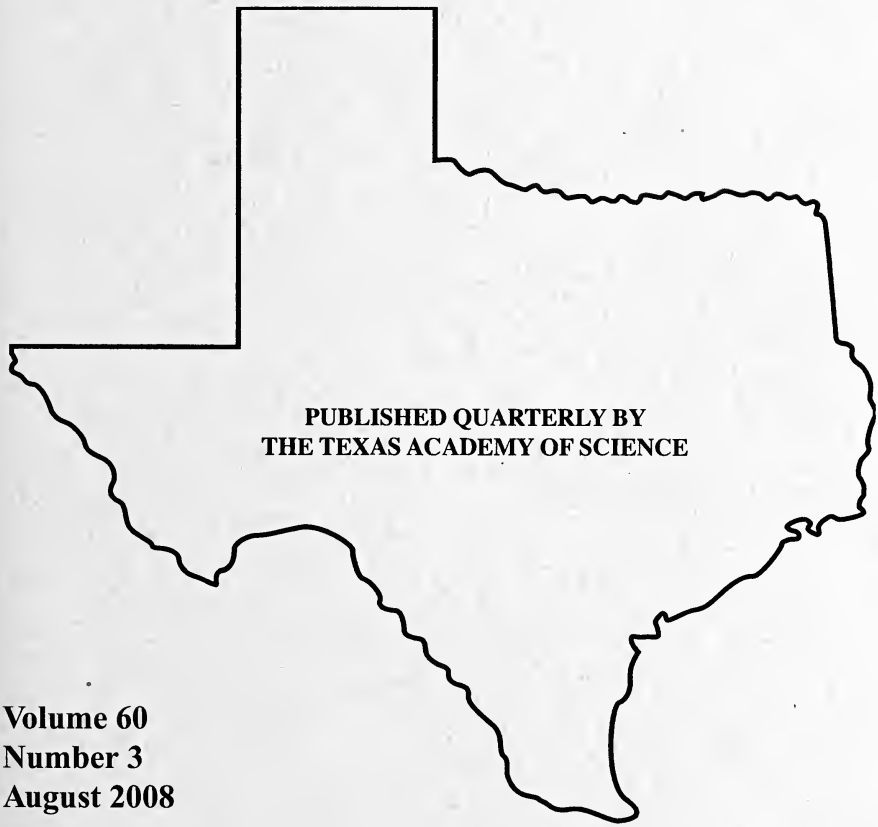

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REPRODUCTIVE CYCLE OF THE COMMON ROUGH-SCALED LIZARD, *ICHNOTROPIS SQUAMULOSA* (SQUAMATA: LACERTIDAE) FROM SOUTHERN AFRICA

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Abstract.–The reproductive cycle of the common rough-scaled lizard, *Ichnotropis squamulosa* from southern Africa was studied from a histological examination of gonads. The smallest reproductively active male and female *I. squamulosa* measured 47 mm and 58 mm SVL, respectively. Males began (spermiogenesis) sperm production and females began yolk deposition in February (summer). The reproductive cycle of *I. squamulosa* differs from that of other lacertid lizards from southern Africa which typically begin sperm production in late winter or early spring concurrent with the onset of yolk deposition. This difference in timing of reproduction may enhance survival of *I. squamulosa* as its young appear in spring by which time neonates of other lacertid species are larger and have different dietary preferences.

The common rough-scaled lizard, *Ichnotropis squamulosa* frequents arid and mesic savannah and ranges through Maputaland, Northern Cape, extreme south and central Mozambique, through Botswana, Zimbabwe and eastern Namibia to Angola and Tanzania (Branch 1998). It is a sit and wait predator that brumates during winter (Pianka 1971) and has a short life span of only eight to nine months (Broadley 1967; Schmidt 2001). There are previous reports on its reproductive biology (Fitzsimons 1943; Broadley 1967; 1974; 1979; Jacobsen 1987; Schmidt 2001). The purpose of this paper is to present additional information on the reproductive cycle of *I. squamulosa* gathered from a histological examination of gonadal material from museum specimens. The reproductive cycle of *I. squamulosa* is compared with those of other lizards from southern Africa.

METHODS AND MATERIALS

One hundred and one lizards were examined from the herpetology collection of the Natural History Museum of Los

Angeles County, (LACM), Los Angeles, California. The sample consisted of 32 females (mean snout-vent length [SVL] = 56.3 mm ± 4.4 SD, range: 48-66 mm), 37 males (mean SVL = 53.0 mm ± 5.4 SD, range: 39-63 mm) and 32 juveniles (mean SVL = 33.4 mm ± 3.7 SD, range: 28-39 mm). *Ichnotropis squamulosa* were collected during 1969 and 1970 as part of an ecological study by Pianka (1971; 1986) or in 1972 and 1973.

For histological examination, the left testis and epididymis were removed from males. The stages in the testicular cycle were identified. The left ovary was removed from females for histological examination to check for the presence of vitellogenesis (yolk deposition) and/or corpora lutea. Tissues were embedded in paraffin and cut into sections at 5 µm. Slides were stained with Harris hematoxylin followed by eosin counterstain (Presnell & Schreibman 1997). An unpaired *t*-test was used to compare *I. squamulosa* male versus female mean body sizes (SVL) and mean body sizes of November versus January juveniles (Instat vers. 3.0b, Graphpad Software, San Diego, CA).

Material examined.–Samples consisted of the following specimens of *Ichnotropis squamulosa*:

BOTSWANA (*n* = 82) KGALAGADI DISTRICT, 11 km S. Tsabong (26°08'S, 22°28'E) 80272-80280, 80282, 80284, 80288, 80290-80292, 80295-80301, 80304, 80305, 80307-80318, 80320-80322, 80326-80335, 80337-80342, 80345-80351, 80353, 80354, 80356-80358, 80360-80364, 80369, 80371-80374, 80376-80381.

NAMIBIA (*n* = 12) OTOZONDJUPA REGION, 40 km WNW Grootfontein (19°34'S, 18°07'E) 77833, 77835, 77836; 30 km ENE Otavi (19°40'S, 17°24'E) 77879-77887.

REPUBLIC OF SOUTH AFRICA (*n* = 7) NORTHERN CAPE PROVINCE, 31 km N, 100 km E. Upington (28°13'S, 22°16'E) 80265-80271.

Results and Discussion

Stages observed in the testicular cycle of *I. squamulosa* are given in Table 1: (1) Regression, seminiferous tubules contain mainly spermatogonia and primary spermatocytes; (2) Recrudescence, there is an increase in numbers of germ cells and cell divisions are noted. Secondary spermatocytes are abundant, a few spermatids may be present; (3) Late recrudescence, secondary spermatocytes and spermatids predominate; no sperm are present; (4) Early spermiogenesis, clusters of metamorphosing spermatids line portions of the lumina of the seminiferous tubules, occasional spermatozoa are seen; (5) Spermiogenesis, borders of seminiferous tubules are lined by rows of metamorphosing spermatids and spermatozoa are abundant. Monthly stages of the testicular cycle are in Table 1. While data is lacking from each month, a large February sample (n = 33) which contained 7/33 (21%) testes in recrudescence (germ cell renewal prior to the next period of sperm formation) and 21/33 (64%) in spermiogenesis indicates that *I. squamulosa* probably commences sperm formation during that month. Spermiogenesis continues at least through April (Table 1). The smallest reproductively active male (spermiogenesis underway) measured 47 mm SVL (LACM 80337) and was from February.

Females of *I. squamulosa* were larger than males (unpaired t-test = 2.7, df = 67, P = 0.008). Three stages were observed in the ovarian cycle (Table 2): (1) No yolk deposition, ovary is quiescent; (2) Early yolk deposition, scattered vitellogenic granules are present; (3) Both corpora lutea from a previous clutch and concomitant yolk deposition for a subsequent clutch are present in the same female. It appears that the *I. squamulosa* female population commences reproductive activity in February as two females (2/29, 7%) exhibited early yolk deposition (Table 2). However, Jacobsen (1987) reported 1 of 3 *I. squamulosa* females from January contained enlarged ovarian follicles. Yolk deposition continued through April (Table 2). Laying of eggs was underway by May as one female (LACM 80381, SVL = 66 mm) exhibited corpora lutea from a recent clutch. The same female was con-

Table 1. Monthly distribution of reproductive conditions in the seasonal testicular cycle of 37 *Ichnotropis squamulosa* from southern Africa. Values are the numbers of males exhibiting each of the five conditions.

Month	*n*	Regression	Recrudescence	Late recrudescence	Early spermio-genesis	Spermio-genesis
January	1	1	0	0	0	0
February	33	1	1	6	2	23
April	3	0	0	0	0	3

Table 2. Monthly stages in the ovarian cycle of 32 *Ichnotropis squamulosa* from southern Africa. Values are the numbers of females exhibiting each of the three conditions.

Month	*n*	No yolk deposition	Early yolk deposition	Corpora lutea & yolk deposition
February	29	27	2	0
April	2	1	1	0
May	1	0	0	1

currently undergoing yolk deposition for an additional clutch indicating *I. squamulosa* females have the potential for producing two egg clutches in the same year. The smallest reproductively active *I. squamulosa* (early yolk deposition) measured 58 mm SVL and was from February (LACM 80357).

Juveniles were collected in November ($n = 12$, mean SVL = 32.3 mm ± 4.1 *S.D.*, range: 28-38 mm) and January ($n = 20$, mean SVL = 34.1 mm ± 3.4 *S.D.*, range: 28-39 mm). There was no significant size difference between the two months (unpaired t-test, $t = 1.3$, $df = 30$, $P = 0.21$). Presumed neonates of 28 mm SVL were collected in both November and January. There is a report of hatchling *I. squamulosa* measuring 28-30 mm SVL (Broadley 1979).

Histological observations indicate that spermiogenesis likely commences in February concomitant with males acquiring breeding coloration (Schmidt 2001). The smallest male to undergo

spermiogenesis in this study (47 mm SVL) was smaller than the minimum SVL (55 mm) in Schmidt (2001).

Broadley (1979) reported a gravid female from 10 April. Eggs are laid in April-May with isolated cases as late as July (Jacobsen 1987). Pianka (1986) reported two *I. squamulosa* females, each with a clutch of 4 eggs; a range of 10-12 eggs are deposited (Fitzsimmons 1943). This value of 58 mm SVL for the smallest breeding female in this study was identical with that of Schmidt (2001).

Ichnotropis squamulosa has a short life span of only eight to nine months; young are born October to November after a five month incubation period (Schmidt 2001). November collections reported herein consisted of only juveniles. Broadley (1974; 1979) similarly reported only juveniles in November and December. Hatchlings reach adult sizes in four to five months; breeding occurs in March and April with eggs being deposited in April and May (Broadley 1979; Schmidt 2001).

Ichnotropis squamulosa is sympatric with *Ichnotropis capensis* however, the timing of their reproductive cycles differ so as to avoid competition for food (Broadley 1967, 1979; Jacobsen 1987). *Ichnotropis capensis* mate in October-December (Branch 1998) at which time *I. squamulosa* have not yet reached maturity.

The reproductive cycle of *I. squamulosa* differs from that of other lacertid lizards from southern Africa which typically begin sperm production in late winter or early spring concurrent with the onset of yolk deposition (Goldberg 2006a; 2006b; 2006c; 2006g; 2006h; Goldberg & Robinson 1979; Nikosi et al. 2004). This difference in timing of reproduction may enhance survival of *I. squamulosa* as its young appear in spring at which time neonates of other species of lacertids have reached sub-adult or adult size and have different dietary preferences. *Aporosaura anchietae* is an apparent exception as some reproduction occurs throughout the

year (Goldberg & Robinson 1979). This may, in part, be due to its ability to utilize seeds in their diet which are available year-round, (Robinson & Cunningham 1978).

Conclusions

Reproduction in *I. squamulosa* is synchronous in that spermiogenesis and ovarian reproductive activity occur at the same time, however each take place in autumn whereas they occur primarily during spring in other lizards from southern Africa (Goldberg & Robinson 1979; Flemming 1994; Flemming & Bates 1995; Nkosi et al. 2004; Goldberg 2006a; 2006b; 2006c; 2006d; 2006e; 2006f; 2006g; 2006h). There are, however, exceptions in which male and female reproductive activity are asynchronous as in *Cordylus giganteus* (cf. Van Wyk 1991; 1995) and *Pseudocordylus melanotus* (cf. Flemming 1993a; 1993b). In addition, females of the agamid lizard, *Agama atra* follow a condensed reproductive cycle in which ovarian activity occurs only in September to November (Van Wyk 1984).

There is a report in a field guide (Branch 1998) that the sympatric congener, *I. capensis* may produce two clutches in a year. However, additional work is needed to verify whether *I. squamulosa,* in fact, may produce more than one egg clutch per year. Follicular atresia (spontaneous degeneration of oocytes with reabsorption of yolk) is common late in the reproductive season (Goldberg 1973). It is thus uncertain if the one *I. squamulosa* female from the May sample with corpora lutea and concomitant yolk deposition would have produced a second egg clutch or undergone follicular atresia.

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SECRETORY VESICLE REFILLING AND TOXIN PRODUCTION IN *OLLOTIS NEBULIFER* (COASTAL PLAIN TOAD) AND *ANAXYRUS SPECIOSUS* (TEXAS TOAD) FOLLOWING TRANSCUTANEOUS ELECTRICAL STIMULATION OF THE PAROTOID GLANDS

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Abstract.–Parotoid glands in two species of toads, *Ollotis nebulifer* and *Anaxyrus speciosus* were expressed using transcutaneous electrical stimulation. Toads were euthanized at various time stages following gland expression. Of the toads that had parotoid glands expressed, microscopic examination revealed the majority of the vesicles in the glands had emptied significantly with little to no contents remaining. General morphology of the vesicles was similar in both species with vesicles in *A. speciosus* somewhat smaller and more numerous than in *O. nebulifer*. Unexpressed vesicles were generally ovoid in shape with smooth cell walls. Emptied vesicles displayed numerous invaginations of the cell wall, resulting in the decrease of the vesicle volume. No gross injury or damage to the emptied vesicles or surrounding structures was noted. Hypertrophy of the inner (secretory) cell wall layer was evident at 24 h in both species. The majority of vesicles in both species had completely refilled and resumed normal size and shape within 5 to 7 days following gland expression.

Amphibians possess two major types of integumentary glands that are widespread throughout the head, body, and limbs (Duellman & Trueb 1985; Zug et al. 2001). The mucous glands, which are more abundant dorsally, secrete a clear slimy fluid that maintains a thin protective layer over the skin to prevent dehydration of the animal. The granular glands, which produce a biochemical mixture of poisonous or noxious secretions, tend to be more concentrated on the head and shoulder regions (Duellman & Trueb 1985; Zug et al. 2001). Granular glands are commonly aggregated into large macrogland structures (e.g., parotoid gland; see Tyler et al. 2001 for an in-depth analysis on the nomenclature and spelling) and are present in various degrees throughout the anurans and occur in some salamanders (Duellman & Trueb 1985).

Each granular gland consists of numerous alveoli or secretory vesicles that are surrounded by connective tissue (Hostetler & Cannon 1974; Cannon & Hostetler 1976; Alvarez et al. 2005). The vesicles produce and store toxic secretions and are the functional units of the granular glands. Microstructure of the vesicle is made up of a double cell layer consisting of an outer layer of myoepithelial cells that function as a contractile component and an inner layer of secretory cells that is continuous with the vesicle contents (Hostetler & Cannon 1974; Cannon & Hostetler 1976). Each vesicle has a duct that allows the biochemical mixture access to the skin surface (Hostetler & Cannon 1974; Cannon & Hostetler 1976; Toledo et al. 1992) where it acts as a defensive mechanism against predators (Duellman & Trueb 1985; Zug et al. 2001).

Amphibian granular glands produce numerous biologically active compounds including; biogenic amines, peptides, proteins, bufadienolides, tetrodotoxins, and lipophilic alkaloids (see review Daly et al. 1987; Erspamer 1994). Because of the abundance of various biochemicals it contains and their application to human health, amphibian integument has generated a continual interest among researchers (Barthalmus 1994). The most common and "traditional" method of obtaining granular gland secretion has been a non-conservative technique that requires sacrificing the animal and subsequent mechanical and chemical processing of the skin (Roseghini et al. 1976; Erspamer et al. 1984; Erspamer et al. 1986; Roseghini et al. 1988; Roseghini et al. 1989; Daly et al. 2004). A much less common, but conservative method (the animal is not sacrificed) for the collection of amphibian granular gland secretion utilizes electrical stimulation (Conlon et al. 1999; Grant & Land 2002; Bevier et al. 2004). Electrical stimulation involves applying a low voltage of electric current (10-15 V) directly to the skin of the animal which causes contraction of the granular glands and ejection of their contents onto the skin surface where it can be collected (Tyler et al. 1992; Grant & Land 2002).

Clearly, an advantage of using electrical stimulation is that the animal is not sacrificed and can be released back into its natural

habitat, or if necessary, utilized again for serial extractions. However, currently there is no information available on the length of time required to refill or produce toxin in the granular glands after vesicle emptying. The present report describes a histological time study using electrical stimulation to express the granular gland contents in two species of anurans to examine vesicle emptying and refilling.

Materials and Methods

Adult specimens (N=10 for each species) of the Coastal Plain Toad, *Ollotis nebulifer* and the Texas Toad, *Anaxyrus speciosus* (taxonomy follows Frost et al. 2006a, b) were utilized because of their abundance, large size, and prominent parotoid glands. All specimens were collected by hand at night within the city limits of Kingsville, Kleberg County, Texas USA and were housed individually in appropriately sized plastic Sterlite® containers which meet the requirements and guidelines for housing live amphibians (Pough 1991; National Academy Council 1997).

Parotoid glands were expressed in live animals using a transcutaneous electrical stimulation device following Grant & Land (2002). Prior to electrical stimulation, the head and parotoid gland region was rinsed with deionized water; which aided in moistening the skin (to facilitate conduction of electrical current) and reduced contaminates in the collected glandular product. Each animal received three to four sessions of electrical massage directly to the parotoid glands for ~ 30 sec at 10 to 15 V. After each 30 second massage the expelled glandular contents was collected with a microspatula. The glandular secretion was frozen immediately and lyophilized for future analysis.

Following gland expression, the animals were euthanized by standard approved methods (American Veterinary Medical Association 2001; Simmons 2002) at various time stages (control, 0 h, 12 h, 24 h, 2 d, 3 d, 5 d, 7 d) and the entire parotoid gland structure was formalized in situ with a 10% solution (3.7% formaldehyde) for one hour. Following formalin fixation the

parotoid glands were excised and soaked in Davidson's solution (Kiernan 2000) for 12 hours then rinsed with deionized water. The glands were placed in an automated tissue processor and dehydrated through a graded series of 70–100% ethanol and embedded in paraffin. Transverse and longitudinal sections were made for light microscopy examination at a thickness of 5 μm. The sections were mounted on glass slides and stained using standard hematoxylin and eosin methods (Sheehan & Hrapchak 1987; Kiernan 2000).

Results

Size range for *A. speciosus* was 46-83 mm (total length), parotoid gland range; width 4.46-5.17 mm, length 7.34-12.17 mm. Size range for *O. nebulifer* was 74-102 mm (total length), parotoid gland range; width 5.48-6.79 mm, length 6.66-11.42 mm. The electrical stimulation procedure did not appear to cause any noticeable discomfort or injury to the animals. Parotoid glands from both species produced a cream colored viscous fluid that was easily collected. The product from *A. speciosus* was much thinner and noticeably more glutinous than in *O. nebulifer*. Microscopic examination of normal unexpressed vesicles in both *A. speciosus* and *O. nebulifer* were similar and appeared generally ovoid in shape with smooth cell walls (Fig. 1a & b). The unexpressed glands appeared full with morphology of the vesicles comparable in both species. In *A. speciosus* the vesicles were somewhat smaller than in *O. nebulifer*. Vesicle size in *O. nebulifer* was ~ 0.75 mm in diameter with vesicle size in *A. speciosus* ~ 0.5 mm in diameter. The contents of the full vesicles appeared homogenous in both species and cell morphology of the outer and inner layers of the vesicles were unremarkable.

Following transcutaneous electrical stimulation, the majority of the storage vesicles in the glands had emptied significantly with little to no contents remaining. In addition, the general morphology of the vesicles had changed dramatically. In contrast to the ovoid shaped full vesicles, the emptied vesicles displayed numerous invaginations or infolding of the cell wall (Fig. 1c & d). The empty

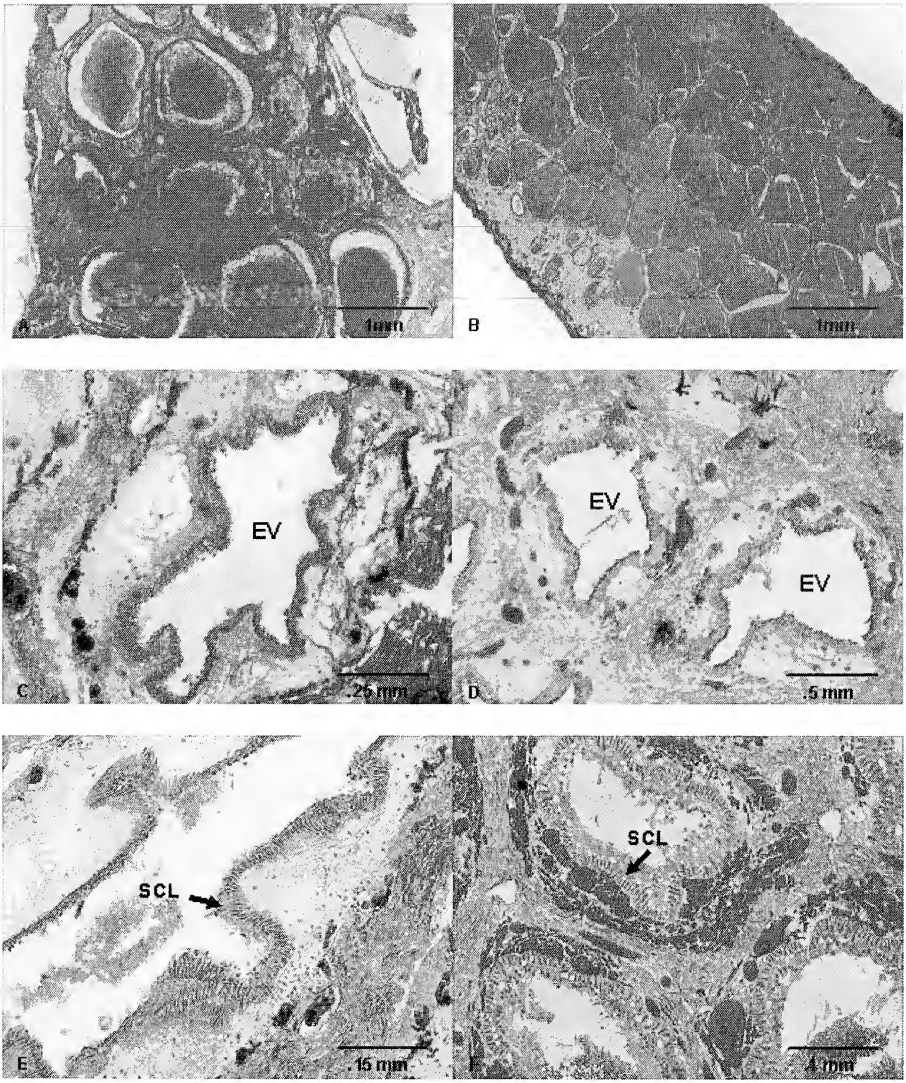

Fig. 1. Full unexpressed vesicles with smooth cell walls (a) *Ollotis nebulifer* (Coastal Plain Toad) (b) *Anaxyrus speciosus* (Texas Toad); Empty vesicles (EV) at 12 h showing numerous infolding of the cell wall, flattened angular shapes, and decrease volumes (c) *Ollotis nebulifer* (Coastal Plain Toad) (d) *Anaxyrus speciosus* (Texas Toad); Vesicles with marked hypertrophy of the inner (secretory) cell layer (SCL) with toxin production at 24 h (e) *Ollotis nebulifer* (Coastal Plain Toad) (f) *Anaxyrus speciosus* (Texas Toad).

vesicles displayed somewhat flattened angular shapes with marked decrease in volumes. There appeared to be no gross injury or damage to the cell walls, vesicles, or surrounding structures. Following vesicle emptying there was a marked hypertrophy of the inner (secretory) cell wall layer that was evident at 24 h in both species (Fig. 1e & f). In both *A. speciosus* and *O. nebulifer*, there was a noticeable increase of fluid material within the vesicles after two to three days. Gradual refilling of the vesicles continued and normal "unexpressed" size and shape was resumed within five to seven days following gland expression.

Discussion

Transcutaneous electrical stimulation is a viable non-lethal method for the extraction and collection of anuran parotoid gland secretion. The process does not appear to cause any harmful effects to the animal as no gross or microscopic injury was evident during the procedure or revealed in histological examination. While there was a small difference in the average size of the vesicles between the two species examined, the time intervals for vesicle refilling was comparable. Empty vesicles refilled within seven days after the contents was extracted. However, it is important to note that the contents in the recently refilled vesicles (i.e., concentration of the toxins) were not analyzed and could possibly differ over time as the vesicles age or "mature". The transcutaneous electrical stimulation method produced a relatively "clean" sample from the contents of the parotoid vesicles and does not require the time and expense of mechanical and chemical processing of the dried skin extraction method. Moreover, this conservative method would be preferred when dealing with rare or endangered species, it also allows for ontogenetic studies to be performed on the same animal, and would make feasible the use of anurans for venom production or farming similar to venom extraction facilities for venomous snakes.

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TEMPERATURE TOLERANCE OF GREEN SUNFISH (*LEPOMIS CYANELLUS*)

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Abstract.–Mean (± *SD*) critical thermal maxima of green sunfish (*Lepomis cyanellus*) acclimated from 10 to 35°C ranged from 33.5±0.83°C to 41.3±0.26°C while the critical thermal minima of individuals acclimated over the same temperatures extended from 0.2±0.28°C to 11.2±0.73°C. Temperature tolerance was significantly related to acclimation temperature and the latter accounted for a majority of variation in both lower (97%) and upper (98%) temperature tolerance. A measured upper lethal temperature of 41.3°C and estimated temperature tolerance polygon of 1270°C^2 confirm that the green sunfish is among the most eurythermal native North American fish species. The tolerance polygon was partitioned into low and high temperature acclimation dependent areas of 384 and 205°C^2, respectively, and when combined, essentially doubled the tolerance area from 618 to 1270°C^2. The low temperature acclimation dependent area is approximately 1.4 times larger than the high temperature dependent area, suggesting that acclimation has a greater effect on tolerance of lower temperatures. The overall temperature tolerance ability of green sunfish may play an important role in this species ability to cope with global climate change.

Green sunfish (*Lepomis cyanellus*) are native to the U.S. central plains between the Appalachian and Rocky Mountains, south to the Gulf Coast and north to the Great Lakes (Pflieger 1997). They are distributed throughout Texas and the U.S. with exceptions of Florida and parts of the northwest. The successful spread of green sunfish has been aided by their tolerance of physical/chemical variables (Smale & Rabeni 1995) which is an outcome of their origin and evolution within environmentally variable Great Plain streams. For example, water temperature in Great Plain streams reach 0°C in winter, and summer temperatures of 40°C have been reported (Matthews & Zimmerman 1990). Although a few studies (see Table 1) have presented upper temperature tolerances of green sunfish at selected acclimation temperatures; neither low temperature tolerance data nor a temperature tolerance polygon

Table 1. CTMaxima (Mean ± SD) of green sunfish. Entries include acclimation temperature, rate of temperature change during trials, test endpoint (Loss Of Equilibrium or Onset of Muscle Spasms) and source.

T_{ACCL} (°C)	Δ T (°C min^{-1})	Endpoint	CTMax (°C)	Source
25	0.3	LOE	37.4±0.53	Carveth et al. (2004)
30	0.3	LOE	40.2±0.50	Carveth et al. (2004)
26	0.017	LOE	37.9±0.75	Smale & Rabeni (1995)
10	1.0	LOE	31.1±1.45	Lutterschmidt & Hutchison (1997a)
10	1.0	OMS	34.2±2.06	Lutterschmidt & Hutchison (1997a)
20	0.3	LOE	35.8+0.69	Carrier & Beitinger (1988)
20	0.3	LOE	35.8+0.40	Carrier & Beitinger (1988)
20	0.3	LOE	35.9+0.56	Carrier & Beitinger (1988)

across a range of acclimation temperatures has been published for this species.

The objectives of this study were to quantify the acute upper and lower temperature tolerances of green sunfish acclimated to four constant temperatures. These data were used to generate an ecological temperature tolerance polygon following Bennett & Beitinger (1997) to more fully characterize the thermal tolerance of green sunfish.

Materials and Methods

Green sunfish were seined from a pond in Denton, Texas, transported to the laboratory in aerated containers and randomly allocated to one of four, well-aerated, glass aquaria containing dechlorinated tap water. Fish were held under a LD 12:12 photoperiod. Water temperature at the time of fish capture was approximately 25°C, and laboratory water temperature in each of the four holding aquaria (originally set to correspond with capture temperature) was changed 1°C d^{-1} until the desired acclimation temperature (10, 20 30 or 35°C) was attained. Haake® circulating thermoregulators (Models E-52 and D-1) were used to maintain 30°C and 35°C while the thermoregulators were used to heat

against constant cooling generated by a submerged cooler (Living Stream, Inc.) to maintain acclimation temperatures of 10 and 20°C. Once acclimated to 10, 20, 30 or 35°C, fish were held for 14 d before temperature tolerances were measured.

The water quality of the holding tanks, measured twice weekly, was within ranges that should have produced no adverse effects to the fish (conductivity 312-700 μmhos cm^{-1}, dissolved oxygen 6.4-11.0 mg $L^{-}1$, pH 7.4-8.0, ammonia (0.0-0.0 mg L^{-1}), and nitrite (0-0.0 mg L^{-1}). Fish were fed Tetramin® tropical flake food twice daily during acclimation, but food was withheld one day prior to temperature trials. Daily water exchanges (5-25%) by siphon removed excess food and feces without changing water temperature.

Critical thermal methodology, CTM, (Cox 1974) was used to estimate lower (CTMinima) and upper (CTMaxima) temperature tolerance of green sunfish acclimated to 10, 20, 30, and 35°C. The most recent reviews of this technique are provided by Lutterschmidt & Hutchison (1997b) and Beitinger et al. (2000). Specifics of the experimental CTM used in this research are identical to those of Cortemeglia & Beitinger (2005). During trials, the rate of temperature change was 0.3°C min^{-1}, and water temperature was recorded each minute within $\pm$ 0.1°C with an ASTM calibrated mercury thermometer. Final loss of equilibrium was the endpoint criterion during CTMaxima trials and lack of response to prodding with a glass rod was the endpoint for CTMinima trials. Different test endpoints were required since fish exposed to increasing or decreasing temperature respond differently. Once each fish reached the endpoint, the temperature was recorded, the fish was removed from the CTM chamber, returned to its acclimation temperature, and assessed for survival for 24 hours. Each fish experienced either a heating or cooling trial, i.e., each fish was tested only once. Subsequently, each fish was weighed (wet, ± 0.05 g) and measured (total length, ± 0.1 cm). Trials were performed between 1400 and

1800h to minimize possible diel fluctuations in temperature tolerance (Hutchison 1976).

The CTMaximum and CTMinimum for each acclimation group were calculated as the arithmetic mean temperature at which the endpoint was observed. Thermal scope was calculated at each acclimation temperature and is defined as the arithmetic difference between CTMaximum and CTMinimum at a specific acclimation temperature. A dynamic temperature tolerance polygon combining both measured and extrapolated tolerance was developed following Bennett & Beitinger (1997). The polygon was partitioned following Beitinger & Bennett (2000) into three distinct zones: an upper acclimation temperature dependent zone that represents heat gain via acclimation, a lower temperature dependent zone that represents gain in low temperature tolerance and a middle tolerance zone that is independent of acclimation temperature. One-Way *ANOVA* followed by Student – Newman –Keuls (SNK) multiple comparison analyses tested for significant differences in mean CTMaxima and CTMinima at the four acclimation temperatures, and regression analyses determined if a linear relationship existed between acclimation temperature and either lower or upper temperature tolerance. Finally, simple linear regression analyses were used to determine if lower or upper temperature tolerances were related to either fish length or weight in each of the eight trials. All statistical decisions were based on an α of 0.05.

