Section 6 (Texas Traditional) Report Review

Attachment to letter dated JAN 05 2005

Project Title: Griffith League Ranch Habitat Conservation Plan

Final or Interim Report? Final

Job #: **WER** 87 **Grant #:** E-20

Reviewer Station: Austin ESFO

Lead station was contacted and concurs with the following comments:
☐ Yes ☐ No ☒ Not applicable (reviewer is from lead station)

Interim Report (check one):

☐ is acceptable as is

☐ is acceptable as is, but comments below need to be addressed in the next report

☐ needs revision (see comments below)

Final Report (check one):

☐ is acceptable as is

☒ is acceptable, but needs minor revision (see comments below)

☐ needs major revision (see comments below)

Comments:

This report was well-written and complete, the only request we have is that you please provide a black and white reproducible version of the map (Figure 1) found on page 1:10 of the report.

FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. E - 20

Endangered and Threatened Species Conservation

Project WER 87:

Griffith League Ranch Habitat Conservation Plan

Prepared by:

Michael J. Forstner

TEXAS PARKS & WILDLIFE

Robert Cook
Executive Director

John Herron
Program Director, Wildlife Diversity

Mike Berger
Division Director, Wildlife

27 September 2004

PERFORMANCE REPORT

STATE: Texas **GRANT NUMBER:** E - 20

GRANT TITLE: Endangered and Threatened Species Conservation

REPORTING PERIOD: 1 Sept 01 – 31 Aug 04

PROJECT NUMBER: WER 87

PROJECT TITLE:

Griffith League Ranch Habitat Conservation Plan

OBJECTIVE(S):

As per Project Statement attached to Grant Agreement E-20:

- obtain additional funding to implement conservation measures for the endangered Houston toad on Griffith League Ranch.
- continue and expand the on-going monitoring and research program on Griffith League Ranch to include dispersal and distribution of the species on the tract.
- apply research-based adaptive management strategies to management of the Houston toad.
- prepare and implement education and outreach programs which support the eventual recovery of the Houston toad, including development of "best management practices" which can be shared with other land managers in the region.

As per Project Statement attached to TPWD Contract #100656:

a) to continue and expand the on-going monitoring and research program on Griffith League Ranch to better define:
- habitat use by the Houston toad.
- distribution and density of adults and sub-adults.
- larval survivorship, distribution and the effects of predation.
- post-metamorphic dispersal and survivorship.
- vegetation and wildlife baseline data across habitats on the tract.

b) based upon results of these studies, to apply research-based adaptive management strategies to management of the Houston toad.

Segment Objectives:

The segment objectives below are from TPWD Contract #100656; accomplishments are summarized in Attachment A:

1.0 Continue the current ecological and population study on the Houston toad and the herptofaunal assembly with which it is sympatric, begun by SWTSU on Griffith League Ranch in 2001.

1.1 Provide funding for SWTSU to employee one full-time Ph.D. candidate and two half-time Master-level graduate students to enable continuation of on-going cohort studies for data on recruitment, dispersal and survivorship of the Houston toad. **(Years 1 - 3)**

1.2 Establish additional drift fences and an array of experimental Houston toad breeding ponds and purchase telemetry equipment. **(Year 1)**

1.3 Monitor capture and pond arrays, track dispersal of Houston toads and document results. **(Years 1 - 3)**

1.4 Complete herptofaunal survey and prepare final report. **(Year 3)**

2.0 Initiate collection of baseline data for the vegetation and wildlife (mammals and birds) components of habitats across Griffith League Ranch.

2.1 Provide funding for SWTSU to employee one half-time Master-level graduate student to establish and monitor vegetation and wildlife transects. **(Years 1 - 3)**

2.2 Monitor vegetation and wildlife transects. **(Years 1 - 3)**

2.3 Complete vegetation and wildlife surveys and prepare final report. **(Year 3)**

Summary Of Progress:

Please see Attachment A.

Significant Deviations:

Due to reduced funding, the Objectives were renegotiated for the TPWD Contract with this subgrantee. As such, the last of the objectives from the Project Statement attached to the E-20 Grant Agreement; specifically,

- prepare and implement education and outreach programs which support the eventual recovery of the Houston toad, including development of "best management practices" which can be shared with other land managers in the region.

was deleted from the TPWD Contract #100656 with the subgrantee. Nonetheless, much of this work was done and is summarized in a memo to Dr. Mike Forstner from Martin Payne (dated 26 August 2004; included with Attachment A).

Preliminary Findings:

Please see Attachment A.

Location: Griffith League Ranch, Bastrop County, Texas

Cost: $117,250.36 (Federal)

Prepared by: Michael J. Forstner **Date:** 27 September 2004

Approved by: Neil (Nick) E. Carter **Date:** 11/15/2004

THE HOUSTON TOAD IN CONTEXT 2000-2004

EDITORS:

MICHAEL R.J. FORSTNER[1] AND TODD M. SWANNACK[2]

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AUGUST 1, 2004

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PREFACE

This volume is a compilation of the results from an integrated research design conducted on the Griffith League Ranch, Bastrop County, Texas during the period from 2001-2004. That research program sought novel field and laboratory data on the Houston toad, its sympatric flora and fauna, and factors affecting toad occupancy across the system within which the toad persists today. That ecosystem, the Lost Pines, represents a unique region of Texas and one containing a significant number of endemic and/or unique taxa in addition to the Houston toad. As a consequence, while our focus was *Bufo houstonensis*, we developed an integrated, but broadly focused program seeking data on the toad in context with the landscape in which it survives today.

The chapters that follow are primarily investigations with specific attention on the Houston toad (Chapters 1-3), but also include chapters on the wildlife and vegetation (5-6). We sought to provide all of these data in one volume, in a user-friendly format containing all of the information we gathered. To achieve part of this goal, we have included a comprehensive literature cited and substantial appendices. The appendices include data tables, the current group of peer reviewed publications resulting from this project thus far, the annual technical reports for the project, and the public presentations made by the researchers during this project. The scope of the project was large and several graduate students were able to gather enough data to write their respective theses. Where possible these form chapters contributed to this compilation. Our goal was to provide a single resource documenting our current research being carried out in the Lost Pines Ecosystem as the final report for the project. Obviously the work and the analyses continue and these future publications are also a direct result of the collaborative efforts of the management and funding agencies, collaborative partners, and the researchers themselves.

Timely peer reviewed publication of the results from scientific endeavors is an important and necessary part of academic life, and the format of this document reflects those needs. Each section was written with the intent of submitting it to a peer-reviewed journal, so the format among chapter sections will vary, according to the requirements of the target journal.

Each graduate student employed by the funds from the Section 6 grant were required to present their research at scientific meetings. Those presentations, plus any other presentation resulting from this research, are included at the end of the document, as they provide succinct summaries of each individual project. In one case the work has contributed to an on-line course, sponsored by the National Wildlife Federation, and the relevant world wide web address and title is provided for it, as well.

The scope of this project was larger than a handful of hard-working graduate students and professors could handle by themselves. Many people donated considerable amounts of time, and deserve special thanks: Jim and Mary Dixon, Josephine Duvall, Phil

Koepp, Ruth Forstner, Chris Nice, Dan Morris, and especially the members, candidates and Elangomats in the Tonkawa Lodge of the Order of the Arrow. This project would not have been as successful if these folks had not been so dedicated to the conservation of the Houston toad and its habitat.

We would not have been able to accomplish as much as we did without the generous support from the Boy Scouts of America, Capitol Area Council. Their dedication to the conservation of both the Houston toad and Lost Pines ecosystem is unparalleled. The United States Fish and Wildlife Service, ALCOA, Texas Parks and Wildlife, and the United States Geological Survey were also critical partners and funding sources throughout this study.

Michael R. J. Forstner, Ph.D.
Todd M. Swannack, M.Sc.

August 1, 2004

Introduction to the Houston toad and its sympatric fauna and flora with a description of the Study Area (Griffith League Ranch, Bastrop Co., TX)

Phil Koepp[1], Michael R.J. Forstner[2], and James R. Dixon[3]
[1]4805 Fieldstone Dr, Austin, TX, 78735; [2]Department of Biology, Texas State University at San Marcos, San Marcos, TX 78666; [3]Department of Wildlife and Fisheries Sciences, Texas A & M University, College Station, Texas 77843

1.1 Endangered and threatened species on the Griffith League Ranch

The Houston toad is currently the only species in Bastrop County on the federal endangered species list. The State of Texas (Texas Parks and Wildlife Department) also lists the species as endangered. The bald eagle (*Haliaeetus leucocephalus*) is listed as threatened both by the Service and the State of Texas. The Service considers the reddish egret (*Egretta rufescens*), white-faced ibis (*Plegadis chihi*), Audubon's oriole (*Icterus graduacauda audubonii*), loggerhead shrike (*Lanius ludovicianus*) and Texas horned lizard to be "species of concern". Currently, available data do not support federal listing of any of these species. The State of Texas recognizes the reddish egret, white-faced ibis, Texas horned lizard and the canebrake (timber) rattlesnake as threatened. Other than the Houston toad, the canebrake rattlesnake is only state-listed species known to occur on the Griffith League Ranch. In Bastrop County, it has been found only on the Griffith League Ranch and in Bastrop State Park (pers. obs. Forstner, 2002). No federal or state-listed plants are known to occur on the Griffith League Ranch or in Bastrop County at the current time.

1.1.1 The Houston Toad

The Houston toad is endemic to south-central Texas. John C. Wottring first noted the toad near Houston, Texas in the late 1940's. In 1953 Ottys Sanders described it as a distinct species. On-going habitat destruction and a severe drought in the 1950's raised concerns for the future of the species (Seal, 1994). The Houston toad was first listed as endangered in 1970 under the Endangered Species Conservation Act of 1969 (35 FR 16047). The endangered classification was continued with passage of the Endangered Species Act of 1973. The Service designated critical habitat for the Houston toad in Bastrop and Burleson counties in 1978 (43 FR 4022). The southern half of the Griffith League Ranch lies within federally designated critical habitat.

The species is currently known to occur in only nine Texas counties: Austin, Bastrop, Burleson, Colorado, Lavaca, Lee, Leon, Milam and Robertson. The Bastrop County population is considered to be the most robust and sustainable of the remaining populations (Seal, 1994; U.S. Fish and Wildlife Service, 1995). The Houston toad has been extirpated from Fort Bend, Harris and Liberty counties (Price, 1990a). Primary threats to survival of the Houston toad include habitat destruction and degradation, fragmentation of habitat, predation, inter-specific competition and hybridization, contamination by chemical herbicides, pesticides and fertilizers and prolonged drought.

There is a high correlation between the occurrence of the Houston toad and outcrops of the Eocene epoch Sparta Sand, Weches, Queen City Sand, Reklaw and Carrizo Sand formations (Seal, 1994). A large area of eastern Bastrop County is underlain by these formations. The Carrizo Sand and Reklaw formations underlie the eastern 73 percent of the Griffith League Ranch. The Calvert Bluff formation of the Wilcox Group underlies 27 percent of the property on its western side (Procter, *et al,* 1974).

Houston toads are usually associated with deep friable sandy soils. Ninety-eight percent of the Griffith League Ranch is covered with Patilo-Demona-Silstid and Axtell-Tabor soils, both series being characterized by deep sands with relatively shallow perched water tables. Sayers soils, on another 2 percent of the tract, are a deep fine sandy loam (Baker, 1979). The Houston toad is thought to burrow into all of these sandy soils to escape winter cold (hibernation) and summer heat and drought (aestivation).

The typical adult Houston toad is two to three inches long, with females being larger and bulkier than males. Coloration is generally speckled, light brown varying to black, sometimes with yellow patches. Some individuals may appear to have a slightly reddish, yellowish or grayish hue overall. Small dark spots are often found on the pale undersides. There may be a variable white stripe down the back and irregular white streaks along the sides. Dark bands extend from each eye to the mouth and also occur on the legs. Males have a dark throat that appears bluish when distended. The species' mating call is a high-pitched ululating trill lasting for four to eleven seconds (U.S. Fish and Wildlife Service, 1984).

Life expectancy of the Houston toad is about four years (Price, 1992). Males can reach sexual maturity in captivity at about one year, females at two years (Quinn, 1981). The toads are generally active between January 15 and June 1, but may emerge as early as late December and remain active until late June, depending upon environmental conditions. Rainfall and warm nighttime temperatures initiate breeding activity, usually in February and March (Hillis, Hillis and Martin, 1984; Dixon, 1982; Dixon, Dronen, Godwin and Simmons, 1990; Price, 1990; Price and Yantiss, 1993). Dark phases of the moon influence nighttime activity (Price, 1990b).

For breeding and maturation of tadpoles, the species requires shallow, non-flowing ephemeral (lasting 30 to 60 days) pools, or permanent bodies of water with shallow, slow-flowing pools or eddies. Successful breeding and survival of tadpoles requires good water quality, availability of food and protection from predators. Female toads lay 500 to 6,000 eggs (Kennedy, 1962; Quinn and Mengden, 1984; Quinn and Mays, 1987). Less than one percent of the eggs survive to maturity (Seal, 1994).

Houston toad activity has been observed on warm, wet, humid nights during both its breeding and non-breeding season. However, little is known about its life history during the non-breeding season. On the Griffith League Ranch, native loblolly pine-oak woodland-savannah covers most (88 percent) of the tract. Native forbs and grasses provide shelter and insects for forage. Ground cover allows the Houston toad easy travel in this vegetation type. Individuals have previously been documented to travel up to 0.95 of a mile between breeding ponds (Price, 1992). The species is known to seek protection under rocks, logs, leaf litter, refuse piles, and in small animal burrows during daytime hours. While preferring deep sandy soils and woodlands, the toad will also breed and

travel in open areas and on non-sandy soils provided there are woodlands and sandy soils nearby.

In 1993, Dr. Andrew Price of Texas Parks and Wildlife Department (TPWD) documented Houston toads at three ponds on the Griffith League Ranch (Price, 1993). Between February 7, 2000 and the spring of 2004, Dr. Michael Forstner has conducted a presence/absence survey for Houston toads on the tract. He documented the species at 15 of 19 existing ponds, two of seven drainage systems (below Pond 12 and Alum Creek) and at one location northwest of Pond 8. In addition to his presence/absence surveys, grant funding provided for studies of the Houston toad during its non-breeding season from March 2001 through August 2004. In reporting the results from the study, he and his coauthors document dispersal, mortality and relevant ecology of juvenile Houston toads, population studies of the adult Houston toads on the Griffith League Ranch, the sympatric vertebrate fauna, and under the direction of Drs. Randy Simpson and John Baccus respectively, characterized floral, avian, and game animal components of the Houston toad's habitat on the ranch.

1.1.2 State-Listed Species on the Griffith League Ranch

The canebrake rattlesnake, listed as threatened by the State of Texas, is the only state-listed species other than the Houston toad that occurs on the Griffith League Ranch. It has been found only on the Griffith League Ranch and within Bastrop State Park in Bastrop County (pers. obs. Forstner, 2002). This seldom-seen snake occupies moist lowland and hilly pine and mixed hardwood forest. It is normally found less than a mile from permanent water sources (Werler and Dixon, 2000). State law prohibits take (injury, killing, capturing), possession, transportation or sale of any state-listed species. Texas law does not protect habitat of state-listed threatened and endangered species.

1.2 WILDLIFE

Invertebrate fauna on the property have not been systematically inventoried. However, eight species of tiger beetle (*Cicindela spp.*) that are geographically separated from their east Texas pineywoods populations are known to occur in the vicinity and some of these have now been recognized at the species level (Taber and Fleenor, 2003). Numerous mounds of leaf-cutter ants (*Atta sp.*) have been observed in wooded areas and the red imported fire ant (*Solenopsis invicta*) has been noted in all large pastures and along roadways inside the property.

Many migratory bird species common to the central flyway are found in the area. Birds observed on the tract include the black vulture (*Coragyps atratus*), turkey vulture (*Cathartes aura*), red-shouldered hawk (*Buteo lineatus*), red-tailed hawk (*B. jamaicensis*), wild turkey (*Meleagris gallopavo*), barred owl (*Strix varia*), blue jay (*Cyanocitta cristata*), Carolina chickadee (*Parus carolinensis*), northern mockingbird (*Mimus polyglottos*) and northern cardinal (*Cardinalis cardinalis*). Other common birds likely to occur include the eastern screech owl (*Otus asio*), ruby-throated hummingbird (*Archilochus colubris*), red-bellied woodpecker (*Melanerpes carolinus*), tufted titmouse (*Parus bicolor*), Carolina wren (*Thyrothorus ludovicianus*), white-eyed vireo (*Vireo griseus*), northern parula (*Parula americana*), summer tanager (*Piranga rubra*), indigo bunting (*Passerina cyanea*), painted bunting (*P. ciris*), lark sparrow (*Chondestes*

grammacus) and white-throated sparrow (*Zonotrichia albicollis*) (Freeman, 1996; Scott, 1987). The southwestern-most range of the pileated woodpecker (*Dryocopus pileatus*) and pine warbler (*Dendroica pinus*) and the western range extension of the Kentucky warbler (*Oporornis formosus*), hooded warbler (*Wilsonia citrina*) and Swainson's warbler (*Limnothlypis swainsonii*) occur in Bastrop County (Bastrop County Environmental Network, undated).

Mammals observed on the Griffith League Ranch include the white-tailed deer (*Odocoileus virginianus*), raccoon (*Procyon lotor*), striped skunk (*Mephitis mephitis*), jackrabbit (*Lepus californicus*), coyote (*Canis latrans*), red fox (*Vulpes vulpes*), gray fox (*Urocyon cinereoargenteus*), bobcat (*Lynx rufus*), ringtail cat (*Bassaricus astutus*), opossum (*Didelphus virginiana*), fox squirrel (*Sciurus niger*), eastern cottontail (*Sylvilagus floridanus*) and nine-banded armadillo (*Dasypus novemcinctus*). The red bat (*Lasiurus borealis*), eastern mole (*Scalopus aquaticus*), plains pocket gopher (*Geomys bursarius*), Attwater's pocket gopher (*G. attwateri*), hispid pocket mouse (*Perognathus hispidus*), white-footed mouse (*Peromyscus leucopus*), northern pygmy mouse (*Baiomys taylori*), hispid cotton rat (*Sigmodon hispidus*) and eastern woodrat (*Neotoma floridana*) are known to occur in the area and may occur on the tract. A disjunct population of short-tailed shrew (*Blarina* sp.), found in an area of sandy soils, new growth loblolly pine and old fallen logs within Bastrop State Park, also occurs on the Griffith League Ranch (Dixon, Dronen, Jr. and Schmidly, 1989; Dixon, Dronen, Jr., Godwin and Simmons, 1990; Dixon, 1987; Davis, 1960). The previous ambiguity in taxonomic identification for the Bastrop County shrew population is another aspect that is now resolved based on work completed during this study (see Chapter 6 below)

Amphibians documented on the property during the study period include the tiger salamander (*Ambystoma tigrinum*), southern leopard frog (*Rana sphenocephala*), bullfrog (*R. catsbeiana*), cricket frog (*Acris crepitans*), gray treefrog (*Hyla versicolor*), green treefrog (*Hyla cinerea*) two narrowmouth toads (*Gastrophryne olivacea* and *G. carolinensis*), spadefoot toad (*Scaphiopus hurteri*), Gulf Coast toad (*Bufo valliceps*), Woodhouse's toad (*B. woodhousei*) and Houston toad (*B. houstonensis*). The Texas toad (*Bufo speciosus*), Rio Grande leopard frog (*Rana berlandieri*) and chorus frogs (*Pseudacris streckeri* and *clarki*) might be found on the tract.

Reptiles observed include turtles, lizards, and snakes. Two turtles have been found on the ranch, the common snapping turtle (*Chelydra serpintina*) and three-toed box turtles (*Terepene carolina*). Numerous lizards including the ground skink (*Scincella lateralis*), the green anole (*Anolis carolinensis*), the Texas spiney lizard (*Scleroporus olivaceaous*), eastern fence lizard (*S. undulatus*), and six-lined racerunner (*Cnemidophorus sexlineatus*). Snakes found on the site include the blind snake (*Leptotyphlops dulcius*), ground snake (*Storeria dekayi*), Ribbon snake (*Thamnophis proximus*), blotched water snake (*Nerodia erythrogaster*), broadbanded water snake (*Nerodia fasciata*) coachwhip (*Masticophis flagellum*), flat-headed snake (*Tantilla gracilus*), Eastern hognose (*Heterodon platirhinos*), Texas rat snake (*Elaphe obsoleta lindheimeri*) broad-banded copperhead (*Agkistodon contortrix*), western cottonmouth (*A. piscivorus leucostoma*), Texas coral snake (*Micrurus fulvius tenere*) and canebrake rattlesnake (*Crotalus horridus atricaudatus*) (Forstner pers.comm.. 2002,). Other reptiles could include the mud turtles (*Kinosternon flavescens* and *subrubrum*), soft-shelled turtle (*Trionyx sp.*), large skinks

(*Eumeces sp.*), Glass lizards (*Ophiosaurus attentuatus*), Texas horned lizard (*Phrynosoma cornutum*), Mediterranean gecko (*Hemidactylus turcicus*), Texas glossy snake (*Arizona elegans*), Eastern racer (*Coluber constrictor*), Corn snake (*Elaphe guttata*), Prairie kingsnake (*Lampropeltis calligaster*), speckled kingsnake (*Lampropeltis getula*), Louisiana milksnake (*Lampropeltis triangulum*), rough green snake (*Opheodrys aestivus*), Texas lined snake (*Tropidoclonion lineatum*), and rough earth snake (*Virginia striatula*) (Ahlbrandt and Forstner, 2002; Dixon *et al.*, 1989, Dixon *et al.*, 1990; Dixon, 1987)

1.2.1 Species of Concern potentially occurring on the Griffith League Ranch

Species of concern are species for which there are indications of vulnerability, but for which there is insufficient information to support their listing as threatened or endangered. Species in this category receive no protection under the Endangered Species Act of 1973. Of the species of concern noted for Bastrop County, the Audubon's oriole is an uncommon tropical resident in south Texas. It is not likely to occur on the Griffith League Ranch. Neither the reddish egret (except as a transient) nor the white-faced ibis are likely to occur on the tract as suitable habitat is lacking. Suitable habitat does exist for the loggerhead shrike and it is possible that this species could be recorded on the property in the future. The Texas horned lizard is not known to occur on the Griffith League Ranch. Given its association with sandy soils, however, it could potentially occur on the tract.