Results

Increasing acclimation temperature resulted in green sunfish gaining heat tolerance and losing "cold" tolerance. The Critical Thermal Maxima, CTMaxima, (mean ± *SD*) of green sunfish acclimated from 10°C to 35°C ranged from 33.5±0.83 to 41.3±0.26°C (Table 2). Mean CTMaxima were significantly different (One-way *ANOVA*, $p < 0.0001$) at all four acclimation temperatures (SNK multiple range analysis). Also a highly significant linear relationship ($r^2 = 0.96$, $p < 0.0001$) exists: CTMaxima (°C) = 30.1 + 0.32(T_{ACCL}, °C).

Table 2. Mean (± *SD*) critical thermal maxima and critical thermal minima and thermal scope of green sunfish at four acclimation temperatures. Sample size was ten in each group except the 35°C-CTMaximum group, which was 9. The thermal scope equals the difference between CTMaximum and CTMinimum at a particular acclimation temperature.

T_{ACCL} (°C)	CTMaxima (°C)	CTMinima (°C)	Thermal Scope (°C)
10°C	33.5±0.83	0.2±0.28	33.3
20°C	36.2±0.59	3.2±0.84	33.0
30°C	39.9±0.67	8.6±0.24	31.3
35°C	41.3±0.26	11.2±0.73	30.1

Mean (± *SD*) CTMinima of green sunfish acclimated to the same temperatures extended from 0.2± 0.28 to 11.2± 0.73°C. One-way *ANOVA* ($p < 0.0001$) and SNK multiple range analyses determined that mean CTMinima also were significantly different at all four acclimation temperatures. A highly significantly ($r^2 = 0.97$, $p< 0.0001$) relationship exists: CTMinima (°C) = -4.8 + 0.45(T_{ACCL},°C).

Thermal scopes ranged from 30.1 to 33.3°C. The area of the green sunfish's dynamic (ecological) temperature tolerance polygon encompassed 1270°C^2 (Figure 1). The upper and lower acclimation dependent tolerance areas were 268°C^2 and 384°C^2, respectively. Combined (652°C^2) they are slightly larger than the area of the acclimation independent zone, 618°C^2. The lower acclimation dependent zone was 1.43 times larger than the upper acclimation dependent zone.

Fish were between 3.9 and 9.0 cm long and weighed between 0.5 and 11.2 g, and no linear relationship was found between either length or weight and temperature tolerance for any of the eight trials, i.e., fish size did not significantly influence temperature tolerance in this study.

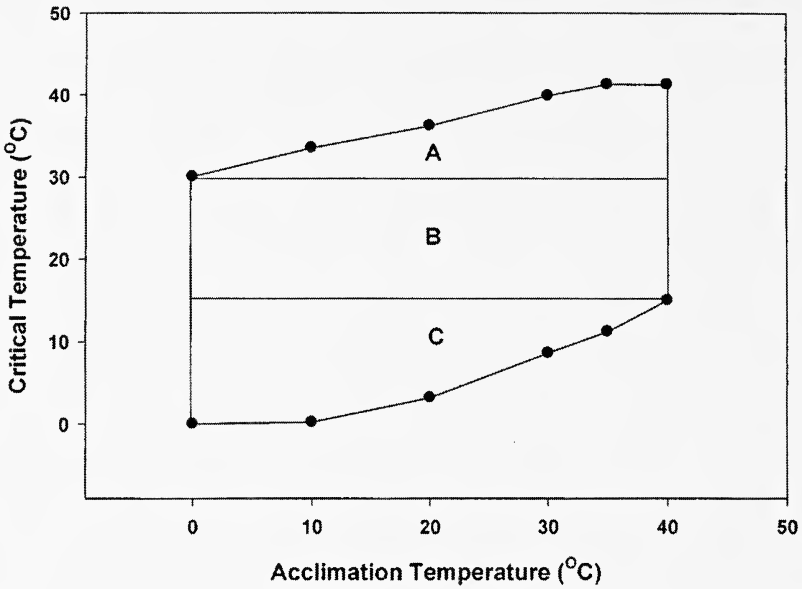

Figure 1. Ecological temperature tolerance polygon for green sunfish. The area (618°C^2) of the middle zone, B, represents temperature tolerance that is independent of acclimation temperature. Zones A and C represent upper (205°C^2) and lower (384°C^2) temperature tolerance areas produced by acclimation. Combined these two acclimation dependent areas account for slightly more than one-half of the entire ecological temperature polygon of 1270 C^2.

Discussion

Owing to its multiple effects (Fry 1947) on poikilotherms such as fish, temperature has been termed the abiotic master factor (Brett 1971). The most dramatic abiotic effect is lethality, i.e., too little or too much heat can kill an organism. Temperature tolerance determinations quantify the thermal limits of a species, offer insights into a species' physiology, biology and ecology, and provide data to model possible effects of global climate change. Data generated by this research suggest that the success of green sunfish within Great Plains streams and their widespread dispersion throughout North America has been aided by their temperature tolerance ability. The large thermal scopes, extensive temperature tolerance polygon and highest CTMaximum (41.3°C) confirm that

the green sunfish is both eurythermous and heat tolerant. A comprehensive review of temperature tolerances of North American freshwater fishes, indicated that only 22 species have a reported CTMaximum equal to or greater than 40°C. Of these only two are centrarchids: bluegill, *Lepomis macrochirus,* 41.4°C (Holland et al. 1974 and Murphy et al. 1976) and largemouth bass, *Micropterus salmoides*, 40.9°C (Fields et al. 1987).

The mean CTMinimum of green sunfish acclimated to 10°C was 0.2°C. Loss of equilibrium was not observed in 7 of the 10 fish during this trial and the CTMinima of those seven individuals was recorded as 0.05°C (the lowest temperature attainable with the available cooling system). If acclimated to temperatures below 10°C, it is hypothesized that their low temperature tolerance allows green sunfish to survive the coldest temperatures attainable in freshwaters.

The largest thermal scope and dynamic thermal tolerance zone reported for a North American fish are 34°C and 1470 C^2 for the sheepshead minnow, *Cyprinodon variegatus*, by Bennett & Beitinger 1997. Comparable variables for green sunfish are 33.3°C and 1270°C^2. The 14% difference in thermal tolerance polygon area is explained by the 45.1°C CTMaximum for sheepshead minnow.

A temperature tolerance polygon not only pictorially describes the temperature tolerance ability of a species, Beitinger & Bennett (2000) developed a geometrical approach to quantify the effect of acclimation on temperature tolerance. In it, a species temperature tolerance polygon is partitioned into three distinct zones: an upper acclimation temperature dependent zone which represents head gain via acclimation, a lower acclimation temperature dependent zone which represents gain of "cold" tolerance, a middle tolerance zone that is independent of acclimation temperature, termed the intrinsic temperature tolerance zone. This technique was applied first to polygons generated from incipient lethal temperature (ILT)

methodology for 21 species of fish (Beitinger & Bennett 2000). Although this is the first application of this partitioning technique to CTM data, our results for green sunfish are consistent with those for ILT generated tolerance data. First, the combined areas of the acclimation dependent zones comprised approximately one-half (51.2%) of the total tolerance polygon. This compares favorably with the mean 51.1% for the 21 species in the original study. Second, the lower acclimation dependent zone was larger (1.4 times) than the upper acclimation dependent zone. This suggests that acclimation plays a larger role in tolerance of low rather than high temperatures. Finally, applying a regression model to predict polygon area solely as a function of ultimate upper temperature yielded a predicted polygon area for green sunfish of $1258.4°C^2$. This is within 99% of this study's empirical determined value of $1270°C^2$. *In toto*, these finding suggest that this approach can be applied to CTM as well as ILT generated data, and reaffirms claims (see reviews by Lutterschmidt & Hutchison 1997b and Beitinger & Bennett 2000) that acclimation plays a major role in a species' temperature tolerance.

In addition to enhancing the area of the temperature tolerance polygon, variation in acclimation temperature accounted for 96% and 97% of the variation in upper and lower temperature tolerance, respectively. For each 1°C increase in acclimation temperature, green sunfish gained 0.32°C in heat tolerance, and for each decrease of 1°C in acclimation temperature, the mean CTMinimum decreased by 0.45°C. Species such as the green sunfish with large slopes relating acclimation temperature and temperature tolerance should be able to adjust lethal endpoints quickly when confronted with exposures to extreme temperatures (Beitinger & Bennett 2000).

In conclusion, depending on its previous acclimation temperature, green sunfish are able to withstand temperatures from 0 to 41.3°C. This high degree of eurythermicity appears to have arisen from evolving within thermally labile streams of the U.S.

Great Plains, and may allow this species to survive extreme temperatures expected by ongoing global climate change.

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EVALUATION OF THE UTILITY OF THE AMERICAN COLLEGE TESTING PROGRAM EXAMINATION IN PREDICTING THE PROBABILITY OF SUCCESS IN CALCULUS I IN SELECT TEXAS UNIVERSITIES

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Abstract.–The efficacy of the American College Testing (ACT) Program examination in predicting success in a standard Calculus I course is analyzed. Using an ex-post facto design and the method of binary logistic regression, a significant relationship exists ACT scores and Calculus I final grades of the 981 students examined during this study. Specifically, the data indicate that the ACT mathematics score was the most important predictor of success in Calculus I. Other covariates included in the final analysis were the ACT English score and student gender. Although ethnicity was considered in the analysis, it was found to be statistically insignificant to the final results. Colleges and universities participating in this study included Abilene Christian University, Angelo State University, Lamar University, Texas State University, and the University of Texas at San Antonio.

Program planning and curriculum development require an accurate assessment of the student entering the educational system. A fundamental concern is the development of a program that is sensitive to accurate placement and evaluation.

Students are traditionally asked by institutions to submit their American College Testing (ACT) Program scores, or other similar scores, to help institutions determine admission status. Scores are also often used to determine the initial placement of students in certain courses. This study is concerned with the relationship between the ACT Mathematics score and the grade earned in Calculus I.

The primary research question was, for students enrolled at the institutions considered in this study for a five-year period, who had completed Calculus I and taken the ACT, is there a relationship between the final grade in Calculus I and the score on the ACT Mathematics test? Secondary analysis was conducted on several

other factors including the ACT composite score, ACT science reasoning score, ACT reading score, ACT English score, student ethnic group, student gender group, and academic institution.

Justification for using standardized tests as a device for placement and predicting achievement of new college students generally has centered on the possible effects of poor placement with respect to their motivation and performance (Dunn 1966). The ACT program scores are used throughout the United States as well as in several other countries as an indicator of potential academic success at the tertiary level. It differs from its closest competitor, the SAT in a number of ways. The SAT is more popular in the eastern and far western United States, whereas the ACT is used more frequently in the Midwestern, southern, and southwestern United States. In addition to the verbal and mathematical sections found on the SAT, the ACT emphasizes reading and science reasoning. A standard scale is used for reporting total test scores on the ACT ranging from 1 (lowest) to 36 (highest). The overall mean standard score on the ACT Mathematics Test for entering college freshman during the course of the study was 20.7. Internal consistency reliability data of the ACT Mathematics Usage Test for students considered in this study was 0.91. The overall mean standard score on the ACT English, Math, Reading and Science Reasoning Test for entering college freshman during the course of this study was 20.4, 21.7, 21.3 and 21.0 respectively with an overall composite score of 21.0. According to these results, the ACT Assessment Test has remained stable over the course of this study.

The selection of Calculus I as the specific course was predicated on the belief that undergraduate mathematics courses (at the level of Calculus and above) have been looked upon as an insurmountable barrier by many students. Students who would like to pursue study in the areas of medicine, dentistry, optometry, engineering, and computer science as well as other disciplines may fail to enter that career track because of a poor score in Calculus I (Lowe, 1981). Since mathematics is the primary tool for problem

solving in many academic areas, a strong relationship between a student's ACT Mathematics score and the final grade earned in Calculus I may provide evidence to support a change of the Mathematics curriculum at the secondary school level. In this study, the ACT Mathematics Usage Test was selected as the measuring device to predict success in Calculus I at five selected institutions of higher learning within the State of Texas.

While there have been extensive studies of a related nature, few have looked at a student's ACT score and their final grade in Calculus I, and none have used the method of binary logistic regression to evaluate the relationship. Binary logistic regression is appropriate in this instance because the response variable takes on only two values: success or failure. Ordinary least squares regression assumes the response variable is continuous, so would be inappropriate in this instance. For an overview of logistic regression, see Peng & So (2002).

Hudson & McIntire (1977) reported a weak correlation between performance in introductory non-Calculus physics and mathematics skills at the beginning of the course, and concluded that such skills alone are not sufficient to guarantee success in physics. In a more comprehensive study, Hudson & Rottman (1981) found a significant correlation (*r-square = .175*) between scores on a pre-course test of algebraic and trigonometric skills and performance in non-Calculus, pre-professional physics for those who completed the course. Wollman & Lawrenz (1984) subsequently verified the correlation between mathematics skills at the time of entry into the course and achievement in non-Calculus physics for those who completed the course. Another previous study, which was conducted at Pima College, showed that there is a weak correlation (*r-square = .106*) between the Nelson-Denny (N-D) Reading Test and the grade which was earned in introductory non-calculus-based physics (Iadevaia 1989).

Calculus I, although fundamental in nature, draws heavily upon a student's mathematical preparation at the secondary school level. Thus, working from the assumption that the ACT Mathematics Assessment test is the best indicator of the mathematical preparation of the student, there should exist a moderately strong correlation between the ACT Mathematics Assessment score and the grade earned in Calculus I at five institutions of higher learning within the State of Texas.

Another, conducted at Southern Illinois University at Edwardsville, focused on the differential effects of initial Chemistry course placement as a function of ACT Mathematics scores and secondary school rank-in-class percentile (Reiner 1971). It was found that students who were initially placed in the first course of the sequence would be expected to attain higher grade point averages if their rank-in-class was in the middle or lower, regardless of the student's ACT Mathematics score.

Data Collection and Statistical Analysis

Data used in the analysis were obtained from each university's Office of Institutional Research, and included results from 981 students attending five different universities in the state of Texas (Table 1) including Abilene Christian University (ACU), Angelo State University (ASU), Lamar University (LU), Texas State University (TSU) and the University of Texas at San Antonio (UTSA). Each university represented a mix of public, private, large, small, suburban, and rural universities as well as both competitive admissions and open admissions universities. Each of the schools was randomly selected from colleges and universities in Texas and each student who took Calculus I during the study timeline in each of these schools were included.

Several variables were included in the analysis: ACT composite score (COMP), ACT English score (ENGL), and mathematics score (MATH) all with a continuous value range between 1 and 36; student gender (GEND) which was a dichotomous variable where 0 indicated male and 1 indicated female; and Calculus I final grade

Table 1. Total university descriptive statistical data for five Texas universities.

Classification	*N*	Abilene Christian University	Angelo State University	Lamar University	Texas State University	University of Texas at San Antonio
African American/ Black	38	7	5	6	16	0
Asian/Pacific Islander	22	6	6	0	7	3
Hispanic	158	5	46	1	91	15
Caucasian	757	296	254	23	173	11
Other	6	0	0	3	3	0
Male	609	103	200	11	277	18
Female	372	63	111	22	165	11

Table 2. Average ACT scores of students in the population tested from 1998–2002.

Students	English	Math	Reading	Science	Composite
981	22.5	24.3	23.4	23.5	23.4

Table 3. Variables in the logistic regression model.

	B	S.E.	Wald	Df	Sig.	Exp(B)
ACTENG	.057	.023	6.398	1	.011	1.059
ACTMAT	.106	.028	14.558	1	.000	1.112
GENDER	-.351	.145	5.888	1	.015	.704
Constant	-.755	1.696	.198	1	.656	.470

(GRAD) which was also dichotomous for this study where 1 indicated successful completion of Calculus I (a final grade of A, B or C) and 0 indicated unsuccessful completion of Calculus I (a final grade of D, F or W). Student ACT data is shown below (Table 2).

The models that were successfully fitted will be discussed. All data was analyzed using SPSS version 14.0. The initial analysis considered all variables for which data were available. As a result, it was easy to establish a baseline from which variables shown to be non-significant could be eliminated. The roles of the parameters in the model may be summarized by observing the variables in the logistic regression model (Table 3). In the table, B is the estimated coefficient. If the parameter is significant ($p < 0.05$) then the parameter is useful to the model.

Table 4. Calculus I successful completion classification table.

	Non-successful Completion	Successful Completion	Percentage Correct
Predicted Outcome Non-successful Completion	177	227	43.8
Actual Outcome Successful Completion	100	642	86.5
Totals	277	876	$\Sigma = 1153$

The next step involved determining how success was defined. An unfavorable response was coded as a 0, while a favorable response was coded a 1. In this study, a final grade of A, B, or C was classified as successful while a grade of D, F, or W was classified as unsuccessful. A positive coefficient indicated that increasing values of the variable resulted in an increased likelihood of a favorable response.

Initially the overall significance of the model was determined. The p-value was < 0.001 for the step, block, and model, which indicates a model that is statistically significant. The Cox & Snell R-Square statistic had a value of .146 and the Nagelkerke R-Square statistic was .201. This indicates that the model performed in an acceptable manner.

The classification table (Table 4) shows that successful completion of Calculus I could be predicted 86.5% of the time. The overall prediction ability average was 71.5%.

In order to visually confirm the results, the data set was transformed into graphical output. A cross-tabulation of final grade in Calculus I and ACT Math score was performed. The data in the resulting frequency table was used to calculate the pass rate. A scatter plot was then created with the ACT Math score on the x-axis and the pass rate on the y-axis (Figure 1).

Figure 1. Calculus I pass rate vs. ACT mathematics score.

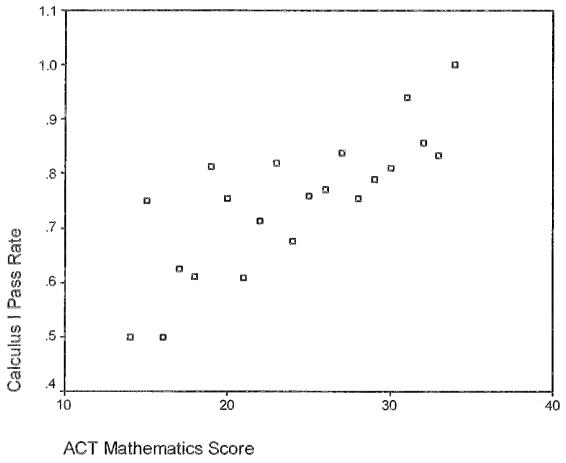
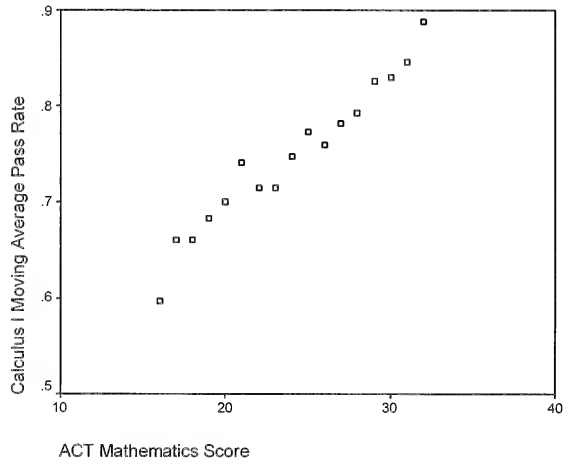


Figure 2. Calculus I moving average pass rate vs. ACT mathematics score.



The graph shows a positive correlation of the data. After smoothing the data a scatter plot was created with the moving average pass rate on the y-axis and the ACT Math score on the x-axis (Figure 2).

Figure 3. Final overlay scatter plot for ACT mathematics score.

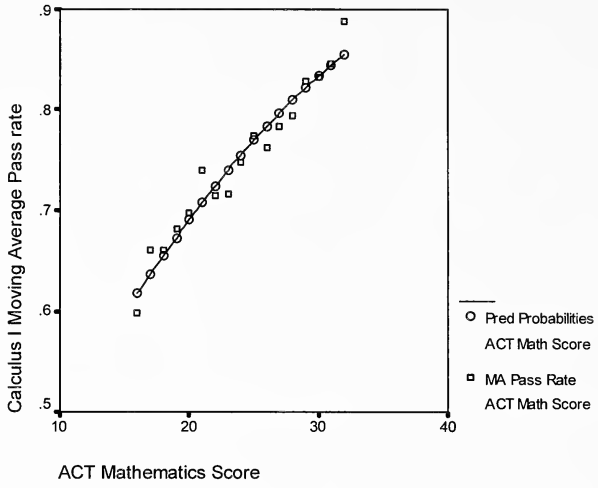
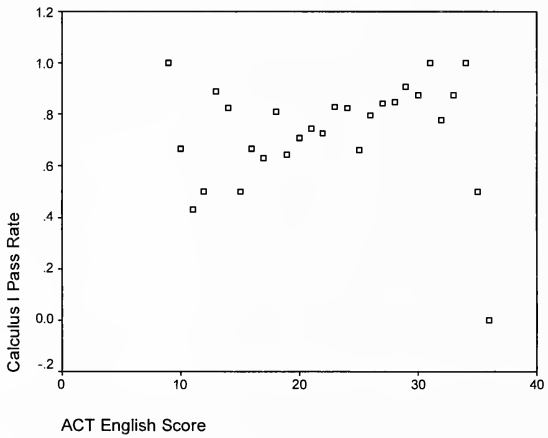


Figure 4. Calculus I pass rate vs. ACT English score.



A binary logistic regression was performed with grade as the dependent variable and ACT Math score as the covariate. An overlay scatter plot was created which specified ACT Math score and pass rate as a pair and ACT Math score and the predicted probabilities as the second pair with the ACT Math score defined on the x-axis each time (Figure 3). This graph shows a significant relationship between the ACT Math score and the final grade earned in Calculus I. The ACT Math score is a crucial factor in this study and shows a relationship that makes sense.

Figure 5. Calculus I moving average pass rate vs. ACT English score.

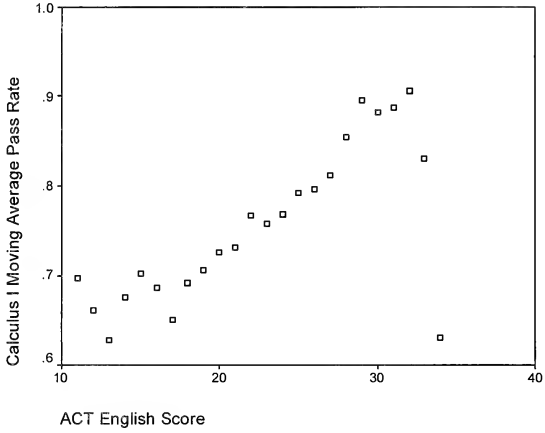
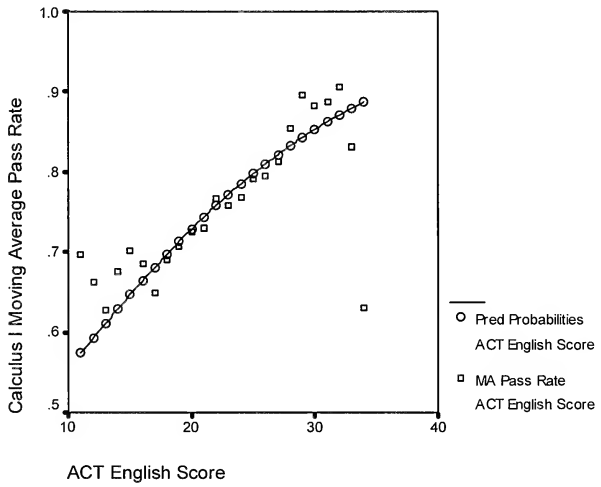


Figure 6. Final overlay scatter plot for ACT English score.



The same statistical analysis technique was used for the ACT English scores yielding the following results (Figures 4-6).

Based upon the statistical analysis a binary logistic regression model was fit that includes the dependent variable GRAD and the independent variables MATH, ENGL, and GEND. The general logistic regression model and corresponding predictive equation took the following form:

$$\ln\left(\frac{\pi}{1-\pi}\right) = \alpha + \beta_1(gend) + \beta_2(math) + \beta_3(engl)$$

Therefore,

$$\pi = \frac{e^{\alpha+\beta_1(gend)+\beta_2(math)+\beta_3(engl)}}{1+e^{\alpha+\beta_1(gend)+\beta_2(math)+\beta_3(engl)}}$$

where α is the intercept or constant, and β_1, β_2, and β_3 represent the partial regression coefficient for x_1, x_2, and x_3 respectively, holding the remaining predictors constant.

A sample calculation is included below for reference. Consider a male university student with an ACT Mathematics score of 23 and an ACT English score of 21. This student's probability of success (e.g., obtaining a grade of C or above) is obtained as follows.

$$\pi = \frac{e^{\alpha+\beta_1(gend)+\beta_2(math)+\beta_3(engl)}}{1+e^{\alpha+\beta_1(gend)+\beta_2(math)+\beta_3(engl)}}$$

$$\pi = \frac{e^{-0.755+(-0.351)(0)+(0.106)(23)+(0.057)(21)}}{1+e^{-0.755+(-0.351)(0)+(0.106)(23)+(0.057)(21)}}$$

$$\pi = \frac{e^{2.88}}{1+e^{2.88}} = 0.9468$$

Therefore, the student's probability of success is 0.9468. If we consider a female student with the same ACT characteristics, the following is obtained.

$$\pi = \frac{e^{\alpha+\beta_1(gend)+\beta_2(math)+\beta_3(engl)}}{1+e^{\alpha+\beta_1(gend)+\beta_2(math)+\beta_3(engl)}}$$

$$\pi = \frac{e^{-0.755+(-0.351)(1)+(0.106)(23)+(0.057)(21)}}{1+e^{-0.755+(-0.351)(1)+(0.106)(23)+(0.057)(21)}}$$

$$\pi = \frac{e^{2.529}}{1+e^{2.529}} = 0.9261$$

In this case, the female student's probability of success is 0.9261. While this equation was quite successful in this study it must be noted that the results presented can only "safely" extrapolate to the universities sampled, however, similar studies at other schools would be easy to do and are encouraged.

Results and Discussion

Binary Logistic Regression was conducted to determine which independent variables (MATH, ENGL, COMP, GEND, ACU, ASU, LU, TSU, and UTSA) were predictors of the final grade earned in Calculus I (GRAD). The statistics for overall model fit indicate a fairly good fitting model. The overall model correctly classified 86.5% of the cases.

The analysis suggests that there is a positive relationship between the covariates under consideration and a student's final grade in Calculus I within the State of Texas. As predicted earlier, it was found that the strongest relationship occurred between a student's ACT mathematics score and the final grade earned in Calculus I. In fact, compared to previous studies that have been done, the results obtained here are as good as or better than the previous research results.

This study has several shown that a student's predicted success in a standard Calculus I course in the State of Texas is directly related to their success on the American College Testing Program Examination (ACT). Colleges and universities can use these results to correctly place incoming students into an appropriate sequence that will enhance student's chances for success. This will provide the student with best possible chance of being successful in collegiate level mathematics.

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ALGAL COMMUNITY STRUCTURE OF THE EAST AND WEST FLOWER GARDENS, NORTHWESTERN GULF OF MEXICO

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Abstract.–The Flower Garden Banks are located approximately 200 km south of the border between Texas and Louisiana, on the outer continental shelf, beginning at a depth of approximately 20 m. The algal community has not been comprehensively evaluated and only checklists of a few dominant macroalgal species have been reported. This study utilized both destructive and non-destructive techniques to characterize the algal community structure. Harvest and photogrammetric samples were collected during two trips to the Flower Garden Banks in October 1998 and March 1999. A total of forty 0.25 m^2 quadrats of standing stock material were randomly collected, curated and stored for further evaluation. Photogrammetric samples (161) were obtained using an underwater camera and were evaluated by a grid of 25 random points. A total of 4,025 points of information were used to calculate percent composition and cover. Harvest sampling was used to characterize the algal composition of the 'red algal mat' which was the dominant feature comprising 38.4% of the photogrammetric samples. A total of 44 species were identified from the samples. The 'red algal mat' was primarily composed of members of the Order Ceramiales (*Centroceras, Ceramium, Hypoglossum, Polysiphonia* and *Anotrichium*).

The Flower Garden Banks are deep-water coral habitats located on the edge of the outer continental shelf in the northwestern Gulf of Mexico and cover an area of 143 km^2. The Flower Garden Banks are found at approximately 18-36 m below the water surface and extend down to 100-150 m (Lugo-Fernandez et al. 2001). The Flower Garden Banks are separated into the East Flower Gardens and the West Flower Gardens and are 12-15 km apart. They were designated as a protected National Marine Sanctuary in 1992. The East Flower Garden Bank is located at 27° 54.5' N latitude and 93° 36.0' W longitude, approximately 193 km southeast of Galveston, Texas. The West Flower Garden Bank is located approximately 172 km southeast

of Galveston, Texas at 27° 52.4' N latitude and 93° 48.8' W longitude (Fig. 1).

Both banks are topographic features created by the uplift of underlying salt domes of Jurassic, Louann origin (Rezak 1981). Bedrock material above the domes is covered with an overgrowth of calcareous marine organisms and represents the largest charted calcareous banks in the northwestern Gulf of Mexico (Bright et al. 1985) and the northernmost coral reefs on the continental shelf of North America (Bright et al. 1984). The Flower Garden Banks are part of a widely dispersed and discontinuous arc of reef material located along the outer continental shelf of the Gulf of Mexico (Rezak et al. 1985). The diversity of the coral community at the Flower Garden Banks has been described as depauperate as it supports only 20 species of hermatypic corals (Bright et al. 1984; Lugo-Fernandez et al. 2001). Although low diversity communities exist on neighboring banks, the reefs adjacent to Cabo Rojo, approximately 100 km south of Tampico, Mexico are the closest extensively developed coral reefs in the Gulf of Mexico and make up the Tuxpan Reef System (Universidad Veracruzana 1996; 2003; Tunnell 2007). The Tuxpan Reefs are approximately 644 km south of the Flower Garden Banks and are composed primarily of stony corals; 31 species of scleractinia coral and two hydrocorals (Universidad Veracruzana 1996; 2003; Tunnell 2007).

Monitoring of the Flower Garden Banks began in 1989 (Gittings et al. 1992) for the purposes of: (1) documenting long-term natural changes in reef growth and associated communities, (2) providing information and scientific data on the effect of gas and oil exploration in the vicinity of this sensitive ecosystem, and (3) encouraging and coordinating research efforts with agencies and institutions in order to better evaluate and/or determine causes of environmental change in the vicinity of the Flower Garden Banks (Dokken et al. 2001). Taxonomic lists of benthic

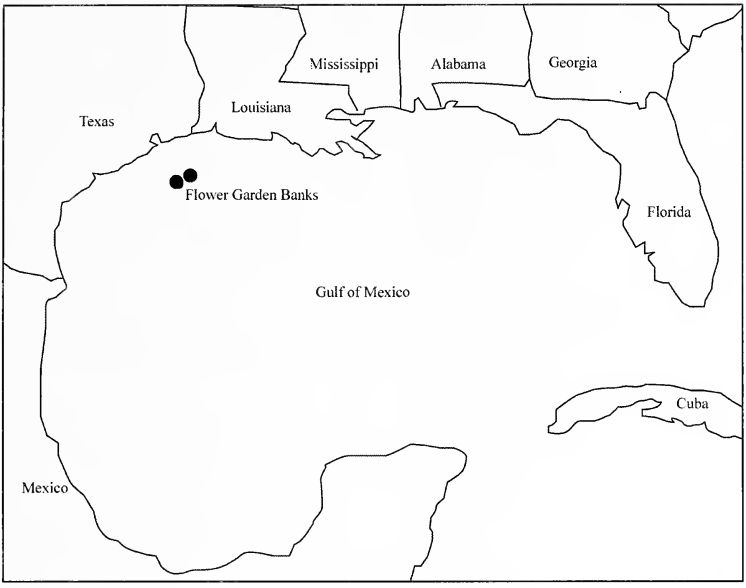

Figure 1. Map showing location of East and West Flower Garden Banks.

marine algae from the Flower Garden Banks were included in investigations by Bright and Pequegnat (1974) and Rezak et al. (1985; 1990). This study characterizes and provides an updated taxonomic assessment of the algae, coral and algae associations and algal distribution of the East and West Flower Garden Banks.