1.3 Vegetation on the Griffith League Ranch

Vegetation on the Griffith League Ranch is typical of the Lost Pines area of Bastrop County: a loblolly pine (*Pinus taeda*) and mixed deciduous woodland interspersed with open, grassy areas. This loblolly pine woodland is disjunct from the "pineywoods" region of east Texas, being separated geographically by over 100 miles. Although rainfall in the Bastrop area averages 8 to 20 inches per year less than in the pine forests of east Texas, loblolly pines occur in Bastrop County because of high humidity, the timing and amount of rainfall, occurrence of deep sandy acid soils and the ability of the species to efficiently utilize available water. The loblolly pine and several associated plant and animal species reach their westernmost range extensions in this area. This loblolly pine-post oak-savannah ecosystem is an outstanding example of a fire-adapted, fire-climax community (Baker, 1979; Gould, 1962). It offers excellent opportunities for studies and discussions related to biogeography and plant and animal dispersal.

The dominant overstory on the Griffith League Ranch is composed of loblolly pine, post oak (*Quercus stellata*), blackjack oak (*Q. marilandica*) and eastern red cedar (*Juniperus virginiana*). Some sandjack oak (*Q. incana*) can also be found. Typically the pines are found in drainages and the oaks on ridge tops. However, they are components of mixed forests in many locations on this particular tract. American elm (*Ulmus americana*), cedar elm (*U. crassifolia*), hackberry (*Celtis spp.*) and hickory (*Carya spp.*) are found along drainages. Cottonwood (*Populus deltoides*) occurs in wetter drainages such as Alum Creek and the unnamed tributary of Piney Creek on the west side of the property.

Understory vegetation contains yaupon (*Ilex vomitoria*), possumhaw (*I. decidua*), southern wax-myrtle (*Myrica cerifera*), American beautyberry (*Callicarpa americana*),

surface is a loose loamy fine sand about 10 inches thick. Below the surface layer is about 18 inches of loamy fine sand over a thicker layer (40 inches) of sandy clay loam. Below the sandy clay loam is a 70-inch thick layer of clay loam, 40 inches of sandy clay loam and 80 inches of a fine sandy loam. Permeability is moderate, runoff is slow and available water capacity is medium. Erosion hazard is moderate. Silstid soils commonly support blackjack oak, post oak and yaupon with an understory of mid- and tall grasses. These soils are useful for recreation and wildlife habitat (Baker, 1979).

Demona Series soils occur mostly on foot slopes and in drainages across uplands, but can also be found on ridge tops. These are deep, gently sloping (1 to 5 percent) moderately well-drained sandy soils. The surface is typically a 5-inch layer of loamy fine sand overlying a thicker layer (23 inches) of loamy fine sand. Permeability is slow and runoff is slow to medium. After heavy rains, Demona sands can have a perched water table at 24 to 36 inches. Erosion hazard is moderate. Blackjack oak, post oak and bunchgrass are typically associated with Demona soils, providing range and wildlife habitat (Baker, 1979).

Three Axtell series phases occur on the property: Axtell fine sandy loam (1 to 5 percent slopes), Axtell fine sandy loam (2 to 5 percent slopes) and Axtell-Tabor Complex (1 to 8 percent slopes). These well-drained to moderately well-drained soils occur on nearly level to strongly sloping side slopes, eroded ridge tops and in drainages. A 5 to 14 inch surface layer of fine sandy to gravelly sandy loam characterizes Axtell soils. Lower layers are slowly permeable, runoff is slow to rapid and available water capacity is high. Permeability, corrosivity and shrink-swell potential limit development on Axtell soils. Erosion hazard is moderate to severe and widely spaced gullies are typical. Axtell soils support post oak, blackjack oak and bunchgrass. These soils are often are associated with native grass pastures, crops and woodland range (Baker, 1979).

Tabor fine sandy loams comprise only a small percentage (about 2 percent) of the soil found on the ranch. These deep, nearly level to sloping (1 to 3 percent) moderately well-drained loamy soils occur on ridge tops, foot slopes and in drainages. The surface is a 6-inch layer of sandy loam over a 9-inch layer of fine sandy loam and a thicker layer (38 inches) of clay. Permeability is very slow, runoff is slow to medium and available water capacity is high. Erosion hazard is moderate. Associated vegetation is post oak, blackjack oak, elm, hackberry and bunchgrass. These soils are normally used for range and pasture (Baker, 1979).

Sayers series soils are deep and nearly level (less than 1 percent slopes) excessively drained sandy soils that occur on floodplains and bottomlands that are subjected to frequent flooding. They formed in recent sandy alluvium and can occur in areas 100 to 500 feet wide and several miles long. The surface is a fine sandy loam about 10 inches thick, with some areas having a surface layer of loam, loamy fine sand or fine sand. Beneath the surface layer is up to 24 inches of slightly stratified loamy fine sand and about 60 inches of fine sand. Permeability is rapid, runoff slow, and available water capacity is low. A perched water table can be found at 60 to 120 inches during spring and fall. Erosion hazard is slight. Native vegetation on Sayers soils includes tall grasses, elm and cottonwood. These soils will support a few crops and are used as wooded and improved pasture and hayfields, native wildlife and livestock range and wildlife habitat (Baker, 1979).

Only five small areas (42 acres) of Jedd Series soils occur on the property. These are moderately deep, sloping to moderately steep (5 to 20 percent), well-drained stony loamy soils found on small narrow ridge tops and short hilly side slopes in uplands. A 4-inch surface layer ranges from a gravelly sandy loam to a gravelly loamy sand. This layer is composed of 30 to 70 percent small siliceous pebbles and as much as 35 percent platy sandstone cobbles and stones. It can contain about 5 to 10 percent sandstone outcrops. A gravelly sandy loam about 8 inches thick with cemented sandstone fragments above a clay and sandy clay is found below the surface layer. Permeability is moderately slow and available water capacity is medium. Erosion hazard is severe. Associated vegetation is typically post oak and blackjack oak with an understory of yaupon, mulberry and bunchgrass supporting woodland and wildlife habitat (Baker, 1979).

2.3 Critical Habitat on the Griffith League Ranch

Approximately 2,712 acres (56 percent) of the Griffith League Ranch are included in federally designated critical habitat for the Houston toad. The Service designated critical habitat for the species in Bastrop and Burleson counties in 1978 (43 FR 4022). Critical habitat in Bastrop County is delineated on the west by State Highway 95 and on the south by the Colorado River. The eastern limit, 97 degrees 7 minutes 30 seconds west longitude, is over four miles from the eastern corner of the Griffith League Ranch. The northern limit, latitude 30 degrees 12 minutes 00 seconds north, bisects the Griffith League Ranch so that its northern half is excluded from critical habitat while the southern half is within federally designated critical habitat.

Determination of critical habitat for the Houston toad pre-dates the Service's 1984 regulations and procedures for designating critical habitat. Therefore, the primary elements of the species' habitat were not detailed at the time critical habitat was listed. Primary elements of critical habitat for the Houston toad would likely include: shallow, non-flowing ephemeral pools or permanent water bodies with slow flowing pools or eddies for breeding and development of tadpoles; good water quality; cover of grasses and forbs that provide for availability of food and protection from predators; deep, friable, sandy soils for burrowing and aestivation or hibernation; and native pine and post oak woodlands-savannah (Seal, 1994; U.S. Fish and Wildlife Service, 1995). There is also a high correlation between occurrence of the Houston toad and outcrops of the Eocene Reklaw and Carrizo Sand formations (Seal, 1994). These elements are all present on the Griffith League Ranch.

No federal critical habitat has been designated for the bald eagle in Bastrop County. Texas law does not provide for protected habitat for state-listed species.

2.4 Wetlands on the Griffith League Ranch

Nineteen ponds are known to occur on the Griffith League Ranch. Thirteen ponds and the headwaters of one creek are noted on the 1993 U.S. Department of the Interior Fish and Wildlife Service's National Wetlands Inventory maps (U.S. Fish and Wildlife Service, 1993). One of the thirteen ponds, classified as palustrine, open water and permanently flooded, was not located. It appears that this site was either mapped in error or has been lost due to changes of a meandering stream channel. The head of the unnamed creek that flows directly into Lake Bastrop from the southern corner of the tract

is designated as riverine, intermittent, streambed and seasonally flooded. The portion of this drainage on the Griffith League Ranch does not meet these criteria and probably should not be listed as a wetland.

Four of the thirteen listed ponds are classified as palustrine, open water and permanently flooded. Three of these are one acre or less in size; the fourth is approximately three acres. Seven of the thirteen ponds are classed as palustrine, open water, permanently flooded and diked. Of these seven, one is about three acres in size, another about two acres and the other five are one acre or less. Two ponds of one acre or less are listed as palustrine, emergent, persistent, temporarily flooded and diked. All 13 ponds mapped as wetlands appear to be constructed stock ponds, possibly natural depressions that were scooped out to enlarge them. It was not possible to determine if any of the ponds are related to naturally occurring seeps or springs.

Two streams on the Griffith League Ranch are not listed on the National Wetlands Inventory, but probably should be. Alum Creek, in the eastern corner of the tract, has year-round water flowing along its length within the property boundaries. This stretch of Alum Creek is impacted by livestock activity upstream of the ranch. The area where Alum Creek flows off the property is a low-lying, marshy area. The streambed downstream of the Finger Pond (Pond 12) appears to meet wetland criteria as it has permanent pools along more than a mile of its reach toward the northwestern boundary of the property. The water source for this stream appears to be one or more natural seeps or small springs. Some of the pools along its run contain persistent perennial aquatic and semi-aquatic vegetation (Forstner, 2000). Likewise both of these locations have supported Houston toad chorusing during the study period.

During the wet winter of 2002, it was noted that almost all of the drainages on the ranch flow for some time after periods of heavy or extended rainfall. Also, shallow depressions in uplands appear to hold water after heavy or extended rainfall. Shallow pools along the intermittent drainages and the upland depressions, while not considered wetlands, could serve as breeding sites for the Houston toad provided that steep banks do not present a barrier for the species or the pools do not dry too fast.

2.5 WATER RESOURCES AND WATER QUALITY

A north-south trending ridge with elevations of 600 to 650 feet divides the Griffith League Ranch hydrologically. The western and northwestern portions of the ranch are drained by intermittent tributaries of Piney Creek, which empties into the Colorado River upstream of Bastrop. Spicer Creek and an unnamed creek drain the southwestern portion of the property. These two creeks are intermittent, head on the property and drain into Lake Bastrop about 1.5 miles to the southwest. Spicer Creek continues below the dam on Lake Bastrop and empties into Piney Creek. Alum Creek, a short segment of which passes through the easternmost corner of the tract, is the major drainage east of the Griffith League Ranch. Several unnamed intermittent branches of Alum Creek head on the property, as does Price Creek, the only named tributary on the tract's east side. Alum Creek empties into the Colorado River below Bastrop. Water flows year-round in this stretch of Alum Creek. While its quality has not been determined, it appeared eutrophic in 2000, probably due to livestock grazing on and upstream of the tract. Despite removal of all livestock from the property in 2001, the creek has remained eutrophic in character.

Of the 19 known ponds on the ranch, many appear to hold water year-round. Most of the ponds appear to be diked ponds, probably constructed to provide water for livestock. Judging from the size of pine trees growing in the dikes, most of the ponds appear to be old construction. In 2000, those ponds having heavy stock use were eutrophic, devoid of vegetation on their perimeters and had little evidence of diverse aquatic life. Water quality in the ponds ranged from excellent to poor during the 2000 season, depending on the amount of stock use each received. With heavier rainfall and removal of livestock during the 2001 season, water quality in all the ponds improved and vegetation covered their banks. In addition to the ponds, several dry upland depressions were noted in 2000. Vegetation associated with these dry depressions indicated that they might hold water for some time after precipitation events, particularly during wet periods. This was, in fact, observed during the winter of 2001, summer of 2002, and spring of 2003. At least one of these depressions served as a breeding site for the Houston toad. However, it dried before the tadpoles emerged (Forstner 2001).

Griffith League Ranch is underlain by two major aquifers of the region: the Wilcox Group and the Carrizo Sand. Only one well, State Well No. 58-55-402, has been recorded on the property. This well, drilled in 1952 for domestic and stock use, taps the Wilcox Group. Water from this well, which was apparently never used, was described as "soft" by the driller. Quality of the water from this well is unknown (Palafox, 1996). Several other wells, probably used to water livestock, are known to exist on the tract but no information related to them has been located. A hand-dug, stone-line well was reported by a Boy Scout Troop in January 2000. Its exact location is unknown and quality and quantity of its water is undetermined.

2.6 Land use and Ranch history

The present Griffith League Ranch was originally granted to Jacob Large by the Board of Land Commissioners, Sabine County, Republic of Texas, in 1838. Jacob Large, upon being certified as "a married man...and the head of a family" and "being a resident citizen of Texas at the date of the Declaration of Independence" was granted "one league and labour of land in said Republic" (*one league equals 4,428.4 acres; one labour equals 177.1 acres*). Survey notes dated June 28, 1838 describe the property as "containing eight labours of temporal land and eighteen labours of pasture land" (Board of Land Commissioners, Republic of Texas, 1838).

In 1846, Jacob Large sold the tract to Alfred Griffith, a "native of the state of Maryland" (Bastrop County, Deed Record E, 1846). Additional adjacent acreage may have been purchased and some of the ranch was evidently sold and later reclaimed in the early 1900's. The ranch passed to Mary Lavinia Griffith Sanders, a direct descendent of Alfred Griffith, in 1950. At about the same time that Mrs. Sanders received title to the ranch, she purchased an additional 50.5 acres from Mrs. Ella Fleming of Travis County. This 50-acre addition provided access to the Griffith League Ranch from Oak Hill Cemetery Road. Knox's 1950 survey recorded the ranch at "4,847.5 acres, more or less"(Knox, 1950). The Griffith family owned the property until 1993 when Mrs. Sanders bequeathed it to BSA/CAC.

Griffith League Ranch remained predominantly vacant and undeveloped since the time of the original grant to Jacob Large in 1838. Aerial photographs dated 1974, 1981, 1991

and 1999 show little change on the property. Topographic maps indicate that the tract has been heavily wooded for at least the past 50 years (Palafox, 1996; Texas Natural Resource Information System, 2000). Approximately 565 acres (about 12 percent) were in improved pastureland in 1999 and at least 17 constructed stock ponds were associated with these pastures. The pastures and adjacent woodlands were used for grazing cattle through 1999. Small areas of the ranch could have been farmed in past years. Fire scars on trees indicate that at least one widespread wildfire occurred on the ranch at some time in the past. A small sawmill operated in the southern corner of the property during the 1960's. Several unimproved roads, skidder trails and the remains of the sawmill evidence past logging activity on much of the ranch (Palafox, 1996; Texas Natural Resource Information System, 2000).

During World War II and the Korean War (prior to 1955), the U.S. Army utilized most of the land between Elgin, Texas and Bastrop (State Highway 21) as a military training camp, Camp Swift. The eastern portion of the Griffith League Ranch was used as an artillery range impact zone. Knox noted military roads in his 1950 survey of the ranch (Knox, 1950). Some of the old military roads now provide access to various sections of the ranch. Archeologists surveying the tract have found evidence of military activities on the tract (Parkhill, 2000). The two existing ranch houses were moved from Camp Swift to the ranch's central pasture in the late 1950's (Palafox, 1996). The larger main residence was refinished with a stone facade and the smaller was used as a ranch worker's residence. Several outbuildings and sheds were built adjacent to the houses.

Adjacent lands to the north of the Griffith League Ranch are heavily wooded. Land to the east and northeast and to the west and northwest appear to be used for agricultural purposes at the present time. To the southeast, south and southwest lands are platted for residential development and are rapidly being converted to that use.

Executive Summary from the Research conducted on the Griffith League Ranch, Bastrop County, Texas from 2000-2004.

1. Extensive research in Bastrop County and on the Griffith League Ranch has re-emphasized the uniqueness of the Lost Pines ecosystem and its endemic fauna.

2. Unfortunately, our work has also revealed an extensively fire suppressed forest system with negative impacts to the Houston toad and the system as a whole. Indeed, we feel that the current environment on the Griffith League Ranch has a very high potential for catastrophic fire should a wildfire occur.

3. Extensive trapping results and habitat sampling strongly statistically support toads avoiding pastures, indeed relatively small openings or pastures (>15m) may pose significant barriers. Likewise reproduction occurring in ponds within, or even adjacent to, pastures is nearly always wasted, as all juveniles emerging into that environment perish.

4. Houston toad chorusing does not indicate successful reproduction, nor even the presence of female toads, much less actual emergence of juveniles from a pond.

5. Houston toad activity is correlated with both moon phase (toads are active only during 'dark of the moon' periods) and rainfall (Houston toad activity was correlated with rainfall events of at least 10 mm).

6. Competition between the Houston toad and the Gulf Coast toad, *Bufo valliceps* (its numerically dominant congener at the GLR) is reduced consequent of temporal isolation, decreasing the probability of hybridization as a significant factor.

7. Houston toads have a significantly skewed sex ratio as a natural consequence of their life history. Females are, at minimum, three times more rare than males, with consequent negative effects on population recruitment, growth, and effective size.

8. Successful reproduction (eggs deposited and hatched) and even more importantly juvenile emergence (recruitment) occur with success in ponds within mature forests with significant canopy. Even under perceived 'perfect' conditions juvenile survivorship may be as low as 0.0001 and thus potentially two orders of magnitude less than that used in the latest Houston toad modeling study (1% was required in that model in order to prevent extinction in less than ten years).

9. Extensive upland buffer zones surrounding breeding ponds are not just important, but absolutely critical during the first 8 weeks of the terrestrial stage for juvenile toads. The same area may well be as critical for adults during at least half of the annual cycle.

10. Based on 4 years of work on the GLR the number of toads captured over the past four years has a negative slope. After a peak in 2002, the total number of adult toads captured is decreasing. Each year a significant number of recaptures occur, supporting the idea of a fairly small overall population size localized to the GLR.

11. Survivorship estimates from the capture – recapture data from the GLR were lower than the estimates made from field data collected at Bastrop State Park.

12. Radio telemetry and fluorescent pigment tracking methods indicated both male and female Houston toads remain within 150 m of the edges of chorusing ponds during the active season (March – May). Likewise, during the same time period, toads do not burrow more than 3 cm from the surface of the soil. Houston toads use hollows beneath fallen logs as hibernacula.

13. Houston toads were not typically found in areas with dense stands of regrowth forest, nor were they found to occur outside of forests. The majority of Houston toads captured during this study were captured in areas reflecting a native mixed hardwood and loblolly pine forest with significant canopy and low duff layers.

14. Creating a more natural environment through fire management or manual clearing of trees and removal of heavy duff on the GLR, would not only increase the quality of habitat for the Houston toad, but also for game species such as White-tail deer and wild turkey.

15. Modern forest management methods that include considerations for the specific life history timing of the Houston toad would positively impact the remaining populations of this species while also benefiting the health of the ecosystem and its components alongside diminishing risks of catastrophic fire.

Chapter 1:
ADULT HOUSTON TOAD ECOLOGY

ACTIVITY PATTERNS OF THE DOMINANT HERPETOFAUNA OF THE GRIFFITH LEAGUE RANCH WITH EMPHASIS ON THE HOUSTON TOAD

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From fairly low density populations of the late 1980s and early 1990s, the Houston toad *Bufo houstonensis* crashed to its current extremely low densities (Price, 2003). Given the present state of human-mediated development and population growth in Bastrop County, conservation efforts must be both comprehensive and cohesive in order to save the species. Even with a plan for recovery, efforts must be accelerated and reemphasized if the species is to be kept from extinction in the wild. Although the Houston toad has received substantial media attention consequent of its endangered status, there are still several aspects of its biology that remain unknown, and unfortunately most of those factors are critical to effective management decisions.

Most of the studies have focused on the breeding behavior and reproductive ecology of *Bufo houstonensis* when the toads are most active (Hillis et al., 1984; Price, 2003); however, most of the individuals were captured at breeding ponds while males were in chorus. This manuscript is meant to supplement the current knowledge regarding the activity patterns of the Houston toad both within and outside of its breeding season based on the results from a four-year study, beginning on 12 March 2001 and ending on 01 August 2004. The work included both breeding pond surveys and an extensive system of drift fence – pitfall traps allowing the presentation of these data in correlation with other sympatric herpetofaunal species captured in the traps.

MATERIALS AND METHODS

Study system

The Griffith League Ranch (GLR) is a 1951 ha property owned by the Boy Scouts of America (BSA) in Bastrop County, 91% of the property is underlain by deep sandy soils of the Patilo, Demona, or Silstid series, and the GLR was historically a pine and mixed hardwood forest. Three large tracts of approximately 200 ha each were cleared for cattle grazing early in the 20th century; however, cattle were removed from the property in 2001. *Bufo houstonensis* was originally detected on the property during the early 1980s (A. Price, pers comm., 2002), but no program of monitoring was established until the BSA acquired the property in 2000. Audio surveys were conducted each year from 2001 – 2004 to determine the distribution of Houston toads on the GLR. *B. houstonensis* choruses were heard at 12 of the 17 ponds on the property.

Trap design and data collection

Based on the results of the 2000 call survey a trapping design was conceived to both maximize the number of toads captured and to determine how *B. houstonensis* utilized the landscape by evaluating 5 treatment groups (Table 1). This design had to be implemented over time, and from the first installation to the last these were: March 2001 – 5 linear drift fences (two 121 m, three 153 m), with 1.9 liter pitfalls every 30 m, were placed along the border of the forest and pasture to determine if toads utilized pasture habitats (henceforth referred to as treatment 5); in addition to the pasture traps, three Y-shaped drift fence arrays were placed in 3 habitats (one trap per habitat): 30 m from a breeding pond (Pond 2) in pine forest, in mixed oak woodland, and in a small (~2 ha) natural clearing. In February 2002, the remaining traps from the original conceptual

design were added – seven additional Y-shaped arrays and pitfalls, completing the following trapping design: 4 traps surrounding Pond 2, one at each cardinal point, at randomly chosen distances from the pond's edge (10m, 30m, and 2 at 50m) (henceforth referred to as treatment 1); two treatments of 3 traps placed 150 m apart, with the first traps in each treatment being equidistant from a known *B. houstonensis* breeding pond (treatments 2 & 3, placed at ponds 5 and 6, respectively). Additional funding allowed us to add another treatment (treatment 4) identical to treatments 2 & 3, at another pond (Pond 12) with known *B. houstonensis* chorusing (Figure 1).

Traps were checked every morning beginning on 12 March 2001 and ending 31 July 2004, with the exception of 1 August 2003 through 9 August 2003, and 20 August 2003 through 1 September 2003 when the traps were closed due to excessive temperatures (greater than 37° C) in order to prevent trap mortalities. Snout-urostyle length (SUL), head width (HW), and weight were recorded for all anurans. Standard measurements were taken for all other vertebrate taxa. Each adult *B. houstonensis* received a passive integrated transponder (PIT) tag. Juvenile *Bufo sp.* and all other vertebrate taxa were toe-clipped. All organisms were released near their capture site shortly after collection. Any dead specimens were cataloged; a tissue sample was retained in the Forstner tissue collection, and when relevant, vouchers were deposited in either the Texas Co-operative Wildlife Collection at Texas A & M University, College Station Texas, or the Museum at Texas Tech University.

Nightly surveys were conducted at each of the 17 ponds at the GLR during the breeding seasons of 2001 – 2004. Any *B. houstonensis* captured were measured and marked accordingly, and released at the spot of capture within 10 minutes. Other anuran vocalizations were noted.

Outdoor min-max thermometers and rain gauges accurate to .01 inches were added in October of 2001. Climatic data were recorded daily, and missing data were taken from the National Climatic Data Center's weather station in Elgin, Texas (station number: 412820), located approximately 10 miles west of the GLR. The daily moon phase was taken from the Naval observatory's online database.

The calendar year was divided into 7-day increments (weeks), with the first increment being Julian days 1 – 7 (January 1st – 7th, regardless of day of week). In order to seek trends within toad activity across years, the number of toads captured during each 7-day time period from January through June was summed across years then graphed in order to determine peak activity; precipitation was summed across years and averaged for each week and summed toad activity was plotted with it; finally, the daily moon phase was plotted against the sum of the number of toads across years captured during that phase, regardless of method (breeding pond captures or caught in pitfall traps).

RESULTS

Between 12 March 2001 and 31 July 2004, 156 adult B. houstonensis (132 M: 24F) were captured at either breeding ponds (or near the pond during walking surveys) or in the drift fence / pitfall traps, refer to table 1 for a breakdown of the captures based on treatment groups. Fifteen Houston toads were captured at treatment 5, this maybe misleading, however as treatment 5 is primarily in open pastures. All of the individuals captured in this treatment were either within a canopied drainage leading to a known chorusing pond, or in the terminal buckets of the entire treatment group, which bordered on the forests no farther than 15 meters from the forest edge. There were not any mid-pasture captures, indeed, no captures occurred outside of 15 meters from the forest's edge for this treatment group.

The number of Houston toads captured increased from 2001, when 13 toads were captured, to 2002, when 77 toads were captured, and decreased in both 2003 (40 individuals) and 2004 (29 individuals) (Figure 2). Several other species of anurans were captured at the GLR during the study with enough data accumulated on 4 other species (*Bufo valliceps*, *Scaphiopus hurteri*, *Rana sphenocephala*, and *Gastrophryne olivacea*) to allow comparisons to *B. houstonensis*. Abundances of *B. houstonensis* and *B. valliceps* peaked in 2002 and decreased each subsequent year; other anuran species either increased in numbers captured (*R. sphenocephala* and *S. hurteri*) or remained constant (*G. olivacea*) (Figure 3).

Five lizard species were captured in the drift fence arrays; two highly abundant species (*Cnemidophorus sexlineatus* and *Sceloporus undulatus*) and the other three species were captured infrequently (Figure 4); lizard densities increased until 2004 when fewer lizards were captured; however, the active period for lizards, based on weekly captures from 2001 – 2003, is late summer, and this would be after the trapping array was beginning to be closed during 2004 (Figure 5). Sixteen species of snakes (157 total individuals) were captured; the abundance of each species varied across years (Figures 6 & 7). In 2002, a timber rattlesnake *Crotalus horridus*, a state-threatened species was captured in a funnel trap near pond 6. This was a county record, and a photograph of the individual was placed in the University of Texas at Arlington's Natural History Archive.

Male *Bufo houstonensis* activity began in January and extended through May, peaking in March; female activity began in late February and extended through May, also peaking in March (Figure 8). The Julian-week activity of *B. houstonensis* peaked at week 10, with continued activity during week 11, which is March 5th – 19th; a second bout of activity occurred during weeks 13 and 14, which is March 26th – April 8th (Figure 9). Six

B. houstonensis were captured outside of the breeding season (June – December) throughout the entire 4-year study.

Few Houston toads were captured when the moon was full, or when there was over 50% lunation; most *B. houstonensis* were captured during the 'dark of the moon' (Figure 10).

Bufo valliceps activity did not begin until week 14, at the tail end of *B. houstonensis* activity; there was very little temporal overlap between the two species, and *B. valliceps* activity did not begin until after the peak of *B. houstonensis* (Figure 11). Both bufonid species were active after significant rainfall; however, *B. houstonensis* did not exhibit much activity after week 14, while *B. valliceps* showed bursts of active after rainfall events over 0.5 inches (Figure 11).

DISCUSSION

The initial pitfall traps were opened on 12 March 2001, which is in the middle of the peak activity for Houston toads, which could explain the low number of toads (13 individuals) captured in 2001; this is supported in part by significantly more *B. valliceps*, which is active later in the year (Figure 11), collected in 2001 than *B. houstonensis* (Figure 3). This was the only year in which these two species differed dramatically in their respective number of captures. The number of Houston toads peaked in 2002 and decreased every year thereafter (Figure 2), in correlation with the trends seen in *B. valliceps*. The trapping effort remained consistent during the study. Traps were checked every day and, in addition, when Houston toads are most active, during the spring months, nightly surveys were performed at each pond on the GLR.

Other species of anurans increased in abundance during the study (Figure 3); however, as mentioned above, *B. valliceps*, like *B. houstonensis*, peaked in 2002 and

decreased each year after that. The dearth of bufonids captured in 2003 and 2004 indicate a biological shift in the number of toads utilizing the GLR. This can be interpreted in a variety of ways. We may be seeing a normal flucuation in mean population activity across these years. It is also possible that inherently low population numbers could explain the decreasing trend. Price (2003) also reported decreasing numbers of Houston toads throughout his 12-year study at Bastrop State Park. Other species did not generally show a decreasing trend across these years at the GLR.

The lizard fauna was dominated by two species (*C. sexlineatus*, and *S. undulatus*). *Cnemidophorus sexlineatus* increased in abundance each year until 2004, when a sharp decline resulted from closing the traps midway through the year. *Anolis carolinensis* and both *S. undulatus* and *S. olivaceous* were probably under-represented in this sample as those species have arboreal tendencies and would not be caught in terrestrial drift fences as often. *Scincella lateralis* were captured frequently, but several individuals were observed escaping through the holes in the bottom of the pitfall traps, so they were probably also under-represented.

While the number of snake species captured at the GLR is representative of the snake fauna of the county, the actual densities are probably under-represented. Our trapping system was certainly able to capture snakes, including rare species (Ahlbrandt et al., 2002); however, the trapping design was intended for Houston toads, which may exclude some snakes.

Male *B. houstonensis* activity began in late January and extended through May (Figure 6). Females were active in February, and peaked in March. Examining Houston toad activity per month (Figure 6) indicates a decreasing slope of activity from March through April; however, when Houston toad activity is plotted against weeks, there is a

dramatic decrease in activity during the middle of March (Figure 7), which is associated with the phase of the moon (Figure 8). This second peak of activity is not as strong as the first and most likely represents a small bout of reproductive activity and post breeding foraging. The *B. houstonensis* captured outside of the breeding season were all captured after rainfall events greater than 20 mm; and were probably active because they were flooded out of their hibernacula.

Houston toad activity at the GLR was correlated on both the phase of the moon (Figure 8) and rainfall (Figure 9). Houston toads were most active in March after substantial rainfall in February (Figure 11). In 2003, which had a wet winter (16 inches from December 2002 – March 2003) – based on the rain gauges stationed at the GLR), toad activity increased throughout Bastrop County, and activity decreased in 2004 – a drier year (10 inches during the same time period); however, even with increased activity throughout the county, fewer toads were captured on the GLR in 2003 than 2002. Likewise, the density of Houston toads appears to be decreasing at Bastrop State Park. (Price, 2003).

Bufo valliceps activity began after the peak in *B. houstonensis* activity (Figure 11). This reduces competition between the two sympatric congeners; however, unlike *B. valliceps*, which is active throughout the year, *B. houstonensis* breeds during a six-week period, limiting recruitment to a small temporal window. Based on rainfall data from the GLR, Houston toads require late winter rainfall (Figure 11), and if the appropriate weather conditions are not present during consecutive breeding periods (years) then toad densities will dramatically decrease. If Houston toad populations are allowed to drop below threshold levels during bad years, then the remnant individuals may reach densities so low that the population may not be able to rebound or recover during good years.

It has long been known that Houston toad chorusing at a given location, can "wink out" for a period of years and then restart at that location after a period of absence. This cycling of breeding locations is undoubtedly tied to expansions and contractions of these populations over time. This is very likely to have been part of the normal ecology of this species and potentially many amphibian species on boom or bust cycles. Unfortunately, such life history strategies are reliant upon reservoirs of individuals enabling the boom portion of the cycle in good years. For the Houston toad, it is possible that as the population reaches a trough during one of these cycles, its continuing decline may prevent the population from being capable of rebound even when environmental conditions would otherwise allow it. Indeed this seems a particularly obvious depiction of how extinction happens.

Figure 1. Map of the trapping design on the Griffith League Ranch. Stars represent ponds where Houston toads have either chorused or bred. Boxes represent Y-shape drift fence arrays. Lines represent linear drift fence arrays. The circle represents an array of 24 artificial ponds. Numbers represent the numbers used for the treatment groups of the traps.

Table 1. Number of toads captured per treatment group (refer to Figure 1 for the treatment groupings) and at breeding ponds at the Griffith League Ranch from 12 March 2001 through 31 July 2004. Type of traps refers to drift fence design. Installation date refers to when traps were placed into the ground. GLR Reference Pond indicates the closest pond(s) near the treatment group. The *Ponds* treatment group refers to the ponds at the GLR.

Treatment group	Habitat type	Type of traps	Installation Date	GLR Reference Pond	Number of toads Captured
1	Oak – Pine woodland	4 Y-shape arrays	Mar. 2001 (1) Jan. 2002 (3)	2	21
2	Oak woodland	3 Y arrays	Mar. 2001 (1) Jan 2002 (2)	5	9
3	Oak woodland	3 Y arrays	Mar. 2001 (1) Jan 2002 (2)	6 & 7	9
4	Oak – Pine woodland	3 Y arrays	Mar. 2001 (5)	12	2
5	Pasture	5 linear arrays	Mar. 2001	9 – 11	15
Ponds	Ponds	NA	NA	NA	97

Figure 2. Number of Houston toads captured per year at the Griffith League Ranch from 12 March 2001 – 31 July 2004. Trapping efforts were the same for each year, with the exception of 2001 when the project began after the first bout of Houston toad choruses.

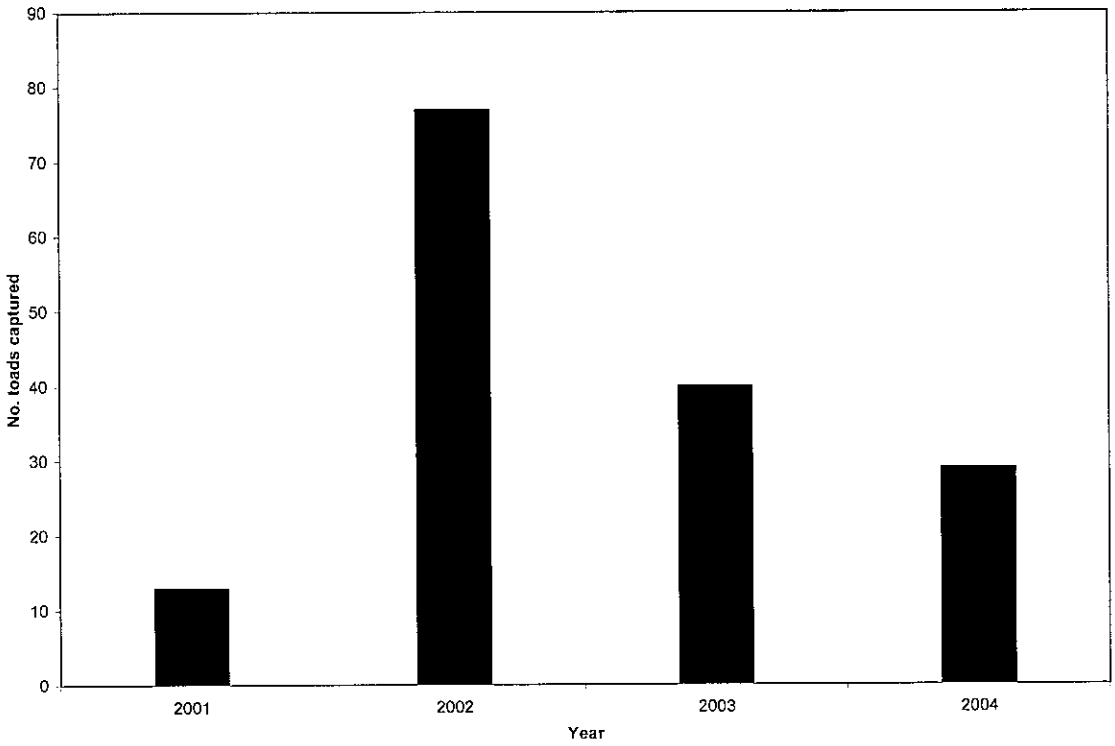


Figure 3. Number of anurans captured on the Griffith League Ranch from 12 March 2001- 31 July 2004. Triangles represent *Scaphiopus hurterii*, diamonds represent *Bufo valliceps*, x's represent *Rana sphenocephala*, squares represent *Gastrophryne olivacea*, and asterisks represent *Bufo houstonensis*.

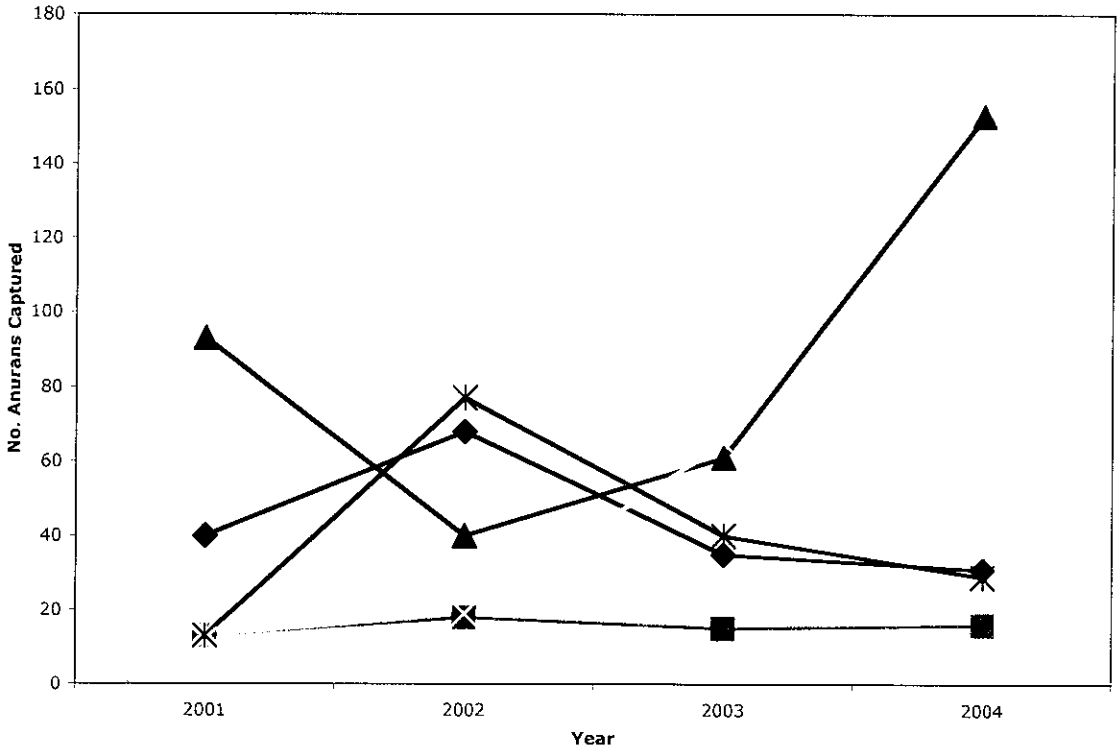


Figure 4. Number of lizards captured at the Griffith League Ranch from 12 March 2001 – 31 July 2004. Triangles represent *Sceloporus olivaceous*, diamonds represent *Cnemidophorus sexlineatus*, X's represent *Anolis carolinensis*, squares represent *Sceloporus undulatus*, and asterisks represent *Scincella lateralis*.

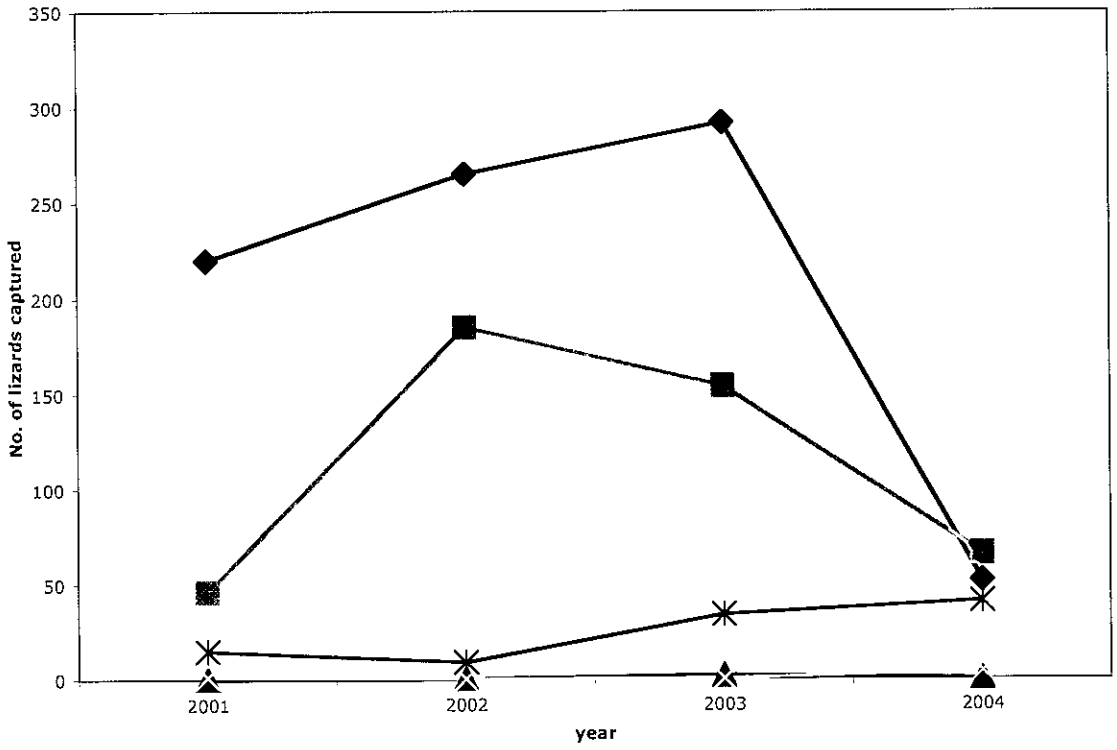


Figure 5. Number of *Cnemidophorus sexlineatus* and *Sceloporus undulatus* captured per Julian-day week at the Griffith League Ranch from 12 March 2001 through 31 July 2004. Lizards were summed across years. Diamonds represent *C. sexlineatus* and squares represent *S. undulatus*