Materials and Methods

Ecological field methods followed those described by Littler & Littler (1985) and Brower et al. (1989). A herbarium voucher collection of benthic macroalgae (seaweeds) was completed and is located at the Ruth O'Brien Herbarium at Texas A&M University-Corpus Christi, Corpus Christi, Texas. Voucher specimens of macroalgal samples were also preserved in 2% gluteraldehyde and microscopic material was preserved on microscope slides sealed with Flo-Texx.

Photogrammetric and harvest samples were collected during two trips to the Flower Garden Banks in October 1998 and March 1999. Random quadrats were photographed using a Nikonos V equipped with a 28 mm lens, close-up kit, and Nikonos strobe. Each transparency represented a 0.25 m^2 quadrat that was evaluated by projecting onto a grid containing 25 random points (modified point quadrat) and evaluated based on (1) Bare Substrate, (2) Cyanobacteria, (3) Green Algal Mat, (4) Red Algal Mat, (5) Coralline Algae, or (6) Live Coral coverage. Values are expressed as the number of 'hits' for each species or feature divided by the total number possible. This number varied with each bank and season measured. A total of 4,025 points of data were identified and used to calculate percent composition, species richness, dominance, diversity and cover. Such samples can be used to generate precisely detailed and highly reproducible quantitative information, including cover, density, frequency and diversity (Littler & Littler 1985). Diversity and other indices were calculated from relative abundance of both, algae and total species observations. Diversity values were determined for each bank and sampling season using the Shannon Index (H', natural log) (Shannon 1948), Richness, Evenness (J') (Pielou 1966) and Simpson's Index of Dominance (Simpson 1948). Relative Community Similarity was determined by species or feature abundance from each bank and season and compared with total percent similarity and Morisita's Index of Community Similarity (Morisita 1959). Morisita's Index is based on Simpson's Index of Dominance and determines the probability that two randomly selected individuals will be the same species. The range of results are 0 (no similarity) to approximately 1 (identical).

Macroalgal mat samples were harvested using paint scrapers and placed into nylon net bags. A total of forty 0.25 m^2 quadrats of standing stock material were randomly collected, preserved

and stored for evaluation. This sampling was used to characterize the algal composition of the 'red algal mat'.

RESULTS

A total of forty 0.25 m^2 quadrats of standing stock material were randomly collected along with one hundred sixty-one 0.25 m^2 photo-quadrats from an average depth of 27 m. A systematic list of marine algae from the East and West Flower Garden Banks has been compiled (Table 1). The systematic organization follows that of Wynne (1998). Taylor (1960) and Schneider & Searles (1991) were referenced when species were encountered that were uncommon to the region.

Photogrammetric sampling.–The feature 'red algal mat' had the greatest coverage comprising 38.4% of all photogrammetric samples followed by Bare Substrate (14.6%), Coralline Algae (9%), Green Algal Mat (8.1%), and Cyanobacteria (4%) (Figure 2). Species richness totaled 25 with both banks combined. The East Bank had the greatest species richness October 1998 with 19 and lowest March 1999 with 14 (Table 2).

Diversity measures using Shannon's Diversity Index and Evenness show little differences in diversity between years and between the East and West Banks (Table 2). The West Bank had the greatest diversity October 1998 (H' = 2.10) and the East Bank had the highest diversity March 1999 (H' = 2.06). Overall, species diversity with both banks combined was H' = 2.20. Morisita's Index of Community Similarity indicated the East and West Banks in 1999 and the total of the East and West Banks from 1998 & 1999 were almost identical (0.94). Percent (Proportional) similarity supported these results (Table 3).

Association between corals and algal mat communities were investigated to determine if a particular coral species had a partiality with the red algal mat. Within all quadrats, each time a coral species was encountered, the presence or absence of the red

Table 1. Taxonomic list of algae collected from East and West Flower Garden Banks, northwestern Gulf of Mexico. (E=East, W=West).

DIVISION CYANOBACTERIA	
Order Oscillatoriales	
Family Oscillatoriaceae	
Lyngbya sp.	E & W
Oscillatoria sp.	E & W
Schizothrix calcicola (C. Agardh) Gomont	E & W
Spirulina sp.	W
Unknown (3 species)	E & W
DIVISION RHODOPHYTA	
Order Compsopogonales	
Family Erythropeltidaceae	
Erythrocladia sp.	E
Erythrotrichia sp.	E
Order Bonnemaisoniales	
Family Bonnemaisoniaceae	
Asparagopsis taxiformis (Delile) Trevisan	E & W
Order Corallinales	
Family Corallinaceae	
Amphiroa fragilissima(Linnaeus) Lamouroux	E
Corrallina spp. (3 species)	E & W
Jania adhaerens Lamouroux	E & W
Order Ceramiales	
Family Ceramiaceae	
Anotrichium tenue (C. Agardh) Nägeli	E & W
Antithamnion sp.	E & W
Antithamnionella sp.	E
Callithamniella sp.	E & W
Callithamnion sp.	E & W
Centroceras clavulatum (C. Agardh) Montagne	E & W
Ceramium spp. (2 species)	E & W
Griffithsia globulifera Kützing	W
Plenosporium flexuosum (C. Agardh) De Toni	E & W
Ptilothamnion occidentale Searles	W
Rhododictyon bermudense W. R. Taylor	E
Spyridia spp. (2 species)	E & W
Family Delesseriaceae	
Hypoglossum hypoglossoides (Stackhouse) Collins et Hervey	E & W
Hypoglossum sp.	W
Myriogramme sp.	W
	E & W
Family Dasyaceae	
Dasya sp.	E & W

Table 1 (Cont.)

Family Rhodomelaceae	
Chondria sp.	E & W
Herposiphonia sp.	W
Polysiphonia spp. (3 species)	E & W
Order Rhodymeniales	
Family Rhodymeniaceae	
Botryocladia occidentalis (Børgesen) Kylin	E & W
Chrysymenia enteromorpha Harvey	E & W
Chrysymenia sp.	W
Rhodymenia divaricata Dawson	E
Order Gigartinales	
Family Dumontiaceae	
Dudresnaya sp.	W
Family Hypneaceae	
Hypnea sp.	W
Order Gelidiales	
Family Belidiaceae	
Gelidium sp.	W
DIVISION OCHROPHYTA	
Class Phaeophyceae	
Order Dictyotales	
Family Dictyotaceae	
Dictyota dichotoma (Hudson) Lamouroux	E & W
Dictyota sp.	E & W
Dictyota sp.	E & W
Lobophora variegata (Lamouroux) Womersley	E & W
Spatoglossum schroederi (C. Agardh) Kützing	E & W
DIVISION CHLOROPHYTA	
Order Ulotrichales	
Family Ulotrichaceae	
Ulothrix flacca (Dilwyn) Thuret	E & W
Order Dasycladales	
Family Polyphysaceae	
Polyphysa polyphysoides (P. and H. Crouan in Maze and Schramm) Schnetter	E & W
Order Ulvales	
Family Ulvellaceae	
Entocladia viridis Reinke	E
Family Ulvaceae	
Enteromorpha sp.	E & W
Order Caulerpales	
Family Bryopsidaceae	
Bryopsis pennata Lamouroux	E & W
Derbesia sp.	E & W

Table 1 (Cont.)

Family Caulerpaceae	
Caulerpa sp.	W
Order Cladophorales	
Family Cladophoraceae	
Chaetomorpha sp.	E
Cladophora sp.	E & W
Family Anadyomenaceae	
Anadyomene stellata (Wulfen) C. Agardh	W

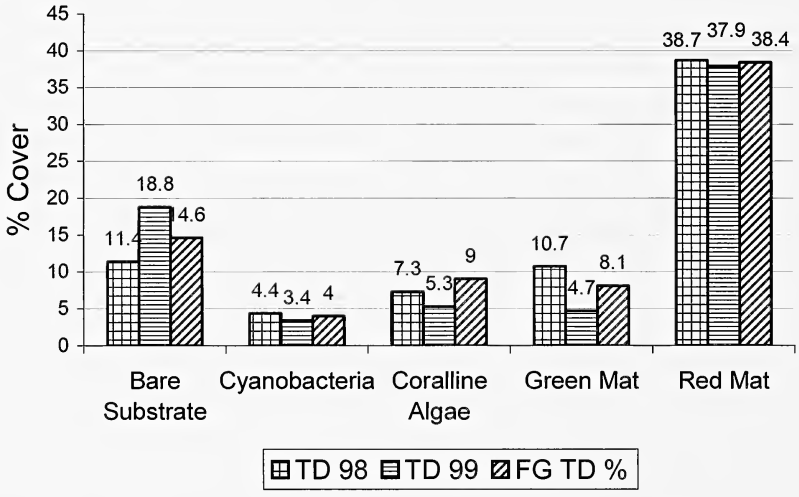

Figure 2. Percent algal coverage of photogrammetric samples from 1998, 1999, and both years combined. (TD = total; FG = Flower Gardens)

algal mat was assessed. A proportion of coral species to total red algal mat was computed. *Montastrea cavernosa* was most often associated with the red algal mat at 54.2% with *Diploria strigosa* following at 27.1%. Additional corals that were also associated with the red algal mat were *Diplora clivosa* at 11.9% and *Montastrea annularis* at 10.1%.

Harvest sampling.–Harvest samples were evaluated to characterize the species composition of the algal mat found at the coral/algal interface. The most common species collected were

Table 2. Species richness, diversity, dominance and evenness of the East and West Flower Gardens Banks, 1998-1999.

Bank/ Year	Species Richness	Shannon's Diversity	Shannon's Evenness	Simpson's Dominance	Simpson's Evenness
West 98	15	2.10	0.77	0.15	0.93
East 98	19	1.90	0.65	0.26	0.77
West 99	16	1.94	0.70	0.23	0.82
East 99	14	2.06	0.78	0.18	0.88
Total	25	2.20	0.68	0.19	0.84

Table 3. Community similarity using Percent Similarity and Mortisita's Index of the East and West Flower Garden Banks, 1998 & 1999.

Bank/Years	Percent Similarity	Mortisita's Index
East 98 vs. West 98	53.01	0.72
East 99 vs. West 99	72.26	0.94
East 98 vs. East 99	61.54	0.85
West 98 vs. West 99	58.94	0.79
East vs. West Total	73.59	0.94

from the Division Rhodophyta. A 'red algal mat' was the dominant algal and non-algal coverage comprising 38.4% of all photogrammetric samples. The mat was composed primarily of members of the Order Ceramiales comprising 37.6% of coverage and was represented by species of *Centroceras*, *Ceramium*, and *Polysiphonia* (Table 3). The Order Ceramiales was also the dominant group at each bank; 38.3% at the East, and 36.9% at the West. The East bank was represented by 13 algal families and the West Bank by 14.

A total of 44 species were identified from harvested samples collected from the East and West Flower Gardens Banks (Table 4). *Bryopsis pennata* and *Derbesia*, both in the Order Caulerpales, composed 12.0% of the 'green algal mat' on the East Flower Gardens and 11.0% on the West Flower Gardens. The Rhodophyta, *Jania adhaerens*, was the most common

Table 4. Total percentage of each algal species from the East and West Flower Garden Banks 1998-1999 contributing to total coverage.

Species	East Bank	West Bank
Amphiroa fragilissima	3.4	0
Anotrichium tenue	0	2.3
Antithamnionella sp.	1.5	0.4
Asparagopsis taxiformis	4.1	2.7
Botryocladia occidentalis	1.1	1.1
Bryopsis pennata	6	6.5
Callithamnion sp.	1.5	2.3
Caulerpa sp.	0	1.1
Centroceras clavulatum	7.5	2.7
Ceramium sp.	6.4	3.8
Chaetomorpha sp.	0.8	0
Chondria sp.	3.8	2.7
Chrysymenia enteromorpha	1.5	2.3
Cladophora sp.	4.9	3.4
Corallina sp.	0.4	0
Dasya sp.	1.1	1.5
Derbesia sp.	6	3.4
Diatom mat	0	0.4
Dictyopteris membranaceae	0	1.1
Dictyota sp.	3.8	3
Dudresnaya sp.	0	1.9
Enterocladaia virdis	0.8	0
Enteromorpha sp.	0.8	2.7
Erythrocladia sp.	0.4	0
Erythrotrichia sp.	1.5	0
Gelidium sp.	0	1.1
Griffithsia globulifera	0	2.7
Herposiphonia sp.	0	1.5
Hypnea sp,	0	0.8
Hypoglossum hypoglosoides	1.9	2.7
Jania adhaerens	7.1	6.1
Lobophora variegata	4.5	1.5
Lyngbya sp.	7.5	5.7
Myriogramme sp.	0	0.4
Oscillatoria sp.	0	6.8
Plenosporium flexuosum	3	3.8
Polyphysa polyphysoides	1.5	0
Polysiphonia sp.	4.9	5.3
Ptilothamnion occidentale	0	0.4
Rhodymenia divaricata	0.8	0
Spatoglossum schroederi	1.9	0.4
Spirulina sp.	0	0.8
Spyridia sp.	3.4	4.9
Ulothrix flacca	2.6	3.4
Unknown	3.8	6.5

coralline alga found at both Banks with coverage of 6.1% at the West and 7.1% at the East Bank.

Cyanobacteria; primarily Oscillatoriales, were common in samples, representing 7.5% at the East Flower Gardens, and 11.0% at the West Flower Gardens. Oscillatoriales was represented by only a few taxa with *Lyngbya* as the most common genus at both banks.

DISCUSSION

It is difficult to determine causes or effects of algal populations affecting the health of particular coral species of the Flower Garden Banks because the limited opportunity of sampling during this study. When given the opportunity to collect algal material from the Flower Garden Banks, especially at the interface of coral, algal material should be photographed *in situ* and samples collected, preserved in 2% gluteraldehyde, and returned to the laboratory for identification and evaluation. Coral species, depth, temperature and other environmental factors at the site should be recorded.

Ceramiales is an Order composed mainly of annual species that dominate as opportunists at sites with irregularly fluctuating conditions (i.e., temperature) (Luning 1990). These opportunists are quick growing algae that, after bacteria and diatoms, are the first multicellular algae to appear on bare, nonliving or damaged substrates. These algae exhibit variability in biomass depending upon herbivorous grazing or changes in environmental and seasonal conditions. Guimaraens & Coutinho (1996) evaluated the effect of an upwelling region near Rio de Janeiro, Brazil on the spatial and temporal variation of benthic marine algae. The survey of benthic algae yielded groups including the Ceramiales with temperature affinities occurring in sites directly influenced by upwelling waters. Their data showed water temperature affects distribution and abundance of algae enabling warm

temperate species (18-20° C) to survive under otherwise tropical conditions.

A seven year mean of water temperature recorded at a depth of 24 m at the West Flower Garden Banks resulted in a range of 18-30°C with low temperatures occurring in February (18-20°C) and highs in July-August (29-30°C) (Lugo-Fernandez 1998). This temperature range at the Flower Garden Banks is optimum for the growth of the 'red algal mat' composed primarily of members from the Order Ceramiales (Guimaraens & Coutinho 1996). The increased growth of these algae may become an environmental problem, possibly global, that would be practically impossible to mediate. The best action is to monitor changes over time to determine the magnitude and effect to the reef resulting from competition of the 'red algal mat'. Little is known about the community dynamics of benthic marine algae and their effect on the character of biotic reefs. Information on the role of inter-specific and intra-specific competition, recruitment, natality and mortality phenomena is lacking and it is difficult to make definitive statements concerning the role of algae and their effect on other organisms.

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A COMPARISON OF MANDIBULAR STRUCTURE IN RUDDY AND MASKED DUCKS (*OXYURA JAMAICENSIS* AND *NOMONYX DOMINICUS*)

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Abstract.–In order to test inferred differences in foraging behavior of both the masked duck (*Nomonyx dominicus)* and the ruddy duck (*Oxyura jamaicensis)*, this study examined nine characters and three ratios in the mandibles of 25 male and 25 female museum specimens of both species. Statistical analysis indicated that *O. jamaicensis* had longer lamellae and a slightly broader bill than *N. dominicus* (data for both sexes combined), which may adapt *O. jamaicensis* for efficiently sifting through bottom silt. Specimens of *N. dominicus* exhibited a stouter bill (greater height/length ratio of the maxilla), supporting the premise that this species is principally adapted for grazing on tough plants. Most bill characters were greater in length or width in *O. jamaicensis* as opposed to the masked duck and in ruddy duck males as opposed to ruddy duck females. Also, maxilla height (absolute as well as relative to dorsal maxilla length) was greater in male than in female masked ducks, suggesting a possible signal function in bill shape. Based on bill morphology, this study supports the premise that the genus *Nomonyx* is less advanced than the *Oxyura/Biziura* group.

Ducks of the tribe *Oxyurini* are distinguished from all other waterfowl by their elongated and pointed tail feathers and stiffened shafts (Johnsguard & Carbonell 1996). Within the tribe Livezy (1995) proposed the generic addition of *Nomonyx* (originally proposed by Salvadori 1895) differentiating the masked duck (*Nomonyx dominicus*) from other *Oxyura* based on a variety of characters including morphology and behavioral traits. Considering the premise that morphology represents aspects of the relationships between the organism and its environment (Bock 1977), this study examined mandibular traits of masked and ruddy ducks as a reflection of their foraging ecology.

Methods and Materials

The following measurements were made on 50 museum specimens (25 male and 25 female) of both *Nomonyx dominicus* and *Oxyura jamaicensis*: length of mandible, length of maxilla

(dorsal), length of maxilla (lateral), depth of maxilla, width of maxilla, width of mandible, length of tarsus (as an indicator of general body size), and number and size of lamellae (Table 1). Lamellar height was measured in the most rostral 30 lamellae. Using a low-power dissecting microscope, flattened insect pin tips that were calibrated to 0.4, 0.6, 0.8, 1.0 mm (with a stage micrometer) were inserted between adjacent lamellae (Fig. 1). All other measurements were made with dial calipers. Due to the difficulty in many specimens of exposing the most caudal lamellae, the authors estimate an accounting error of some 4-8 lamellae. Descriptive statistics and student's *t*-test were done with Statview (1996) version 4.51 for Macintosh.

Material examined.–Fifty museum specimens (25 male and 25 female) of *Oxyura jamaicensis* from nine countries and fifty specimens (25 male and 25 female) of *Nomonyx dominicus* from 12 countries were examined during this study. Accession numbers of specimens are from the following museums: Museum of Vertebrate Zoology, University of California, Berkeley (MVZ); Texas Cooperative Wildlife Collection-College Station (TCWC), Field Museum of Natural History-Chicago (FMNH), Louisiana Natural History Museum-Baton Rouge (LSUMZ) and Texas Tech University Museum-Lubbock (TTU).

Oxyura jamaicensis.–USA (LSUMZ 6791, 10406, 21507, 25271, 39136, 43115, 43116, 80214, 154354, 155831, 157507, TTU 178, 618, 3076, 4012); MEXICO (LSUMZ 10697, 14900, 27127, 43118); PUERTO RICO (LSUMZ 10336, 23185); BAHAMAS (LSUMZ 141425, 141426, 141427); DOMINICAN REPUBLIC (LSUMZ 141430, 141431, 141432, 141433); HAITI (LSUMZ 141428, 141429); EL SALVADOR (LSUMZ 50481); PERU (LSUMZ 34550, 34551, 63848); BOLIVIA (LSUMZ 35633, 35634, 35635, 35636, 35637, 37023, 37024, 37025, 37026, 37027, 37028, 37029, 37030, 37031, 37032, 37033).

Nomonyx dominicus.–USA (FMNH 96714, TCWC 11150); JAMAICA (FMNH 96719); CUBA (FMNH 96717, 96718, 156703, 411026, 411028, 411029); COSTA RICA (FMNH 126953, 127846,

Table 1. Measurements of bill dimensions in ruddy and masked ducks. Numbers are means (mm). The greater value is listed in bold-faced type when the probability is less than 0.05 that the two samples are from the same population.

	Ruddy mf	Masked mf	*t*	Ruddy m	Ruddy f	*t*	Masked m	Masked f	*t*
No. Specimens	50	50		25	25		25	25	
Length of Lamellae	**0.80**	0.47	13.20	0.81	0.78	0.80	0.46	0.47	0.81
S.D.±	0.14	0.05		0.12	0.15		0.05	0.05	
Mandibular Length	**35.10**	29.49	12.80	**35.70**	34.50	2.10	29.90	29.10	1.30
S.D.±	2.05	2.41		1.89	2.05		1.39	3.08	
Dorsal Length Maxilla	**40.90**	33.15	21.90	**41.60**	40.30	2.30	33.00	33.30	1.00
S.D.±	2.22	1.28		2.36	1.87		1.26	1.3	
Lateral Length Maxilla	**44.40**	36.87	19.00	**45.20**	43.50	2.50	36.80	37.00	0.70
S.D.±	2.05	2.41		2.36	2.63		0.89	1.3	
Height of Maxilla	**18.20**	15.90	9.80	**18.70**	17.80	2.40	**16.20**	15.70	2.10
S.D.±	2.22	1.28		1.25	1.45		0.97	0.79	
Width of Maxilla	**23.30**	17.20	24.80	**23.70**	22.80	2.00	17.10	17.30	1.10
S.D.±	2.02	1.11		1.43	1.68		0.75	0.63	
Width of Mandible	**13.80**	10.90	17.50	**14.10**	13.50	2.00	10.80	10.90	0.20
S.D.±	1.1	0.52		1.14	0.99		0.44	0.6	
Length of Tarsus	**33.80**	26.30	17.10	**34.50**	33.10	2.07	26.30	26.30	0.01
S.D.±	2.62	1.8		3.01	1.94		1.22	2.27	
No. Lamellae	**43.60**	40.20	7.70	42.90	**44.40**	2.10	40.40	39.90	1.00
S.D.±	2.63	1.94		2.41	2.67		1.98	1.9	
No. Lamellae/mm	**1.25**	1.21	2.10	1.20	**1.30**	3.20	1.20	1.20	1.30
S.D.±	0.11	0.08		0.08	0.12		0.07	0.08	
Maxilla Height/Dorsal Length	0.45	**0.48**	5.90	0.45	0.44	0.70	**0.49**	0.47	2.40
S.D.±	0.11	0.08		0.02	0.03		0.04	0.03	
Maxilla Width/Dorsal Length	**0.60**	0.50	9.00	0.57	0.57	0.40	0.42	0.43	0.80
S.D.±	0.03	0.03		0.03	0.03		0.02	0.02	

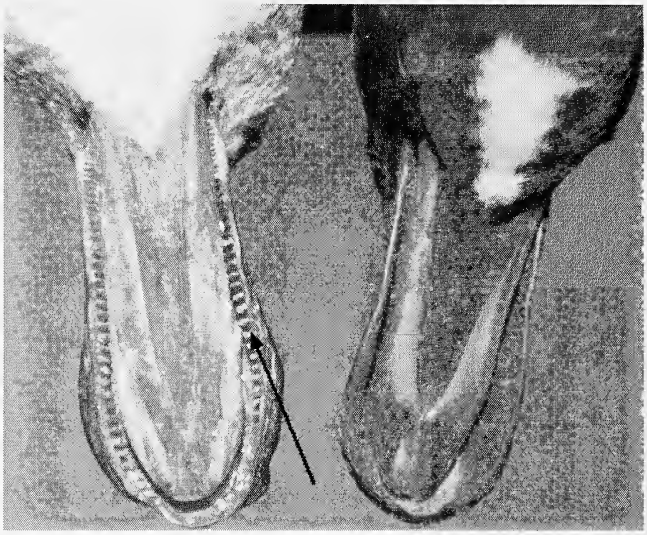

Figure 1. Ventral view of ruddy duck (left) and masked duck (right) to show lamellae (arrow). Lamellar length was measured by inserting calibrated pins in the spaces between the lamellae.

127847, 156705, 156706, 156707, 156708, 408630, 408631, 409925); PANAMA (FMNH 409924); COLOMBIA (FMNH 13611, 416473, 418705, 422612; MVZ 140619); VENEZUELA (FMNH 400007, 400008); GUYANA (FMNH 32190, 32191); SURINAME (MVZ 123495); ECUADOR (FMNH 414321); PARAGUAY (FMNH 411008, 411212, 412533, 412534, 413272, 413273, 413274, 413275, 413276, 414416, 416533, 416534, 416536, 417165; MVZ 99594, 99595, 99596); BRAZIL (FMNH 410472, 411414).

Results and Discussion

Table 1 shows that both species are sexually dimorphic with regard to bill size, and that the density of lamellae is approximately the same in both species, suggesting that if the ruddy duck is better adapted for sifting through silt, the adaptive advantage relates to lamellar size rather than number. On the other hand, the difference in scale between lamellar size and body size is so great that the larger lamellae may simply be a function of the overall larger size of the ruddy duck. The relative width of the bill is slightly larger in the ruddy duck: the ratios of maxilla width to dorsal maxilla length are 0.57 (ruddy duck)

and 0.52 (masked duck), both sexes combined. Relative bill height (maxilla height/dorsal maxilla length) is greater in the masked duck (0.49) than in the ruddy duck (0.45), sexes combined, suggesting that the masked duck possesses a stouter bill.

Where morphological and ecological measurements have been compared morphological generally predicts a greater portion of the variation in ecology than ecology does of morphology (Wainwright & Reilly 1994). Field data documents that adult breeding ruddy ducks feed almost exclusively on invertebrates by inserting their bills into the substrate, and moving the head in a lateral arc about 1.5 times the width of the bill, forcing the substrate out of the bill through the lamellae (Johnsgard & Carbonell 1996). During the non-breeding season ruddy ducks feed on a more varied diet that includes vegetative materials (Brua 2001). While little information is available on masked ducks specimens collected indicate that this species feeds primarily on seeds, stems, leaves and roots of aquatic plants. Nesting adults may feed on aquatic insects and crustaceans, but few data are available (Eitniear 1999). The literature on masked ducks also documents its preference for bodies of water with dense emergent vegetation often residing within the vegetation itself. The ruddy duck, however, prefers open areas of shallow water (Anderson & Tacha 1999; Eitniear & Colon 2005).

While the masked duck's bill is high and broad at the base, as in other stifftails, it is not as depressed at the tip; nail is less narrow and recurved, suggesting that perhaps roots and stems are a key part of the diet (Johnsgard & Carbonell 1996). The ruddy duck's longer lamellae and slightly broader bill may adapt this species for efficiently sifting through bottom silt. The resulting analysis of this study supports the premise that the masked duck is more primitive in structure being less adapted for sifting but retaining a stout mandible whose anatomy facilitates aquatic plant extraction. Ecology and morphology provide alternative, but mutually consistent expressions of the outcome of ecological and evolutionary adjustments between phenotype and environment (Bock 1977).

Based on bill morphology, this study supports the premise that the genus *Nomonyx* is less advanced that the *Oxyura/Biziura* group

(Johnsgard & Carbonell 1996). Additional field and captive studies should provide insights as to whether morphology reflects feeding ecology or if mandibular morphology is less important than behavioral foraging strategies (Guillemain et al. 2002) in this cryptic, elusive species of stifftail duck.

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GENERAL NOTES

THE OCCURRENCE OF *AGARDHIELLA RAMOSISSIMA* (GIGARTINALES) AND *ACANTHOPHORA SPICIFERA* (CERAMIALES) IN THE TEXAS COASTAL BEND

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The algal flora of the Texas coast is largely of tropical affinity; however, a distinctive cool temperature element develops during the winter and early spring (Edwards & Kapraun 1973). This range in water temperatures gives the Texas coast a variation of macroalgae characteristic of both surrounding areas and the tropics. Studies along the Texas coastline over the past few decades have contributed to new taxa for those areas (e.g.Kapraun 1980, Medlin 1984, Wardle 1992).

The Corpus Christi Bay area is now known to support at least 112 species of algae (Lehman 1999). Within this list, 24 additional species of macroalgae are reported that had not been previously noted in the Corpus Christi Bay area by past research, nine of which have a tropical affinity.

A study was recently completed of the colonization of macroalgae along the rocky jetties of Packery Channel (27° 36.836′ N; 97° 12.044′ W), a new man-made tidal inlet opened in 2006 near Corpus Christi, Texas. Macroalgae were sampled during bimonthly destructive sampling from September 2006 thru March 2007. Specimens were obtained by scraping clear quadrats (20 X 30 cm) into 500 μm biobags at a depth of < 1 m. By the end of the year-long sampling event, 40 species of macroalgae had been collected from the inlet.

This study reports the occurrence of *Agardhiella ramosissima* (Harv.) Kylin, a tropical species found occurring on coral reefs. In the Atlantic, this species has been reported from as far north as North Carolina (Schneider and Searles 1973; 1991) and south as Rio de Janeiro, Brazil (Pedrini 1984), but has never been reported from the northwest Gulf of Mexico. Less than 20 individual plants were collected, with size ranging from 1–5 cm. Specimens were further confirmed by the presence of zonate tetraspores embedded in surface patches within the thalli. Dry-pressed specimens are housed with the Phycological Collections of the Center for Coastal Studies Herbarium of Texas A&M University-Corpus Christi (accession number PCK 019 -*A. ramosissima*).

Cheney's Floristic Ratio (Cheney 1977) was used to indicate the floristic type represented by the algae growing at Packery Channel and was found to be 9.0 for this study. This is exceptionally high when compared to other macroalgal communities from the Gulf Coast. Temperature is the major factor controlling geographical distribution of marine algae (Edwards and Kapraun 1973) and, therefore, high ratios (meaning a more tropical flora) should be found progressing from north to south. This finding for Packery Channel is likely to be inaccurate due to incomplete development of the community. The authors are of the opinion that Cheney's Floristic Ratio, while useful in classifying communities, should be avoided in developing habitats.

Jetties are likely to draw at least part of their populations from the floating pool of individuals in the Gulf of Mexico (Britton & Morton 1989). Until jetties were constructed along the Texas coast, our outer shores were limited in algal growth, for they generally lack the necessary hard substrate. The inhibition model for structural assemblages states that any species can potentially colonize bare space (Connell & Slatyer 1977). With increased human impact in coastal zones and the increase in numbers of

jetties and seawalls, both the algal diversity and documentation of this change have increased significantly.

In a related study conducted simultaneous to that along the Packery Channel jetties, *Acanthophora spicifera* (Vahl) Børgesen was identified from the recessed portion of the channel. Several (10-15) specimens of this species were collected near the intersection of the Laguna Madre and the Gulf Intracoastal Waterway (GIWW), found growing up to 15 cm in length (accession number PCK 041 -*A. spicifera*). This species has been reported from the Gulf of Mexico (Schneider & Searles 1991; Littler & Littler 2000), but has never been reported from the Texas Coastal Bend. This species is noted to be an early colonizer in areas of calm waters (Littler & Littler 2000), which is characteristic of the protected segment of the newly-dredged Packery Channel. This study proposes a range extension for the species to include the Upper Laguna Madre near Packery Channel.

Hurricane Emily, a category five storm, opened Packery Channel prematurely on 22 July 2005, before completion of the jetties. Large-scale disturbance such as hurricanes are thought to deliver terrestrial-derived nutrients in concentrations sufficient to trigger and sustain macroalgal blooms (Cooper 1966; McCook 1999). Hurricanes often carry with them foreign species, trapped within eddies created by the storm. Increased storm activity over the past several years may further explain the initial presence of some tropical flora uncommon to the area.

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FIRST RECORD OF THE GRAY FOX (*UROCYON CINEREOARGENTEUS*) ON TEXAS BARRIER ISLANDS

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Barrier islands are dominant features of many coastal areas (Pilkey 2003). Approximately two-thirds of the Texas coastline is bordered by six major islands, the largest of these being Padre Island. Padre Island extends ca. 180 km from Corpus Christi south to Port Isabel, and although it was once continuous, it is now divided into North and South Padre Islands by the Mansfield Channel. Although parts of Padre Island are extensively developed, Padre Island National Seashore is the largest (ca. 110 km) stretch of undeveloped barrier island in the world (Pilkey 2003).