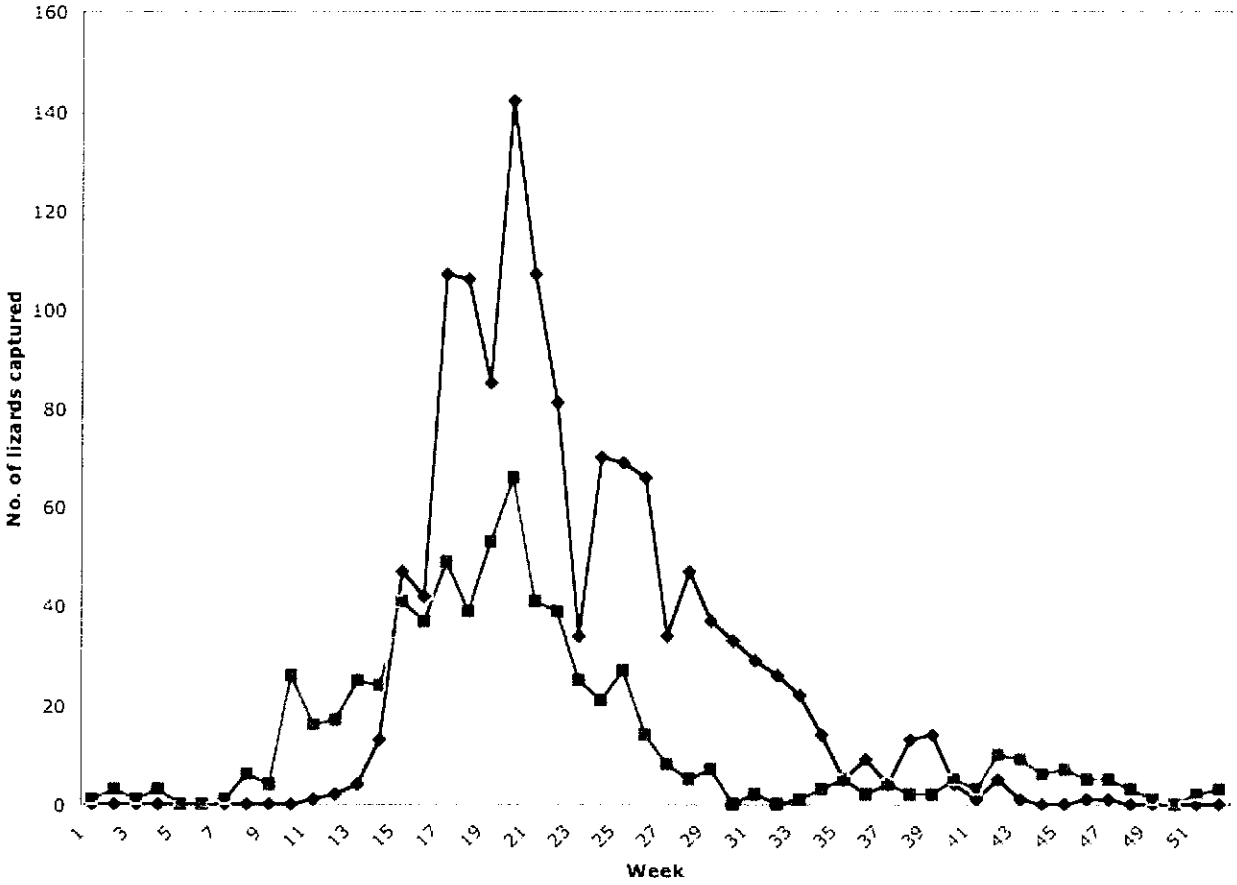


Figure 6. Number of snakes captured at the Griffith League Ranch from 12 March 2001 – 31 July 2004. Triangles represent *Elaphe obsoleta*, diamonds represent *Agkistrodon contortrix*, X's represent *Heterodon platirhinos*, squares represent *Crotalus horridus*, asterisks represent *Leptotyphlops dulcis*, closed circles represent *Masticophis flagellum*, | represents *Masticophis f. testaceous*, _ represents *Micrurus fulvius*

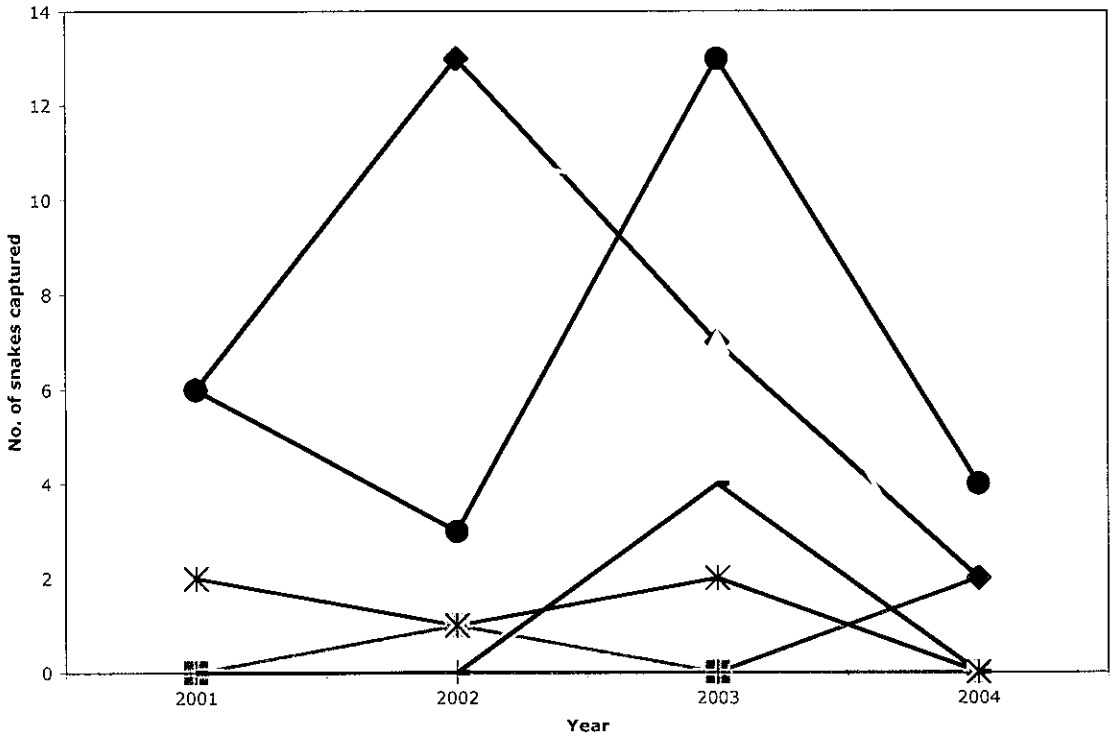


Figure 7. Number of snakes captured at the Griffith League Ranch from 12 March 2001 – 31 July 2004. Triangles represent *Nerodia fasciata confluens*, diamonds represent *Nerodia erythrogaster*, X's represent *Storeria dekayi*, squares represent *Nerodia rhombifer*, asterisks represent *Tantilla gracilis*, closed circles represent *Tantilla nigriceps*, | represent *Thamnophis proximus*, _ represent *Virginia striatula*

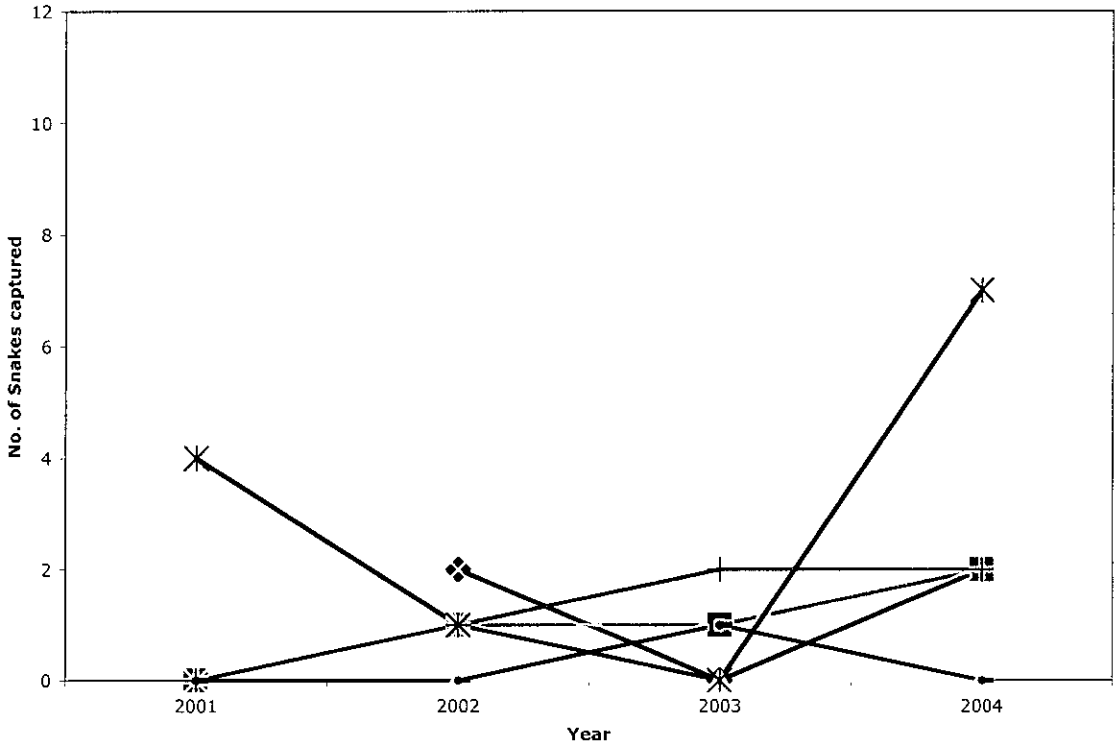


Figure 8. Number of Houston toads captured per month at the GLR from 12 March 2001 – 31 July 2004. Open bars represent males, and closed bars represent females.

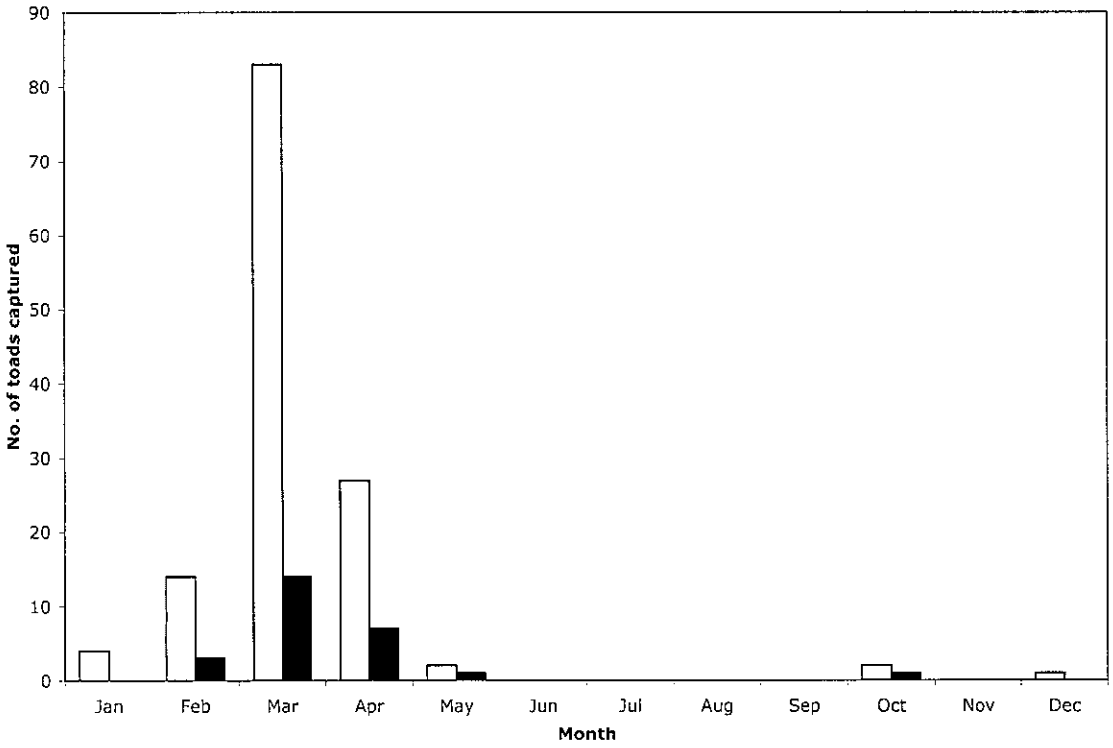


Figure 9. Number of Houston toads captured at the Griffith League Ranch per 7-Julian day period beginning 01 January 2001 ending 31 July 2004. The number of toads was summed for each week across the 4 years of the study.

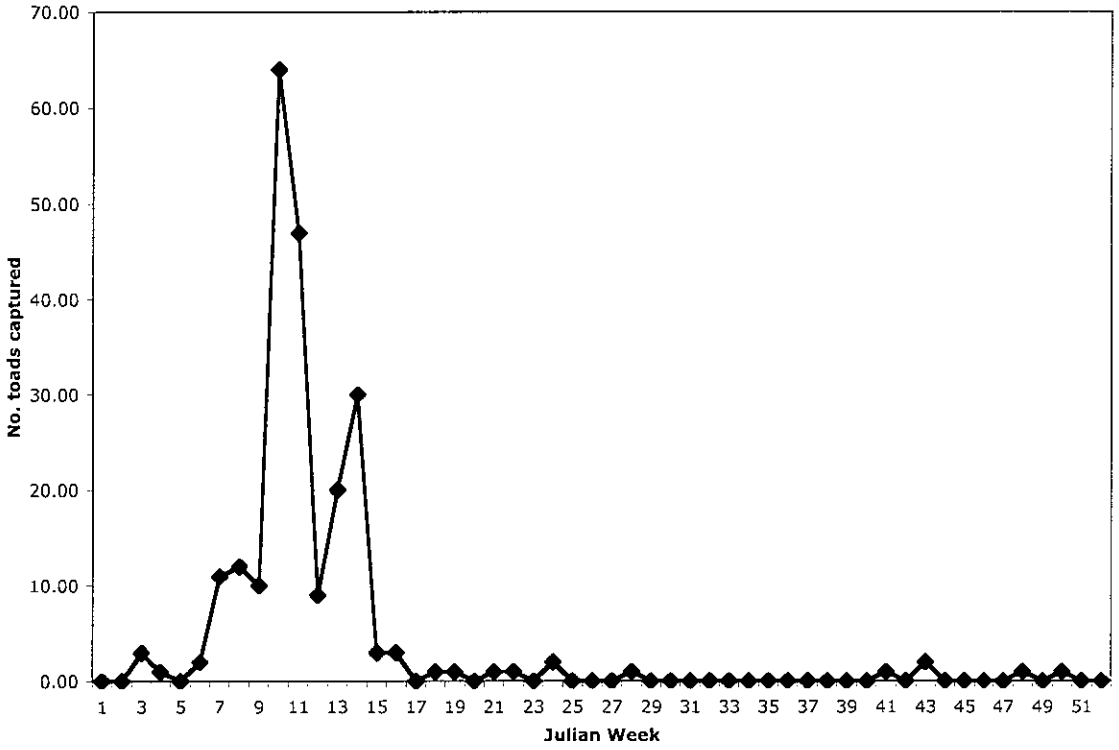


Figure 10. Number of toads captured at the Griffith League Ranch per moon phase. Toads occurrences were summed across years from 2001 – 2004. *New* represents a new moon (0% lunation), *waxcres* represents waxing crescent moons (1 – 49% lunation), *First* represents the first quarter moon (50% lunation), *waxgib* represents waxing gibbous moons (51-99% lunation), *Full* represents a full moon (100% lunation), *wangib* represents waning gibbous moons (99 – 51%, *Last* represents a last quarter moon (50% lunation), *wancres* represents waning crescent moons (49 – 1% lunation).

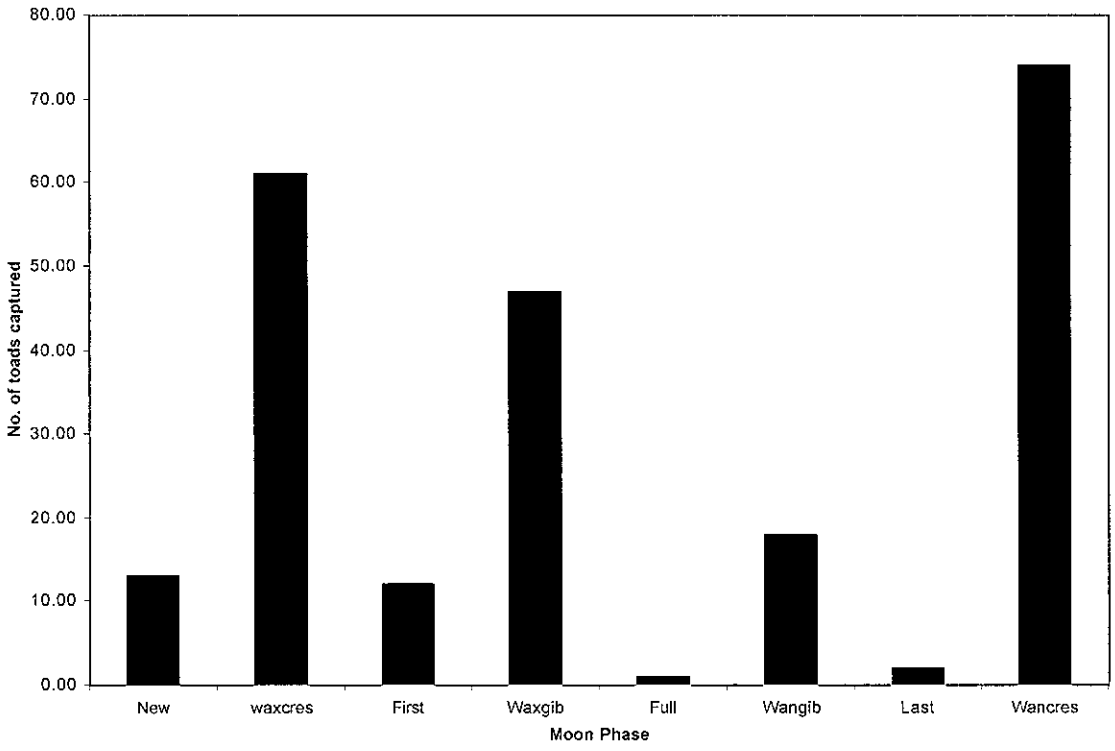
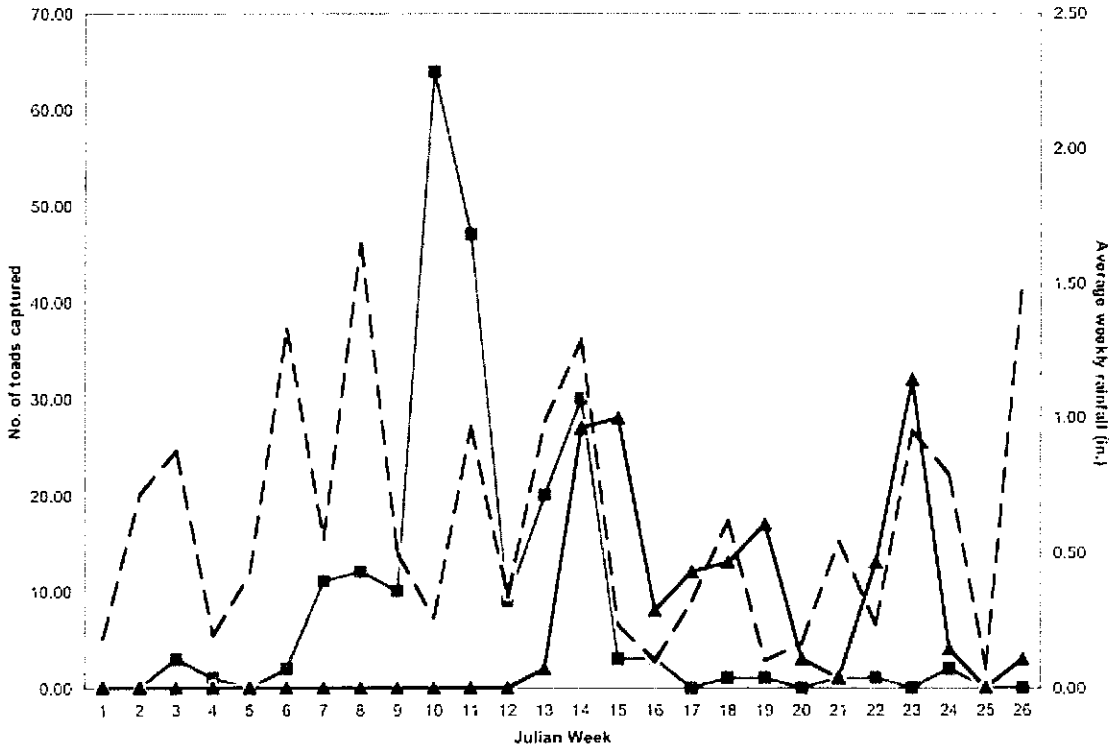


Figure 11. Bufonid activity during the first 26 Julian weeks of the year (the active period of *Bufo houstonensis*), based on toads captured at the Griffith League Ranch (GLR) from 12 March 2001 – 31 July 2004. Squares represent the number of *B. houstonensis*, triangles represent *B. valliceps*, and the dotted line represents the average weekly rainfall (in inches) (graphed on the secondary Y axis) at the GLR during the study.



SPATIAL DISTRIBUTION AND HABITAT ASSOCIATIONS OF ADULT HOUSTON TOADS

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INTRODUCTION

One of the goals of conservation biology is to maintain native biodiversity, regardless of taxonomic affiliation. Amphibians are currently the focus of concern because of the perceived worldwide decline of the group (Barinaga, 1990). Amphibians are important not only on a biodiversity scale, but also because they can be used as "bio-indicator" species that help judge the integrity and health of an entire ecosystem.

As the human population continues to grow exponentially, encroaching and fragmenting the remaining natural areas of the planet, natural resource managers and conservation biologists are faced with an increasingly difficult task to develop management strategies that both maximize biodiversity and provide some sustainable value, economic or aesthetic, to the area of concern (Semlitsch, 2000). This is an especially difficult task in areas with high levels of development, and is further compounded if the area is within the distribution of an endangered species. Conservation tools, such as buffer zones, can help protect native habitat for species; however, since most organisms differ ontogenetically as to how they utilize their habitat, it is necessarily important to understand the natural history of the different life stages of the target species before restricting development to certain areas.