When compared to other island systems, relatively little research regarding mammals has been conducted on barrier islands. However, this research has demonstrated that barrier islands have relatively depauperate faunas when compared to adjacent mainland areas (McAlister & McAlister 1993; Hice & Schmidly 2002). In Texas, several species of mammals that exist in coastal mainland areas, including the gray fox (*Urocyon cinereoargenteus*), have not been reported from adjacent barrier islands (Hice & Schmidly 2002). While gray fox are widely distributed and are common throughout their range in Texas (Schmidly 2004), there have been no previous verified records of this species on Padre Island or other islands within the Gulf of Mexico (Selander et al. 1962; Hall & Dalquest 1963; Hice & Schmidly 2002). Bent (1940) reported observing gray fox on Cumberland Island, Georgia, but Ruckdeschel et al. (1986) later dismissed these accounts. This study reports the first confirmed record of the gray fox from Texas barrier islands.

On 15 March 2005, National Park Service personnel found a dead female gray fox on North Padre Island, 11.0 km south of the end of Park Road 22 (Kleberg County, Padre Island National Seashore, Pan Am Road, 14 0664484E, 3023097N). The specimen was prepared as a

standard skin and skeleton voucher specimen (Padre Island National Seashore catalog number 3744). Measurements of this specimen were: total length, 960 mm; tail length, 402 mm; right hind foot, 129 mm; mass, 3.0 kg. Mammae showed no obvious signs of lactation. Seacoast bluestem (*Schizachyrium scoparium*) and gulf dune paspalum (*Paspalum monostachyum*) dominated the vegetation where the specimen was found.

Since August 2005, five observations of gray fox have been made on Texas barrier islands (Jones 2008). On North Padre Island: 14 August 2005 (five specimens together), 14 August 2006 (one specimen), 9 September 2006 (one specimen). On South Padre Island: 15 August 2005 (one specimen), 17 may 2006 (one specimen). Gray fox also appear to be well established in residential areas at the north end of North Padre Island. Residents of Corpus Christi have regularly reported foxes in yards and on porches (Ellis pers. comm.). Crooks (2002) found that the relative abundance of gray fox in California was highest at the edge of urban areas. Gray fox are known to utilize and perhaps benefit from residential development through the use of anthropogenic resources (Harrison 1993; 1997) and it is possible that gray fox are taking advantage of available resources while avoiding predators in residential areas. Although the abundance of gray fox around residential areas on Padre Island is unknown, these individuals may be dispersing into surrounding undeveloped areas. Whatever the reason for their presence on Padre Island, it is possible that gray fox inhabit other barrier islands and have not yet been discovered.

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* * * * *

FIRST REPORT OF THE BROWN ALGA *PADINA GLABRA* (OCHROPHYTA: DICTYOTALES) FROM THE COAST OF TEXAS AND THE GULF OF MEXICO

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Up to now the only species of the brown algal genus *Padina* that has been recognized as occurring on the Texas coast has been *P. gymnospora* (Kütz.) Sonder (Taylor 1960; Humm & Hilldebrand

1962; Earle 1969; Edwards 1970; Sorensen 1979, all as *P. vickersiae*). This species had been known as *P. vickersiae* Hoyt in Howe (Howe 1920), until Allender & Kraft (1983) recognized that the "*Padina gymnospora*" of various authors had been misinterpreted to be usually three cells in thickness, whereas the type (*Zonaria gymnospora* Kütz.) is four cells thick in mid frond and 6-8 cells thick closer to the base, in agreement with Kützing's (1859) original depiction. Therefore, *Padina vickersiae* Hoyt is a later taxonomic synonym of *P. gymnospora*.

Some new species of red, green, and brown algae have been described from the Texas coast in the past few decades (Wynne & Edwards 1970; Wynne 1993; Scott et al. 2006) or newly reported species added to the marine algal flora (Baca et al. 1977; Kaldy 1977; De Yoe & Hockaday 2001; Strenth 2001; Kowalski et al. 2007). The primary source of new records has been from work carried out at the Flower Garden Coral Banks (National Marine Sanctuary) south of Galveston (Eiseman & Blair 1982; Gavio & Fredericq 2005). In the present study it was a matter of a more careful examination of *Padina* specimens from Texas in the University of Michigan Herbarium (MICH) that allowed for the recognition that two collections (one from Nueces County in south central Texas and one from Cameron County in southernmost Texas) were not the customary "*Padina gymnospora*", the common species on the Texas jetties, but a distinct species representing not only its first report from the coast of Texas but also from the Gulf of Mexico.

Padina glabra Gaillard 1966: 226

Type locality.–Pointe de Fann, Dakar, Senegal, West Africa. Holotype in the Muséum National d'Histoire Naturelle, Paris (PC).

Texas.–Port Aransas jetty, Mustang Island, Nueces County, 27 July 1969, coll. M. Wynne 2588 [MICH] (Fig. 1). Isla Blanca Beach State Park, jetty, South Padre Island, near Port Isabel, Cameron County, 24 March 1975, coll. M. Wynne 4293 [MICH].

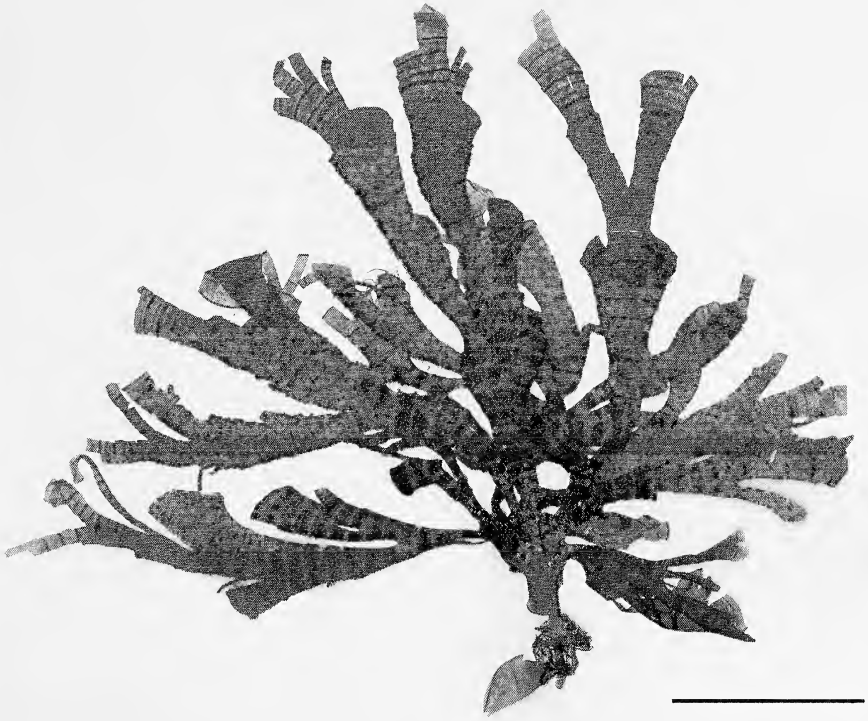

Fig. 1. *Padina glabra*. Specimen from Port Aransas jetty, Texas (MICH *2588*). Scale bar: 4 cm.

Padina glabra was described from a site near Dakar, Senegal, and was characterized as the only species in the genus that lacked multicellular hairs that are typically arranged in concentric rows in other species of the genus (Gaillard 1966). The species was described as having non-lacerated blades attached by rhizoids, only 1.0-1.5 cm tall, 3 or 4 cell layers in thickness, rarely with calcification, and with non-indusiate tetrasporangial sori arranged in concentric zones. The species was subsequently reported from Mandapam, southern India, by Rengasamy & Anand (1986), who reported blades to be 4.0-5.0 cm tall, divided down the middle and bearing plantules. The important point is that their specimens

lacked hairs. Wynne & De Clerck (1999) made the first report for the occurrence of this species from the western Atlantic, namely, several collections from the vicinity of St. Augustine, St. Johns Country, Florida, U.S.A. Wynne & De Clerck (1999) also pointed out that Dangeard (1952) had reported on several collections of a hairless *Padina* (that he called "*Padina* sp.") from the very same location where Gaillard (1966) was later to describe *P. glabra.* Gaillard, however, did not cite the Dangeard (1952) publication.

The Isla Blanca Beach State Park specimens, of which there were six, reached 8-12 cm in height, and the Port Aransas species, of which there were two, reached up to 14 cm in height. The plants from Isla Blanca are much branched axes ending distally in rounded blades. The plants from Port Aransas seem more mature and are divided into elongate, lacerate segments (Fig. 1). Blades in both collections are usually four cell layers in thickness. Some sections show local regions of three cell layers in thickness. Calcification is either lacking or just barely present on the blades. The base of the plant is a well developed stupose portion reaching to 1 cm in thickness and with a spreading attachment area. Sporangial and oogonial plants were observed. In both collections, however, plants bear propagula. These are also referred to as plantules and brood buds (Thivy 1945). Such propagula have been reported in this same species by Rengasamy & Anand (1986) in Indian material and by Wynne & De Clerck (1999) in Florida material. They appear to develop from sporangia at a very young stage or possibly in place of sporangia. Such propagula have been noted in other species of the genus: in *P. pavonica* (Linn.) Thivy by Bitter (1899), *P. durvillaei* Bory de St.-Vincent and *P. distromtica* Hauck by Thivy (1945), *P. gymnospora* (Kütz.) Sonder by Hoyt (1920) and *P. antillarum* (Kütz.) Piccone by Hauck (1887), Gaillard (1967), and Lawson & John (1977). Finally, in the Port Aransas plants the sporangia and propagula were observed to occur in two closely positioned rows on the inferior blade surface and in more irregularly arranged sori on the superior side of the blade. This pattern is essentially the same as the pattern present in *P.*

antillarum, in which the inferior surface of the blade bears sporangia in two rows straddling each line of hairs (Jaasund 1976, as *P. tetrastromatica*; Tseng 1983, as *P. tetrastromatica*; Wynne 1998; Wynne & De Clerck 1999). In the Port Aransas plants identified as *P. glabra*, however, these parallel rows of sporangia/propagula do not have hairs present separating them. A similar arrangement of propagula lying in two closely aligned rows present in Florida material of *P. glabra* was depicted by Wynne & De Clerck (1999: fig. 12).

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REPRODUCTION IN THE SIAMESE LEAF-TOED GECKO, *DIXONIUS SIAMENSIS* (SQUAMATA: GEKKONIDAE) FROM THAILAND

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The Siamese leaf-toed gecko (Siam-blattfinger), *Dixonius siamensis* is known from Thailand where it occurs from Songkhla, south to the Isthmus of Kra and north to at least Chiang Mai, from southern Myanmar to the Lao People's Democratic Republic, Vietnam and likely Cambodia (Bauer et al. 2004). It occurs from sea level to at least 700 m (Manthey & Grossman 1997) and inhabits a variety of forest types and occasionally occurs in developed areas; females deposit clutches of two eggs during the rainy season (Cox et al. 1999). The purpose of this note is to provide information on the reproductive cycle of *D. siamensis* from a histological analysis of monthly gonad samples from museum specimens collected in Thailand. Minimum sizes for reproduction in males and females of *S. siamensis* are given. This paper represents the first detailed account of reproduction in *D. siamensis.* Comparisons are made with the timing of reproduction of other lizards from the tropics.

A total of 105 *D. siamensis* from Thailand including 49 females (mean snout vent length, SVL = 42.5 mm ± 4.9 *SD*, range = 33-52 mm), 50 males (mean SVL = 41.5 mm ± 5.3 *SD*, range = 29-52 mm) and 6 presumed neonates (mean SVL = 21.0 mm ± 1.1 *SD*, range = 19-22 mm) were examined from the herpetology collection of the Field Museum of Natural History (FMNH), Chicago, Illinois. Geckos were collected 1957-1960, 1967 and 1969.

For histological examination, the left testis was removed from males and the left ovary was removed from females. Enlarged follicles (> 4 mm length) or oviductal eggs were counted. Tissues were embedded in paraffin and cut into sections of 5 μm. Slides

were stained with Harris hematoxylin followed by eosin counterstain (Presnell & Schreibman 1997). Slides of testes were examined to determine the stage of the spermatogenic cycle. Slides of ovaries were examined for the presence of yolk deposition or corpora lutea. Histology slides were deposited in the Field Museum of Natural History (FMNH) herpetology collection. An unpaired *t*-test was used to compare *D. siamensis* male and female mean body sizes (SVL) using Instat (vers. 3.0b, Graphpad Software, San Diego, CA).

The following specimens of *D. siamensis* from Thailand (by province) from the herpetology collection of the Field Museum of Natural History (FMNH), Chicago, Illinois, comprise the basis for this study: CHIANG MAI: 177752. CHON BURI: 177696, 177697, 177707, 177708, 177710, 177711, 177713, 177714, 177716, 177719, 177720, 177722-177725, 177728, 177731-177733, 177735, 177736, 177738, 177739-177741, 177743-177745, 181568, 181569, 181583, 181597. KANCHANABURI: 177698-177701, 177704. LOEI: 177747, 177748, 177762, 177763. NAKHON RATCHASIMA: 181474-181478, 181480-181484, 181486-181488, 181491, 181493-181495, 181497, 181498, 181537, 181538, 181543, 181547, 181553, 181555, 181561, 181562, 181571, 181573-181576, 181580, 181585, 181589, 181593, 181596, 181602-181604, 181606, 181608, 181609, 181614-181616, 181625, 181627, 181630, 181633-181637, 181640, 181641, 181644, 181645, 181647, 181652, 181664. PRACHUAP KHIRIKHAN: 177694. UBON RATCHATHANI: 177695.

There was no significant size difference (mean SVL) between *D. siamensis* males and females (unpaired *t*-test). Two stages in the testicular cycle were observed. Recrudescence in which cellular renewal for the next period of sperm formation (= spermiogenesis) occurs, was typified by spermatogonia and primary spermatocytes. Spermiogenesis (sperm production) was characterized by groups of spermatozoa and metamorphosing spermatids lining the lumina of the seminiferous tubules. The presence of males undergoing spermiogenesis in twelve months of the year (Table 1) indicates a continuous testicular cycle. Males in recrudescence were found

Table 1. Monthly stages in testicular cycle of *Dixonius siamensis* from Thailand.

Month	*n*	Recrudescence	Spermiogenesis
January	5	0	5
February	2	0	2
March	4	0	4
April	5	0	5
May	3	0	3
June	2	0	2
July	6	1	5
August	2	1	1
September	5	0	5
October	9	0	9
November	3	0	3
December	4	0	4

only in July (FMNH 177701, SVL = 33 mm) and August (FMNH 181537, SVL = 36 mm). However, samples of *D. siamensis* from these months are too small to speculate on the significance of this finding, i.e., whether they might suggest a "resting" period in the testicular cycle. The smallest reproductively active male (FMNH 181641) measured 29 mm SVL and was collected in April.

Mean clutch size for 14 gravid females was 1.86 ± 0.36 *SD*, range = 1-2. Females with either enlarged ovarian follicles (> 4 mm), soon to be ovulated, or oviductal eggs were found in all months except September to December (Table 2). Nevertheless, the presence of females exhibiting early yolk deposition (basophilic yolk granules within the follicle) in September, October and November suggests ovarian activity is still occurring, but perhaps at a slower rate than January to August. The smallest reproductively active female (FMNH 181627), collected in June, measured 37 mm SVL and was undergoing early yolk deposition. One female from April (FMNH 181495) that exhibited no yolk deposition contained a

Table 2. Monthly stages in the ovarian cycle of *Dixonius siamensis* from Thailand.

Month	*n*	No yolk Deposition	Early yolk deposition	Follicles > 4 mm	Oviductal eggs
January	3	1	1	0	1
February	3	2	0	1	0
March	6	1	2	0	3
April	6	2*	1	1	2
May	2	1	0	0	1
June	4	0	2	0	2
July	3	0	1	1	1
August	3	1	0	1	1
September	4	2	2	0	0
October	10	9	1	0	0
November	3	3	0	0	0
December	2	1	1	0	0

* one female contained a corpus luteum.

corpus luteum indicating an egg clutch had recently been deposited. There was no evidence (corpus luteum and/or oviductal egg) and concomitant yolk deposition to indicate that *D. siamensis* produces more than one clutch in the same year. However, this may reflect small sample sizes. Presumed neonates were collected in July (n = 1, SVL 20 mm), September (n = 4, SVL = 20, 21, 21, 22 mm) and October (n = 1, SVL = 19 mm).

The reproductive cycle of *D. siamensis* appears similar to that of other tropical lizards as it exhibits an extended period of sperm formation and egg production (Fitch 1982). This pattern appears typical for other gekkonid species studied in the tropics as reported for *Cosymbotus platyurus* (currently *Hemidactylus platyurus*), *Hemidactylus frenatus* and *Peropus mutilatus* (currently *Gehyra mutilata*) from West Java, Indonesia (Church 1962) and *Cyrtodactylus malayanus* and *C. pubisulcus* from Borneo (Inger & Greenberg 1966). In contrast, gekkonids from temperate areas

exhibit a seasonal cycle with most reproduction occurring in spring (Flemming & Bates 1995; Goldberg 1997; Goldberg 2006a; Goldberg 2006b). This pattern is seen in many other species of temperate zone lizards (see for example, Goldberg 1974; Goldberg 1975) indicating the local environment plays a role in adjusting life history characteristics of lizards (Vitt 1986). *Dixonius siamensis* also produces small clutches of eggs (1-2) as is typical for other gekkonid species (Vitt 1986).

Kluge (1967) categorized gecko reproductive cycles as: (1) no definite seasonal reproductive cycle and mating may occur throughout the year, and (2) breeding is cyclic and restricted to a short period during the year. *Dixonius siamensis* clearly fits into the former category. However, with at least 39 species of geckos recorded from Thailand (Chan-Ard et al. 1999), subsequent studies are needed before the variations in their reproductive cycles can be ascertained.

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RIPARIAN BIRD COMMUNITY FROM THE RIO SABINAS, COAHUILA, MEXICO

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Abstract.–Riparian areas have been identified as key habitats for wildlife, especially for the North American arid landscape; however, similar areas in northern México have been poorly studied. This study was conducted in a riparian habitat dominated by Montezuma bald cypresses (*Taxodium mucronatum*) of the Río Sabinas, Coahuila, México, from December 2004 to November 2005. Monthly samples were conducted, using a total of 443 point counts, in three river sections characterized by distinct levels of disturbance, from lesser to greater: Melchor Múzquiz, San Juan de Sabinas and Sabinas. A total of 168 species were recorded. The Melchor Múzquiz section exhibited the smallest number of species corrected by rarefaction and a significantly smaller number of species and individuals per point count. The Olmstead-Tukey diagrams per section also showed differences in the dominance of the species. From a conservation point of view, noteworthy species were recorded such as Wood Stork and Painted Bunting.

Resumen.–Las zonas ribereñas han sido identificadas como hábitats clave para la vida silvestre, especialmente para el paisaje árido de Norteamérica; sin embargo en el norte de México dichas zonas han sido poco estudiadas. El presente estudio se llevó a cabo en el hábitat ribereño dominado por sabinos (*Taxodium mucronatum*) del Río Sabinas, Coahuila, México, de diciembre de 2004 a noviembre de 2005. Se llevaron a cabo muestreos mensuales utilizando un total de 443 puntos de conteo de radio fijo en tres zonas del río caracterizadas por distintos niveles de disturbio, de menor a mayor: Melchor Múzquiz, San Juan de Sabinas y Sabinas. Se registraron un total de 168 especies. La zona Melchor Múzquiz mostró el menor número de especies corregidas por rarefacción y un número significativamente menor de especies e individuos por punto de conteo. Los diagramas Olmstead-Tukey por zona también mostraron diferencias en la dominancia de las especies. Se registraron especies importantes desde el punto de vista de la conservación, como *Mycteria americana* y *Passerina ciris*.

The importance of riparian zones for breeding and migrant birds has been extensively documented (e. g., Knopf et al. 1988; Knopf & Samson 1994; Skagen et al. 1998; Anthony et al. 1996; Finch & Yong 2000; Flannery et al. 2004; Kelly & Hutto 2005; Villaseñor

2006). While riparian woodlands of North America constitute less than 1% of the area of arid landscapes they support more reproductive birds than the extensive surroundings (e.g., Knopf et al. 1988). Unfortunately, it has been suggested that at least 95% of all western riparian habitats have been altered in some way during the past century (Ohmart 1994), and that such destruction may be the most important factor in the decline of some western North American landbird species during the past century (DeSante & George 1994). However, the effects of such alterations on riparian systems may be unnoticed for decades due to longevity of cottonwoods (Rood & Mahoney 1993) and other riparian trees such as Montezuma bald cypresses. This could cause a delay in awareness of the need for conservation, management, and restoration actions.

In northwestern Mexico, there have been few studies conducted on the importance of riparian habitats for migratory birds (Hutto 1995; Villaseñor 2006), but none in the northeastern portion. The Río Sabinas (130 km length) is located in northeastern Coahuila, running from the Sierra Santa Rosa near Melchor Múzquiz to the Venustiano Carranza Dam near Juárez municipality (Fig. 1). Its avifauna has been little studied even though it is a Priority Terrestrial Region for Conservation in México (RTP-152, Arriaga et al. 2000), a State Natural Protected Area (Periódico Oficial 1998), and an important biological corridor linking northern Mexico with the southern United States through the well-conserved Sierra Santa Rosa, Maderas del Carmen and Big Bend National Park. This connection is also maintained downstream from the Venustiano Carranza Dam where the river is named the Río Salado; it flows through Nuevo León and Tamaulipas and enters the Falcon Reservoir which is part of the Río Grande. Its riparian vegetation is dominated by Montezuma bald cypress (*Taxodium mucronatum*, locally known as "sabino"), while pecan (*Carya illinoinensis*), sycamore (*Platanus occidentalis*), sugar hackberry (*Celtis laevigata*), and Mexican ash (*Fraxinus berlanderiana*) are also present. Its shrub stratum is variable but includes spiny hackberry

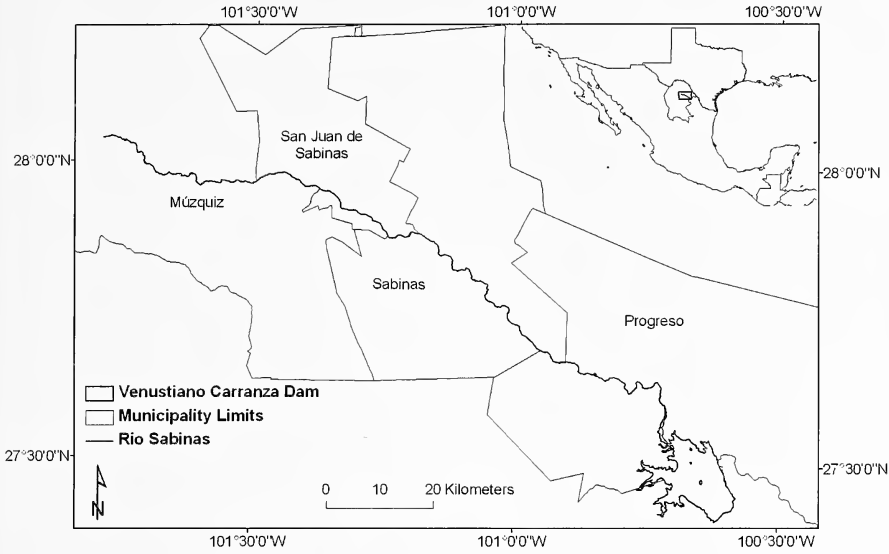

Figure 1. Map of the Río Sabinas, Coahuila, México.

(*C. pallida*), huisache (*Acacia farnesiana*), catclaw (*A. greggii*), blackbrush (*A. rigidula*), honey mesquite (*Prosopis glandulosa*), Texas persimmon (*Dyospiros texana*), and Mexican mulberry (*Morus celtidifolia*). The herbaceous cover is composed principally of straggler daisy (*Calyptocarpus vialis*).

This area faces serious conservation issues such as lack of biological knowledge, overgrazing, deforestation, and open-ceiling coal mining. The latter is especially harmful because it drastically modifies the environment and frequently breaks into the freatic mantle and extracts water (sometimes from very near the river: Treviño-Garza et al. 2002), affecting the hydrologic dynamics of the basin and interrupting the water flow in some sections of the river. Three distinct sections are observed in the riparian habitat qualitatively characterized by different levels of disturbance. The Melchor Múzquiz (MM) municipality section has a well preserved riparian habitat with mature and young woodland patches; the San Juan de Sabinas (SJS) municipality section contains partially preserved habitat, with mature patches but several rural and urban

localities; and the Sabinas (S) municipality section has deteriorated and fragmented riparian woodland vegetation in a narrow corridor which includes dead bald cypresses (due to coal mining activities). Therefore, the objectives of this study were to characterize the bird community of the Río Sabinas and compare communities among sections with different levels of disturbance.

Methods

A systematic sampling of birds was conducted from December 2004 to November 2005 along the Río Sabinas, in localities where Montezuma bald cypresses were present near the municipalities of Melchor Múzquiz (MM), San Juan de Sabinas (SJS) and Sabinas (S). Sections were visited monthly on a rotating basis to obtain a balanced sampling design. A total of 443 point counts (10 min, 25 m radius: Ralph et al. 1996) in 247 randomly selected sites were sampled, covering an area of 34.5 ha and 64 km of river length (approximately 50% of the total). Point counts were conducted by a single observer to reduce bias in detectability. The area was visited from December 2005 to January 2006 and May to August 2006, adding new records to the check-list. Species, including common and scientific names, were listed in phylogenetic order (Tables 1 & 2) following the AOU checklist (1998; 2000) and its supplements (Banks et al. 2002-2007). The residency status of each species was determined on the basis of field observations and information provided by Howell & Webb (1995). Only results from the one-year systematic effort were used for the analysis described below.

To demonstrate that the sampling effort generated species accumulation curves reached the asymptote, the EstimateS program (Colwell 2006) was used (Mao Tau's Model, Colwell et al. 2004). Log-normal (truncated) and log-series models were assessed for goodness-of-fit (Kolmogorov-Smirnov test) to the observed species abundance distribution of the whole community (e.g., Fisher et al. 1943; Preston 1948).

Density (D) was calculated as the number of birds per hectare. Frequency of occurrence (FO) was also obtained based on the percentage of point counts at which each species was observed. In both variables, a total of 443 point counts was considered for permanent resident and occasional species, 234 point counts for winter residents and transients (Oct-Mar), and 209 point counts for summer residents (Apr-Aug). In order to rank the species by dominance, two Olmstead-Tukey Diagrams were used for the whole community (Sokal & Rohlf 1995), one for species present during the breeding season (Apr-Aug) and one for those observed during the migratory-wintering season (Sep-Mar). The average values of FO and abundance (expressed as ln + 1) are used as discriminators for four classes. Common (or dominant) species are those with values equal to or greater than abundance and FO averages. Frequent (or constant) species are those with a value less than the abundance average, but another equal to or greater than FO average. Uncommon (or occasional) species are those with a value less than the FO average, but another equal to or greater than abundance average. Finally, rare species are those with values less than both averages.

Olmstead-Tukey Diagrams were used to compare communities among sections during the breeding season. Also, for an unbiased comparison of number of species, rarefaction curves (±95%CI; Mao Tau's Model, Colwell et al. 2004) were obtained for each section using EstimateS (Colwell 2006). Finally, Shannon's Diversity Index (1948) and the Bray-Curtis Index of Similarity (1957) were calculated for each section and for the entire community.

Results And Discussion

A total of 168 species were observed. Of these, 58 were considered as permanent residents, 32 as summer residents, 40 as winter residents, 30 as transients and nine as occasional. Eight confirmed and three potentially new breeding, and nine new non-breeding species were recorded for Coahuila (further discussed in Ruvalcaba-Ortega & González-Rojas 2009), emphasizing the little

Table 1. List of breeding species in phylogenetic order (AOU 1998, 2000; Banks et al. 2002-2007); their residency status (R): PR=Permanent Resident; SR=Summer Resident; WR=Winter Resident. Density (D, birds/hectare) is shown per species. Olmstead Tukey Dominance for breeding (OT-B) and wintering (OT-W) seasons: C=Common; U= Uncommon; R=Rare. Olmstead-Tukey Dominance (OT) per section: MM= Melchor Múzquiz; SJS= San Juan de Sabinas; S= Sabinas; FO= Frequency of occurrence; A= Abundance. Species in bold were used for quantitative analysis.

Scientific Name	Common Name	R	D	OT-B	OT-W	MM			SJS			S		
						FO	A (ln+1)	OT	FO	A (ln+1)	OT	FO	A (ln+1)	OT
Breeding Species														
Dendrocygna autumnalis[+]	**Black-bellied Whistling-Duck**	**SR**	**2.59**	**C**		1	2(1.7)	R	21.6	78(5.4)	C	13.5	22(4.1)	C
Cairina moschata[*+]	**Muscovy Duck**	**PR**	**0.07**	**R**	**R**				1.1	1(1)	R	1.4	1(1)	R
Anas platyrhynchos	**Mallard**	**PR/WR**	**0.41**	**R**	**U**				3.4	5(2.6)	R	5.4	6(2.8)	R
Meleagris gallopavo*	**Wild Turkey**	**PR**	**0.11**	**R**	**-**	1	4(2.4)	R				1.4	2(1.7)	R
Colinus virginianus	**Northern Bobwhite**	**PR**	**0.16**	**R**	**R**							1.4	2(1.7)	R
Tachybaptus dominicus*	**Least Grebe**	**PR**	**0.02**	**-**	**R**									
Podilymbus podiceps	Pied-billed Grebe	PR												
Phalacrocorax brasilianus	**Neotropic Cormorant**	**SR**	**0.84**	**U**					3.4	4(2.4)	R	12.2	18(3.9)	U
Ardea herodias[+]	**Great Blue Heron**	**PR**	**0.09**	**-**	**R**									
Ardea alba[+]	**Great Egret**	**PR**	**0.16**	**R**	**R**				1.1	2(1.7)	R	5.4	5(2.6)	R
Egretta thula	**Snowy Egret**	**SR**	**0.15**	**R**								2.7	4(2.4)	R
Bubulcus ibis	**Cattle Egret**	**PR**	**0.02**	**R**	**-**							1.4	1(1)	R
Butorides virescens	**Green Heron**	**SR**	**0.38**	**R**		1	2(1.7)	R	4.5	5(2.6)	R	5.4	4(2.4)	R
Nyctanassa violacea[+]	**Yellow-crowned Night-Heron**	**SR**	**0.72**	**U**		5	8(3.1)	U	9.1	9(3.2)	U	1.4	2(1.7)	R
Coragyps atratus	**Black Vulture**	**PR**	**0.66**	**U**	**U**	4	12(3.5)	U	3.4	4(2.4)	R			
Cathartes aura	**Turkey Vulture**	**PR**	**1.31**	**C**	**U**	9	11(3.4)	U	5.7	7(3)	U	16.2	16(3.8)	C
Elanus leucurus[+]	**White-tailed Kite**	**SR**	**0.15**	**R**					2.3	3(2.1)	R	1.4	1(1)	R
Buteogallus anthracinus[*o~]	Common Black-Hawk	SR												
Parabuteo unicinctus*	**Harris's Hawk**	**PR**	**0.11**	**-**	**R**				1.1	1(1)	R			
Buteo lineatus[+]	**Red-shouldered Hawk**	**PR/WR**	**0.36**	**R**	**U**	1	1(1)	R	3.4	4(2.4)	R	4.1	4(2.4)	R
Buteo nitidus[o+]	**Gray Hawk**	**PR**	**0.18**	**R**	**U**	1	1(1)	R	2.3	3(2.1)	R			

Table 1. Cont.

Scientific Name	Common Name	R	D	OT-B	OT-W	MM			SJS			S		
						FO	A (ln+1)	OT	FO	A (ln+1)	OT	FO	A (ln+1)	OT
Breeding Species														
Buteo swainsoni*	**Swainson's Hawk**	**SR**	**0.04**	**R**					1.1	1(1)	R	1.4	1(1)	R
Buteo jamaicensis	Red-tailed Hawk	PR												
Charadrius vociferus	**Killdeer**	**PR**	**0.14**	**R**	**R**							4.1	5(2.6)	R
Zenaida macroura	**Mourning Dove**	**PR**	**1.44**	**C**	**C**	16	20(4)	C	25.0	31(4.4)	C	21.6	24(4.2)	C
Columbina inca	**Inca Dove**	**SR**	**0.31**	**R**					1.1	1(1)	R			
Columbina passerina	**Common Ground-Dove**	**PR**	**0.04**	**U**	**R**	1	1(1)	R	3.4	5(2.6)	R	6.8	9(3.2)	U
Leptotila verreauxi	**White-tipped Dove**	**PR?**	**0.02**	**R**								1.4	1(1)	R
Coccyzus americanus	**Yellow-billed Cuckoo**	**SR**	**0.11**	**R**		1	2(1.7)	R	3.4	3(2.1)	R			
Geococcyx californianus	**Greater Roadrunner**	**PR**	**0.02**	**R**	-							1.4	1(1)	R
Crotophaga sulcirostris	**Groove-billed Ani**	**SR**	**0.08**	**R**								2.7	2(1.7)	R
Tyto alba	**Barn Owl**	**PR**	**0.04**	**R**	-				1.1	2(1.7)	R			
Megascops kennicottii	**Western Screech-Owl**	**PR**	**0.04**	-	**R**									
Megascops asio	Eastern Screech-Owl	PR												
Bubo virgnianus	**Great Horned Owl**	**PR**	**0.02**		**R**				1.1	1(1)	R			
Glaucidium gnoma	Northern Pygmy-Owl	SR												
Chordeiles acutipennis	**Lesser Nighthawk**	**SR**	**0.04**	**R**								1.4	1(1)	R
Lampornis clemenciae	**Blue-throated Hummingbird**	**SR**	**0.04**	**R**		1	1(1)	R						
Calothorax lucifer	Lucifer Hummingbird	SR												
Archilochus alexandri	**Black-chinned Hummingbird**	**SR**	**0.88**	**C**		7	7(2.9)	R	6.8	6(2.8)	R	12.2	10(3.3)	U
Selasphorus platycercus	Broad-tailed Hummingbird	SR												
Megaceryle torquata*	**Ringed Kingfisher**	**PR**	**0.65**			5	5(2.6)	R	4.5	6(2.8)	R	12.2	9(3.2)	U
Chloroceryle americana	**Green Kingfisher**	**PR**	**1.19**	**C**	**C**	14	14(3.6)	C	17.0	16(3.8)	C	14.9	12(3.5)	C
Melanerpes aurifrons	**Golden-fronted Woodpecker**	**PR**	**5.19**	**C**	**C**	30	39(4.7)	C	52.3	75(5.3)	C	82.4	102(5.6)	C
Picoides scalaris	**Ladder-backed Woodpecker**	**PR**	**1.71**	**C**	**C**	18	20(4)	C	13.6	14(3.6)	C	21.6	19(4)	C

Table 1. Cont.

Scientific Name	Common Name	R	D	OT-B	OT-W	MM			SJS			S		
						FO	A (ln+1)	OT	FO	A (ln+1)	OT	FO	A (ln+1)	OT
Breeding Species														
Camptostoma imberbe[o+]	Northern Beardless-Tyrannulet	SR?												
Sayornis nigricans	**Black Phoebe**	**PR**	**2.34**	**C**	**C**	11	11(3.4)	U	35.2	40(4.7)	C	18.9	19(4)	C
Pyrocephalus rubinus	**Vermilion Flycatcher**	**PR**	**0.38**	**U**	**U**	7	10(3.3)	U	1.1	1(1)	R	2.7	4(2.4)	R
Myiarchus cinerascens	**Ash-throated Flycatcher**	**SR**	**0.91**	**U**		8	11(3.4)	U	3.4	5(2.6)	R	8.1	8(3.1)	U
Myiarchus tyrannulus	**Brown-crested Flycatcher**	**SR**	**2.82**	**C**		31	44(4.8)	C	13.6	14(3.6)	C	17.6	18(3.9)	C
Pitangus sulphuratus	**Great Kiskadee**	**PR**	**1.81**	**C**	**C**	19	26(4.3)	C	13.6	15(3.7)	C	13.5	12(3.5)	C
Tyrannus couchii[+]	**Couch's Kingbird**	**SR**	**1.10**	**C**		13	14(3.6)	C	3.4	3(2.1)	R	9.5	11(3.4)	U
Tyrannus vociferans	**Cassin's Kingbird**	**SR**	**0.19**	**R**								4.1	5(2.6)	R
Vireo griseus	**White-eyed Vireo**	**PR**	**1.17**	**C**	**C**	11	14(3.6)	U				24.3	22(4.1)	C
Vireo bellii	**Bell's Vireo**	**SR**	**0.69**	**U**		3	4(2.4)	R	6.8	7(3)	U	10.8	8(3.1)	U
Cyanocorax yncas[-]	**Green Jay**	**PR**	**0.29**	**-**	**R**				3.4	3(2.1)	R			
Corvus sp.	**Raven**		**0.70**	**U**	**R**	8	15(3.7)	U	3.4	3(2.1)	R	12.2	19(4)	U
Corvus cryptoleucus	Chihuahuan Raven	PR												
Corvus corax	Common Raven	PR												
Hirundo rustica	Barn Swallow	SR												
Baelophus atricristatus	**Black-crested Titmouse**	**PR**	**4.60**	**C**	**C**	28	37(4.6)	C	36.4	69(5.2)	C	27.0	26(4.3)	C
Auriparus flaviceps	**Verdin**	**PR**	**0.18**	**R**	**R**							4.1	6(2.8)	R
Thryothorus ludovicianus	**Carolina Wren**	**PR**	**5.28**	**C**	**C**	68	102(5.6)	C	53.4	74(5.3)	C	55.4	48(4.9)	C
Thryomanes bewickii	**Bewick's Wren**	**PR**	**0.63**	**U**	**U**	2	3(2.1)	R	4.5	4(2.4)	R	18.9	18(3.9)	C
Polioptila caerulea	**Blue-gray Gnatcatcher**	**PR**	**0.72**	**R**	**C**	1	1(1)	R						
Mimus polyglottos	**Nothern Mockingbird**	**PR**	**0.31**	**R**	**R**									
Toxostoma longirostre	**Long-billed Thrasher**	**PR**	**0.22**	**R**	**R**	2	3(2.1)	R	2.3	2(1.7)	R	4.1	4(2.4)	R
Toxostoma curvirostre	Curve-billed Thrasher	PR												
Geothlypis trichas	**Common Yellowthroat**	**PR**	**0.11**	**R**	**R**	3	3(2.1)	R						

Table 1. Cont.

Scientific Name	Common Name	R	D	OT-B	OT-W	MM			SJS			S		
						FO	A (ln+1)	OT	FO	A (ln+1)	OT	FO	A (ln+1)	OT
Breeding Species														
Myioborus pictus	Painted Redstart	SR												
Basileuterus rufifrons [+]	**Rufous-capped Warbler**	**PR**	**0.05**	**-**	**R**									
Icteria virens	**Yellow-breasted Chat**	**SR**	**3.16**	**C**		13	13(3.6)	C	26.1	36(4.6)	C	35.1	34(4.5)	C
Piranga flava	Hepatic Tanager	PR?		R	R									
Piranga rubra	**Summer Tanager**	**SR**	**6.36**	**C**		50	98(5.6)	C	43.2	58(5.1)	C	31.1	37(4.6)	C
Arremonops rufivirgatus	**Olive Sparrow**	**PR**	**1.53**	**C**	**U**	24	27(4.3)	C	36.4	44(4.8)	C	32.4	28(4.3)	C
Chondestes grammacus	**Lark Sparrow**	**PR**	**0.57**	**-**	**R**									
Amphispiza bilineata	Black-throated Sparrow	PR		R	R									
Cardinalis cardinalis	**Northern Cardinal**	**PR**	**4.37**	**C**	**C**	21	33(4.5)	C	34.1	53(5)	C	50.0	50(4.9)	C
Cardinalis sinuatus	**Pyrrhuloxia**	**PR**	**0.09**	**-**	**U**									
Passerina caerulea	**Blue Grosbeak**	**SR**	**0.49**	**U**		6	7(2.9)	R	3.4	5(2.6)	R	5.4	5(2.6)	R
Passerina ciris	**Painted Bunting**	**SR**	**1.33**	**C**		14	20(4)	C	8.0	7(3)	U	8.1	7(3)	U
Agelaius phoeniceus	**Red-winged Blackbird**	**PR**	**0.41**			2	2(1.7)	R				12.2	19(4)	U
Quiscalus mexicanus	**Great-tailed Grackle**	**PR**	**0.66**	**R**	**R**				1.1	5(2.6)	R	1.4	4(2.4)	R
Molothrus aeneus	Bronzed Cowbird	PR												
Molothrus ater	**Brown-headed Cowbird**	**PR**	**0.11**	**R**	-				1.1	4(2.4)	R	1.4	2(1.7)	R
Icterus spurius	**Orchard Oriole**	**SR**	**0.27**	**R**		4	5(2.6)	R				2.7	2(1.7)	R
Icterus cucullatus	**Hooded Oriole**	**SR**	**0.08**	**R**					3.4	3(2.1)	R			
Icterus bullockii	**Bullock's Oriole**	**SR**	**0.11**	**R**								4.1	3(2.1)	R
Icterus graduacauda	**Audubon's Oriole**	**PR**	**0.02**	**-**	**R**									
Carpodacus mexicanus	**House Finch**	**PR**	**0.22**	**R**	**R**	1	1(1)	R				5.4	7(3)	U
Carduelis psaltria	**Lesser Goldfinch**	**PR**	**1.92**	**C**	**C**	19	39(4.7)	C	36.4	57(5)	C	9.5	9(3.2)	U

* Species under Mexican law protection (NOM-059-SEMARNAT-2001, DOF 2002);
° Species listed as threatened for Texas (TPWD 2003);
[+] New records for Coahuila (Ruvalcaba-Ortega & González-Rojas 2009).

Table 2. List of non-breeding species in phylogenetic order (AOU 1998, 2000; Banks et al. 2002-2007); their residency status (R): WR=Winter Resident; T=Transient; and O=Occasional. Density (D, birds/hectare) is shown per species. Olmstead Tukey Dominance for wintering season (OT-W): C=Common; U= Uncommon; R=Rare. Species in bold were used for quantitative analysis.

Scientific Name	Common Name	R	D	OT-W
		Non-breeding Species		
Aix sponsa	**Wood Duck**	**WR/PR?**	**0.45**	**U**
Anas strepera	**Gadwall**	**WR**	**0.14**	**R**
Anas discors	**Blue-winged Teal**	**T**	**0.24**	**U**
Anas cyanoptera	Cinnamon Teal	T		
Anas crecca	Green-winged Teal	T		
Phalacrocorax auritus	Double-crested Cormorant	T		
Egretta tricolor	**Tricolored Heron**	**O**	**0.02**	**R**
Nycticorax nycticorax	**Black-crowned Night-Heron**	**WR**	**0.10**	**R**
Plegadis chihi°	White-faced Ibis	T		
*Mycteria americana**°+	Wood Stork	O		
Circus cyaneus	Northern Harrier	O		
Accipiter striatus*	**Sharp-shinned Hawk**	**WR**	**0.07**	**R**
Accipiter cooperii*	**Cooper's Hawk**	**WR**	**0.07**	**R**
***Buteo albicaudatus**°**	**White-tailed Hawk**	**O**	**0.02**	**R**
Falco sparverius	**American Kestrel**	**T**	**0.03**	**R**
Falco columbarius	Merlin	WR?		
Grus canadensis*	**Sandhill Crane**	**T?**	**0.07**	**R**
Actitis macularius	**Spotted Sandpiper**	**WR**	**0.95**	**C**
Calidris minutilla	**Least Sandpiper**	**WR**	**0.17**	**U**
Gallinago delicata	Wilson's Snipe	WR		
Zenaida asiatica	White-winged Dove	O?		
Ara militaris+	Military Macaw	O?		
Megaceryle alcyon	**Belted Kingfisher**	**WR**	**0.54**	**C**
Sphyrapicus varius	**Yellow-bellied Sapsucker**	**WR**	**0.17**	**U**
Sphyrapicus nuchalis	**Red-naped Sapsucker**	**WR**	**0.07**	**R**
Colaptes auratus	**Northern Flicker**	**WR**	**0.03**	**R**
Contopus cooperi	**Olive-sided Flycatcher**	**T**	**0.14**	**R**
Contopus sordidulus	**Western Wood-Pewee**	**T**	**0.03**	**R**
Contopus virens	Eastern Wood-Pewee	T		
Empidonax sp.	**Flycatcher**	**T**	**0.07**	**R**
Empidonax minimus	Least Flycatcher	T		
Empidonax wrightii	Gray Flycatcher	WR		
Empidonax oberholseri	Dusky Flycatcher	T		
Sayornis phoebe	**Eastern Phoebe**	**WR**	**1.90**	**C**
Sayornis saya	Say's Phoebe	WR		
Tyrannus forficatus	Scissor-tailed Flycatcher	T		
Lanius ludovicianus	**Loggerhead Shrike**	**WR?**	**0.03**	**R**
Vireo vicinior	**Gray Vireo**	**T?**	**0.07**	**R**
Vireo cassinii	**Cassin's Vireo**	**T**	**0.03**	**R**
Vireo solitarius	**Blue-headed Vireo**	**WR**	**0.14**	**R**

Table 2 cont.

Scientific Name	Common Name	R	D	OT-W
		Non-breeding Species		
Stelgidopteryx serripennis	**Northern Rough-winged Swallow**	**WR/PR?**	**0.61**	**C**
Troglodytes aedon	**House Wren**	**WR**	**0.78**	**C**
Cistothorus palustris	Marsh Wren	WR		
Regulus satrapa	**Golden-crowned Kinglet**	**WR**	**0.24**	**U**
Regulus calendula	**Ruby-crowned Kinglet**	**WR**	**2.58**	**C**
Sialia sialis	**Eastern Bluebird**	**WR**	**0.58**	**U**
Catharus guttatus	**Hermit Thrush**	**WR**	**0.03**	**R**
Turdus grayi[+]	Clay-colored Robin	O?		
Turdus migratorius	American Robin	WR		
Anthus rubescens	American Pipit	WR		
Bombycilla cedrorum	**Cedar Waxwing**	**T**	**0.48**	**U**
Vermivora celata	**Orange-crowned Warbler**	**WR**	**0.41**	**U**
Vermivora ruficapilla	**Nashville Warbler**	**T**	**0.14**	**R**
Dendroica petechia	**Yellow Warbler**	**T**	**0.10**	**R**
Dendroica coronata	**Yellow-rumped Warbler**	**WR**	**5.27**	**C**
Dendroica nigrescens	**Black-throated Gray Warbler**	**T**	**0.03**	**R**
Dendroica virens	**Black-throated Green Warbler**	**T**	**0.03**	**R**
Dendroica townsendi	**Townsend's Warbler**	**T**	**0.07**	**R**
Dendroica occidentalis	Hermit Warbler	T		
Dendroica dominica	**Yellow-throated Warbler**	**O**	**0.02**	**R**
Dendroica pinus	**Pine Warbler**	**O**	**0.02**	**R**
Mniotilta varia	**Black-and-white Warbler**	**WR**	**0.07**	**R**
Seiurus aurocapilla	**Ovenbird**	**T**	**0.03**	
Seiurus noveboracensis	Northern Waterthrush	T		
Oporornis tolmiei*	**MacGillivray's Warbler**	**T**	**0.03**	**R**
Wilsonia pusilla	**Wilson's Warbler**	**WR**	**0.24**	**U**
Piranga ludoviciana	**Western Tanager**	**T**	**0.14**	**R**
Spizella passerina	Chipping Sparrow	WR		
Spizella pallida	**Clay-colored Sparrow**	**WR**	**0.03**	**R**
Spizella pusilla	**Field Sparrow**	**WR**	**0.78**	**U**
Pooecetes gramineus	Vesper Sparrow	WR		
Passerculus sandwichensis	Savannah Sparrow	WR		
Melospiza melodia	Song Sparrow	WR?		
Melospiza lincolnii	**Lincoln's Sparrow**	**WR**	**1.29**	**C**
Melospiza georgiana	**Swamp Sparrow**	**WR**	**0.37**	**U**
Zonotrichia leucophrys	**White-crowned Sparrow**	**WR**	**0.24**	**U**
Pheucticus melanocephalus	Black-headed Grosbeak	T		
Icterus galbula	Baltimore Oriole	T		
Carduelis pinus	**Pine Siskin**	**T**	**0.14**	**R**

* Species under Mexican law protection (NOM-059-SEMARNAT-2001, DOF 2002);
° Species listed as threatened for Texas (TPWD 2003);
[+] New records for Coahuila (Ruvalcaba-Ortega & González-Rojas 2009).

attention previously given to this area. Fifteen of the species are under protection by Mexican laws (NOM-059-SEMARNAT-2001, DOF 2002), such as Muscovy Duck, Wood Stork, and Red-shouldered Hawk. In Texas as well, six of the species are listed as threatened for the state, e.g., White-faced Ibis, Gray Hawk, and Northern Beardless-Tyrannulet (TPWD 2003). Painted Bunting, a near threatened species on the Red List of Endangered Species (IUCN 2007), was also recorded as a common summer breeder in the area (Table 1). Based only on the systematic sampling effort, 126 species (75% of the total) were recorded and used for further quantitative analysis.

The accumulation curve of observed and expected species reached the asymptote assuring a representative sampling (Fig. 2). The species abundance distribution for the whole community was best fitted by the log-series distribution ($D=0.035$, $P>0.05$), which is suggested to be associated with a community dominated by one or a few factors (Magurran 2004). This would be expected for a restricted vegetation type, such as the riparian woodlands of northern Mexico, which are generally immersed in a matrix of semi-arid scrubland.

During the breeding season, 40 common, 12 uncommon, and 42 rare species were observed for the Río Sabinas bird community (Table 1). The most dominant species were the Carolina Wren (D=5.28 birds/ha; A=173 inds.; FO= 62.2%) and Golden-fronted Woodpecker (D=5.19 birds/ha; A= 198 inds.; FO=57.9%) as permanent residents, and Summer Tanager (D=6.36 birds/ha; A=167 inds.; FO=50.2%), as a summer breeder. During the migrating/wintering season, 21 common, 21 uncommon, and 59 rare species were observed (Table 2). The most dominant species were permanent residents such as the Carolina Wren (A=93 inds.; FO= 48.2%), Black-crested Titmouse and Northern Cardinal (both D=0.18 birds/ha; A=76 inds.; FO=39.8%), but wintering residents were also present among common species, e. g., Yellow-rumped Warbler (D=2.78 birds/ha; A=155 inds.; FO=26.5%) and Ruby-crowned Kinglet (D=1.37 birds/ha; A=76 inds.; FO=23.5%).

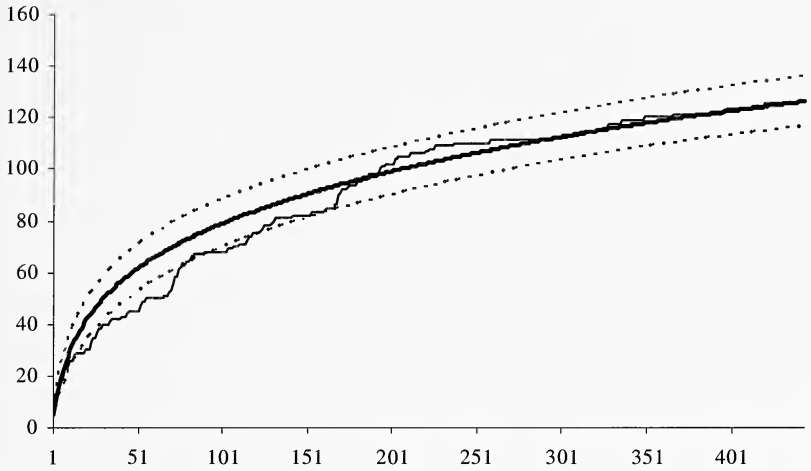

Figure 2. Observed (solid line) and expected (bold line) ±95%CI (dotted lines) species accumulation curves of the Río Sabinas bird community sampled from December 2004 to November 2005.

Important differences among communities were found when separating the analysis by sections. During the breeding season, MM (43 species, 694 individuals) showed the Carolina Wren (A=102 inds.; FO= 68%) and Summer Tanager (A=98 inds.; FO= 50%) as the dominant species; SJS (49 species, 800 individuals) also showed the Carolina Wren as the most dominant species (A=74 inds.; FO= 53.4%) but included the Golden-fronted Woodpecker (A=75 inds.; FO= 52.3%) as the second. On the other hand, S (58 species, 736 individuals) showed a community mostly dominated by the Golden-fronted Woodpecker (A=102 inds.; FO= 82.43%), a species that adjusts well to human-altered environments (Husak & Maxwell 1998). Also, the Painted Bunting was determined to be a common species only for the MM section (A=20 inds.; FO= 14%), where as SJS (A=7 inds.; FO= 8%) and S (A=7 inds.; FO= 8.1%) included this species as uncommon (almost rare).

Rarefacted species richness for MM was significantly lower than SJS and S; however SJS and S were not distinct from one another (Fig. 3). A Kruskal-Wallis test among sections, comparing number of species and individuals per point count, showed highly

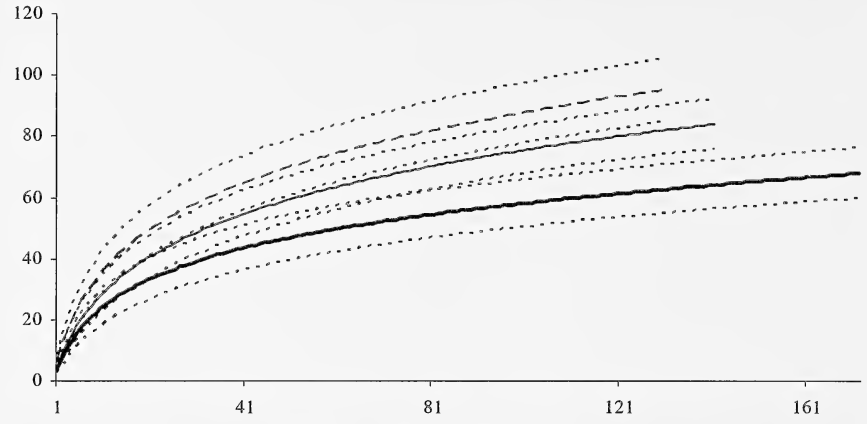

Figure 3. Rarefacted (by sample) species accumulation curves (±95%CI) for each riparian section: MM (bold line), SJS (solid line), S (dashed line) in the Río Sabinas, Coahuila (Mao Tau's Model, Colwell *et al.* 2004).

significant differences in both cases (richness: $KW_{2,443} = 39.61$, $p< 0.01$; abundance: $KW_{2,443} = 27.90$, $p< 0.01$). A posterior Mann-Whitney test between each section showed statistically significant differences in all cases, showing the highest mean number of species and individuals for S, and the smallest for MM (Richness: MM vs. SJS, U=10332, $p<0.05$; MM vs. S, U=5418, $p<0.01$; SJS vs. S, U=6095, $p<0.01$; abundance: MM vs. SJS, U=10196, $p<0.05$; MM vs. S, U=5789, $p<0.01$; SJS vs. S, U=6645, $p<0.01$). Higher numbers of species and individuals in S could be a consequence of a more open habitat with better visibility and a higher detection rate. Also, a more fragmented habitat with a greater influence of arid scrubland species such as the Scissor-tailed Flycatcher, Loggerhead Shrike, and Pyrrhuloxia, which were recorded only in this section.

Using Bray-Curtis' Index, the highest value of similarity was obtained for the S and SJS sections (66.3%), followed by MM with S (63.7%) and SJS (63.5%). Diversity values showed the same pattern across sections, the highest was S (H' = 3.8, J'=0.84), followed by SJS (H' = 3.6, J' =0.83), and M (H' = 3.5, J'=0.82). The whole community showed a higher diversity (H'=3.8), but a slightly lower evenness (J'=0.79), than the individual sections.

Although the MM section did not show the highest value of diversity or evenness, nor the highest number of species or individuals per point count, its bird community shows important differences among species' dominances. It is also the best preserved section, and the one that shows the greatest regeneration of bald cypresses at all stages. In addition, it is the only section with the Painted Bunting as a common species, which had the highest number of individuals and frequency of occurrence. This is noteworthy because the species has shown a negative population trend (1966-2006) in the Breeding Bird Surveys of U. S. (Sauer et al. 2007). On the other hand, the consequences of habitat deterioration in S and SJS sections may not have been fully revealed yet, due to the longevity of Montezuma bald cypresses, causing a possible delay in the consequences of alteration of the riparian habitat for several decades (Rood & Mahoney 1993).

The Río Sabinas is an important site for breeding (87) and migratory (72) birds. All of the breeding species of the Río Sabinas are confirmed (82), probable (4), or possible (1) breeders for southern Texas (south of the 31st parallel; Benson & Arnold 2001, Lockwood & Freeman 2004). This was expected due to the fact that these rivers have similar riparian forests dominated by bald cypress, pecan and sycamore, especially in the Plateau Region such as the Nueces, West Nueces, Frio, and South Llano Rivers (El-Hage & Moulton 2007). In northeastern Mexico, as well, there are rivers dominated by bald cypresses such as the Río Nazas in Durango and Coahuila, and the Río Sabinas Hidalgo and Río Ramos in Nuevo León; therefore, similarities in avian communities should be expected for these riparian habitats. Twenty-six of 35 common migrant species of the riparian systems of the southwestern U.S. (Skagen et al. 2005) were found in the area, highlighting its importance as a corridor and a stopover site. It is also noteworthy that migrants from both the eastern (i.e., Blue-headed Vireo, Yellow-throated, Pine and Black-throated Green warblers and Yellow-bellied Sapsucker) and western U.S. (i.e., Orange-crowned and Hermit warblers, and Red-naped Sapsucker) were recorded. Finally, these data should promote management plans leading to the conservation and restoration of the riparian habitats and water flow of the Río Sabinas especially in the disturbed sections of San Juan de Sabinas (SJS) and Sabinas (S).

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REPRODUCTION IN SEVEN SPECIES OF *MICROLOPHUS* (SQUAMATA: TROPIDURIDAE) FROM SOUTH AMERICA

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Abstract.–Information on the reproductive cycles of seven species of *Microlophus* (*M. koepckeorum, M. occipitalis, M. peruvianus, M. stolzmanni, M. theresiae, M. thoracicus* and *M. tigris*) from coastal northern South America gathered from a histological examination of gonadal material is presented. All species exhibited extended reproductive activity present in both austral spring-summer and autumn. Histological evidence is presented that *M. peruvianus* may produce multiple clutches in the same year. Comparisons are made with the reproductive cycles of other species of *Microlophus*.

The genus *Microlophus* consists of some 20 species and is restricted in distribution to South America and the Galapagos Islands (Peters & Donoso-Barros 1986). *Microlophus koepckeorum, M. peruvianus, M. theresiae, M. thoracicus, M. tigris* are endemic to Peru (Lehr 2002). *Microlophus occipitalis* is known from southwestern Ecuador and northern and central Peru, and *M. stolzmanni* is known from northwestern Peru and possibly southwestern Ecuador (Peters & Donoso Barros 1986). Information on reproduction of *M. peruvianus, M. theresiae, M. thoracicus* and *M. tigris* (all as *Tropidurus*) are in Dixon & Wright (1975) and *M.* (as *Tropidurus*) *occipitalis* is in Watkins (1996). The purpose of this paper is to add information on the reproductive cycles of seven species of *Microlophus* lizards from coastal northern South America.

Materials and Methods

Histological examinations were performed on 313 museum specimens of seven species of *Microlophus* on deposit with the Natural History Museum of Los Angeles County (LACM), Los Angeles, California (Table 1). Counts were made of oviductal eggs or enlarged follicles (> 4 mm length). The left testis and

epididymis were removed from males; the left ovary was removed from females for histological examination. Tissues were embedded in paraffin and cut into sections at 5μm. Slides were stained with Harris' hematoxylin followed by eosin counterstain (Presnell & Schreibman 1997). Testis slides were examined to determine the stage of the male cycle; ovary slides were examined for the presence of yolk deposition. Histological slides of most gonads from 45 juveniles: *M. koepckeorum* ($n = 4$), *M. occipitalis* ($n = 6$), *M. peruvianus* ($n = 3$), *M. stolzmanni* ($n = 13$), *M. theresiae* ($n = 6$), *M. thoracicus* ($n = 8$), *M. tigris* ($n = 5$) were made to determine the minimum size at which maturity was reached. In cases where the gonads were extremely small, immaturity was assumed and no histology was performed. The relationship between female SVL and clutch size was examined by linear regression analysis and an unpaired *t* test was used to compare male and female mean body sizes (SVL) (Instat vers. 3.0b, Graphpad Software, San Diego, CA).

Specimens examined.–The following specimens of *Microlophus* were examined from the herpetology collection of the Natural History Museum of Los Angeles County (LACM).

Microlophus koepckeorum ($n = 46$) PERU: Lambayeque Department (49083, 49084, 49086, 49087, 49089-49098, 49100, 49104, 109569, 122583-122585, 122587-122592, 122594-122606, 122608-122613, 122615); collected 1968, 1970, 1976.

Microlophus occipitalis ($n = 67$) PERU: La Libertad Department (48853-48861, 48866-48869, 48902-48904, 48906-48908, 122639, 125348, 136012); Lambayeque Department (48870, 48872, 48873, 48897-48901, 122617-122622, 122627-122630, 122632, 122633, 122635-122638); La Libertad Department (136013); Piura Department (48876, 48877, 48891-48895): Tumbes Department (48878-48890); collected 1968, 1976.

Microlophus peruvianus ($n = 45$) CHILE: Antofagasta Province (122000); ECUADOR: Esmeraldas Province (154385-154387,

154389-154394); PERU: Ancash Department (49021, 49037) Ica Department (49018, 49019, 49040, 49042, 49044-49046); La Libertad Department (136004-136011); Lima Department (9342-9345, 9347, 49023, 49024, 49026, 49028-49030, 49032, 49034, 49036, 49050, 49055-49057); collected 1965, 1966, 1968, 1984.

Microlophus stolzmanni (*n*= 50) PERU: Cajamarca Department (49107, 49108, 49110-49116, 49119-49123, 49126-49129, 49131, 49132, 122641, 122646, 122649-122651, 122653-122657, 122659, 122660, 122662-122667, 122669, 122670, 122672-122676, 122678-122682); collected 1968, 1976.

Microlophus theresiae (*n* = 29) PERU: Ica Department (49060-49063, 49075-49077); Lima Department (LACM 49064, 49068-49072, 49074, 49079, 122692-122698, 122700-122706); collected 1968, 1976.

Microlophus thoracicus (*n* = 50) PERU: Ancash Department (48911, 48914-48918, 48953, 48954, 48956-48963, 48965-48967, 48969-48974, 48976-48979, 122737, 122738, 122742, 122746, 122747); Ica Department (122726-122731, 122733-122736); La Libertad Department (48951); Lambayeque Department (48922, 48924); Lima Department (48980, 48983, 48984); collected 1968, 1976.

Microlophus tigris (*n* = 26) PERU: Lima Department (48830-48838, 48841-48847, 48849, 122712-122714, 122716, 122718-122722); collected 1968, 1976.

Results And Discussion

Microlophus koepckeorum.–The mean SVL of males was significantly larger than that of females (Table 1). All 21 males examined (May = 10, June = 2, August = 1, November = 8) were undergoing spermiogenesis (= sperm formation). In this stage, the seminiferous tubules were lined by spermatozoa or groups of metamorphosing spermatids and the epididymides contained sperm. The smallest reproductively active male (122592) was undergoing spermiogenesis, measured 52 mm SVL and was from May.

Table 1. Sample sizes (*n*), mean sizes (snout-vent length, mm) ± *SD* and range for males of seven species of *Microlophus* from South America. *Indicates mean snout-vent length of males was significantly larger than that of females (unpaired *t*-test).