The Lost Pines region of Central Texas is the westernmost remnant of the southern pine forests of the United States, and is a unique ecosystem due to its isolation from the East Texas pineywoods (approx. 130 km separates the two regions). The Lost

Pines is home to several Texas endemics (Elliot's short-tailed Shrew, Texas long-lipped beetle, and the endangered Houston toad, to name a few); human-mediated development and urban sprawl has fragmented the pine forests, leaving only patches of suitable habitat within which these species thrive. In Bastrop County, the heart of the Lost Pines, two large patches (over 1900 ha each), exist – Bastrop State Park (BSP) and the Griffith League Ranch (GLR); there are currently no plans to significantly fragment either area. While both BSP and GLR are large enough to provide suitable habitat for these endemics, neither are pristine. Fire suppression and poor management have created habitats with increased tree density and thick leaf-duff layers that do not represent the natural condition of the region, and to which native species would be poorly adapted.

The Houston toad *Bufo houstonensis* is an endemic to South Central-East Texas, and currently found in only 9 counties, with the largest known group being within the Lost Pines region of Bastrop County. Due to its limited distribution, the toad was listed as endangered in 1970 (Honegger, 1970). While *B. houstonensis* has received considerable media attention, several critical aspects of its biology remain unknown, and in order for conservation efforts to move forward, these components of the toad's natural history must be addressed.

Within Bastrop County, the Houston toad is currently being threatened by encroaching development, yet only the chorus pond behavior of *B. houstonensis* has been documented (Hillis et al., 1984; Price, 2003). Not only must the post-breeding movement patterns of the toad must be documented, including how toads utilize their upland habitat, where they go after the breeding season, but a quantitative characterization of their habitat must be completed in order to determine the types of habitats *B. houstonensis* utilize.

The purpose of this manuscript is to detail how the toad utilizes its habitat both during and after the breeding season using radio telemetry and fluorescent powder, and to also compare the habitat around known breeding ponds to determine if toads utilize certain areas / habitat types more than others.

MATERIALS AND METHODS

This study took place at the Griffith League Ranch (GLR), which is within the Lost Pines eco-region, located in Bastrop County, Texas (refer to Ch. 1 – Activity Patterns for a complete description of the GLR).

Radio telemetry and fluorescent powder

Houston toads were captured for radio telemetry in 2003 & 2004. Toads were either captured in the drift fence / pitfall array (refer to Ch. 1 – Activity Patterns for a description of the trapping design) or at ponds during choruses. All individuals were marked with PITs and radio telemeters (BD-2; 1.8g; Holohil Systems, Ontario Canada) were placed on the individual within 30 minutes after capture. Only toads weighing above 20 g received telemeters to stay within the 10% rule (White and Garrott, 1990). In 2003, spandex jackets (Figure 1A) were used to attach the telemeter to the toad; each jacket weighed 0.5 grams. In 2004, a stainless steel beaded chain "belt" was created (Rathburn and Murphey, 1996); the telemeter was attached to the belt with epoxy resin and allowed to set overnight; the belts were made with extra beads and tailored (beads were cut off) for individual toads. Males were generally collected while chorusing, and females were collected in the pitfall traps; only non-gravid females were fitted with telemeters.

Toads were located at least once every two days and locations were marked using red marking flags. GPS coordinates were taken weekly using a Garmin GPS V. The habitat immediately surrounding the radio-telemetered toad was recorded.

A black spandex jacket was placed on a single non-gravid female, captured on 2 May 2004. Inert fluorescent pigment (T1 series pigment, Radiant Color, Richmond, CA) was placed underneath the jacket, against the dorsum of the toad. The female was tracked each subsequent night until the jacket fell off, 48 hours from initial release.

Vegetation analysis

Woody vegetative cover was assessed using the line intercept method (Higgins et al., 1996). The habitat immediately surrounding the drift fence / pitfall arrays (refer to Ch. 1 – Activity Patterns) was measured in June 2004. Two 50 m intercepts were spaced at each trap array. A randomly chosen bucket was used for the starting point; buckets were assigned a number and a 4-sided die was rolled to determine the buckets used for the analysis. Percent cover was calculated by summing canopy cover for the two intercepts. Kiefer & Baccus (Ch. 5) and White & Simpson (Ch. 5) assessed the vegetative cover for the rest of the GLR, using 25 additional reference points. A principal component analysis was used to examine the habitat types of the traps and the rest of the ranch to determine what type of canopy cover *B. houstonensis* utilized.

RESULTS AND DISCUSSION

Radio telemetry

Between 2003 and 2004 twelve B. houstonensis (7M, 5F) were fitted with radio telemeters and 63 total observations were recorded. While the jackets were lightweight, they did not stay on the toad for more than 4 days. The shortest monitoring period for an individual was two days before the toad removed the jacket. The beaded chain belt

method (Rathburn and Murphey, 1996) worked better; the longest period of observation was 6 weeks. Both males and females were released at the edge of a pond. Two of the 7 males stayed within 15 meters of the ponds edge, while the other 5 moved between 20 – 50 meters away from the pond. Females did not stay at the pond's edge. Within two days after release, all females had moved at least 50 m away from the ponds edge. Both sexes chose hibernacula under fallen trees, generally oak stumps, and remained there. Houston toads did not burrow more than 3 cm. Two females captured in 2004 were observed in hibernacula, then observed foraging within two meters of their hibernacula, and were re-located in the same hibernacula the following day. Foraging did not occur every night; two toads were monitored every hour during a night of high anuran activity (10 April 2004) and did not leave their hibernacula throughout the night. Toads did not make any long excursions and generally remained in the same place for several consecutive days. One individual, a female, moved from one hibernacula to another less than 1 meter away and throughout the life of the telemeter (40 days), moved exclusively between those two spots. While there are not enough data to determine a home range, these data are valuable for qualitative descriptions of the post-breeding behaviors of the females, and to a lesser degree males.

The fluorescent powder method provided a different type of result than the radio telemetry. The female moved 3.2 m (straight line distance) from the release point, and situated herself underneath a fallen oak tree. She did not move in a straight line, however. The powder marks on the ground indicated she moved from the release point to the tree and moved along the tree, paused (indicated by a higher concentration of fluorescent powder), and then moved further down the length of the tree until she found a

hollow spot large enough to fit in. The female remained in the hollow for 48 hours after release (confirmed visually), and then left, leaving the jacket, and all the powder.

Each of the telemetered individuals, regardless of sex, were found underneath fallen logs; we assumed *B. houstonensis* uses the dead-fall for hibernacula. Several of the hibernacula were lifted in order to determine if the toad was still present. Toads were not buried more than 3 cm below the ground, and could usually be seen once the log was removed. Most of the tree-fall utilized by toads were oak species; however, this may be because the majority of toads were found in oak dominated habitat.

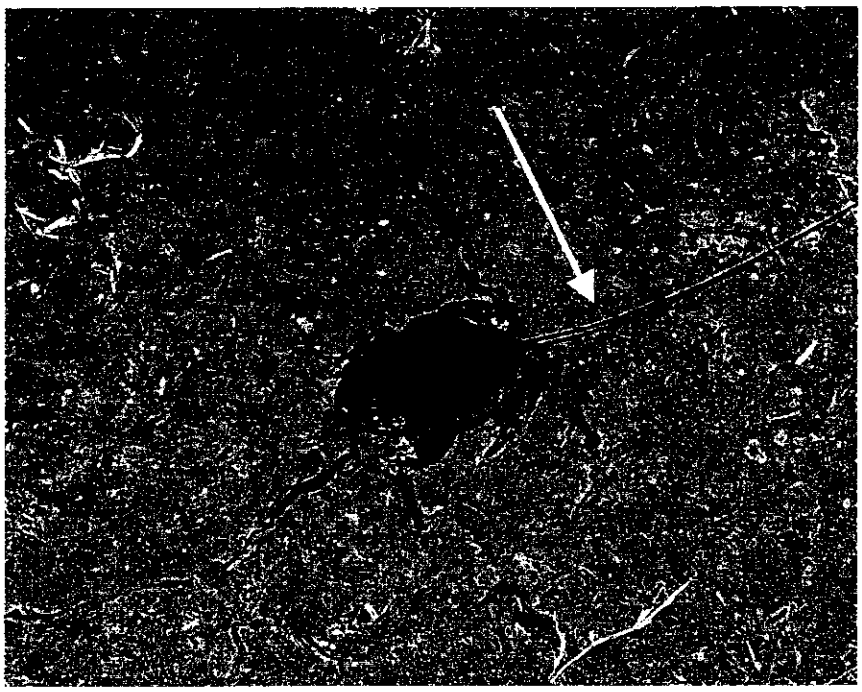
The woody vegetation was dominated by two species of oak: *Quercus stellata*, and *Quercus marilandica*, loblolly pine *Pinus taeda*, Eastern red cedar *Juniperus virginiana*, and yaupon *Ilex vomitoria*. The principal component analysis yielded three distinct groupings, essentially, a grouping with dense canopy cover (dense pine stands and yaupon), a grouping with moderate canopy cover (mostly oak), and a grouping zero canopy cover (pasture land) (Figure 2). The majority of Houston toads were captured in moderate canopy forest (all but 18 of the 159 individuals were captured in oak-pine woodlands with low density of trees). During the study three individual males were heard chorus, albeit infrequently, in areas with dense canopy; however, no egg strands or post metamorphic juveniles were detected. The toads captured (15 individuals) in the pastoral areas were only captured in at the border of the pasture and woodlands in buckets no more than 15 meters away from moderate canopy forest, and near a drainage that led to a pond, which has had *B. houstonensis* choruses every year since 2000.

While the system (the GLR) we have is one of the best remaining areas to study the ecology of *B. houstonensis* within its historical distribution, it is very artificial. The dense pine forest at the Southeastern edge of the ranch (refer to Figure 1 – Activity

Patterns) represents a 23 yr old clear cut (A. Sanson, pers. comm. to MRJF), which was allowed to grow back without any management. The best habitat on the ranch for Houston toads, based on the PCA (Figure 2) is a moderate canopy oak woodland. This may be a consequence of oak duff decomposing quicker than pine duff. The pine duff at the Southeastern edge is dense (Kiefer, Ch. 5) and even fire management would not remove the duff layer quickly. If the GLR is going to be used as a refuge for the last remaining Houston toad populations, then the property must be returned to its natural, historical state. Both fire management and manual removal of the duff are recommended in order recompose the natural integrity of the forest habitat on the GLR.

Figure 1. A) Picture of *Bufo houstonensis* with a radio telemeter attached to the individual with a custom-made spandex jacket. The arrow is pointing to the whip antenna of the radio telemeter. B) Picture of two *B. houstonensis* with radio telemeters attached via a beaded chain "belt."

A



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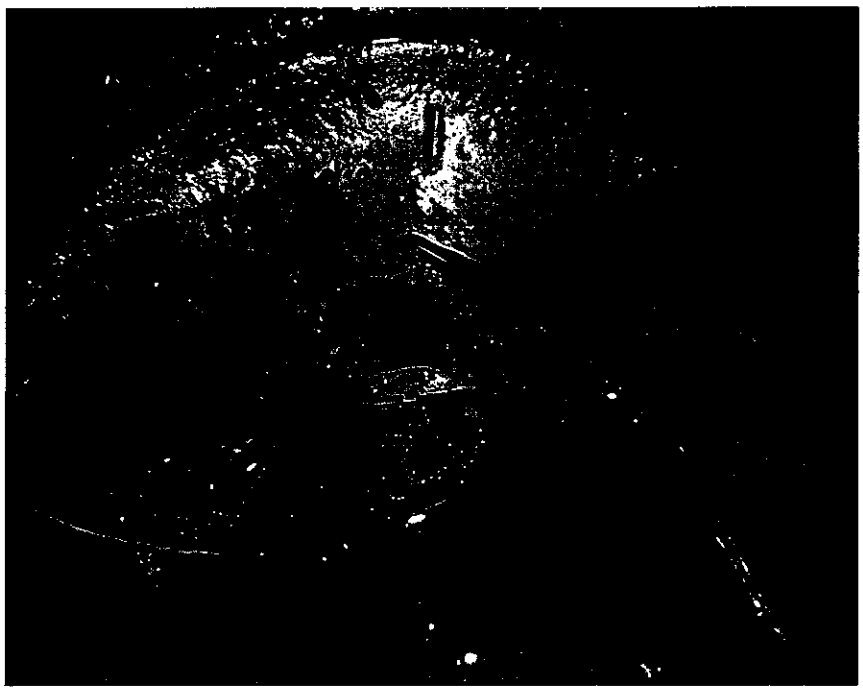
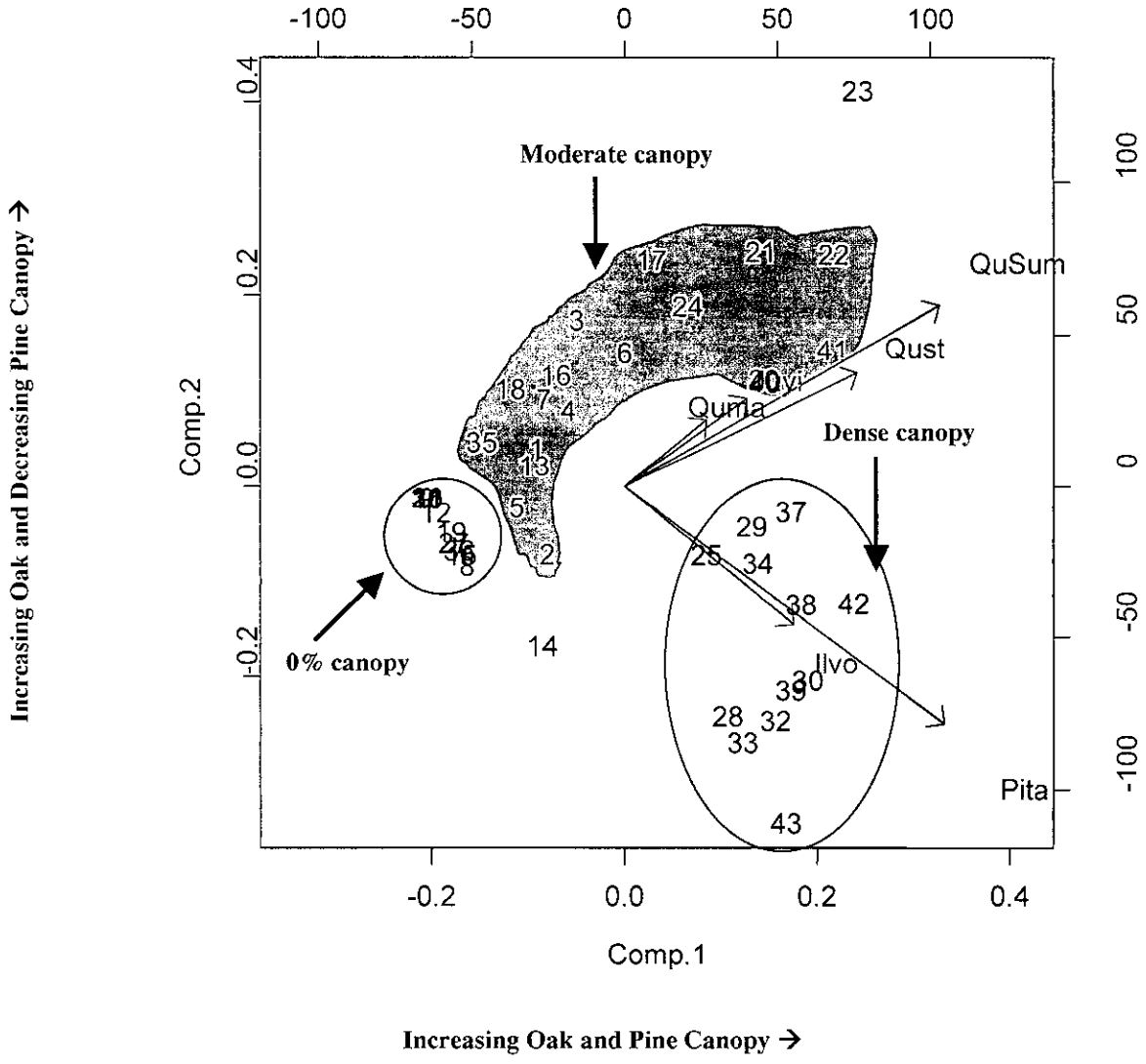


Figure 2. Results from a principal component analysis on the dominant vegetation of the GLR. Component 1 is canopy cover dominated by oak and pine; component 2 is canopy cover dominated by oak and negatively correlated with pine. The PCA yielded 3 separations, an area with 0% canopy cover (blue), an area with moderate canopy cover (orange) and an area with dense canopy cover (gray). The majority of Houston toads (greater than 90%) captured during this study were captured in areas with moderate canopy cover. *Pita* represents canopy cover (CC) from *Pinus taeda*, *Quma* represents CC from *Quercus marilandica*, *Qust* represents CC from *Quercus stellata*, *Qusum* represents CC from the sum of the oak component, *Ilvo* represents CC from *Ilex vomitoria*, and *Juvi* represents CC from *Juniperus virginiana*. Numbers represent either vegetation from the trap lines (1 – 18) or transects (19 – 43)



POPULATION DYNAMICS OF ADULT HOUSTON TOADS

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INTRODUCTION

While congeneric species may have similar life history strategies (Frazer et al., 1990), detailed studies of the population dynamics of individual species remain important, especially in the case of threatened or endangered species, as each species possesses unique life history attributes, which affect its demographic dynamics. The current concern over the decline of the amphibian populations (Barinaga, 1990; Pechmann et al., 1991) re-emphasizes this importance – as amphibian species become more rare, it becomes more important to fill in the knowledge gaps about the remaining populations as quickly as possible.

The population dynamics of several species of Bufonid toads have been thoroughly studied including the Gulf Coast toad *Bufo valliceps*, (Blair, 1961); the American toad *B. americanus* (Christein and Taylor, 1978); Fowler's toad *B. woodhousei foweleri*, (Clarke, 1974) and the Common toad *B. bufo* (Gittins et al., 1985); however, such data do not exist for most species of toads, including the highly endangered Houston toad *Bufo houstonensis*. The purpose of this manuscript is to examine the dynamics of the *B. houstonensis* population at the Griffith League Ranch in Bastrop County, Texas. We further sought to place the annual dynamics of *B. houstonensis* in context with other species of herpetofauna on the site for the same period in order to demonstrate the different population trends occurring among herptile species on the GLR.

MATERIALS AND METHODS

Trap design and data collection

Refer to Ch 1. Activity Patterns for a detailed description of the trapping regime at the GLR.

Population dynamics

In order to examine the population dynamics of the Houston toad alongside other amphibian and reptile species at the GLR, captured individuals were divided into two categories: initial captures and recaptures. The time period for the mark-recapture analyses was year, individuals were only counted once per year, either as an initial capture or recapture. The percentage recapture per year was calculated for the numerically dominant species collected during this study. Enough data were gathered for *Bufo houstonensis, B. valliceps, Scaphiopus hurteri, Cnemidophorus sexlineatus*, and *Sceloporus undulatus* to allow analyses.

In addition to the above analyses the *B. houstonensis* mark-recapture data was examined using a full Jolly-Seber model (Krebs, 1999) in POPAN (Arnason et al., 1998). The assumptions of the Jolly-Seber model include an open population (populations that constantly change size because of differential rates of birth, death, immigration, and emigration), equal catchability of marked and unmarked individuals, individuals are clearly marked, and marks are not overlooked. An open population was assumed since *B. houstonensis* are known to occur on several adjacent properties (e.g., *B. houstonensis* were heard calling at a pond at the property bordering the Northern corner of the GLR), both marked and unmarked individuals had an equal probability of being captured on any given sample night; throughout the study we did not notice a difference among these categories, and finally, for this analysis only adults with PITs (113 individuals) were

used, and since all *B. houstonensis* were scanned with a PIT reader, it was highly unlikely that marks were overlooked.

RESULTS

From 2001 – 2004, 159 individual adult Houston toads were captured. Fourteen toads, all males, were recaptured during subsequent years, an overall recapture rate of 8%. Females were not recaptured during this study. The percent recapture varied among years, and other herpetofauna were not recaptured as frequently as *B. houstonensis* (Table 1). The recaptured Houston toads were distributed temporally as follows: two toads were originally captured in 2001, and recaptured in 2002; six toads were initially captured in 2002 and recaptured in 2003; five toads initially captured in 2002 were recaptured in 2004. One individual was initially captured in 2002, and recaptured in 2003 and again in 2004.

The results from the Jolly-Seber model predicted adult *B. houstonensis* survivorship at 12.47% from 2001 – 2002 and 12.19% from 2002 – 2003. Estimates from 2003 – 2004 were unavailable due to the time period being too close to the end of the sample chain. The population size estimate from the model for 2002 was 2373 individuals, and 2764 individuals for 2003, although these are obviously larger than the actual population size. Estimates of abundance were unavailable for the first and last time step.

DISCUSSION

Bufo houstonensis had the highest recapture rate among the herpetofaunal species collected at the GLR during the 4-year study. This result maybe misleading, as this study was specifically designed to capture *B. houstonensis*, so more effort went into collecting Houston toads than any other species. Individuals initially captured in 2001 had a higher

recapture rate than individuals initially captured any other year. *Scaphiopus hurteri* is an exception in that only 2.15% of the 2001 individuals were recaptured, other species had over a 7% recapture rate, on average. Fewer species were recaptured after 2001, although the trapping and collecting effort remained consistent and continuous. One hypothesis is animals became trap shy and began avoiding the traps. If this is true, it further emphasizes the importance of multiple collection techniques, which could explain the higher recapture rates of *B. houstonensis* because without both techniques (collecting at breeding ponds and pitfall traps), the number of recaptures would be less, which would dramatically affect the results of any mark recapture model.

Price (2003) reported severe declines in the population of *B. houstonensis* at Bastrop State Park (BSP) during the early 1990s when several hundred B. houstonensis were captured each year, peaking in 1995 at 437 individuals. During the time period from 2001 – 2004; however, less than 100 toads were captured each year, and the percentage recaptured decreased as well. The trends from BSP (Price, 2003) mirror the decreasing trends observed at the GLR.