				Males			Females		
	DF	*t*	*P*	*n*	X±SD	(Range)	*n*	X±SD	(Range)
*M. koepckeorum**	40	6.9	<0.001	21	67.6±6.8	(52-78)	21	55.7±4.1	(50-67)
*M. occipitalis**	59	4.9	<0.001	28	62.8±11.9	(35-79)	33	51.5±5.1	(44-65)
*M. peruvianus**	40	2.9	<0.001	17	86.8±18.8	(50-109)	25	73.0±12.6	(55-93)
*M. stolzmanni**	35	6.1	<0.001	19	88.2±17.4	(65-120)	18	62.1±5.3	(53-73)
*M. theresiae**	21	4.7	0.0001	6	68.0±3.9	(62-72)	17	59.8±3.6	(53-67)
*M. thoracicus**	40	4.7	<0.0001	23	63.2±7.8	(43-77)	19	53.9±4.3	(47-64)
M. tigris	–	–	–	16	61.9±13.8	(40-80)	5	55.0±8.5	(45-67)

Monthly stages in the ovarian cycle are in Table 2. Reproductively active females were found in May-June and November. Mean clutch size (n = 13) was 3.2 ± 0.69 *SD*, range = 2-5. Linear regression analysis revealed a significant positive correlation between female body size (SVL) in mm and clutch size for 13 *M. koepckeorum* females (r = 0.82, P = 0.0007). The smallest reproductively active females (both from May) each measured 50 mm SVL (122595, early yolk deposition and 122601, three follicles > 4 mm). Dixon & Wright (1975) reported *M. koepckeorum* mature at 50 mm SVL, and reproductively active females were found in August, November and December with 2-4 yolked ovarian follicles or oviductal eggs.

Microlophus occipitalis.–The mean SVL of males was significantly larger than that of females (Table 1). All 28 males examined (May = 6, June = 2, November = 7, December = 13) were undergoing spermiogenesis. The smallest reproductively active male (spermiogenesis) measured 35 mm SVL (48853) and was from November. Reproductively active females were found June, August, November and December (Table 2). The smallest reproductively active female (48906, two follicles > 4 mm, measured 44 mm SVL and was from December). Mean value for nine clutches was 4.5 ± 1.8 SD, range = 2-8. Linear regression

Table 2. Monthly stages in ovarian cycles of seven species of *Microlophus* from Peru.

	Month	*n*	No yolk deposition	Early yolk deposition	Follicles > 4 mm	Oviductal eggs
M. koepckeorum	May	12	2	1	5	4
	Jun	5	0	2	1	2
	Nov	4	0	2	1	1
M. occipitalis	Jun	9	2	2	4	1
	Aug	1	0	1	0	0
	Nov	7	3	0	3	1
	Dec	16	15	0	1	0
M. peruvianus	May	4	1	1*	0	2
	Jun	2	0	0	2	0
	Aug	1	0	0	1	0
	Oct	1	1	0	0	0
	Nov	10	4**	1	0	5
	Dec	7	2**	2	2	1
M. stolzmanni	Jun	13	0	1	10	2
	Nov	5	0	0	3	2
M. theresiae	Jun	11	0	2	9	0
	Dec	6	5	0	0	1
M. thoracicus	May	5	1	1	2	1
	Dec	14	2	0	4	8
M. tigris	May	1	1	0	0	0
	Dec	4	2	0	1	1

* = one *M. peruvianus* female from May contained corpora lutea from a previous clutch and concomitant yolk deposition for a subsequent clutch; ** = one *M. peruvianus* female from November and another from December each contained corpora lutea but were not undergoing yolk deposition.

analysis revealed a significant positive correlation between female body size (SVL) in mm and clutch size for 9 *M. occipitalis* females ($r = 0.75$, $P = 0.021$). Dixon & Wright (1975) reported oviductal eggs in females from July, August and November, clutches of 2-4 eggs were produced. Males mature between 50-55 mm SVL and females between 45-47 mm SVL (Dixon & Wright 1975). Watkins (1996) studied a population of *M. occipitalis* at Guayas Province,

Ecuador. He reported males (mean SVL = 63.0 ± 15.7 mm) were larger than females (mean SVL = 55.1 ± 7.5 mm) and the smallest female with oviductal eggs or enlarged follicles measured 48 mm SVL. Burt (1935) reported that a series of 15 *M. occipitalis* from Ecuador collected in January exhibited gradation in size from juveniles to adults suggesting an extended breeding season.

Microlophus peruvianus.–The mean SVL of males was significantly larger than that of females (Table 1). Sixteen of 17 males examined were undergoing spermiogenesis (May = 5, June = 6, November = 4, December = 1). One November male (SVL = 66 mm) appeared to have completed spermiogenesis. The germinal epithelium was reduced to a few layers in thickness, occasional metamorphosing spermatids but no spermatozoa were present. The smallest reproductively active male (spermiogenesis) measured 63 mm SVL (49021) and was from November. Reproductively active females were present May, June, August and November (Table 2). The smallest reproductively active *M. peruvianus* female contained two oviductal eggs (49028) measured 56 mm SVL and was from November. Mean clutch size for 13 gravid females was 3.5 ± 1.3 SD, range = 2-6). Linear regression analysis revealed a significant positive correlation between female body size (SVL) in mm and clutch size for 13 *M. peruvianus* females (r = 0.60, P = 0.032). Dixon & Wright (1975) reported *M. peruvianus* females were reproductively active during July-September and November and December. One *M. peruvianus* female from May (154389) contained corpora lutea from a previous clutch and concomitant vitellogenic follicles for a subsequent clutch indicating *M. peruvianus* can produce multiple clutches in the same year. One female each from November (49032) and December (49055) contained corpora lutea indicating eggs had recently been deposited. There was no concomitant yolk deposition for a subsequent clutch. Leyton et al. (1981) reported males and females of *M.* (as *Tropidurus*) *peruvianus* were reproductively active throughout the year in northern Chile.

Microlophus stolzmanni.–The mean SVL of males was significantly larger than that of females (Table 1). All 19 males examined were undergoing spermiogenesis (May = 1, June = 8, November = 10). The smallest reproductively active male (spermiogenesis) measured 65 mm SVL (122672) and was from June. Reproductively active females were found in June and November (Table 2). The smallest reproductively active female (49128, measured 53 mm SVL) and was from November. Mean clutch size for 17 females was 2.6 ± 0.86, range = 1-4. Linear regression analysis revealed a significant positive correlation between female body size (SVL) in mm and clutch size for 13 *M. stolzmanni* females ($r = 0.53$, $P = 0.029$). Dixon & Wright (1975) reported *M. stolzmanni* females were reproductively active in November, matured between 65-76 mm SVL and produced 3-4 oviductal eggs.

Microlophus theresiae.–The mean SVL of males was significantly larger than that of females (Table 1). All 6 males examined were undergoing spermiogenesis (June = 3, November = 1, December = 2). The smallest reproductively active male (spermiogenesis) measured 62 mm SVL (49072) and was from December. Reproductively active females were found in June and December (Table 2). Mean clutch size for 10 females was 3.4 ± 0.84, range = 2-5. Linear regression analysis revealed no significant positive correlation between female body size (SVL) in mm and clutch size for 10 *M. theresiae* females. The smallest reproductively active female (122696, 3 follicles > 4 mm), measured 58 mm SVL and was from June. Dixon & Wright (1975) reported *M. theresiae* females contained oviductal eggs or enlarged follicles in December.

Microlophus thoracicus.–The mean SVL of males was significantly larger than that of females (Table 1). Seventeen of nineteen males were undergoing spermiogenesis: (May = 3, November = 6, December = 8). One male from May was undergoing late recrudescence (renewal of germinal epithelium for the next period of spermiogenesis). A few metamorphosing spermatids were noted.

Another May male was in late spermiogenesis, clusters of metamorphosing spermatids and spermatozoa were present. The germinal epithelium was reduced in cellularity. The smallest reproductively active male (spermiogenesis) measured 43 mm SVL (48983) and was from December. Reproductively active females were found in May and December (Table 2). The smallest reproductively active female (48966, 2 oviductal eggs) measured 46 mm SVL and was from December. Mean clutch size for 15 females was 2.5 ± 0.83, range = 1-4. Linear regression analysis revealed a significant positive correlation between female body size (SVL) in mm and clutch size for 15 *M. thoracicus* females (r = 0.60, P = 0.018). Dixon & Wright (1975) reported *M. thoracicus* females were reproductively active in November and December, mature at 45-50 mm SVL and produce 1-5 eggs per clutch with a mean of 3.1 eggs for 40 females.

Microlophus tigris.–There was no significant difference between mean males and females in SVL (Table 1). Fourteen of 16 males examined were undergoing spermiogenesis: (May = 8, December = 6). Two males from December were in recrudescence prior to the next period of sperm formation. Germinal epithelium contained sufficient numbers of cells to support spermiogenesis, A few metamorphosing spermatids but, as yet, no spermatozoa were present. The smallest reproductively active male (spermiogenesis) measured 40 mm SVL (122713) and was from May. Reproductively active females were found in December (Table 2). The smallest reproductively active female (48834, 2 follicles > 4 mm) measured 56 mm SVL and was from December. Mean clutch value for two females was 3.0 ± 1.4 SD, range = 2-4. There was insufficient data to perform regression analysis to examine the relation between body size and clutch size. Dixon & Wright (1975) reported *M. tigris* females were reproductively active in September, November and December and produced clutches of 2-5 eggs.

It appears that other species of *Microlophus* from Peru and elsewhere have extended reproductive cycles. Goldberg & Rodriguez (1986) reported on reproduction in *M. quadrivittatus* and

M. theresioides (both as *Tropidurus*) from northern Chile. In both species maximum numbers of males were undergoing spermiogenesis in spring although varying numbers of spermiogenic males were found in most months indicating an extended reproductive cycle. Some males with regressed testes were found in summer; female reproductive activity (enlarged follicles or oviductal eggs) was limited to spring (Goldberg & Rodriguez 1986). This contrasts with six of the seven species of *Microlophus* presented herein which were reproductively active in both austral winter and spring (spring female data was limited to only one specimen of *M. tigris*). Furthermore, Vitt & Goldberg (1983) similarly reported some males of *Microlophus* (as *Tropidurus) torquatus* from northeastern Brazil were undergoing spermiogenesis and females were reproductively active in all months. Subsequent examination of the reproductive biology of other *Microlophus* species are needed before the diversity of reproduction exhibited by these lizards can be ascertained.

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NOTES ON FOOD ATTRACTION AND THE DEMOGRAPHICS OF BLUE CRAB (*CALLINECTES SAPIDUS*) TRAPPING IN TRES PALACIOS BAY, TEXAS

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Abstract.–The null hypothesis that blue crabs which enter typical commercial-style baited traps are random draws from populations with equal sex ratios and physical characteristics was evaluated. Trap demographic data (sex, size and number of crabs) were collected during ten single-day trials comprising six traps each (n = 60) over a 3-week period in April 2006. These data indicate that blue crabs tend to be caught in same-sex assemblages more frequently than expected by chance, and that males caught in crab traps have different width-weight ratio trajectories than do females. Interestingly, blue crabs in Tres Palacios Bay are more readily attracted to remains of fish that are endemic and common in the Gulf of Mexico, rather than those that are exotic and/or relatively rare. These data relate directly to previously unexplored aspects of the blue crab fishery in Texas and therefore will be useful to fishery managers.

Blue crabs (*Callinectes sapidus* Rathbun) are subject to a significant commercial fishery on the coast of Texas, harvested at a rate surpassed only by shrimp and oysters (in total organisms landed) (Suttton & Wagner 2007). Landings have declined considerably since 1987, due to a combination of many factors including overharvest (Sutton & Wagner 2007). Currently, Texas Parks and Wildlife (TPW) regulates the harvest of blue crabs by a minimum-size limitation of five inches (127 mm), a ban on harvest of egg-bearing females, commercial trap limitations (200 traps) and design specifications, and a 10-day to 30-day closure of the fishery each year (February or March). To evaluate trends in blue crab abundance and biomass, TPW typically relies on fishery-independent data collected during annual routine monitoring. However, sparse data is available that pertains to the demographics of crabs specifically targeted by current trapping methodologies. Although abundant data is available detailing the effects of trap design on retention of various crab size ranges (Guillory & Prejean

1997; Guillory 1998; Guillory & Hein 1998a; 1998b) there is a lack of available data on the size and sex ratios of crabs typically retained in traps in the western Gulf of Mexico. Behavioral and ecological characteristics such as mutual antagonism during foraging (Mansour & Lipcius 1991; Clark et al. 1999) and intra-population habitat partitioning (Hines et al. 1987) may result in different demographic characteristics between wild crab populations, and what is typically found in commercial traps. Here, the demographics of crabs captured in commercial-style traps in Tres Palacios Bay, Texas are described in an effort to improve the data upon which future management decisions are based.

Methods

Six identical commercial-style crab traps which consisted of a coated wire frame throughout, a bait compartment and four entrance funnels were used in this study. The dimensions of the traps were 60 cm width, by 60 cm length, by 44 cm height. Entrance funnels were approximately oval in shape, were spaced evenly on each of the four sides of the trap and were 26 cm wide by 12 cm high. The bait compartment was located central to the bottom of the trap, and was 22 cm in height by 12 cm in diameter. While commercial traps in Texas are required to include 6.03 cm escape vents in each retaining chamber, traps used in experimental trials contained no escape vents and theoretically retained all crabs captured during the duration of the trial. Traps were deployed in a 2 by 3 grid system adjacent to a rock jetty (Fig. 1). The first row of traps was located approximately 100 m from the shoreline, with each additional row located 20 m apart. In each row, traps were located 20 m and 40 m from the face of the jetty.

During the first three weeks of April 2006, traps were baited and set between 0730 h and 0800 h on each day of the trial. Trials were conducted Mon-Thurs of the first two weeks and Mon-Tue of the final week, for a total of ten trials. Bait was prepared by measuring an equal amount of cut bait menhaden (200g) into the bait compartment of each trap. Three species of menhaden were used

for bait; finescale menhaden (*Brevoortia gunteri*), Gulf menhaden (*B. patronus*) and Atlantic menhaden (*B. tyrannus*). Bait was distributed into traps in randomized design each day in order to test for differences in attraction among bait species. Traps were emptied and crabs collected following 24 h of exposure. Traps were checked as quickly as possible by two researchers in order to limit the amount of time between traps.

Hydrological data were recorded prior to checking traps. Hydrological data consisted of water temperature (°C), salinity (practical salinity units, or psu) and dissolved oxygen (mg/L). Tidal data for each day of the three-week trial was obtained from the National Oceanic and Atmospheric Administration's online tidal database (NOAA 2007), and included high tide and low tide measured as the difference between the recorded water level and the mean water level on site. The difference between high and low tide for each day of the trial was calculated and used as a proxy for the volume of water movement into and out of the site.

Crabs were extracted from the sampling area without return for the duration of the study. Each crab was weighed to the nearest g, and the greatest width of the carapace in mm was recorded. Sex was determined by the shape of the abdomen ventrally. In the case of escape, crabs were recorded as "lost" and were used only for demographic count data. In addition to blue crabs, the occurrence of other species, such as hermit crabs and (rarely) fishes were recorded by species, but no measurements were taken and these organisms were released back into the study site.

The assumption that the sex ratio of crabs caught in single traps is likely to be representative of the sex ratio in the population was tested by constructing contingency tables with single-sex and multiple-sex trap counts (rows) by the number of crabs caught within a specified trap (columns, $n = 2$, 3, 4 and 5-crab traps). The expected frequency of single-sex traps for each treatment was calculated using the estimated frequency of males and females in the population. For instance, the expected frequency of traps

containing two crabs of the same sex was calculated as $(n\ (p^2 + q^2))$, where n = the overall number of traps containing two crabs, p = the overall frequency of males in the study and q = the overall frequency of females. This value was then subtracted from 1.0 to obtain the expected frequency of traps containing both sexes. Similar expected frequencies were obtained for traps containing three, four and five crabs. Cell values for traps which collected more than three crabs were combined into a single treatment to minimize the effects of small expected frequencies as described by Cochran (1952). Observed and expected frequencies of same and multi-sex traps were compared using a χ^2 test of homogeneity, and significance was assessed via comparison to the χ^2 null distribution with an arbitrary value of $\alpha = 0.05$ for all significance tests.

The relationship between crab width and total weight was assessed via the general linear model, as implemented in SAS (version 8.02, SAS Inst., Inc., Cary, NC). Weight was treated as the response variable, and the predictor variables considered in the model were sex, width and sex/width. Sex was included as a predictor after observing what appeared to be different trajectories of the width-weight curves of males and females.

Results

Through the course of the study, salinity (range 30.0 – 31.4 psu), and dissolved oxygen (5.8 – 6.9 mg/L) remained relatively consistent at the study site. Female crabs tend to be found in higher salinity than males (Tagatz 1971, and references therein), and more females than males were captured in this study. However, because of the relative consistency of high salinity throughout all trials, the effects of variability in salinity on crab sex ratios could not be evaluated. Temperature also remained relatively consistent among trials (18.9 – 24.5 °C) although fewer crabs were captured at the lowest temperature trial than any other trial. Otherwise, there was not a significant relationship between the number of crabs captured and either temperature ($r^2 = 0.075$, $p = 0.446$) or tide ($r^2 = 0.017$, $p = 0.737$).

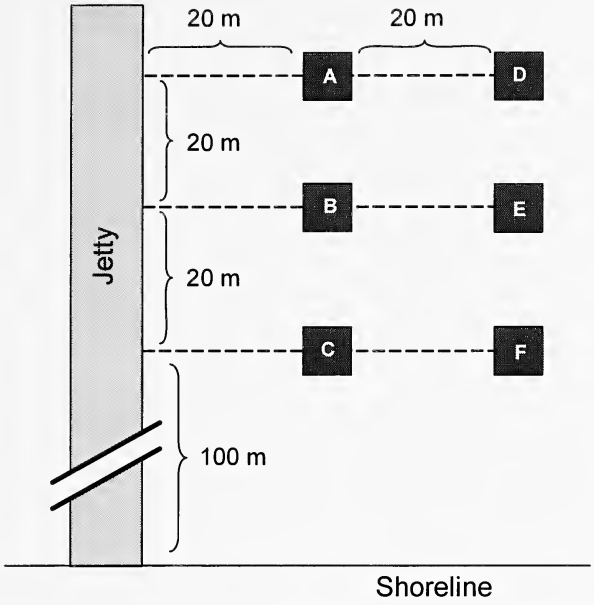

Figure 1. Study design of *Callinectes sapidus* study. Traps are labeled A-F and were located at indicated distances from the shoreline and jetty.

A total of 86 crabs were captured through the duration of the study, including 50 females, 35 males and a single individual that was of undetermined gender prior to escape. Overall trap success throughout the study ranged from 12 crabs in trap B (Fig. 1) to 20 crabs caught in trap E. Heterogeneity in success among trap positions was not significantly different from the distribution expected under equal success ($\chi^2 = 3.02$, $p = 0.696$), suggesting that crabs entered each trap with equal probability. However, there was a significant difference in success between bait types ($\chi^2 = 7.19$, $p = 0.027$). Whereas crabs entered traps baited with *B. tyrannus* at approximately the rate ($n = 26$) expected under equal distribution (equal distribution = 86 crabs/3 bait types, or 28.7 crabs per bait), traps baited with *B. patronus* were visited more frequently ($n = 38$), and traps baited with *B. gunteri* less frequently ($n = 22$), than would be expected under equal distribution. Both the total number of positive traps (traps capturing at least one crab) and the total number of crabs captured were highest in traps baited with *B. patronus* (Fig. 2).

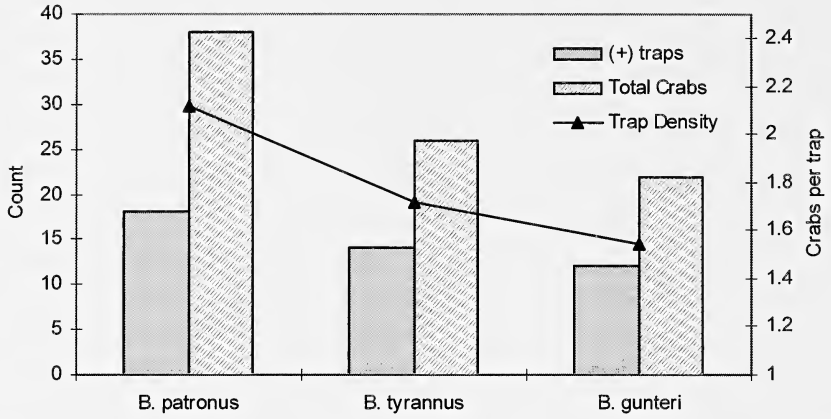

Figure 2. The trap success of each of three species of crab bait, Gulf menhaden *Brevoortia patronus*, Atlantic menhaden *B. tyrannus* and finescale menhaden *B. gunteri*. The number of positive traps (traps with at least one crab) and overall number of crabs per bait are counted on the left axis, whereas the trap density (crabs/trap) is indicated on the right axis.

Crabs tended to be captured in same-sex assemblages more frequently than would be expected assuming entrance at rates proportionate to the local sex ratio, calculated from the expected ratio of females to males over all samples ($\chi^2 = 7.89$, $p = 0.005$). For instance, the frequency of females in the study was 0.588, and the frequency of males was 0.412; however, of the two traps that captured four crabs, each contained only females. Similarly, three of five 3-crab traps contained single-sex assemblages.

The width-weight relationship trajectory was also different between male and female crabs ($p = 0.004$). Much of the variance in weight could be accounted for by differences in width ($F = 193.51$, $p < 0.001$), as compared to sex ($F = 30.59$, $p < 0.001$). However, males tended to be heavier than females of equal width, especially in larger (and presumably, older) individuals (Fig. 3).

In addition to blue crabs, other species were captured during the duration of the study, including stiped hermit crabs (*Clibanarius vittatus*, $n = 82$), hardhead catfish (*Arius felis*, $n = 6$) and a single red drum (*Sciaenops ocellatus*). While the effect of these species

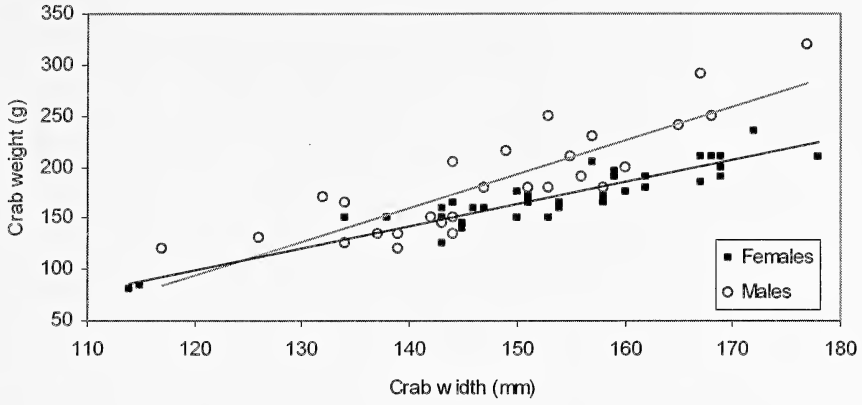

Figure 3. Width-weight relationship of male (open circles) and female (closed boxes) blue crabs, *Callinectes sapidus*.

on the entrance of blue crabs into traps was untested, the capture of these species after only a single night of trap placement has implications for ghost fishing (Guillory 1993) with commercial-style blue crab traps.

Discussion

In previous studies, the number and size of crabs entering commercial-style traps was shown to vary seasonally (Guillory 1993). However, to the author's knowledge, sex-ratios within individual traps have not previously been examined. Here, blue crabs tended to be captured in same-sex groups more often than would be expected by chance. While this result seems extraordinary, the finding is not without biological basis. For instance, Hines et al. (1987) demonstrated significant habitat partitioning by size, molt stage and sex. Broad movements of both sexes, but females in particular, are affected primarily by reproductive cycles and result in significant differences in resource usage. As a result, it is not surprising to find that males and females may be collected in same-sex assemblages. These data also indicate divergent width-weight trajectories for males and females. The finding that males tend to be heavier than females of equal width was first reported by Newcombe et al. (1949) and has since

been corroborated by numerous authors working in Texas (Pullen & Trent 1970), South Carolina (Olmi & Bishop 1983), Florida (Tagatz 1965) and even Turkey (Atar & Secer 2003). If weight can be used as an indication of crab maturity, this finding indicates that mature males of market size will be encountered less often than females in baited traps. However, in the duration of the current study, only four crabs were encountered that were smaller than market size; of these, two were male and two were female. Thus, if these numbers are reflective of the fishery as a whole, it is likely that crabs are harvested equally regardless of gender. Additionally, Olmi & Bishop (1983) reported that in addition to sex and maturity, molt stage was a considerable source of variance in weight of individual crabs. Thus it seems unlikely that size requirements for commercially captured crabs in Texas result in biased exploitation of either male or female crabs, although this finding could potentially be reevaluated with fishery-dependent data.

The finding that blue crabs were statistically more likely to be caught in traps baited with Gulf menhaden *B. patronus* is very significant. The Gulf menhaden is one of the more common fishes in the western Gulf of Mexico and census estimates for this species suggest that the Gulf population may number in the hundreds of millions (Avise et al. 1989) or more. In contrast, the finescale menhaden *B. gunteri* exist in smaller population sizes and are encountered much more infrequently (Dahlberg 1970, Anderson & McDonald 2007), whereas the Atlantic menhaden *B. tyrannus* is generally not encountered in the Gulf. Thus, the implication is that blue crabs in Tres Palacios Bay exploit species which are endemic and common in the Gulf of Mexico, rather than species that are exotic or rare. Blue crabs rely heavily upon olfactory-mediated chemical cues for foraging (Weissburg & Zimmer-Fuast 1994, Keller & Weissburg 2004), and thus may be more readily attracted to cues which are frequently encountered. However, this result must be tempered by at least one major caveat. Both Gulf and Atlantic "large-scaled" menhaden species tend to have softer flesh and are oilier than their small-scaled menhaden counterparts (such

as the finescale menhaden, Dahlberg 1970). The authors are in agreement that rotten tissue from finescale menhaden tends to be much less malodorous than either of the large-scaled baits, although this was not quantified scientifically. Nevertheless, the difference in attraction noted here may be simply attributed to the strength of odor plumes caused by physiological differences between species. Behavioral studies examining bait preference in a closed system would be helpful but were not conducted. Irrespective of the cause, the finding of differences between bait effectiveness is significant for the following reason. During the course of this study, the authors purchased a box of "crab bait" from a local fish dealer, and were given a box of fish that was primarily (> 99%, by weight) menhaden. Using previously described molecular and morphological characters (Anderson 2007, Anderson & McDonald 2007), it was determined that the box was filled with Atlantic menhaden, rather than the local (and presumably more effective) Gulf menhaden.

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TEXAS J. OF SCI. 60(4):281-298 NOVEMBER, 2008

SPATIAL AND TEMPORAL DISTRIBUTIONS OF PLANKTONIC DIATOMS IN A SUBTROPICAL BAYOU, ALONG THE UPPER TEXAS COAST

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Abstract.–A study of planktonic diatom (Bacillariophyceae) community spatial and temporal distributions was carried out in Offatts Bayou, a small embayment within the larger Galveston Bay complex in southeast Texas. The dominant diatom genera were *Chaetoceros, Ditylum, Rhizosolenia, Coscinodiscus, Guinardia, Dactyliosolen, Odontella* and *Lithodesmium*, in this order. Over 20 other diatom genera were represented at different times including some known to produce toxins (e.g., *Pseudonitzschia*), while others only appeared in surface waters after strong wind induced mixing events (e.g., *Navicula*). The spatial and temporal patterns observed for the dominant planktonic diatoms followed changes in salinity and temperature. Other environmental variables (light, nutrients, grazing) were not measured as part of this initial investigation but may have been important. Mean *Chaetoceros, Ditylum, Odontella* and *Lithodesmium* standing crops were highest at the open end of the bayou, while *Guinardia* and *Dactyliosolen* numbers were highest in the most flow-restricted regions of the bayou. Spatial distributions of *Rhizosolenia* and *Coscinodiscus* were homogenous throughout Offatts Bayou. Understanding natural diatom succession patterns may provide important insights into normal variations in community composition. Diatoms are potentially a powerful biomonitoring tool for future assessments of the impacts of eutrophication, climate change and/or human induced ecosystem dysfunction.

Climate change and sea level rise will pose complex effects on phytoplankton communities, particularly those in coastal environments. Eutrophication induced decline in ecosystem function in coastal areas has resulted in algal blooms, red and/or brown tides (Hallegraeff 1993; Nixon 1995; Howarth et al. 2000), fish disease, fish kills, and the development of hypoxic areas (Paerl et al. 1998; Thronson & Quigg 2008). Like bays and estuaries, coastal bayous are especially susceptible to contamination because they have intrinsically low flushing rates and limited freshwater inflow. In their tidal reaches, bayous are high in suspended sediments and dissolved organic carbon, and low in dissolved oxygen, especially

from late spring to early fall (e.g., Dickinson Bayou, Texas; Quigg et al. 2009).

Given that diatoms (Division Bacillariophyta) are important primary producers in coastal waters, making up a large fraction of the phytoplankton community in many places (Örnólfsdóttir et al. 2004a; Örnólfsdóttir et al. 2004b; Bukyates & Roelke 2005; Lavoie et al. 2006), changes in their dynamics may be used as a sensitive biomonitor of the influence of the above processes on ecosystem function. Diatoms respond quickly to physical, chemical and biological perturbations (e.g., Örnólfsdóttir et al. 2004a; Örnólfsdóttir et al. 2004b). Yet few studies have examined diatom community composition and succession in coastal systems (Resende et al. 2005; Cetinić et al. 2006) despite growing concerns for ecosystem health in these, the most densely populated, developed and growing areas in the world.

Galveston Bay (Fig. 1) is the second largest estuary (ca. 1550 km^2) on the Texas coast. It faces conservation issues due to high density industrialization and urbanization throughout its watershed (GBEP 2001; TWDB 2007). A human population of four million live within its five bordering counties; this figure doubles when considering the entire watershed which encompasses two of the largest metroplexes in Texas: Houston and Dallas-Fort Worth (TWDB 2007). Galveston Bay is the most productive of all Texas' estuaries with an oyster production that is unsurpassed in the U.S., and a recreational fishery that is worth millions of dollars annually (GBEP 2001; Lester & Gonzalez 2002; TWDB 2007).

Phytoplankton biomass (measured as chlorophyll *a*) has been declining in Galveston Bay since the 1970's (Lester & Gonzalez 2002). The bay now sustains a moderate to low phytoplankton biomass of 4 to 15 ug chlorophyll *a* L^{-1} (Santschi 1995; Quigg et al. 2007). The decrease is thought to be a response of the bay to improved water quality as a result of the Clean Water Act of 1970 (H-GAC 2006). Concurrent decreases in nitrogen and phosphate loading have also been recorded (Santschi 1995; Lester & Gonzalez

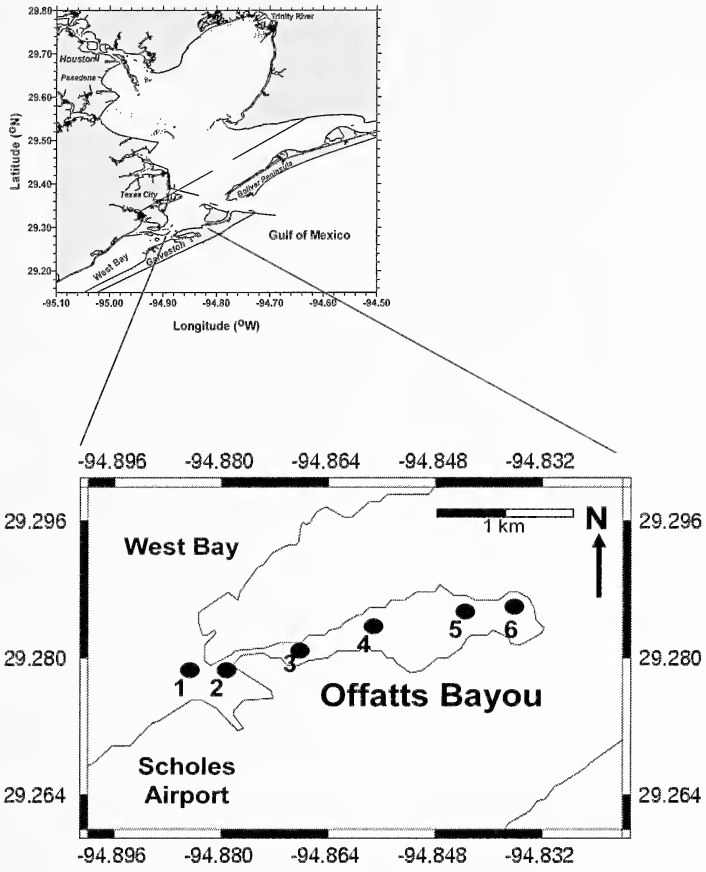

Figure 1. Galveston Bay is the largest estuary along the Texas coast. Offatts Bayou is a small embayment within this complex. Sampling site locations for water collected in the study area are indicated with filled circles.