The 12% survivorship estimated for this population of *B. houstonensis* was lower than the 20% survivorship estimated through computer simulation (Hatfield et al., *In press*). Our survivorship estimate was also lower than other allopatric congeners: survivorship for *B. woodhousei* was estimated at 22% (Clarke, 1977), and 34% for *B. hemiophrys* (Duellman and Trueb, 1994).

The estimate of *B. houstonensis* density can be misleading. The Jolly-Seber model predicted a density of 2764 individuals in the population; however, this assumes an equal sex ratio. The sex ratio of any organisms with differential maturation between the sexes will be inherently biased to some degree (refer to Ch. 1 – Sex ratios). The

effective population size (N_e) quantifies breeding population sizes for populations with biased sex ratios (Freeman and Herron, 1998), and from the data gathered at the GLR, assuming a 4M : 1F sex ratio, N_e is 1769 individuals in the population. While this estimate is significantly lower than what the Jolly-Seber model predicted, it is more accurate, as the sex ratio in *B. houstonensis* is significantly male-biased. These results indicate that toad densities at the GLR are incredibly low, and conservation strategy that increase the number of toads, especially adult females, are vitally important to the survival of the Houston toad.

Table 1. Mark-recapture results from a 4-year continuous study (12 March 2001 – 21 July 2004) at the Griffith League Ranch, Bastrop County, Texas. Adult individuals were marked with either a PIT (most *B. houstonensis*) or toe-clipped. An individual was considered 'recaptured' if it was captured in a subsequent year; individuals captured more than one time per year was only counted once. *In. Cap.* represents initial captures for that year; *Recap* represents the number of individuals initially captured during that year that were recaptured during subsequent years (e.g., 2 BH captured in 2001 were recaptured during a subsequent year); *% recap* represents the percentage of recaptures. *BH = Bufo houstonensis; BV = B. valliceps; SH = Scaphiopus hurteri; CS = Cnemidophorus sexlineatus; SU = Sceloporus undulatus.*

Year	Species	BH	BV	SH	CS	SU
	In. Cap.	13	41	93	220	46
2001	Recap	2	3	2	23	5
	% recap.	15.38	7.32	2.15	10.45	10.87
	In. Cap.	77	68	40	265	185
2002	Recap	6	0	0	6	7
	% recap.	7.79	0	0	2.2	3.78
	In. Cap.	40	35	61	292	154
2003	Recap	6	0	0	2	6
	% recap.	15	0	0	0.68	3.89
	In. Cap.	29	32	0	52	0
2004	Recap	NA	NA	NA	NA	NA
	% recap.	NA	NA	NA	NA	NA

A POSSIBLE CAUSE FOR THE DISPARITY IN THE SEX RATIO OF THE EXPLOSIVELY BREEDING HOUSTON TOAD

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INTRODUCTION

Anurans exhibit several different temporal breeding strategies, from continuous year-long breeding (prolonged) to short, seasonal breeding (explosive) that last from a few days to a few weeks (Wells, 1977). Several species on the explosive end of the breeding strategy continuum may have a breeding season lasting for several months, but even then breeding activity actually occurs in intermittent bursts, termed breeding periods, throughout the season, usually depending on favorable climatic conditions (Wells, 1977; Zug, 1993; Duellman & Treub, 1994).

Most male anurans congregate and vocalize to attract females to a breeding area. This breeding strategy most resembles a lek, where males are displaying to attract females (Emlen, 1976). Males are concentrated at the breeding ponds and chorusing; females do not visit the ponds simultaneously, but rather as individuals become receptive. The adult sex ratio at any given instant at a breeding pond will most likely be male-biased as a result of this breeding strategy. The breeding individuals function under an operational sex ratio (OSR), which is the ratio of potentially mating males to receptive females at any instant (Emlen, 1976). While the OSR is a valuable tool for examining reproductive dynamics, it is not a good indicator of the population structure. Unfortunately, accurate sex ratio estimates are difficult to obtain for explosively breeding anurans, especially those species that retreat to hibernacula as soon as the breeding

season is over. An accurate sex ratio estimate of a population is an important demographic measurement because of the potential influence each gender has on the population dynamics (Gibbons, 1990). An intrinsically biased sex ratio lowers the effective population size (N_e) (Freeman and Herron, 1998), which is the number of individuals that actually contribute to reproduction in the population (Wilson, 1975). Accurate sex ratio estimates are necessary to examine the population and genetical dynamics of any species, and given the current concern for declining amphibian populations (Barinaga, 1990; Blaustein and Wake, 1990) these data are especially important in the cases of threatened or endangered amphibbians where they may provide valuable direction for conservation and management efforts.

The Houston toad *Bufo houstonensis* is a relict descendant of the narrow-skulled *Bufo americanus* species group, diverging from southernmost populations of ancestral *B. americanus* within the last 10,000 years (Blair, 1972). Sanders (1953) first described *B. houstonensis*, and its specific identity was reconfirmed genetically by Hillis et al. (1984). *Bufo houstonensis* is an endemic Texas amphibian, historically distributed in South-central Texas in areas underlain with deep, sandy soils associated with pine – mixed-oak woodlands (Brown, 1971). The paucity of these habitats and the destruction of these habitat types associated with urban expansion during the last half of the 20th century have decreased the geographic distribution of *B. houstonensis* to 9 Texas counties. The largest remaining group occurs in the Lost Pines region of Bastrop county; smaller, further isolated groups are still found in adjacent Burleson and Lee counties (Dixon, 2000; Gaston et al., 2001). This limited distribution alongside perceived declines caused *B. houstonensis* to be listed on both national and international endangered species lists (Gottschalk, 1970; Honegger, 1970). Although the Houston toad has received

considerable media attention due to its endangered status, the majority of previous studies mainly focused on the toad's reproductive biology (Hillis et al., 1984; Price, 2003).

The breeding strategy of the Houston toad falls onto the explosive side of the anuran breeding continuum. The breeding season extends from late January through early May (Hillis et al., 1984); however, *B. houstonensis* does not breed continuously throughout the season, instead breeding activity and chorusing occurs intermittently in 3 – 5 day spurts, correlated in part with the dark of the moon (Price, 2003; pers. obs.). *Bufo houstonensis* is an excellent model for examining the sex ratio of an explosive breeding anuran not only because both Hillis et al., (1984) and Price (2003) reported male-biased sex ratios (from data gathered from breeding locations), but also because the Houston toad has been the focus of an extensive 3-year study encompassing both breeding pond surveys and drift fences placed throughout Houston toad habitat (Forstner and Ahlbrandt, 2003). The objectives of this study were: 1) to establish an accurate sex ratio of *B. houstonensis* captured at the Griffith League Ranch and to determine if the sex ratio was the result of sampling error or a natural phenomenon, and 2) to explore, through computer simulation, possibilities as to what could cause the sex ratio to be naturally biased.

MATERIALS AND METHODS

Study System and Field Research

The Griffith League Ranch (GLR) is a 1951 ha property owned by the Boy Scouts of America (BSA) in Bastrop County, 91% of the property is underlain by deep sandy soils of the Patilo, Demona, or Silstid series, and the GLR was historically a pine and mixed hardwood forest. Three large tracts of approximately 200 ha each were cleared for cattle grazing early in the 20th century. *B. houstonensis* were originally detected on the

property during the early 1980s (A. Price, pers comm). Beginning in 2000, audio surveys were conducted to determine the current distribution of Houston toads on the GLR. *B. houstonensis* choruses were heard at 12 of the 17 ponds on the property.

In March 2001, 5 linear drift fences (two 121 m, three 153 m), with 5-gallon pitfalls every 30 m, were placed along the border of the forest and pasture to determine if toads utilized pasture habitats. In addition to the pasture traps, three Y-shaped drift fence arrays were placed in 3 habitats (one trap per habitat): 30 m from a breeding pond (Pond 2) in pine forest, in mixed oak woodland, and in a small (~2 ha) natural clearing. In February 2002, seven additional Y-shaped arrays and pitfalls were added, creating the following trapping design: 4 traps surrounding Pond 2, one at each cardinal point, at randomly chosen distances from the pond's edge (10m, 30m, and 2 at 50m); 2 treatments of 3 traps placed 150 m apart, with the first traps in each treatment being equidistant from a known *B. houstonensis* breeding pond (FIG. 1).

Traps were checked every morning throughout the study period. Snout-urostyle length (SUL), head width (HW), and weight were recorded for all anurans. Standard measurements were taken for all other vertebrate taxa. Each adult *B. houstonensis* received a passive integrated transponder (PIT) tag. Juvenile *Bufo sp.* and all other vertebrate taxa were toe-clipped. All organisms were released near their capture site shortly after collection.

Nightly surveys were conducted at each of the 17 ponds at the GLR during the breeding seasons of 2001 and 2002. Any *B. houstonensis* captured were measured and marked accordingly, and released at the spot of capture within 10 minutes.

Sex ratios were established for the total sample, pitfall traps, and breeding ponds. Each observed ratio was tested for differences from parity using a χ^2 test corrected for

continuity, and a minimum sex ratio that was not significantly different from the data was established for each sample. The sex ratios established from the trapping data and breeding pond data were tested for differences between each other. Critical statistical values were taken from Draper and Smith (1998).

Simulation model

According to data gathered from a captive study (Quinn and Mengden, 1984), male *B. houstonensis* mature in 1 year while females mature in 2 years. We were interested to quantitatively determine how the adult sex ratio was affected by delayed maturation and the associated differential mortality of the 1-year age class. One-year old males are sexually mature and exhibit behaviors associated with reproduction, while one-year old females are still juveniles and do not exhibit reproductive behavior and would therefore experience a different mortality regime. In order to examine the hypothesis that the bias in the adult sex ratio is, at least in part, a result of delayed maturation, a simulation model was created using STELLA® v7 (High Performance Systems, 2001). The model was represented mathematically as a discrete-time compartment model with a 1-year time step. Recruitment (R) into the terrestrial population was the driving variable (see Grant et al., 1997 for specific definitions of variable types) of the model and was parameterized as constant, adding the same number of new individuals to the system at the beginning of each time step; eggs and tadpoles were included together. The sex ratio was assumed equal at birth since gender is determined genetically in toads (Duellman and Trueb, 1994), and mortality was not calculated as immature individuals are not thought to possess behavioral differences which would significantly alter the adult sex ratio (Gibbons, 1990). The state variables were the four terrestrial life stages of *B. houstonensis*: post-metamorphic juveniles (PMJ), sexually mature adult males (AM),

immature females (IF), and sexually mature adult females (AF). Mortality for PMJ was once again assumed not to affect the adult sex ratio and not parameterized for the metamorphs. After the first time step, 50% of the individuals in the PMJ state variable were transferred to the AM state variable and the other half went into IF; assuming an equal sex ratio at parturition, within a given cohort half of the individuals will be males and mature in a single time step, the remaining individuals will be the immature females. A conservative assumption of this model was that the adult mortality was constant between the genders. Male *B. houstonensis* have a higher predation risk during the active period (breeding season) since they will return to a breeding site multiple times in a season, while females visit a breeding location once per season. Our data indicate a significant male-biased sex ratio, which cannot be explained by higher predation on males. IF mortality (ζ) was a constant, which was re-parameterized for each simulation – ζ was calculated and immature females were removed for each time step. The state variables were calculated as follows:

$$1)\ PMJ_{t+1} = PMJ_t + \left(R - PMJ_t\right)\Delta t$$

$$2)\ AM_{t+1} = AM_t + \left(.5PMJ_t\right)\Delta t$$

$$3)\ IF_{t+1} = IF_t + \left(.5PMJ_t - \zeta IF_t - (IF_t - \zeta IF_t)\right)\Delta t$$

$$4)\ AF_{t+1} = AF_t + \left(IF_t - \zeta IF_t\right)\Delta t$$

Five simulations were executed based on maturation data from a captive population with males maturing in 1 year and females maturing in 2 (Quinn and Mengden, 1984); ζ was set at either 0.01, 0.1, 0.25, 0.5 or 0.75 for each simulation. Another 5 simulations were executed, delaying female maturation by another time step

(males matured in 1 year, females in 3) to simulate the effects of an extended growing season (i.e., harsh environmental conditions preventing maturation within two years). The same values for ζ were used for the second set of trials. Each simulation was run for 10 years to allow ample time for the model to stabilize. Adult sex ratios were established as the proportion of AM : AF and expressed as the proportion of during each time step. The resulting sex ratios were tested for differences from parity using χ^2 test corrected for continuity.

RESULTS

Between 5 March 2001 and 30 November 2003, a total of 129 (116M : 13F) individual adult *B. houstonensis* was captured at the GLR; collection at the breeding ponds yielded 84 (79M : 5F) toads, and the drift fence / pitfall traps captured 45 (37M : 8F). The sex ratios for the total sample and the two different collection methods (hand collection at the ponds and toads captured in traps) were all male biased and significantly different from parity ($P > .05$) (Table 1). Minimum sex ratios were established for which the data were not significantly different, e.g., the actual sex ratio for the total sample was 8.92M : 1F, which is not significantly different from 5M : 1F; however, it is significantly different from 4M : 1F (Table 1). The actual sex ratio established from the trapping results (4.6M : 1F) was significantly different from the sex ratio of toads captured at breeding ponds (15.8M: 1F) ($\chi^2 = 8.33$, $P = .003$)

The results of the 10 simulations are shown in Figure 1. The model stabilized two time steps after female maturation. When females matured in 2 years, adult sex ratio was not significantly different from parity at the three lowest values of ζ (0.01, 0.1, and 0.25) ($P > .15$); at higher values of ζ (0.5 & 0.75), the adult sex ratio was significantly

biased ($P < .001$). When females matured in 3 years the sex ratios were not significantly different from 1: 1 at the two lower values of ζ (0.01, 0.1); the three higher values of ζ (0.25, 0.5, & 0.75) produced significantly biased sex ratios ($P < .006$). An interesting property of the model emerged – the sex ratio increased exponentially between the two models. The final sex ratio at $\zeta = 0.5$ and females maturing in 2 years and was 2M : 1F, and 4M : 1F at females maturing in 3 years. This pattern holds when comparing each pair of ζ values across the two maturation times.

DISCUSSION

The sex ratio for the overall sample of *B. houstonensis* at the GLR was significantly male-biased. Previous studies on *B. houstonensis* reported male-biased sex ratios as well (Hillis et al., 1984; Price, 2003); however, in those studies toads were only collected at chorusing ponds, which could artificially bias the sample as the mating system of *B. houstonensis* involves multiple males congregating and chorusing at ponds to attract females. While previous studies (Hillis et al., 1984; Price, 2003), and the chorusing pond capture data from this study were only initial captures, males are more conspicuous and therefore easier to capture at breeding sites. The sex ratio of *B. houstonensis* captured at the drift fence / pitfall arrays should more accurately reflect the true sex ratio of the population as the traps were placed to capture toads as they moved across the landscape, regardless of gender, minimizing collection site-mediated error. The sex ratio of toads captured in traps was less biased, but still significantly different from parity; the minimum sex ratio for this sample was 3M : 1F, and was significantly different from the pond data. This indicated that the bias in the sex ratios from both previous studies is indeed better explained by an operational sex ratio (Emlen, 1976).

Survivorship for juvenile toads is low (Zug and Zug, 1979; Greuter, 2004;). The results from the simulation model (FIG. 1) show that higher mortalities associated with the IF life stage would result in a male biased sex ratio. Female *B. houstonensis* raised in captivity matured in 2 years (Quinn and Mengden, 1984). If a female arrived at the breeding site and deposited eggs early in the breeding season, then her female offspring would be mature by their second year; however, if a female deposited eggs late in the season, her female offspring would not be mature for 3 years. The simulations show that there is a large difference in the adult sex ratio at higher mortalities between 2 and 3 years. The longer it takes for females to reach maturity, the more male biased the adult sex ratio will be.

Females can achieve a larger size by delaying maturation, and since fecundity is positively correlated with size in toads (Reading, 1986), longer maturation times result in larger clutch sizes. The trade-off, however, results in fewer females reaching sexual maturity, especially if the mortality of juvenile females is high. A male-biased sex ratio results in a lower effective population size (Freeman and Herron, 1998), which will decrease genetic variation more rapidly than a population with sex ratio at parity.

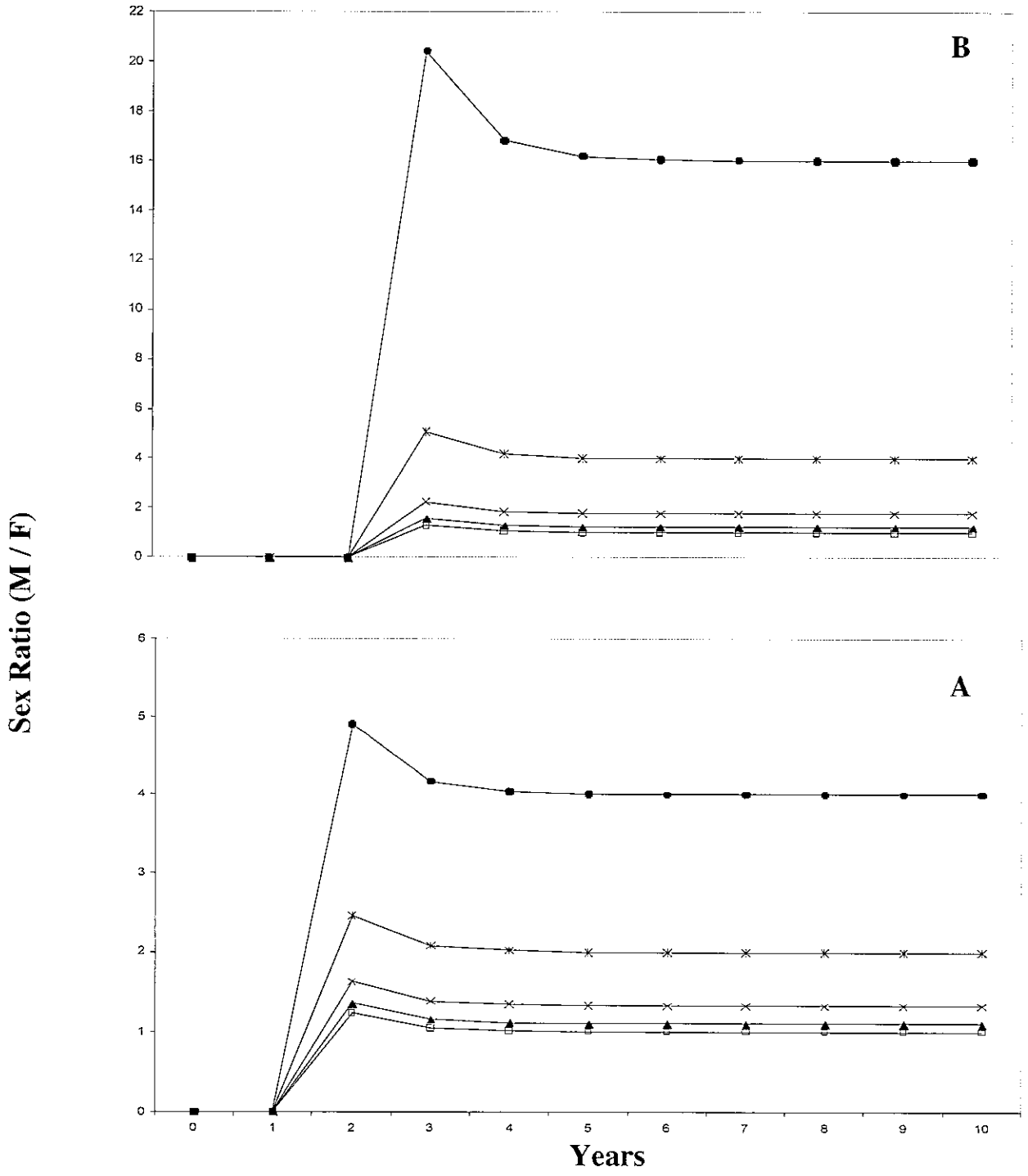
The results from this study indicate the bias in the adult sex ratio of *B. houstonensis* can at least, in part, be explained by differential mortality experienced as a result of delayed maturation by females, which would dramatically lower the effective population size. Using the numbers from this study, N_e at parity would be 129 individuals, 82.56 individuals at the 4M : 1F sex ratio from the trapping data, 46.76 from the 8M : 1F sex ratio from the overall sample, and 29.76 from the 15M : 1F sex ratio established from the chorusing pond data (N_e was calculated from $N_e = 4(N_{males}N_{females}) / (N_{males} + N_{females})$ from Freeman and Herron (1998)). This is further exacerbated for

species with fragmented habitats. In the case of the Houston toad, where migration among patches is incredibly limited, and in the majority of instances almost impossible due to development, a male-biased sex ratio will be a natural, intrinsic property of the dynamics of increasingly smaller and more isolated populations, increasing the importance of each individual female. This dramatically affects management applications as strategies that maximize female toad survival are likely to have significant effects on Houston toad recovery.

Table 1. *Bufo houstonensis* captured at the Griffith League Ranch (GLR) from 2001 – 2003. Sex ratios were established for: the total sample; toads captured in traps only; and toads captured at breeding ponds. Sex ratios were tested for differences from parity using χ^2 goodness of fit test corrected for continuity, and minimum sex ratio is the minimum sex ratio that was not significantly different from the actual (* indicates $p > 0.05$).

Capture Method	Males	Females	Actual Sex Ratio	Minimum Sex Ratio*
Total	116	13	8.92 : 1	5 : 1
Traps	37	8	4.6 : 1	3 : 1
Ponds	79	5	15.8 : 1	7 : 1

FIG. 1. Sex ratios resulting from a 10-year simulation using a model created to examine the effects of 5 levels of yearly mortality (ζ) on immature female *Bufo houstonensis* that mature in either 2 (A) or 3 (B) years. Open squares represent a ζ of 0.01, closed triangles represent a ζ of 0.1, x represents a ζ of 0.25, * represents a ζ of 0.5, and closed circles represent a ζ of 0.75. Sex ratios were expressed as proportion male.



Chapter 2:
JUVENILE HOUSTON TOAD ECOLOGY

THE IMPORTANCE OF JUVENILE ECOLOGY IN THE CONSERVATION AND MANAGEMENT OF THE ENDANGERED HOUSTON TOAD, *BUFO HOUSTONENSIS* (ANURA: BUFONIDAE)

KENSLEY L. GREUTER AND MICHAEL R. J. FORSTNER

The Houston toad, *Bufo houstonensis*, is an endangered species that, despite efforts to obtain life history information, retains many unknowns about of its ecology. These ecological unknowns have inhibited management practices to the extent where only practices to benefit the adult population have been applied (Hatfield 2002). To determine the best management practices for any endangered species, data from all stages of the life cycle are optimal. Data from the juvenile stage are particularly important, as this stage is subject to higher vulnerability because of rapid desiccation (Werner 1986). Anuran juveniles depend on wetlands and the surrounding upland habitat for their survival (Semlitsch 2000a). To document this habitat dependence in *B. houstonensis* juveniles, base line data on survivorship from egg to metamorph are necessary. Once this is achieved, the development of techniques for determining juvenile growth, dispersal, and morphological identification can be made. These techniques will allow more efficient data collection and aid in management practices, including the definition of appropriate buffer zones, for conservation of the species.

THE HOUSTON TOAD

The Houston toad was federally listed as an endangered species in 1970 (Peters 1968 found in Quinn and Mengden 1981). The listing was based primarily on strict habitat requirements, its scarcity in perceived, and since realized, habitat destruction

(Brown 1975). Destruction of habitat has been linked mainly to agricultural use and urban expansion and peripherally to watershed alteration. The decline of the species was also resulted from failure in reproduction and survival during droughts (U. S. Fish and Wildlife Service 1992). Hybridization with sympatric congeners has had an impact on the species as well (Kennedy 1961, Brown 1971, Hillis et al. 1984).

Bufo houstonensis is a Texas endemic species with distribution in Bastrop, Burleson, and Lee counties with scarce subpopulations located in 6 other counties including Austin, Colorado, Lavaca, Leon, Milam, and Robertson counties (Jacobson 1989). Historically, the Houston toad occurred in Fort Bend, Harris, and Liberty counties (U. S. Fish and Wildlife Service 1992); however, no recent records exist for those areas.

The preferred *B. houstonensis* habitat is deep, carrizo sand often in or near pines of the Post Oak Savannah Region of Texas (Kennedy 1962, Brown 1971, Brown and Thomas 1982). Because adult Houston toads are poor burrowers and have difficulty digging into compacted soil (Bragg 1960), they select the soft, pliable sand of central Texas. However, juveniles may not necessarily be confined to this soil type as they might seek moist shelter under leaf litter (Clarke 1974).

Bufo houstonensis is a small toad 5.0 - 8.5 cm long and is similar in appearance to the American toad, *Bufo americanus*. In fact, *B. houstonensis* is a relict descendant of the narrow skulled *B. americanus* group (Hillis et al. 1984). General coloration varies from light brown to gray or purplish gray, sometimes with green patches. They have pale ventral surfaces, which often have small, dark spots. Males have a dark throat coloration (U. S. Fish and Wildlife Service 1992). Figure 1 depicts an adult Houston toad (*B. houstonensis*) with the characteristic mottled coloration and size.

Figure 1. Adult Houston toad *Bufo houstonensis* with the characteristic mottled or blotchy coloration. Photo courtesy of Todd M. Swannack.

The Houston toad's diet consists mainly of insects and other invertebrates. However, Bragg (1961) noted that when given the opportunity, adult *Bufo houstonensis* would consume certain juvenile toads (e.g., juvenile Spadefoot [*Scaphiopus bombifrons*]) and even congeneric juveniles (juvenile *Bufo cognatus*).

The breeding dynamics of the Houston toad include mating in ephemeral rain pools, flooded fields, and permanent ponds (Jacobson 1989). Breeding ranges from January to June, followed by aestivation in shallow underground refugia until the next spring's rains (Jacobson 1989). Stagnant pools that persist for at least 30 days are

required for breeding and for the aquatic larval life stage to develop (Jacobson 1989). Males vocalize from shallow water or from habitat near the breeding pond and can call up to a 100 m radius around the breeding pond. However, pairs have arrived to the breeding sites already in amplexus (Jacobson 1989), indicating terrestrial amalgamation.

Adult toads emerge to breed only when conditions are optimal, but can emerge outside the breeding season if habitat disturbance occurs. Adults do not appear to be faithful to particular breeding ponds. This lack of site fidelity allows genetic exchange to occur, alleviating isolated sink populations from the possibility of inbreeding or bottlenecking (Lacy and Seal 1994). However, Houston toads in Bastrop County are part of a metapopulation where two subpopulations are locally spread out (Semlitsch 2000b) and separated by a four-lane highway. This separation has been a cause of concern for the species as it inhibits the crucial genetic exchange that eventually could lead to extinction.

These problems along with several other factors required a population and habitat viability assessment (PHVA) for *B. houstonensis* to assist with recovery efforts. PHVA is a conclusive assessment of the potential interacting risk factors of a population (Shaffer 1990). PHVA attempts to predict future events using present data. The viability of Houston toad metapopulations is dependent on the size of populations, the rate of exchange among individuals, and threats that affect each small population (Lacy and Seal 1994).

Several recommendations were made from examination of the initial Houston toad population and habitat viability assessment (Lacy and Seal 1994). A geographic information system database was proposed to incorporate annual surveys of the adult

population, soil maps, road maps, known Houston toad population locations, and habitat type. This database would have determined areas of suitable habitat as well as the amount of isolation and continuity among population localities (Lacy and Seal 1994). However, this has not happened.

In the 1994 PHVA analysis, the Conservation Breeding Specialist Group (1994) provided recommendations to improve habitat for the Houston toad, described threats and land use activities, gave management guidelines, and encouraged public outreach. For habitat improvement, the preferred toad habitat used outside of the breeding season must be characterized. In addition, pond construction and restorative techniques must be investigated. The role of travel corridors between and among breeding ponds must also be explored (Lacy and Seal 1994).

Identifying possible threats such as oil run-off, imported fire ants (*Solenopsis invicta*), and UV radiation will contribute to the limited information available for this species. Likewise, identifying water and land use activities such as fish stocking, agricultural practices, cropland/orchard operations, prescribed burning, and planned grazing systems are essential in evaluating the natural history of the toad. Previously, recommendations for management guidelines include minimizing the soil disturbance, pesticide use, and habitat fragmentation, and maximizing the restoration of corridors and potential habitat (Lacy and Seal 1994).

Since the publication of the PHVA in 1994, some recommendations have been implemented. However, all recommendations were based on adult populations. To date, the aquatic and juvenile life stages have not been addressed and therefore, are imperative for inclusion in management plans. A closer inspection of interactions between *B.*

houstonensis life stages and the local environment might increase the support for a greater protection.

HOUSTON TOAD ENVIRONMENT

Adult Houston toads use pond environments for breeding, while tadpoles use ponds for growth and development. Once metamorphosed, emergent anuran juveniles migrate to a terrestrial habitat, where they grow to sexual maturity (Semlitsch 1998) and disperse. Terrestrial habitats are essential for juvenile dispersion and growth. Therefore, both aquatic and terrestrial habitats are crucial to the Houston toad's life history pattern. These habitats tend to be located around small, fragmented wetland ponds, as anthropogenic development has damaged much of the required habitat (Brown 1975).

Protection for small wetlands is not explicitly provided by the federal government for tracts less than 0.4 ha (1 acre) as they are considered insignificant (Snodgrass et al. 2000). Combined, these small wetlands make up a large percentage of total wetland habitat. Figure 2 illustrates the distribution of natural depression wetland sizes (n = 371) from the Savannah River Site on the upper coastal plain of South Carolina where the smallest wetland was 0.2 ha. Three hundred twenty-five of 371 wetland ponds were smaller than 0.4 ha, which indicates that, although presumably insignificant, these wetlands should not be overlooked as each can represent an entire ecosystem to a myriad of species (Snogdrass et al. 2000). This example is just one of many that illustrate the importance of all wetlands, regardless of size.

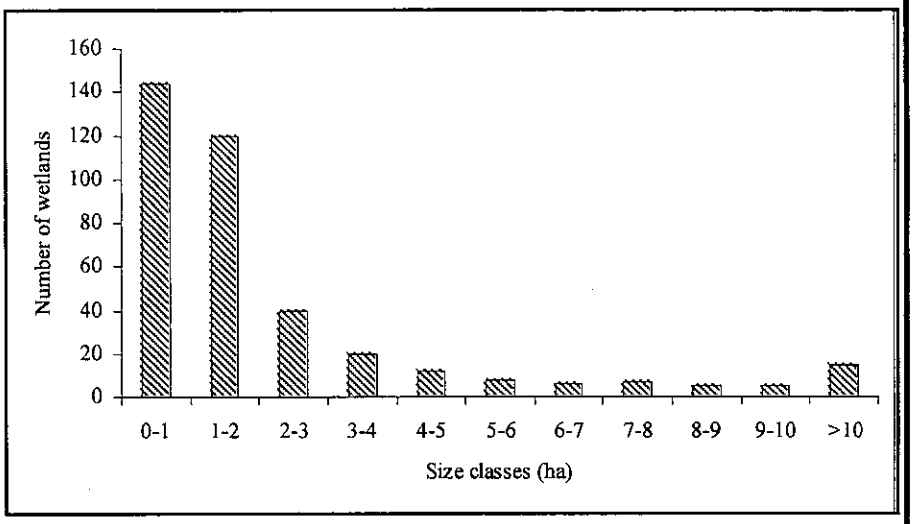


Figure 2. Distribution of natural depression wetland sizes (n=371) from the Savannah River Site on the upper coastal plain of South Carolina (Semlitsch and Bodie, 1998). The lower limit of detection from geographic information system data was 0.2 ha.

Small wetlands are critical habitat requirements for amphibians (Semlitsch and Bodie 1998) providing both aquatic and terrestrial habitats. Both the aquatic and adjacent terrestrial habitats are required to complete amphibian life cycles and maintain viable breeding populations (Semlitsch 1998). Aquatic environments are required for breeding, egg development, and larval growth. Beyond the pond's edge, multiple aspects of the terrestrial life cycles occur. Yearly hibernation and migration patterns are two aspects of the amphibian life cycle that take place beyond wetland boundaries. Adult amphibians make annual migrations from the terrestrial habitat to breeding ponds (Pechmann and Semlitsch 1986, Pechmann et al. 1989). Juveniles have the greatest abundance in wetland habitats because post-emergent dispersal has not yet begun. Once dispersal occurs, juvenile amphibians tend to migrate beyond the water's edge into small ephemeral wetlands rather than to larger wetlands (Semlitsch 2000b) due to the lack of

aquatic predators (Dayton and Fitzgerald 2001). Therefore, to complete their life cycles, the majority of amphibian species require use of not only wetlands, but also lands adjacent to them.

Protection is needed for wetland ponds and the habitat surrounding ponds. Often characterized as buffer zones, these protected areas are important because they not only allow amphibians and various other species the space required for reproduction and survival, but they also minimize human interactions and harmful impacts to these species (Clark et al. 1994). Buffer zones can increase the chances for survival of delicate species that depend on small wetlands and increase the connectivity of each small wetland section to ensure migration and aestivation are successful.

Often insufficient biological information is available for terrestrial habitats surrounding these small isolated wetlands and for the area immediately adjacent to the water's edge, both of which are required for the survival of these species (Semlitsch 1998). This is especially true in the case of the Houston toad. The use of the adjacent terrestrial habitats, the identification of relevant distances for both communities and species, and the area required for the life cycle stages can be inconsistent but necessary in the accurate determination of buffer zones (Burke and Gibbons 1995). Each of those factors will have a direct effect on guiding basic principles for managing the remaining Houston toad habitat.

Adult life stage dynamics are well known as studies have been done on hybridization (Kennedy 1961, Brown 1971, Hillis et al 1984), reproductive ecology (Hillis et al. 1984, Jacobson 1989, Price, pers. comm.) including captive breeding (Quinn et al. 1987), feeding, (Bragg 1960), and conservation (Brown 1975, Brown and Thomas

1982). However, the post-emergent ecology of the Houston toad is poorly characterized. Little research has exclusively focused on juvenile toad dispersal, developmental patterns, and survivorship. Hillis et al. (1984) addressed tadpole development and only briefly mentioned the dispersal of postmetamorphic *B. houstonensis* from the pond site. Thomas and Allen (1997) observed a small number (n = 25) of juveniles over a short sampling period (about 1 month) at a native pond. Quinn and Mengden (1983) evaluated captive raised adults from chemically induced egg strands and observed all subsequent life stages. Developmental growth was recorded, but dispersal of juveniles was not addressed. In all of these studies, dispersal or growth were the only two aspects of juvenile ecology addressed. Survivorship and morphological identification are also needed to better address population survival estimates and field identification. Yet, data on either topic have not been published.

Therefore, the current knowledge of Houston toad juvenile ecology remains incomplete. An investigation is needed to classify and clarify the importance of developmental growth, juvenile dispersal patterns, morphology, and survivorship. Such data are useful for their contribution to survivorship calculations. These data also support the need for buffer zone implementation and better management practices compatible with the toad.

With little preexisting data and current information being neither extensive nor complete, this study was designed to assemble biological and ecological characteristics of the juvenile Houston toad. This research has included developmental growth, dispersal patterns, morphological distinctions and the determination of survivorship from egg to metamorph. The results are an evaluation for the Houston toad juvenile habitat and

developmental requirements, the justification for buffer zone enforcement, and the calculation of survivorship and methods for making those assessments.

The objectives of this research were: (1) to test multiple methods of estimating egg numbers in Bufonid egg strands and apply the successful technique to *B. houstonensis* egg strands in calculating survivorship, (2) to study juvenile ecology including dispersal, growth, habitat choice, and other important factors affecting successful data collection including interspecific morphological characters, and (3) to apply this information in guidelines for management and conservation plans for this species.

FIELD TECHNIQUES WHICH AID IN THE DETERMINATION OF HOUSTON TOAD *BUFO HOUSTONENSIS* (ANURA: BUFONIDAE) JUVENILE ECOLOGY

KENSLEY L. GREUTER AND MICHAEL R. J. FORSTNER

To define ecological parameters needed in designing a management plan for an endangered species, baseline data should be collected for all life history stages (Semlitsch 2000a). This has yet to happen for the endangered Houston toad, *B. houstonensis*, where valuable larval and juvenile ecological data are scarce. The focus of this study is to define Houston toad juvenile ecology, which will aid in better management practices (Chapter 1). Before this can be done, basic life history parameters such as survivorship calculations from egg to metamorph must be available. Once the number of survivors from a cohort is established, characteristics of the juvenile ecology, such as growth, movement, and morphology can be examined to determine which influences juvenile survival to adulthood (Chapter 3).

Survivorship calculations range from simple in nature (number of individuals survived/total number initial individuals) (Shirose and Brooks 1995) to highly complex as those found in MARK (White and Burnham 1999), but the techniques used to determine the raw data for these calculations (i.e., the actual number of individuals) have rarely been explored. Published data on egg and tadpole counting techniques are limited to 3 areas of estimation: counting the number of gravid females, total masses, or individual eggs.

Information on manipulation of females, masses, or eggs exists, but detailed descriptions of methodologies are scarce. Berven and Chadra (1988) and Semlitsch and Gibbons (1990) collected wood frog, *Rana sylvatica*, egg masses. They did not count the eggs, but grouped the masses into categories based on their weight ranges. Crouch and Paton (2000) only counted the total number of wood frog egg masses in a pond to determine female/male population estimate. Reading (1986) determined how many eggs were in an egg mass by subtracting the weight of a female common toad, *Bufo bufo*, (post egg laying) from her gravid weight and dividing the remaining number by the average weight of 1 egg. Light (1974) also used this method with the red-legged frog, *Rana aurora aurora*, and the spotted frog, *Rana pretiosa pretiosa*. He modified the method to include the chemical inducement of ovulation using Rugh's (1941) pituitary method. In other studies, eggs were counted individually, but the methodology was not described (Anderson et al. 1971, Walls and Altig 1986, Semlitsch and Gibbons 1990).

Few techniques avoid handling eggs during counting. Because of clear, shallow water, large eggs, and small masses, Anderson et al. (1971) counted eastern tiger salamander (*Ambystoma t. tigrinum*) eggs without removing masses from the environment and with a minimum of handling. Biologists have used a technique that avoided manipulation of amphibian eggs by using an estimation technique where the number of eggs was counted in multiples of 10 (The Ministry of Environment, Lands, and Parks 1998).

When dealing with an endangered species, such as the Houston toad, manipulation of any part of the embryonic stage could contribute to mortality (Anderson et al. 1971) and, therefore, is generally avoided. However, little research has addressed

egg counts without manipulation or displacement. Therefore, I: (1) designed 4 estimation techniques, (2) tested these techniques on bufonid egg strands (*Bufo valliceps*) so that unnecessary harm to *B. houstonensis* egg strands can be avoided, (3) determined the best technique and apply that technique to future *B. houstonensis* egg strands, and (4) used estimation techniques to determine survivorship calculations from egg to metamorph in *B. houstonensis*.

METHODS

STUDY AREA

The Griffith League Ranch (1,963-ha; 4,848-acres) is located in Bastrop County and is owned by the Boy Scouts of America. This ranch is an ideal site for observing Houston toads due to its numerous ponds, favorable habitat conditions, and known Houston toad populations.

Seventeen ponds on the Griffith Ranch have had Houston toad chorusing and 7 of these are known breeding sites. One pond in particular was chosen for this experiment (pond 2). Its abundance of Houston toads, characteristic habitat of carrizo sand and mixed hardwood/pine forest, and easy access made pond 2 an optimal research site.

Eclosures were built for the experiment in pond 2. Two, 2-m radius aluminum flashing enclosures were built along the eastward pond's edge (0.5 m into the water). These enclosures were used to house adult toads overnight to prevent escape and to provide opportunity for breeding. As only 2 enclosures were made, multiple trials had to be performed in each one. Therefore, multiple cohorts were contained in the enclosures over time.

INVOLVED SPECIES

Only 2 *B. houstonensis* egg strands were found in the breeding pond (pond 2). Half of each egg strand was moved and divided equally into 24 artificial ponds for a separate experiment. Once the egg strand from the artificial arrays hatched, tadpoles were individually counted to determine the number of eggs hatching from that half of the strand. This additional counting was performed to determine the precision of my estimates.

Gulf coast toad egg strands were also counted later in summer due to the small number of *B. houstonensis* strands and the need to practice multiple times. Because *B. valliceps* has analogous egg strands to *B. houstonensis,* is not endangered, and readily available, the Gulf coast toad was an appropriate substitution. Eight *B. valliceps* egg strands were estimated. The actual number of eggs in each egg strand was determined by displacing each egg strand into a 5 gallon bucket until the tadpoles hatched. Once hatched, the tadpoles were individually counted and placed back into the holding enclosure until metamorphosis.

When working on *B. valliceps* strands, 3 strands died after initial development and before hatching (before stage 13, Werner, 1986) due to a lack of fertilization or some other natural cause. One *B. houstonensis* egg strand died in the pond before the eggs could hatch (before stage 13, Werner, 1986) in early March due to extreme cold weather. All 4 bufonid strands were estimated, but were not included in the data due to their incomplete nature where completed survivorship calculations could not be made.

Egg Strand Estimation Techniques

The number of eggs in egg strands were estimated using 4 techniques. Each of these techniques was performed 3 times to determine an average and to examine precision over time. Each technique and trial was timed. A description of each technique follows.

Full Wire Technique

Green craft wire was used to visually model the egg strand. The wire was molded into the shape of the egg strand and then straightened out and measured. This number was compared to a measured number of eggs (the average length of 10 eggs) to determine the total number of eggs from the model. An egg strand was modeled 3 times and then an average of the 3 attempts was taken.

Wire Section Technique

The wire section technique is similar to the full wire technique; but broken into sections, instead of modeling the entire strand. Fifty eggs were measured 3 times. These 3 measurements were averaged. This length became the wire length used to measure the whole strand. The wire section was then held near the egg strand. I counted the number of wire sections it took to estimate an entire egg strand. This method was repeated 3 times and an average number of eggs was recorded.

Spherical Technique

The spherical technique uses the geometric equation for the volume of a sphere to determine the approximate egg strand size. The number of eggs within a cm^3 (*e*) was recorded 3 times and averaged. The egg strand was estimated 3 times by visually condensing the strand into a solid sphere of eggs and measuring the diameter (*d*). This number was then used in the equation for the volume of a sphere, $V_{sphere} = 4/3\Pi r^2$ where r = the radius (cm). This estimation is particularly variable, as the equation tends to increase by orders of magnitude. Therefore, precision is imperative. With the above information, the following calculations can be performed:

1	$e = \#\ eggs/cm^3$	
2	$V_{sphere} = 4/3\ \Pi r^3$	where r is the radius in cm
3	$r = d/2$	where d is the diameter of the estimated egg sphere in cm

so that

4	$E = V*e$	where E is the total number of eggs

Individual Counting Technique

This technique is self-explanatory. Three attempts are made to count all the eggs in an egg strand. This is by far the most variable as counting ability, memory, and vision are crucial. However, I sought to test the difference between total counting and the other techniques as so many studies have reported counting eggs one by one. This method was not initiated until the third *B. valliceps* egg strand.