2002) along with increased water clarity (Lester & Gonzalez 2002). Although phytoplankton blooms occur at different times of the year in the bay, they typically do not reach proportions which may be considered 'harmful'. The appearance of the diatom *Nitzchia pungens* forma *multiseries* in 1989 in Galveston Bay (Fryxell et al. 1990) and in 1990 in Offatts Bayou (Reap 1991) first raised concerns for human health and for the local fisheries nearly two decades ago. The presence of this and other diatoms (and species

of other phytoplankton) capable of producing toxins remains an issue of concern for scientists, resource managers and residents.

In order to develop an effective biomonitoring tool, it must be simple, fast, inexpensive, representative and reliably measured. Planktonic centric diatoms potentially fulfill all these criteria in that they are easily collected, readily identifiable (at least to generic level), and cosmopolitan. Students, volunteers and state agency personal can easily participate in a biomonitoring program involving diatoms. Phytoplankton other than diatoms are often more difficult to identify without sufficient training or molecular methods; some are very fragile and/or sensitive and may be broken by improper handling and/or preservation techniques. Smaller phytoplankton species such as cyanobacteria are often missed by nets or require specialized collection techniques. Filamentous species require certain assumptions in the enumeration process and often form clumps or masses, complicating the process further. Phytoplankton that typically do not occur in surface waters also require specialized collection techniques. Hence, planktonic centric diatoms were selected as a possible biomonitoring tool.

This study reports the findings of a year-long study which examined patterns of diatom (*Chaetoceros, Ditylum, Rhizosolenia, Coscinodiscus, Guinardia, Dactyliosolen, Odontella* and *Lithodesmium*) succession in Offatts Bayou, an embayment of Galveston Bay, Texas (Fig. 1). These eight centric planktonic diatom genera were chosen for their ease of identification and enumeration. They were also chosen because they dominated on the spatial (1 km^2) and temporal (monthly) scales which are typically examined. Shifts in diatom community composition that are not related to natural variables (temperature, salinity) would appear useful as indicators of changing environmental conditions.

Materials and Methods

Study site.–Offatts Bayou was created on Galveston Island early in the twentieth century after the 1900 Hurricane. The area was used as a borrow pit for local construction sites, in particular, the

seawall which now protects one-third of the seaward side of Galveston Island. By the end of the 1960's, Offatts Bayou was subject to extensive human development and became surrounded by homes and businesses, including Houston's most visited tourist attraction, Moody Gardens. Offatts Bayou is now approx. 1 km wide and 5 km long (Fig. 1). It is generally shallow (5-6 m, but mostly less), has poor circulation and restricted exchange with West Bay, its link to Galveston Bay (Fig. 1). Along its northern side is a boat channel which runs west to east; this side of the bayou is generally deeper (> 7 m). Most of the south side is not accessible by boat because of its shallow depths (< 1 m). Hence, sampling was performed along the northern bank with stations indicated on Fig. 1. Station 1 was located in West Bay at the mouth of Offatts Bayou; the remaining stations were spread approximately equidistant to the closed end finishing with station 6 (Fig. 1). The water column at West Bay (station 1) is well mixed in nature and relatively pristine (Roehrborn 2006). In the most restricted parts of the bayou (stations 5 and 6), residence times may be long (several months), water transparency is low (Secchi depths often 1-2 m less than those measured in West Bay (Skinner 2007), and the sediment and nutrient loading high as a result of dense development on its borders. Between these two extremes lies a gradient of water quality conditions and unique characteristics (bathymetry, mixing) that provides a range of environmental conditions for diatoms.

Field procedures.–Sampling was performed over a 12 month period from December 2004 to November 2005. Phytoplankton tows (67 μm mesh net) were conducted at each station shown in Fig. 1 on a monthly basis. Water samples were transferred to 50 mL centrifuge tubes and stored in a cooler until returning to the laboratory. Phytoplankton were then preserved with formalin (final concentration 3%) and kept in a dark cool place until examined microscopically. Salinity and temperature were measured with a Hydrolab Mini Sonde (Hydrolab, Texas, USA) during field collections. All field sampling was conducted in the mornings between 0800 and 1100 hrs Central Standard Time.

Table 1. Characteristics of the dominate diatom genera in Offatts Bayou between December 2004 to November 2005 at the six sampling stations. Species in this table reflect only those most commonly identified (> 90%) for each genera. As all diatoms were cylindrical in shape, biovolumes were calculated according to L*H*D*Pi/4.

Genera	Species ($n \geq 20$)	Average length (μm)	Average diameter (μm)	Relative biovolume (μm^3 $cell^{-1}$)
Chaetoceros	*C. danicum*	12-16	12-14	1,857
	C. curvisetus	15-20	15-30	6,955
	C. lorenzianus	30-48	12-24	9,920
Ditylum	*D. brightwellii*	80-130	12-38	51,515
Rhizosolenia	*R. setigera*	130-440	4-12	14,320
	R. pungens	250-690	4-20	53,130
Coscinodiscus	*C. radiatus*	25-35	45-55	58,875
Guinardia	*G. striata*	50-80	5-12	3,690
	G. delicatula	50	9-22	8,830
Dactyliosolen	*D. fragilissimus*	8-70	42-300	964,076
Odontella	*O. mobilensis*	35-90	24-70	108,379
	O. sinensis	240-320	28-32	197,820
Lithodesmium	*L. undulatum*	38-63	31-35	43,170

Phytoplankton analysis.—Light microscopy was used to identify and enumerate diatoms. Numerical abundance of diatom cells was determined by counting a minimum of five ocular micrometer grids at 400x magnification. The number of grids counted was adjusted according to cell density. Counts were completed upon reaching a minimum of 100 cells. The text used most frequently to aid in taxonomic identification was Tomas (1997). The primary focus of this study was the examination of centric planktonic diatoms which are reported only to the generic level.

Because algal volumes can vary immensely among species, and because many ecological processes are more dependent on biovolumes than on densities, biovolumes were also estimated. Length and width measurements were calculated based on the averages ($n \geq 20$) determined for the species in Table 1. For example, all the *Ditylum* were *D. brightwellii* and the majority of *Coscinodiscus* were *C. radiatus* (>90%) so that biovolume calcula-

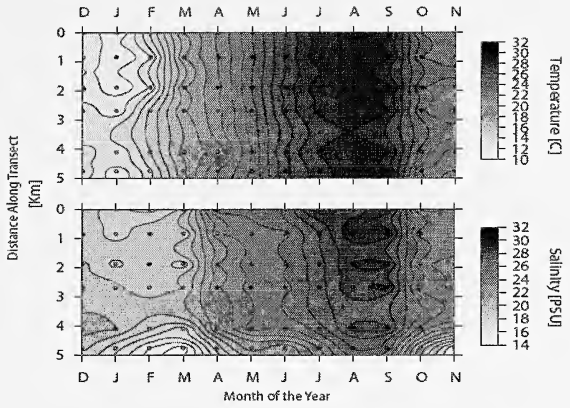

Figure 2. Monthly changes in surface water (a) temperature (°C) and (b) salinity (PSU) at the six stations in Offatts Bayou from December 2004 to November 2005.

tions for these genera were based on dimensions of these species respectively (Table 1). *Chaetoceros* species were most often represented by *C. danicum, C. lorenzianus* and *C. curvisetus* so that an 'average' biovolume for *Chaetoceros* was calculated based on the mean measurements of these three species (Tables 1 & 2). It maybe for *Chaetoceros* that the calculated biovolume is underestimated given the sizes of these species. Biovolumes were calculated assuming all centric species were cylindrical: L*W*H*Pi/4 where L = length (μm), width (μm) and height (μm).

RESULTS

Physical/chemical parameters.–Water temperature did not vary spatially but did vary temporally (Fig. 2). Temperatures ranged from summer highs of 30.5°C (±1°C) between June and August to winter lows of 14°C (±0.3°C) from December through the end of February. Salinity did not vary significantly among stations except for station 6 (ANOVA, $p > 0.05$), which had slightly lower salinities than the other stations. Salinities also varied temporally with highest salinities measured during July through to September (29 ± 0.3) and lowest salinities in the cooler months of December to March (19 ± 0.4) (Fig. 2).

Table 2. Spatial distributions of the eight major diatom genera in Offatts Bayou between December 2004 to November 2005 at the six sampling stations. The biovolume of each genus at each station was calculated by multiplying the number of cells by the average biovolume for that genus.

Station	*Chaetoceros*	*Ditylum*	*Rhizosolenia*	*Coscinodiscus*	*Guinardia*	*Dactyliosolen*	*Odontella*	*Lithodesmium*
1	1.05	2.47	4.18	6.06	0.66	67.49	2.91	0.00
2	0.92	6.54	1.69	0.71	0.61	60.74	4.13	0.78
3	0.93	7.11	2.06	5.77	0.31	45.31	6.28	0.52
4	1.05	8.35	2.60	2.65	0.51	41.46	1.99	0.30
5	1.31	2.16	4.99	2.94	0.19	0.00	5.51	3.76
6	1.55	7.11	4.69	7.71	0.14	0.00	8.27	1.25
Relative biovolume (μm^3 $cell^{-1}$)	6244	51516	33724	58875	6259	964076	153100	43171
Average biovolume (μm^3 mL^{-1})	7.3	33.9	22.1	31.6	1.93	168	30.1	8.1
% Total of a genera	2	11	7	10	1	55	10	3

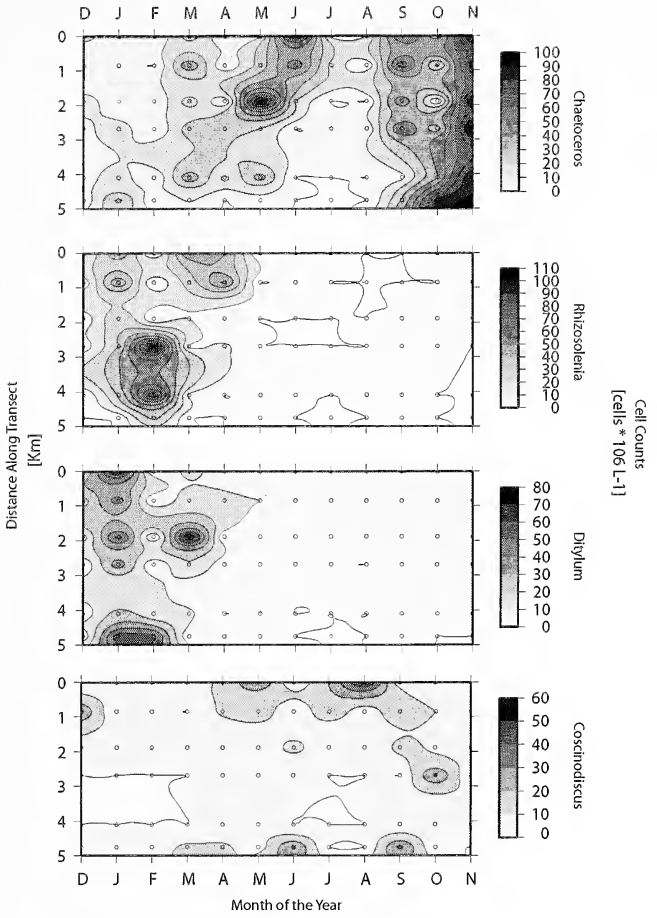

Figure 3. Spatial and temporal distributions of the eight dominate diatom genera in Offatts Bayou from December 2004 to November 2005.

Diatom standing crops – abundance and biovolume.–Of the eight major planktonic diatom genera present in Offatts Bayou (Fig. 3), several different species of *Chaetoceros* accounted for 39% of the standing crop (numerically), while *Ditylum* accounted for 16%. *Rhizosolenia* (14%), *Coscinodiscus* (11%) and *Guinardia* (8%) also accounted numerically for relatively large fractions of standing crops while *Dactyliosolen* (5%), *Odontella* (4%) and *Lithodesmium* (4%) were present in the lowest proportions. Approximately 20

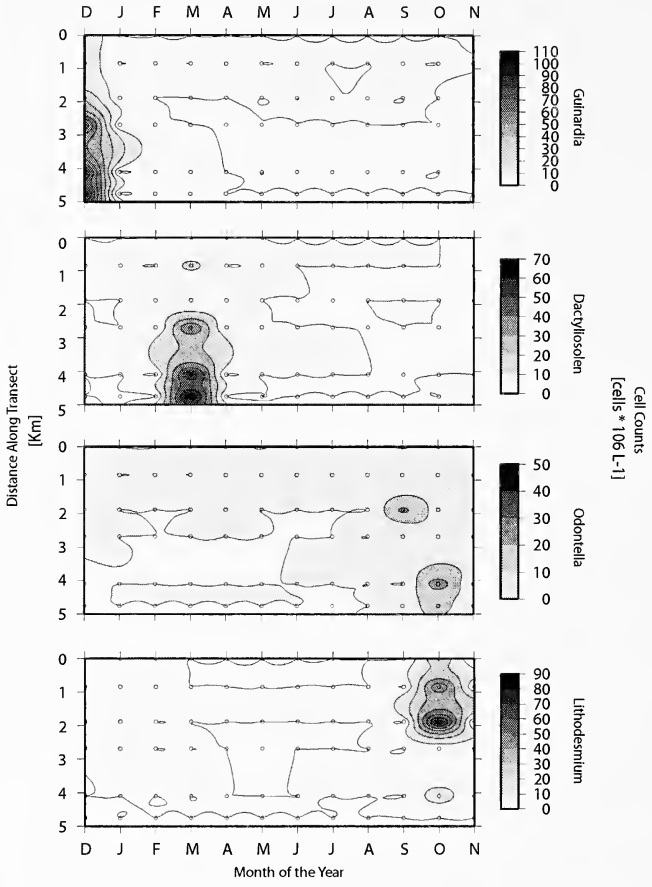

Figure 3. Cont.

other diatoms were present at the six stations during the sampling year; they accounted for < 1% of the total diatom community at anytime and were comprised of both centric and pennate forms, as well as other planktonic and some benthic types. Despite dominating numerically, *Chaetoceros* accounted for only 2% of the total biovolume of diatoms whilst *Dactyliosolen* accounted for the largest fraction (59%) (Table 2). *Ditylum, Coscinodiscus* and *Odontella* each accounted for ~10% of the diatom biovolume in Offatts Bayou.

Diatom distributions – spatial patterns.–Diatom standing crops of *Chaetoceros, Ditylum, Odontella* and *Lithodesmium* were highest at the mouth of Offatts Bayou, near West Bay, and then decreased along the sampling transect to the inner most station (Fig. 3) during the sampling period (December 2004 to November 2005). Wherever, *Odontella* and *Dactyliosolen* were present in high numbers, *Guinardia* and *Lithodesmium* were generally present in low numbers (Fig. 3). While this observation was not statistically significant, it was the pattern observed consistently over the study period as reflected in Fig. 3. *Rhizosolenia* and *Coscinodiscus* did not appear to exhibit any preferences spatially in Offatts Bayou. In the flow-restricted region of the bayou (stations 5 & 6), *Guinardia* and *Dactyliosolen* dominated. At station 1, where flow was not restricted but greatest mixing was generally observed, *Odontella* and *Lithodesmium* were present in highest quantities.

Diatom distributions – temporal patterns.–Eight diatom genera dominated at different times of the year (Fig. 3). While *Chaetoceros* dominated in the fall (September to November), a smaller peak was also observed late in the spring (March to June). These two peaks were associated with different species of *Chaetoceros* but clear species identifications were difficult with current resources. *Ditylum* and *Rhizosolenia* dominated late winter and early spring (December to March), while *Coscinodiscus* was prevalent between May and September (late spring to end of the summer). Each of these diatom genera appear to have dominated at most stations for several months or a season; during other times, they were still present in water samples, albeit at lower numbers.

On the other hand, when *Guinardia, Dactyliosolen, Odontella* and *Lithodesmium* appeared, it was typically for about a month, usually at all stations, and then they essentially disappeared again from surface water samples (Fig. 3). *Guinardia* was present in highest densities in December, *Dactyliosolen* densities peaked from February to March, while *Odontella* and *Lithodesmium* were concurrently found in highest densities in from September to

November (Fig. 3). In this manner, different diatom genera peaked at different times of the year.

Discussion

Understanding natural changes in phytoplankton community composition is becoming more important with the increasing occurrence of eutrophication, changes in climate regulated processes which define circulation patterns, salinity regimes and pressures on ecosystems. Knowledge of the spatial and temporal distributions of estuarine phytoplankton offers insights into the potential effects of eutrophication and provides a tool for establishing relevant management strategies (Conley 2000; Olsen et al. 2001; Arhonditsis et al. 2007). Diatoms play an important role in many ecosystems (e.g., Badylak & Phlips 2004; Buyukates & Roelke 2005; Lavoie 2006) yet the bulk of studies using diatoms as bioindicators have been performed in freshwater systems.

Most of the phytoplankton species identified in Offatts Bayou to date (Roehrborn 2006; Skinner 2007) were diatoms (up to 65 %). Similarly, Örnólfsdóttir et al. (2004a; 2004b) found diatoms dominated in Galveston Bay. They also found cyanobacteria, chrysophytes and cryptophytes were dominant at times while dinoflagellates, chlorophytes and euglenoids appeared occasionally abundant, but were relatively minor components on an annual basis. Buyukates & Roelke (2005) also found diatoms were an important component of the Nueces Delta (south Texas).

While dinoflagellates and cyanobacteria are most often associated with eutrophication issues and are more likely to be associated with 'harmful algal blooms', the representatives in these taxa are often more difficult to identify and require more considerate handling and sampling methods. Because of the interest in developing a biomonitoring tool, diatoms were chosen for the reasons stated above and because of their established utility as biomonitors in other coastal systems (e.g., Resende et al. 2005; Cetinić et al. 2006) and in freshwater systems worldwide.

The eight dominant centric planktonic diatom genera examined in this study are cosmopolitan and can develop into bloom concentrations if conditions are suitable. Blooms of *Dactyliosolen, Odontella, Chaetoceros, and Rhizosolenia* for example, were observed in the Indian River Lagoon, Florida, USA (Badylak & Phlips 2004). Wang et al. (2006) found that *Rhizosolenia* and *Chaetoceros* dominated in Daya Bay (China). Although present, *Skeletonema* and *Nitzschia* were not observed in high abundances in Offatts Bayou (Roehrborn 2006); these genera are however known to dominate in other systems (Badylak & Phlips 2004; Buyukates & Roelke 2005; Wang et al. 2006). The eight planktonic genera examined in this study are not known to be harmful based on their absence from the 'harmful algal bloom' list developed by the Intergovernmental Oceanographic Commission of UNESCO (IOC 2007).

Spatial and temporal patterns in the diatom community.– Similarly to earlier studies on Offatts Bayou (e.g., Cooper & Morse 1996), seasonal variations were observed in the thermohaline structure of Offatts Bayou over the course of the study (December 2004 to November 2005). Temperatures and salinities were also typical of those measured in subtropical bays and bayous (Quigg et al. 2007; Quigg et al. 2009) and lagoons (Badylak & Phlips 2004) in the Gulf of Mexico. Winter waters were cooler, less saline and mixed while summer waters were warmer, saltier and often stratified (Fig. 2; Roehrborn 2006). In Fig. 2, the importance of salinity and temperature in regulating the composition of this diatom community on temporal scales in Offatts Bayou is seen. It is well know that other factors such as light, nutrients, grazing, mixing, would also be important. These however, were not measured comprehensively as part of this initial investigation due to insufficient resources. Light, nutrients and mixing will be measured in on-going studies. Adaptation to osmotic stress is a major factor in dictating the success of a particular species in estuarine environments, and can have broad impacts on phytoplankton composition and standing crops (Kennish 2002).

Resende et al. (2005) and Cetinić et al. (2006) recently also reported that salinity and temperature were the most important factors in defining species composition in the Canal de Mira, Ria de Aveiro (Western Portugal) and in the Krka Estuary (Adriatic coast), respectively. Hence, these two measures alone may not describe in full detail the pressures on the community, but are nonetheless important.

Three possible scenarios may explain the relatively low diatom standing stocks in summer in Offatts Bayou compared to the rest of the year. First, small rapidly growing diatoms may be replaced by slow-growing, filamentous or inedible forms of diatoms and other phytoplankton during low-nutrient conditions such as those that occur in summer as a result of little or no rainfall, and/or freshwater inputs (Sommer 1989). Although, nutrients were not measured as part of this study, earlier findings revealed that nitrate concentrations were below detection (year round), phosphate varying from zero (winter) to 3 mM (summer) and silicate from zero (winter) to 30 uM (summer) in surface waters (top 2-3 m) of Offatts Bayou (Cooper & Morse 1996). Similar nutrient distributions have been observed for Galveston Bay (Santschi 1995; Quigg et al. 2007). While the actual concentrations during this study may not have been the same, similar patterns were likely, particularly the low nutrient concentrations in the summer. Winter diatom populations were likely to be N-limited given the phosphate and silicate concentrations. This is in fact what was observed. Studies of the constraints on primary productivity in Galveston Bay also found the system to be predominately N-limited (Örnólfsdóttir et al. 2004; Quigg et al. 2007). Changes in relative abundances of diatoms under increasing nitrogen loading and/or other human-induced changes to estuarine hydrography thus have important implications for Offatts Bayou and other estuaries.

Second, centric diatoms are nonmotile and prone to sinking out of the water column (Smetacek 1999). Sinking may be exacerbated in the summer in Offatts Bayou given its highly stratified nature

during the warmer months (Roehrborn 2006; Skinner 2007), much like that which occurs in other subtropical estuarine systems (Pinckney et al. 2001; Paerl et al. 2003; Wang 2006). Hurricanes, such as Rita, which came close to Offatts Bayou in the summer of 2005 (September 24), may temporarily inject the system with nutrients and/or disrupt stratification. It was noted however, that stratification had been re-established within two weeks of Hurricane Rita passing. This was surprising given that recorded wind gusts at the nearby meteorological station (Scholes airport; Fig. 1) were >100 km h^{-1} for many hours.

Third, the timing of the decrease in diatom biomass during summer may have coincided with the timing of greatest zooplankton grazer populations in this system. Although zooplankton samples was not quantified during this study, larger numbers of grazers were observed to be present. Also, phaeophytin concentrations, which are sometimes used as a proxy for grazing pressure, were elevated in the summer months (Roehrborn 2006; Skinner 2007). Large centric planktonic diatoms such as those investigated in the present study are an important part of the diet of many copepod species, which are in turn preyed upon by fish, including important commercial fish species. Thus any change in the abundance of centric diatoms could cause changes in the food web structure in this and/or other estuarine systems.

Whether results were presented as relative abundance (Fig. 3) or biovolume (Table 2), the overall findings in terms of spatio-temporal distributions of these planktonic diatoms were the same. Given the inherent difficulties associated with calculating cell size (biovolume and surface area), the additional total analysis time (substantial) due to the numerous measurements required, and the possibility of overestimating the contribution of a particular genera or species with large vacuoles to the overall community, the findings of this study are presented predominantly on the basis of diatom abundances (Fig. 3). Lavoie et al. (2006) came to a similar

conclusion, determining that relative abundance was the most appropriate metric to use for biomonitoring purposes.

This study provides a snapshot of the diatom community structure in Offatts Bayou, Texas. This is a valuable dataset in providing a baseline on the nature of this system, especially given that eutrophication, development and climate change will concurrently modify it in the future. Ongoing studies are being conducted in Offatts Bayou and Galveston Bay with the intent to develop microbial indicators for these aquatic ecosystems. Future studies will involve collecting nutrient data and ultimately modeling efforts such as those presented in Arhonditis et al. (2007). Such tools are required for the development of effective eutrophication management strategies.

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TEXAS J. OF SCI. 60(4):299-308 NOVEMBER, 2008

AN EVALUATION OF MODIFIED IMS SWABS FOR THE SCREENING OF OXIDIZERS AND HOME-MADE EXPLOSIVES

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Abstract.–Two cotton wipe treatments were developed, and a commercial colorimetric wipe was evaluated with the goal of improving airline security screening of suspect articles for chemical oxygen generators (COG) and homemade peroxide-containing explosives (HME). Five redox indicators (2,3'-diphenylamine dicarboxylic acid, diphenylaminesulfonic acid, diphenylamine, iodine:starch, and luminol) were evaluated against hydrogen peroxide and potassium chlorate. Diphenylamine and iodine:starch worked in the dry state but with very slow color-development times – negating their effectiveness as a primary security screening technique. The use of a color-developing spray reduced the detection times of the formulations prepared in this study. A commercial product (Drop-Ex Plus, Mistral Security Inc.) contained color developing solvents and produced similar results. The need for color-developing solvents relegates the colorimetric wipe systems to secondary screening where separate wipes can be used for the nitro-based explosives, COG, and HME.

With the constant threat of terror attacks present in the minds of world travelers it is important to have a fast, cost-effective, and safe way to individually screen luggage and carry-on items for explosive residues and hazardous substances. The current primary security screening method employs ion mobility spectrometry (IMS) to detect nitro-containing explosives such as trinitrotoluene (TNT) and pentaerythritol tetranitrate (PETN) (Ewing et al. 2001) in approximately six seconds per screened article (DHS S&T 2006). Liquids in containers with a capacity greater than 59 mL (two fluid ounces) are currently confiscated because the TSA has not developed or deployed a primary screening procedure for chemical oxygen generators (COG) and peroxide-containing explosives (FAA 2007).

The typical single-tube IMS is set to detect negative ions because the volatile nitro-containing explosives readily form negatively-charged adducts with the reactant gas in the IMS ionization chamber, as illustrated: H-R-NO_2 (g) + Cl^- (g) → [Cl (H-R-NO_2)]$^-$ (g). The chloride-laden reactant gas is produced by beta-

particle-induced dissociation of methylene chloride inside the IMS. (Eiceman & Stone 2004)

Triacetonetriperoxide (TATP) and hexamethylene triperoxide diamine (HMTD) are homemade explosives (HME) that are becoming more widely used in attacks (Buttigieg et al. 2003). These peroxide-based explosives are dangerous to synthesize, but large amounts can be made from inexpensive items such as acetone, sulfuric acid, 3% hydrogen peroxide, citric acid, and hexamethylenetetramine (Shakashiri 1983).

The triperoxides form positive-ion adducts in the IMS and are not detected by single-tube IMS instruments in negative-ion mode (Buttigieg et al. 2003). Dual-mode instruments are now available that can detect HMEs and traditional explosives simultaneously. However, more than 10,000 single-mode IMS instruments are currently deployed at airports worldwide (Eiceman & Karpas 2005), and the expense of replacement with dual-tube IMS as well as commensurate training of IMS operators would significantly postpone large-scale implementation. Even so, current IMS will only detect volatile nitro-based explosives. An inexpensive screening technique that detects oxidizers (i.e. peroxides and chlorates) is needed (FAA 2001).

Since most airport security screening is based upon a wipe sampling technique (Eiceman & Stone 2004), a dry peroxide-detecting colorimetric wipe that does not interfere with the single-tube IMS screening procedure would be a valuable interim solution.

Ideally in primary screening, the security official would swab the suspect article and look for a color change on the swab. If the color indication were negative, the official would use the same swab in the IMS to detect nitro-containing explosives. If the color indication were positive, the article would then be pulled aside for further inspection. A dry swab is also desired as this would minimize interference with the IMS detection of nitro explosives.

In a secondary screening scenario, normal TSA procedures would identify suspect articles to be wiped with the colorimetric swab as a test for the presence of HME or COG residues. Time constraints for secondary screening are not as restrictive as those for primary screening, so a color-development time could be tolerated. Also, IMS interference is not an issue since a different wipe could be used for the primary nitro-based explosive screening.

Concentrated peroxides and COG have been banned from passenger air transport since the 1996 ValuJet accident (FAA 2007; 49 CFR §172.101). A good screening technique for oxidizers will also detect peroxidic HMEs. The TSA has expressed interest in better screening and identification methods for oxidizers (FAA 1998; 2001) that are banned from commercial aircraft (Table 1). The CRC Handbook (Lide & Frederikse 1996) lists the standard reduction potentials of these substances. A high reduction potential represents a strong oxidizing agent.

Several colorimetric redox indicators are given in Table 2 along with the color of the oxidized species. Skoog et al. (1996) report that redox indicators typically depend only upon the cell potential of the system, which is the reduction potential of the oxidizing species minus the reduction potential of the indicator. This reaction is spontaneous when the standard reduction potential of the oxidant is greater than the standard reduction potential of the indicator.

It is anticipated that the standard cell potentials will accurately reflect the spontaneity of the reactions in the solid state. Many of the indicator reactions require water, hydroxide ions, or acid protons as reactants (Skoog et al. 1996), but the spontaneity of these reactions should allow the oxidizing agents to utilize the water, protons, and hydroxyl groups present in the cotton fiber matrix. If the rate of mixing of the reactants in a dry wipe is slow, it may be necessary to employ a liquid developing solution.

Table 1. TSA forbidden oxidants and explosives.

Substance	E_{red} (V)
Triacetone triperoxide (TATP) explosive	
Hexamethylene triperoxide diamine (HMTD) explosive	
Hydrogen peroxide, H_2O_2	+1.8
Chlorate, ClO_3^-	+1.5
Permanganate, MnO_4^-	+1.5
Perchlorate, ClO_4^-	+1.4
Dichromate, $Cr_2O_7^{2-}$	+1.2
Hypochlorite, ClO^- (Bleach)	+0.8

The same cotton swabs used in the Ionscan 400B IMS (Smiths Detection, Inc) were treated with the redox indicators in Table 2 and were evaluated both dry and wet to determine if colorimetric wipes were suitable for primary or secondary security screening. Hydrogen peroxide was tested in this study as a representative of the peroxidic explosives, and potassium chlorate was tested as a representative of the chemical oxygen generators.

Materials and Methods

The indicators 2,3'-diphenylamine dicarboxylic acid, diphenylamine sulfonic acid, and diphenylamine were exposed to potassium chlorate to investigate the result as it applies to visual detection and screening. The individual diphenylamine indicators were prepared as methanol solutions with 1% w/w indicator. One milliliter of the 2,3'-diphenylamine dicarboxylic acid indicator was pipetted into each of three 1.5 mL vials with caps. This was repeated for the other two indicator solutions for a total of nine vials. Each set of three indicators was probed with 100 μL of three different concentrations of potassium chlorate (0.1 M, 0.05 M, and 0.01 M). The solutions were then developed with two drops of hydrochloric acid. The results were evaluated subjectively on the basis of opacity, color, and development time. Based on these

Table 2. Redox indicators tested in this study.

Substance	Indicating color	E_{red} (V)
2,3'-diphenylamine dicarboxylic acid	blue-violet	+1.12
diphenylamine sulfonic acid	red-violet	+0.85
diphenylamine	violet	+0.76
iodine:starch	Blue-black	+0.54
luminol	chemiluminescent	

results the indicator diphenylamine was selected as a candidate for dry wipe testing.

Three indicator solutions were prepared for dry wipe testing. Indicator solution #1 was prepared as a methanol solution with 1% w/w diphenylamine and 5% hydrochloric acid. Indicator solution #2 was prepared as a 0.1 M potassium iodide aqueous solution containing 1% w/w starch. Indicator solution #3 was a luminol indicator prepared from the following reagents: 0.1% sodium carbonate, 1% sodium bicarbonate, 0.01% ammonium carbonate monohydrate, 0.01% copper (II) sulfate, and 66 ppm of 3-aminophthalhydrazide (luminol). The recipe for indicator #3 was taken from Shakashiri (1983). Three IMS swabs (Smiths Detection Inc.;one for chlorate testing, one for peroxide testing, and one for a control) were soaked in each of the indicator solutions. The control would be evaluated for resistance to atmospheric oxidation. The swabs were allowed to dry overnight at room temperature and atmospheric pressure.

A surface was prepared on a clean epoxy resin countertop. An area of 7 by 10 cm was masked off for each swab. A 50 µL drop of either 0.01 M potassium chlorate or 3% hydrogen peroxide was placed in the center of each area and allowed to dry for 5 h. This yielded a contamination of approximately 61 µg $KClO_3$ and 2.2 µg H_2O_2 upon drying.

When both the modified swabs and the test areas were dry a separate swab was used for each test area. The full test area was wiped, and the color change was evaluated over time.

The experiment was repeated in its entirety to evaluate the effect of a moistening step after dry-wiping the test area. The modified swabs were moistened with a single spray of water from a common pneumatic pump spray bottle.

A commercial off-the-shelf (COTS) detection package – Drop-Ex Plus (Mistral Security Inc.) – was tested for the detection of hydrogen peroxide and potassium chlorate residues. The kit provided collection paper and analysis reagents in drop-dispensing bottles. The kit also came with a procedure and chart for analyzing the results. A surface was prepared on a clean countertop as before. Test areas were masked off and similar peroxide and chlorate residues were applied. After the surface reagents were dry a collection paper was taken from the Drop-Ex Plus kit and used to swab one of the test areas. Two drops of the proprietary Drop-Ex Plus chlorates indicator (bottle A) were applied to the test paper to test for chlorate residues. If a dark-blue color change was not observed, the test was continued for peroxide residues on the same wipe. Two drops of the proprietary Drop-Ex Plus peroxides indicator (bottle B) were applied to the collection paper. If a dark-blue color change occurred at this point the presence of peroxides was suggested. This experiment was repeated twice for a total of three experiments. The Drop-Ex Plus kit verification sample was tested, thus ensuring that the indicator solutions were behaving as expected.

Results and Discussion

The diphenylamine species comparison tests were subjectively graded on opacity, color, and development time. The 2,3'-diphenylamine dicarboxylic acid reacted in solution to produce a translucent red-brown color 15 sec after addition of the acid developer. The intensity of the red-brown color increased with

Table 3. COG and HME detection results of the treated wipes

Indicator	Analyte	Solution Response Time (min)	Dry Response Time (min)	Wet Response Time (min)
2.3'-diphenylamine	chlorate	0.25	–	–
dicarboxylic acid	peroxide	–	–	–
diphenylamine	chlorate	0.25	–	–
sulfonic acid	peroxide	–	–	–
diphenylamine	chlorate	instantly	15	3
	peroxide	–	–	–
KI/starch	chlorate	–	–	–
	peroxide	instantly	35	5
luminol	chlorate	–	–	–
	peroxide	instantly	–	–
Drop-Ex Plus	chlorate	–	–	< 1
	peroxide	–	–	< 1

increasing chlorate ion concentration. Diphenylamine sulfonic acid performed in a similar fashion, leaving a light, translucent green solution 10 – 15 sec after development. In contrast, the indicator diphenylamine developed immediately after the addition of hydrochloric acid. All three vials were an intense navy-blue. This color was intense enough to prevent light from passing through the side of the small vial. Because of its fast development time and unmistakable color, diphenylamine was chosen for dry-wipe testing.

Dry, treated wipes modified with the luminol indicator (#3) showed no chemiluminescence in ambient room light or in total darkness (Table 3). Swabs treated with iodine:starch (#2) were able to detect peroxides but only after a swab development time of 30 min or more (Fig. 1). These swabs were totally ineffective at screening chlorate residues from the surface no matter how long they were allowed to develop. Swabs treated with diphenylamine (#1) were able to detect chlorate residues but only with

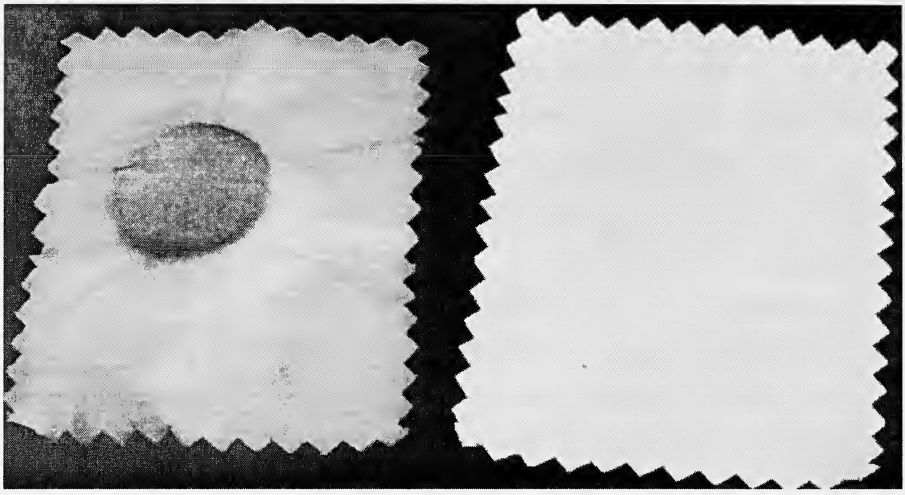

Figure 1. A representative result of the iodine:starch indicator (#2) detecting the presence of peroxides (left) and the untreated control wipe (right).

development times of 15 min or more. These swabs were totally ineffective at detecting peroxide residues, even after a long development time. In summary, the rate of mixing of the dry reactants was too slow to detect COG and HME residues in primary screening.

The addition of water spray to the procedure significantly reduced development times to 5 min for indicator #2 detecting peroxide residues and to 3 min for indicator #1 detecting chlorate residues. Indicator #2 still was not able to detect chlorate residues and indicator #1 still was not able to detect peroxide residues. Indicator #3, the luminol reagent was not able to detect either species in ambient room light or even when modified swabs were inspected in a dark room.

The Drop-Ex Plus kit was able to successfully screen all chlorate and peroxide residues on the surface. Sample collection, testing, and analysis were performed in less than 1 min, and the results were clear and easy to evaluate. Because the collection paper is not

chemically treated and reagents are stored as liquids in plastic bottles, atmospheric oxygen interference was not an issue.

Conclusions

The wipe and look procedure achieved by a chemically-modified dry-wipe will not work for primary security screening because of the limited reactant mobility and long color-development times. The use of a developing solution shortens this time, but excess moisture would not be compatible with the subsequent IMS detection of the nitro-based explosives. Therefore, the wipe treatments and developing solutions described herein are useful only for secondary screening for COG and HME.

In summary, the wipe-treatment preparation instructions in this article would allow production of COG- and HME-detection wipes for secondary screening at low cost. Potassium chlorate and 3% hydrogen peroxide solutions would be suitable for use as verification solutions. A COTS solution similar to the Drop-Ex Plus detection kit would also work well for detection of oxidizers and explosives. This kit contains the required test procedures and verification samples, and it performed as well as the wipes prepared in this study.

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GENERAL NOTES

NOTES ON BEHAVIOR OF THE TEXAS KANGAROO RAT (*DIPODOMYS ELATOR*)

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The Texas kangaroo rat (*Dipodomys elator*) is listed as a threatened species by the Texas Parks and Wildlife Department (Schmidly 2004). Reasons for listing *D. elator* are largely based on its apparent scarcity and small geographic range. Investigators have found *D. elator* in only 10 counties in north-central Texas (Schmidly 2004). The International Union for Conservation of Nature (1986) listed habitat loss and degradation resulting from expanding agricultural and infrastructure development as the major threats to continued existence of *D. elator*. There is general agreement that *D. elator* requires a sparse, short-grassland habitat (Carter et al. 1985; Dalquest & Collier 1964; Goetze et al. 2007; Roberts & Packard 1973; Stangl et al. 1992), but this type of habitat is becoming less common throughout the present range of the Texas kangaroo rat (Goetze et al. 2007).

With the exception of a few brief reports (Dalquest & Collier 1964; Chapman 1972; Roberts & Packard 1973; Packard & Roberts 1973), little information exists on behavior of the Texas kangaroo rat. Information on home ranges and burrow affinity was mostly obtained in catch-and-release studies of this Texas state-threatened species. Sociality and other behaviors have been described for other species of kangaroo rats (Behrends et al. 1986; Randall 1989; 1991; 1993; 2007; Jones 1993; Yoerg 1999), but have not been specifically documented for *D. elator*. A thorough understanding of the social interactions and other behaviors of a species provides vital information for its conservation (Allison & Destefano 2006).

Focal observations on agonistic, dust bathing, foraging, and day burrow usage behaviors for *D. elator* are presented within this note.

During a study involving habitat characterization, burrow use, and population estimation of the Texas kangaroo rat (Goetze et al. 2007), night vision scopes were used to closely observe behavior. *Dipodomys elator* is a nocturnally active species and previous researchers have utilized dim incandescent lighting to observe behavior. The use of infrared scopes disturbs the animals less and is an additional way of observing behavior of this species. In their study of equipment and techniques for nocturnal wildlife studies, Allison & Destefano (2006) pointed out that night vision scope generation model, light pollution due to nearness of urban areas, approach distance to study subjects, ability to distinguish individuals, and inclement weather were potential drawbacks of research utilizing night vision scopes. However, the *D. elator* study site was not located near an urban area, therefore light pollution levels were low. Also, researchers were able to approach to within a few meters of Texas kangaroo rats without significant disturbance, therefore individuals could easily be focally observed. Only two observation periods were interrupted by inclement weather. Because kangaroo rats habituate readily to observers (Randall 1991) and *D. elator* usually occurs in areas with little concealing vegetation, the Texas kangaroo rat proved to be a good subject for study utilizing night vision scopes.

The 15 ha study site is located in Wichita County, Texas, 3 km N Buffalo Creek Reservoir and was previously described by Goetze et al. (2007). The study area is heavily grazed and bordered by wheat fields on its eastern and western sides, and contains short-grasses and low shrubs. Mechanical brush control and herbicide applications have resulted in small (1-2 m in height) and scattered woody vegetation. These habitat features facilitated observations with the night scopes.

Observations were made utilizing a pair of Night Owl Optics model NOLT3 Generation One monocular night scopes. Fourteen observational sessions were conducted during the months of May and June, 2004 and 2005, for a total of 101 hours and 15 minutes. Observation began either before sunset or, in two cases, during early morning hours (330 and 400 hrs.). One observational period extended from sunset to sunrise. Behavioral observations were recorded in field journals. Focal observations were conducted at a distance of approximately three to five meters from the animals. However, observations of agonistic behaviors and dust bathing were often made at closer distances.

Agonistic behavior.–Agonistic behavior of *D. elator* has only been reported from a laboratory setting between males and females (Packard & Roberts 1973). Females harassed males by nipping them on the bulky portion of the body. In this study, agonistic behavior was observed in the field when a striped skunk (*Mephitis mephitis*) ran down a fence-row trail near two foraging kangaroo rats causing the rats to come together. These kangaroo rats jumped into each other several times and pushed away in mid-air until one antagonist disengaged and hopped back toward a burrow. Later, one of the previous individuals hopped to another individual's burrow as the preceding individual foraged some distance away. The intruder dug up seed caches at the burrow entrance and around the base of a shrub until the burrow's occupant returned and aggressively contacted the intruder and chased this rat down an established trail toward its own burrow. These contacts occurred three times during the observational period. Pilfering of seed caches has also been observed in Merriam's kangaroo rat (*D. merriami*) (Daly et al. 1992). An additional incidence of agonistic behavior was observed between two individuals who fought at a dust bathing site along a trail connecting their burrows. The conflict occurred at a point approximately midway (14 m) between the two burrow entrances. In each instance, the intruding individual retreated back to its own burrow location. Texas kangaroo rats may forage in distinct, defended territories along their trails. When one

kangaroo rat entered another's territory, the resident leapt into the air several times possibly indicating to the intruder that conflict was eminent. If the intruder did not retreat, the resident sometimes engaged the intruder and both individuals bumped and pushed against each other. If the conflict continued, the antagonists rolled and scuffled on the ground until one retreated. Similar agonistic behavior has been reported for *D. heermanni* and *D. merriami* (Randall 1989; Yoerg 1999).

Texas kangaroo rats occasionally forage in areas away from established trails and dust bathing sites within two to five meters of each other without conflict. Such behavior has been observed in Merriam's kangaroo rat (Behrends et al. 1986). Dalquest & Collier (1964) and Packard & Roberts (1973) never observed two or more adult *D. elator* above ground together. Observations made during this study may indicate that agonistic behavior occurs only within well-defined territories such as dust bathing sites and trails associated with burrows. Behrends et al. (1986) and Jones (1993) have also reported territorial behaviors in *D. merriami*, *D. heermanni*, and *D. spectabilis*.

Dust bathing.–Use of scratching and dusting areas by *D. elator* has been reported by other researchers (Dalquest & Collier 1964; Packard & Roberts 1973; Carter et al. 1985), but none have described dust bathing behavior for the species. Several *D. elator* conducting dust bathing activities were closely observed. Dust bathing occurred in bare areas containing loose, fine-grained soils immediately adjacent to trails. Dust bathing was observed in five individuals. The kangaroo rats scratched in the soil with their hind legs, rubbed their sides and their bellies in the dust and stroked the sides and front of their faces at and around the cheek pouches with the front feet. This behavior is similar to dust bathing activities reported for other species of heteromyids by Randall (1991; 1993). Deposition of oils from the dorsal skin gland at dust bathing sites plays a role in neighbor recognition, at least in some species of kangaroo rats (Randall 1991; 1993). Although this aspect of

behavior in *D. elator* was not observed during this study, Stangl et al. (2006) found that size and development of the dorsal gland of the Texas kangaroo rat was correlated with an individual's reproductive condition.

Foraging behavior.–Foraging behaviors were observed 12 times. Foraging kangaroo rats moved forward slowly upon all fours and utilized their forelimbs to place food in their cheek pouches, instead of immediately consuming the food items. The rats unloaded their cheek pouches at burrow entrances and pushed the seeds into the burrow with their forelimbs. The Texas kangaroo rat was not observed scatter hoarding seeds as does *D. merriami* (Daly et al. 1992). Foraging kangaroo rats sometimes covered considerable distances in a single bound and often jumped over taller vegetation. Foraging rats occasionally jumped up and backward during their foraging sessions. Similar backward leaping has been reported in *D. deserti*, *D. ingens*, and *D. spectabilis* as an avoidance mechanism against snakes (Randall 1993; 2007), however no snakes were observed near foraging *D. elator*. Movements of foraging cattle near the kangaroo rats caused them to briefly enter their burrows. Howling coyotes (*Canis latrans*) caused the kangaroo rats to stop foraging, freeze, and sometimes rise up on their hind legs.

Although some burrows were located 2 m from an adjacent wheat field, foraging always occurred in the pasture and never in the wheat field. No kangaroo rats were observed within the wheat field area either before or after harvesting of the grain.

Foraging distances were obtained for seven individuals. One individual foraged a distance of 2.5 m from its burrow, another 5 m from its burrow, four individuals foraged 15 m from their burrows, and one ranged 20 m from its burrow. The longest movement occurred when a kangaroo rat left one burrow and traveled 26 m directly to another burrow without foraging. Roberts and Packard (1973), using incandescent lighting, reported maximum distances of

86.89 m for males and 108.53 m for females and found that some *D. elator* traveled more than 300 m along roads at night. Because infrared rather than incandescent light was used during this study, the observations of shorter foraging distances may be more indicative of the Texas kangaroo rat's foraging behaviors.

Temporal, thunderstorm and moonlight effects on foraging behaviors.–Other researchers noted that the Texas kangaroo rat was most active two to three hours after darkness (Packard & Roberts 1973; Carter et al. 1985). Initial activity of Texas kangaroo rats during this study began at one hour or less after darkness. Active kangaroo rats were also observed during early morning hours (330 and 400 hrs). In one case, Texas kangaroo rats were observed from 2230 hrs to sunrise. No differences in activity levels or foraging behavior were noticed during any of these periods of time.

Texas kangaroo rats continuously foraged as lightening from a nearby thunderstorm illuminated the pastureland. No precipitation occurred during this event. During another early morning storm that had high winds and frequent lightening and thunder, a kangaroo rat that had been foraging about 3 m from its burrow entrance ducked back into its burrow after each lightening flash. The kangaroo rat repeated this behavior five times over a period of 20 minutes. Once precipitation began, the rat entered and remained within its burrow until after the storm moved out of the area.

Other researchers have stated that the Texas kangaroo rat is not active during moonlit periods (Dalquest & Horner 1984; Jones et al. 1988), or that moonlight foraging is unusual for the species (Packard & Roberts 1973). During this study, Texas kangaroo rats were observed foraging during new, crescent, half, and full moon phases. Randall (1993) observed that heteromyid rodents shift their activities from open areas to more protected brush and tree covered areas during moonlit nights.

Burrow utilization.–Dalquest & Collier (1964) and Schmidly (2004) stated that Texas kangaroo rat burrows were left unplugged during the day. Of five burrows known to be occupied by *D. elator* during the night, three were found plugged the following morning.

Observational data from this study suggests that *D. elator* may occupy more than one day burrow. Runways were found connecting burrows, and observations of animals leaving one burrow and entering and remaining within an adjacent burrow were documented on three separate occasions (21, 22, and 25 May 2005). Trapping data obtained during an earlier study (Goetze et al. 2007) also supports this hypothesis. Packard & Roberts (1973) reported no use of multiple burrows based only on trapping data. Behrends et al. (1986) reported that *D. merriami* utilized two or more day burrows and occasionally two different individuals may occupy the same burrow at different times.

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REPRODUCTIVE CYCLE OF THE BROWN FOREST SKINK, *SPHENOMORPHUS CHERRIEI* (SQUAMATA: SCINCIDAE), FROM COSTA RICA

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The brown forest skink, *Sphenomorphus cherriei* is an oviparous forest floor inhabitant known from central Veracruz, Mexico to extreme western Panama (Fitch 1983; Savage 2002). In Costa Rica it is an abundant inhabitant of the leaf litter community (Guyer & Donnelly 2004). Previous reports on *S. cherriei* reproduction are in: (Greene 1969; Fitch 1970, 1973a,b; Alvarez del Toro 1982; Köhler 2000; Savage 2002; Watling et al.2005). The purpose of this paper is to provide additional information on reproduction of *S. cherriei* from a histological examination of gonadal material from museum specimens as part of an ongoing study on the reproductive biology of Costa Rican lizards. The first information on the testicular cycle and evidence that *S. cherriei* produces multiple clutches are presented.

Specimens of *S. cherriei* from Costa Rica collected 1959 to 1986 were examined from the herpetology collection of the Natural History Museum of Los Angeles County (LACM), Los Angeles, California. Thirty three males (mean SVL = 49.6 mm ± 4.6 SD, range = 41-57 mm); fifty-three females (mean SVL = 53.4 mm ± 4.8 SD, range = 36-64 mm) and seventeen presumably neonates (mean SVL = 22.2 mm ± 1.4 SD, range = 18-23 mm) were examined. The left testis and epididymis were removed from males and the left ovary was removed from females for histological examination. Enlarged follicles (> 4 mm) or oviductal eggs were counted; no histology was performed on them. Tissues were embedded in paraffin, sectioned at 5 μm and stained with Harris' hematoxylin followed by eosin counterstain (Presnell & Schreibman 1997). An unpaired *t*-test was used to compare *S. cherriei* male and female mean body sizes using non-parametric data and the relationship between female SVL and clutch size was examined by linear regression analysis using Instat (vers. 3.0b, Graphpad Software, San Diego, CA).

Material examined.-The following specimens of *S. cherriei* from Costa Rica (by province) were examined: Alajuela (1): LACM 161700. Cartago (7): LACM 161672, 161673, 161868, 161913, 161914, 162562, 162598. Guanacaste (23): LACM 161615, 161619, 161667, 161691, 162565, 162567, 162571, 162573, 162575, 162580, 162582, 162590, 162592, 162595, 162596, 162601, 162610, 162614, 162616, 162620, 162621, 162623, 162628. Heredia (25): LACM 161618, 161632, 161659, 161690, 161865, 161869, 161878, 161880, 161682, 161694, 161869, 161870, 161882, 161884, 161886, 161895, 161912, 161916, 161917, 161929, 161934, 162586, 162600, 162603, 162612. Limón (22): LACM 161617, 161633, 161644, 161645, 161661, 161675, 161681, 161693, 161872, 161874, 161894, 161899, 161901, 161902, 161903, 161905, 161906, 161910, 161911, 161918, 162569, 162608. Puntarenas (23): LACM 161628, 161629, 161636, 161650, 161653, 161656, 161657, 161671, 161676, 161684-161686, 161692, 161695, 161705, 161876, 161907, 162557, 162560, 162561, 162564, 162599, 162625. San José (2): LACM 161665, 162572.

Stages in the testicular cycle are presented by month in Table 1. Three stages were present: (1) Recrudescent: (renewal) in which there is a proliferation of germinal cells, spermatogonia and primary spermatocytes predominate; (2) Late Recrudescent: numbers of germinal cells have increased; primary, secondary spermatocytes, spermatids, but no spermatozoa are present; (3) Spermiogenesis, clusters of sperm line the seminiferous tubules, metamorphosing sperm are present; epididymidis contains sperm. Males undergoing spermiogenesis were present in each month (Table 1) indicating a continuous testicular cycle. The smallest reproductively active male (LACM 161886) measured 41 mm SVL and was collected in April.

In another reproductive study on a lizard from Costa Rica, Marion & Sexton (1971) reported a seasonal testicular cycle for *Sceloporus malachiticus* from high elevations (800 to 3200 m). In November-January (dry season) testes were in regression (spermatogonia predominate) or recrudescent; by June maximum spermiogenesis was in progress (Marion & Sexton 1971).

Females of *S. cherriei* were significantly larger than males (unpaired t test, $t = 3.7$, 84 *df*, $P = 0.0004$). Reproductively active

Table 1. Monthly distribution of reproductive conditions in the testicular cycle of 33 *Sphenomorphus cherriei* from Costa Rica. Values are the numbers of males exhibiting each of the conditions.

Month	N	Recrudescent	Late Recrudescent	Spermiogenesis
February	1	0	0	1
April	5	0	0	5
May	7	0	1	6
June	3	0	0	3
July	7	1	0	6
August	3	0	1	2
September	2	0	0	2
October	1	0	0	1
November	3	0	0	3
December	1	0	0	1

Table 2. Monthly distribution of reproductive conditions in the ovaries of 53 *Sphenomorphus cherriei* from Costa Rica. Values shown are the numbers of females exhibiting each condition.

Month	*n*	No yolk deposition	Early yolk deposition	Enlarged follicles >4mm	Oviductal eggs	Oviductal eggs and yolk deposition
January	2	0	1	1	0	0
March	6	0	0	3	2	1
April	6	1	0	1	3	1
May	9	1	3	3	2	0
June	7	0	2	1	2	2
July	12	2	2	3	5	0
August	6	2	1	1	2	0
September	3	0	1	1	0	1
October	1	0	0	1	0	0
December	1	0	1	0	0	0

females were present in each month (Table 2) indicating a continuous ovarian cycle. Five females (Table 2) with oviductal eggs were undergoing concomitant early yolk deposition (= basophilic yolk granules in the ovarian follicle) for a subsequent clutch. This is the first definitive evidence that *S. cherriei* produces multiple clutches. Mean clutch size (n = 36) was 2.3 ± 0.65 SD, range = 1-3. Linear regression analysis revealed a significant positive correlation between female body size (SVL) in mm and clutch size for 36 *S. cherriei*

females: Y = - 0.01 + 0.04X, r = 0.35, P = 0.04. The smallest reproductively active female (collected in May) measured 46 mm (LACM 161673), with 2 follicles > 4 mm. My estimation of minimum size for female reproductive activity of *S. cherriei* of 46 mm is close to that of Greene (1969) who reported 44 mm.

Sphenomorphus cherriei of neonate size (18-23 mm) were collected in March, April, May, June, July and August indicating an extended reproductive season.

Fitch (1973a,b) who analyzed samples from Turrialba, Cartago Province, Costa Rica found most females were non-reproductive during the dry season (January to April) indicating there was some seasonality in *S. cherriei* reproduction. One January female from Cartago Province (LAM 162562) was undergoing early yolk deposition, however, it is not known when it would have been completed. According to Fitch (1973b; 1983) *S. cherriei* reproduces throughout the year in the humid Caribbean lowlands.

Alvarez del Toro (1982) reported *S. cherriei* in Chiapas produced clutches of 3-4 eggs in June and July (maximum clutch size reported herein is 3) and Fitch (1985) reported the possibility of geographic variation in clutch sizes. Examination of additional gonadal samples from different populations is required before the degree of geographic variation in the reproductive cycle of *S. cherriei* can be ascertained.

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* * * * *

THE DISTRIBUTION OF SPOTTED SKUNKS, GENUS *SPILOGALE*, IN TEXAS

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Spotted skunks (genus *Spilogale*) are small skunks that occur over much of the continental United States and Mexico, ranging north to British Columbia and south to Costa Rica (Hall 1981). The taxonomic history of members of the genus in the contiguous 48 states has ranged from as many as 10 species (Howell 1906) to their synonymy into one polytypic species, *S. putorius*, by Van Gelder (1959). In Texas, early editions of *The Mammals of Texas* followed

this pattern with recognition of three species of spotted skunks (Taylor & Davis 1947) followed by only one (Davis 1966).

Research by Mead (1968a; 1968b) revealed that eastern and western forms of *S. putorius* had distinctly different breeding times, with western forms breeding in the early fall and having delayed implantation, whereas eastern forms breed in the spring with no delayed implantation. Mead (1968b) argued that this reproductive pattern acted as a temporal reproductive isolating mechanism and suggested the eastern and western spotted skunks be treated as two species, *S. putorius* and *S. gracilis*, respectively. Since that time, several mammalian classifications continued to recognize only *S. putorius* in the United States (Hall 1981; Corbett & Hill 1991; Wozencraft 1993) but the most recent carnivore classification treated both eastern and western spotted skunks as different species (Wozencraft 2005). In Texas, editions of *The Mammals of Texas* since 1974 (Davis 1974; Schmidly & Davis 1994; Schmidly 2004) have followed such a classification recognizing the existence of both species in the state. Although the taxonomic status of spotted skunks in Texas appears resolved, the current distributional ranges of these two species remain unclear.

All depictions of the distribution of the two species of spotted skunks in Texas have left the central part of the state without records and presumably the two did not meet in much of this part of the state (Schmidly 2004). Counties without records (Schmidly 2004) include Nolan, Taylor, Callahan, Eastland, Runnels, Coleman, Brown, Comanche, McCullough, San Saba, Mills, Hamilton, Lampasas, and Burnet. Recent recovery of records, reported herein, for seven counties in west-central Texas clarify the distribution of spotted skunks in Texas (Fig. 1). Specimens are deposited in the Angelo State Natural History Collections (ASNHC), Angelo State University and the Texas Cooperative Wildlife Collections (TCWC), Texas A&M University.

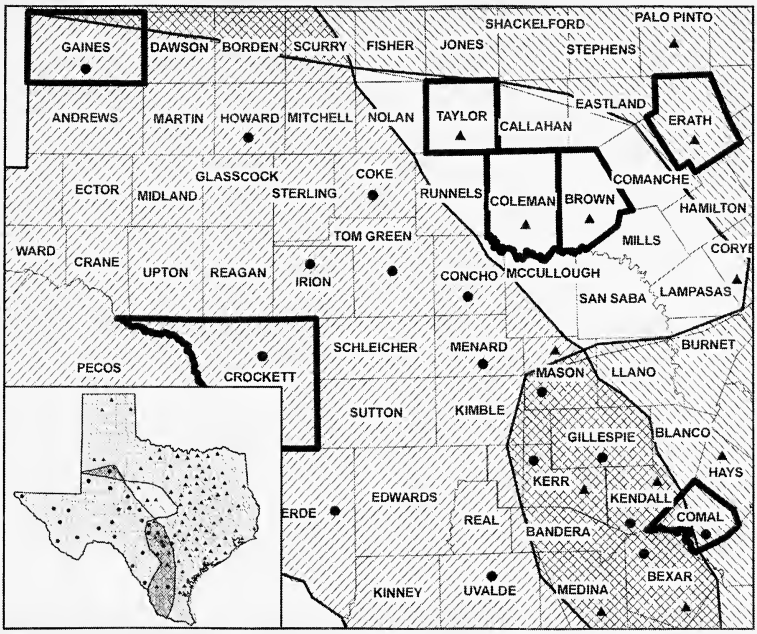

Figure 1. Range map of *Spilogale gracilis* [▨] and *S. putorius* [▧] in Texas adapted from Schmidly (2004) identifying the area in central Texas (white) where *Spilogale* was thought to be absent (inset). Area of potential range overlap between the two species is identified by ▩. Map indicating records of *S. gracilis* [●] and *S. putorius* [▲] from central Texas with county records reported herein outlined in bold. The Erath County record was previously reported in Goetze et al. (2004).

On 21 March 2003, a road-killed specimen (ASNHC 13370) of *Spilogale putorius* was recovered from Coleman Co., 1.6 km E Valera (UTM 14R 449697, 312919). This animal had an intermediate pattern of pelage spots and stripes between typical *S. gracilis* and *S. putorius;* however, white portions of the tail were the same as *S. putorius* from east Texas. The specimen was an adult male with testes measuring 14 mm. Body measurements (in mm) were: total length-470, tail length-175, hind foot length-46, and ear length-24; mass was 0.6 kg.

Another specimen of *S. putorius* was donated to Howard Payne University in Brownwood and subsequently transferred to the

Angelo State Natural History Collections (ASNHC 13369). This animal was salvaged 1.6 km NW Brownwood on Route 84 and was taken on 22 February 2004. It was a scrotal male with testes measuring 24 mm. Measurements of this skunk (as above) were 490-168-47-22 and its mass was 650 g. The pattern of stripes and spots was somewhat intermediate between the two species, but was generally typical of eastern spotted skunks.

A road-killed specimen (ASNHC 13372) of *S. putorius* was recovered in Taylor County, 6.9 km N, 3.7 km E Tuscola on 23 April 2007. This animal, also a scrotal male with testes measuring 20 mm, had body measurements of 469-158-45-23 and a mass of 0.8 kg. Its pelage also was intermediate in striping pattern between *S. gracilis* and *S. putorius*, but shared the pattern of little white on the ventral side of the tail, typical of *S. putorius*.

A road-killed specimen of *S. gracilis* (TCWC 59610) was collected on 27 July 2008 on Interstate 10 in Crockett County, 18.2 km west of Ozona (14 R 270901, 3397611) at 711 m elevation. This western spotted skunk was a male measuring 460-150-46-27 with a mass of 726 g.

In addition to the specimens described above, two photographs verifying records of western spotted skunks, *S. gracilis*, in Gaines and Comal counties have come to the attention of the authors. The first photograph is of a Gaines County animal which was killed in a house 1.6 km S Seminole (UTM 13S 0721230, 3619012) on 2 February 1994. This record is at the northern limit of the range of *S. gracilis* at the southern extreme of the Texas panhandle (Fig. 1). A second photograph is of a western spotted skunk that was trapped and released about 24 km N of New Braunfels in Comal County, (UTM 14R 581695 3310470). This record (Fig. 1) extends the range of the western spotted skunk about 16 km east of its range as depicted by Schmidly (2004). Both photographs have been archived at the ASNHC.

Goetze et al. (2004) reported an additional county record for Erath County, Texas, which is housed in the mammal collection at Tarleton State University (TSU 1264). This specimen was collected 9.6 km WNW Stephenville on 10 November 1971. Its gender was unknown.

All records, excluding Goetze et al. (2004), are new for counties in Texas, based on Schmidly (2004), and four are range extensions. The records of eastern spotted skunks in Brown, Coleman, and Taylor counties fill much of the gap (Fig. 1) in the reported range of spotted skunks in central Texas (Schmidly 2004). Although these records may indicate recent range expansions, it is just as likely that these represent historical occupation by *Spilogale putorius* in this region and the species simply has not been detected previously. Compilation of these *Spilogale* records indicates that the entire state is occupied by at least one species and areas of historical range overlap still remain. Additional monitoring of this genus in Texas should further help increase the understanding of its distribution and conservation status across the state.

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INDEX TO VOLUME 60 (2008) THE TEXAS JOURNAL OF SCIENCE

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