SURVIVORSHIP CALCULATIONS

Once multiple estimation of the egg strands occurred, the number of juveniles emerging could be used in survivorship calculations from egg to metamorph. A simple survivorship formula was used (Krebs, 1999) where the number of metamorphs to emerge is divided by the estimated or actual number of eggs and multiplied by 100 to obtain a percentage (metamorphosis survivorship). The equation is:

$$\hat{S}_0 = \frac{N_t}{N_0} \times 100$$

where $\hat{S}_0$ = finite survival rate
N_t = number of individuals alive at end of time period
N_0 = number individuals alive at start of time period

Survivorship from emergence to juvenile aged 13 weeks was calculated similarly. The Schnabel-Schumacher method (Krebs 1999) was used to determine the population estimate using mark-recapture data after juveniles emerged (data used in Chapter 3). This estimate was then similarly used in the above equation to determine survivorship from egg to 13-week old postmetamorphic juveniles.

RESULTS

EGG STRAND ESTIMATION TECHNIQUES

Results for the 4 egg strand techniques can be found in Figure 1, which is a comparison among all of the estimation techniques to the actual egg count. The standard deviation (σ^2) for each technique was: (1) wire section technique, $\sigma^2 = 1279$ eggs, (2) full wire technique, $\sigma^2 = 1536$ eggs, (3) spherical model technique, $\sigma^2 = 2098$ eggs, and (4) individual counting technique, $\sigma^2 = 1494$ eggs.

Figure 2 individually compares each technique with the actual number of eggs. Statistical analyses (Table 1) via analysis of variance (ANOVA) indicated no significant difference overall among techniques ($P = 0.27$, $\alpha = 0.05$). However, when testing each technique on the individual level against the actual number using Student's T-test, the full wire model had a significant difference ($P = 0.03$) (Fig. 2-A). Comparison of the actual number and the other 3 techniques are shown in Figures 2-B to 2-D.

Some techniques were difficult to perform. Multiple hours were spent on each egg strand. If these techniques are to be used in a realistic situation, they must be practical in nature. The degree of difficulty and the amount of time each technique took on average is explained in Table 2.

SURVIVORSHIP CALCULATIONS

Survivorship for these 7 egg strands was difficult to determine due to the lack of individual enclosures. The close proximity of the two *B. houstonensis* egg strands in the open pond (wild) prevented individual traps. There was no way to determine how many *B. valliceps* egg strands there were going to be in the summer of 2003. Therefore, only two enclosures were made. Table 3 illustrates the estimated and actual percent survival from all 7 cohorts . Survival of *B. houstonensis* metamorphs was overestimated using the estimation techniques (5.29%) when compared to the actual number of juvenile survival (4.73%). When averaged together, the estimation techniques provided a 1.36% and 0.76% survival of *B. valliceps* metamorphs as compared to the actual number which determined to be 1.20% and 0.64%. The estimated percentage was not significantly

different from the actual percentage (P = 0.89). Percent survival for each individual estimation technique ranged from 0.49% to 15.09%.

Survivorship from egg to juvenile aged at 13-weeks is even lower than that of metamorphic survivorship. The Schnabel-Schumacher method revealed an estimated population size of a 15% decline in population from 332 individuals to 281 over the course of 13 weeks. By the age of maturity (1 year for males), the survival rate, if continuously declining, would decline to 2.9%. Survivorship would be near 0 if the 15% decline was continuous for females who mature at an even later date (2 years post-emergence).

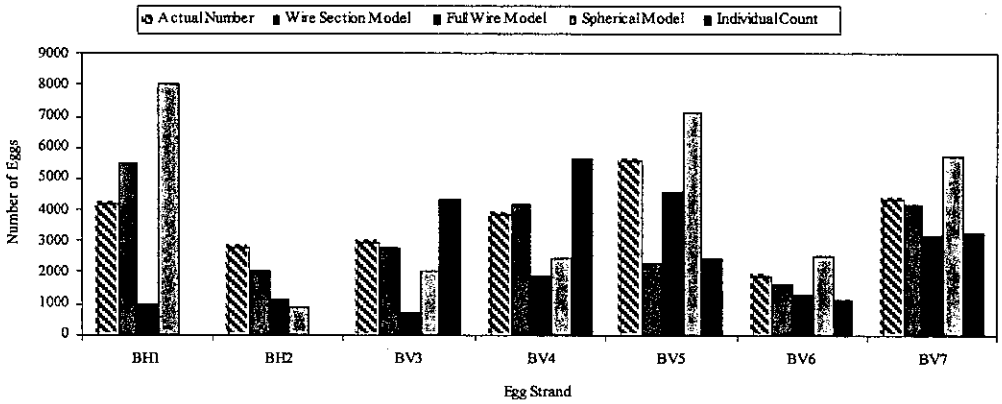


Figure 1. Average Houston and Gulf coast toad egg strand number comparison between four estimation techniques and the actual number counted. BH=*Bufo houstonensis*. BV=*Bufo valliceps*.

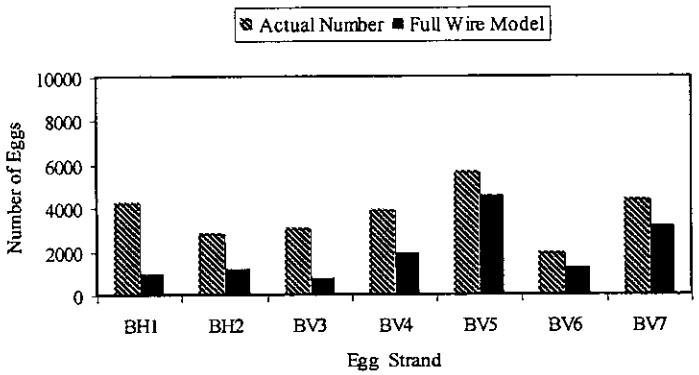


Figure 2a. Average Houston and Gulf Coast toad egg number (No. egg/strand) estimated by the full wire model and compared to the actual egg number of each strand. BH1 and BH2 are *Bufo houstonensis* egg strands. BV3 through BV7 are *Bufo valliceps* egg strands. P = 0.03, Significant difference between actual number and full wire model number.

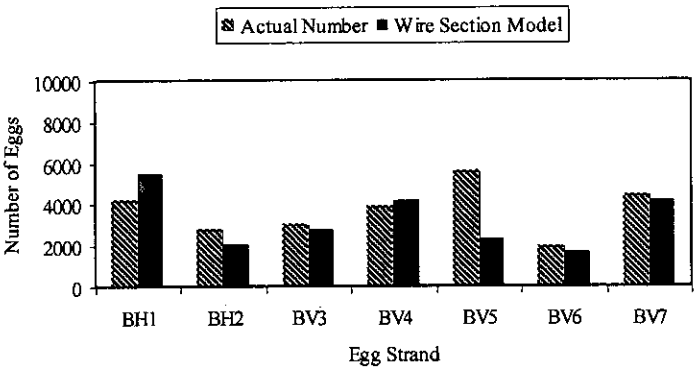


Figure 2b. Average Houston and Gulf Coast toad egg number (No. egg/strand) estimated by the wire section model and compared to the actual egg number of each strand. BH1 and BH2 are *Bufo houstonensis* egg strands. BV3 through BV7 are *Bufo valliceps* egg strands. P = 0.55, No significant difference between actual number and wire section model number.

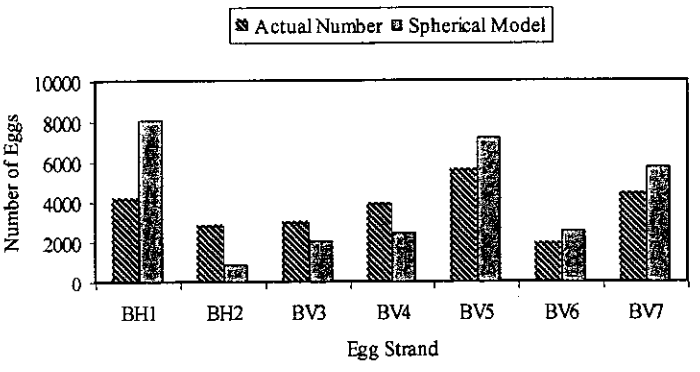


Figure 2c. Average Houston and Gulf Coast toad egg number (No. egg/strand) estimated by the spherical model and compared to the actual egg number of each strand. BH1 and BH2 are *Bufo houstonensis* egg strands. BV3 through BV7 are *Bufo valliceps* egg strands. P = 0.68, No significant difference between actual number and spherical model number.

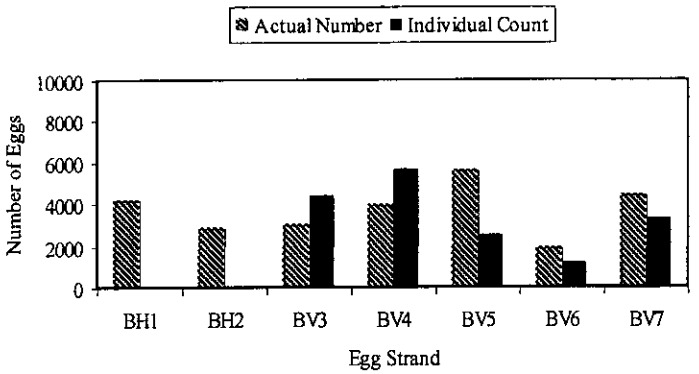


Figure 2d. Average Houston and Gulf Coast toad egg number (No. egg/strand) estimated by the individual count method and compared to the actual egg number of each strand. BH1 and BH2 are *Bufo houstonensis* egg strands. BV3 through BV7 are *Bufo valliceps* egg strands. This method was not used on the Houston toad strands. P = 0.76, No significant difference between actual number and individual count number.

Table 1. Comparison of Egg Strand Estimator Techniques showing there is no significant difference among techniques (P = 0.27) when compared together.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Actual Number	7	25862	3694.571	1513939
Wire Section Model	7	14100	2014.286	1941895
Full Wire Model	7	22850	3264.286	1988929
Spherical Model	7	29200	4171.429	7919048
Individual Count	5	17271	3454.2	3046001

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	18100321	4	4525080	1.371728	0.268953	2.714074
Within Groups	92366865	28	3298817			

Table 2. Technique difficulty on a scale of 1 to 5, 5 being the most labor intensive to estimate. The amount of time each technique approximately takes is also given in minutes.

Technique	Degree of Difficulty	Amount of Time (min.)
Full Wire Model	4	45 to 60
Wire Section Model	2	30 to 40
Spherical Model	1	10 to 30
Individual Count Model	5	90 to 120

The survivorship numbers for *B. houstonensis* egg to metamorph were surprisingly high in both the estimated and actual percent survivorship as juvenile survivorship has been estimated to be between 1% and 2% (Hatfield et al. 2002) for Houston toads during PVA simulations. This is extremely positive as survival was better than expected. *B. valliceps* estimation and actual percentages were very close to that prediction made by Hatfield et al. (2002).

There are two obvious explanations: (1) the 2 *B. houstonensis* cohorts were in optimal conditions for survival and the 5 *B. valliceps* were not. Shade and confinement could have played a role in *B. valliceps* survivorship as it is well documented that lower pond temperature (Wilbur and Collins 1973, Semlitsch 2000) and increased density (Smith 1983, Petranka and Sih 1986, Petranka 1989, Scott 1990, Van Buskirk and Smith 1991, Scott 1994) in larval populations not only decrease body size upon emergence, but survivorship as well (Wilbur 1972, Wilbur and Collins 1973, Wilbur 1976, 1977a, b, Smith-Gill and Gill 1978, Smith-Gill and Berven 1979, Pough and Kamel 1984, Goater et al. 1993); and/or (2) accuracy of estimation increased over time and the five *B. valliceps* cohort estimations are more representative of realistic survivorship numbers for successful egg strands in the wild.

The latter is most likely to occur, as even though shade was present for the 5 *B. valliceps* strands and could have affected survivorship, all 7 strands were in the same pond at relatively the same time. This large difference in survivorship percentages is not as likely to occur. *B. houstonensis* survivorship is overestimated. Because *B. houstonensis* estimation received little practice, *B. valliceps'* survivorship is relatively low, but still has a thriving population, it can be assumed that the endangered *B.*

houstonensis survivorship is closer to that of *B. valliceps'* survivorship. However, It is not known how close.

Regardless of when estimation techniques were perfected and which species benefited from better precision, there was a decrease in *B. houstonensis* survivorship over the course of 13 weeks. This decline is indicative of serious problems for the species given current PVA models. More research is needed to determine the last missing link in the survivorship chain: survival of older juvenile (> 13 weeks) to adult.

By identifying these techniques and making them applicable for realistic situations, more precise survivorship numbers can be calculated. Monitors of Houston toad populations can use these methods to aid data collection related to population dynamics. The survivorship of the remaining population is a better estimate of current population trends for endangered species and future population trends.

POSTMETAMORPHIC BIOECOLOGY ON THE JUVENILE HOUSTON TOAD, *BUFO HOUSTONENSIS* (ANURA: BUFONIDAE)

KENSLEY L. GREUTER AND MICHAEL R. J. FORSTNER

Buffer zones provide protection when anthropogenic activities conducted in surrounding habitat impact wetlands. These areas are not just a buffer; they are core habitat for many semi-aquatic species. Buffer zones are, therefore, essential for the survival of a number of species and for the preservation of biological diversity (Semlitsch 2001). Biologists who study semi-aquatic species have long understood the importance of uplands immediately adjacent to wetlands for the survival of myriad species of toads, turtles, salamanders, and other organisms (Semlitsch 1998).

The endangered species, *Bufo houstonensis*, also requires upland habitat adjacent to its wetland ponds for survival. Anthropogenic activities around vital breeding ponds and core habitat appear to play an important role in the trend toward extinction (Brown 1975). These activities would be greatly reduced with the enforced protection of buffer zones around native ponds. Information from all stages of the toad's life cycle, especially the juvenile stage, could help define Houston toad core habitat for buffer zone enforcement. Once buffer zones are enforced, better management practices for all life stages of the Houston toad could be implemented.

The habitat areas used by the juvenile stage can contribute to the definition of "core habitat" for a species and hence, juvenile dispersal can help determine the

configuration of a buffer zone. Juvenile dispersal is among the most important life-history movements as it connects populations and can thus mitigate against the deleterious effects of genetic isolation (Gill 1978, Berven and Grudzien 1990, DeMaynadier and Hunter 1999). When migrating towards other ponds, juveniles tend to travel further distances than adults (Breden 1987), sometimes extending to 200 m or beyond (Semlitsch 1998). Therefore, juveniles can actually use more of the upland habitat surrounding wetland ponds than adults and can be better indicators of required habitat.

While juvenile dispersal is well recognized for some species of amphibians, relatively little is known concerning individual movements of the Bufonid postmetamorphic stage (Daugherty and Sheldon 1982), especially the Houston toad. This lack of knowledge has spurred interest in postmetamorphic behavior that has a direct impact on the survivorship of adult populations. Breden (1987) observed dispersal in juvenile *B. woodhousei fowleri* and found juvenile migrations to surrounding ponds serve an important resource in maintaining genetic flow, a concept throughout all terrestrial amphibians. Examining postmetamorphic behaviors such as dispersal and movement are not only crucial to the genetic flow of a population, but can serve as a measurement tool in the design of buffer zones. Therefore, monitoring juvenile dispersal helps determine the core habitat for the majority of individuals.

Buffer zones are especially important to juvenile amphibians as this protected habitat allows their development, ultimately resulting in the survival and reproductive success of the species (Goater 1994). Juvenile growth analysis is essential because it can contribute to an understanding of the population processes of the species (Clarke 1974).

This stage is particularly significant for those in the genus *Bufo,* which metamorphose at an extremely small size relative to ranid and hylid frogs (Werner 1986). One physiological advantage of this small size is that juveniles minimize the risk of mortality due to desiccation (Boone et al. 2001). Clarke (1974) demonstrated *Bufo* juveniles grew 6 times as fast in the first year of life as those individuals in four ranid species. Werner (1986) suggested that this selection for a high postmetamorphic growth rate and small body size upon emergence occurs when the mortality and growth is relatively high in the larval environment and low in the adult environment, a pattern found in R-selected species. Therefore, this stage includes not only the most rapid growth (Breckenridge and Tester 1961, Labanick and Schlueter 1976), but also the highest mortality (Pechmann et al. 1991).

Contradictory to the rapidness of juvenile growth, morphological characteristics develop over time. In fact, juveniles of the genus *Bufo* are reported to be morphologically indistinguishable until maturity (Blair 1972). This ambiguity has caused concern as *B. houstonensis* can be confused with *B. valliceps, B. woodhousei,* and other congeners at this stage. In terms of conservation, detection of species' differentiation earlier than maturity would be beneficial. Identification of juvenile toads would provide an opportunity to ensure their survival through better estimation measures. This earlier detection could effectively enhance Houston toad population surveys if male audio calls, amplexus, or egg strands were not directly observed. Therefore, it is beneficial to investigate at what point morphological differentiation among congeneric bufonids occurs.

Since development in congeneric species of *B. houstonensis* have been moderately studied, the lack of juvenile *B. houstonensis* research is astonishing. Hillis et al. (1984) only briefly mentioned the movement of postmetamorphic *B. houstonensis* from their natural pond. Thomas and Allen (1997) observed only a few juveniles from a natural population (maximum of 25 individuals at 1 time) in Bastrop State Park for only 1 month. Quinn and Mengden (1984) studied captive-raised adults from chemically-induced egg strands and, hence, briefly studied the developmental growth of *B. houstonensis* juveniles. Houston toad postmetamorphic development has never been examined in natural populations while juvenile morphology has been overlooked completely.

Examining these aspects of *B. houstonensis* juvenile ecology is essential for understanding of not only Houston toad population dynamics, but for guiding conservation practices. Studying juvenile dispersal would aid in measuring core habitat used for buffer zone implementation. Analyzing juvenile growth would contribute to the overall population dynamics of a species. Identifying morphological characteristics that distinguish *B. houstonensis* would allow for quicker identification of juveniles for correct conservation practices.

Therefore, the objectives of this study were: (1) to determine postmetamorphic growth and development for multiple years and in various cohorts of toads in Bastrop County, (2) to determine when morphological characters become differentiated among bufonid species, and (3) to determine dispersal patterns and compare habitats of a natural pond and an artificially cleared field.

METHODS

STUDY SYSTEM

The study site is located on the Griffith League Ranch (1,1963-ha; 4,848-acre) in Bastrop County owned by the Boy Scouts of America. The Griffith League Ranch (GLR) serves as an ideal site for the study of juvenile Houston toads due to its numerous ponds, favorable habitat conditions, and known Houston toad populations.

Audio surveys began on the Griffith Ranch in 2000. Site-wide sampling Houston toad research began on 12 March 2001. Permanent pitfall arrays were used to capture herpetofauna throughout the Griffith Ranch. Three 23.6 m linear pitfall arrays and two 18.9 m linear pitfall arrays were installed in a cleared, open field (101-ha; 250-acre) on the property near *B.houstonensis* breeding ponds. Fourteen Y-shaped aluminum pitfall arrays were arranged in various habitats also adjacent to *B.houstonensis* breeding sites. One 94.4 m linear pitfall array was placed in marshland habitat. Plant communities associated with the arrays included: loblolly pine forest, pine-oak forest, mixed hardwood-juniper forest, and a small natural clearing.

The permanent traps were checked at dawn each day. Five-gallon buckets flush with the ground were used with bucket lids tilted over the openings to create shade and shelter. These arrays had previously collected juvenile toads.

PROJECT DESIGN

Collection Dates

Data were collected from 26 May 2001 – 17 June 2001, 18 April 2002 – 25 June 2002, and 23 April 2003 – 15 August 2003. The 2001 data were used from a previous collection by M. Gaston on the GLR.

Research Site — Native Pond

Seventeen ponds on the GLR have had Houston toad chorusing and 7 of these are known Houston toad breeding sites. One pond in particular was used for this project (Pond 2). Abundance of Houston toads, characteristic habitat of carrizo sand and mixed hardwood/pine forest, and easy access made it an optimal research site.

Pitfall traps were positioned around the natural pond to capture juveniles for marking as they emerged and traveled throughout the habitat. Traps were 2.5-quart paint mixing buckets with aluminum shading placed over each bucket to prevent desiccation. Aluminum flashing (0.3 m x 15.24 m) was used as a guide to pitfall traps.

Pitfall traps were positioned in groups of concentric ellipses around the natural pond (Figure 1). Egg mass surveys were conducted to determine how many egg masses were in the pond. Sets of ellipses were arranged at the pond's edge around *B. houstonensis* egg strings. The ellipses were placed 2 m, 5 m, and 8 m from the egg strings. Each end of the aluminum flashing was placed 3 m away from the previous end and 1 m into the water. This layout helped determine in what direction and at what rate

the juveniles emerged from the natural pond. Emerging juveniles caught by the first aluminum drift fence were marked and released on the opposite side of the flashing.

Houston toad juveniles were individually/cohort marked with toe clips (Ferner 1979). Standard measurement techniques of Houston toads caught in pitfall traps were body mass (BM), head width (HW), and snout-to-urostyle length (SUL). A maximum of 5 to 10 min. were used in handling toads. An Acculab portable scale (model # PP2060D) was used for weighing to the nearest 0.001 g. A 20 cm vernier caliper was used for obtaining the length and head width to the nearest 0.01 mm.

In 2003, improvements of previous capture methods were attempted. Fluon AD-1 (Asahi Glass Fluoropolymers USA, Inc.), a non-toxic (when dry) "paintable Teflon," had been used in several studies to prevent invertebrates (Petren and Case, 1996; Fagan, 1997; Wirth et al., 1997; Cerda et al., 1998; Losey and Denno, 1998; Lucas et al., 1998), and vertebrates such as geckos (Petren et al. 1993) from escaping pitfall traps. Fluon AD-1 could be a potential method to prevent the escape of juvenile *B. houstonensis* from buckets. Because of possible adverse effects of this product on *B. houstonensis* juveniles, a short experiment was performed. *B. houstonensis* juveniles had adverse affects from recently coated pitfalls. *B. valliceps* juveniles escaped from aged fluon-coated rims of several buckets. Fluon AD-1 did not prove suitable for retaining juvenile amphibian species in pitfalls as it is ineffective in preventing escape and may cause adverse effects in some species.

I used quadrat plots in 2002 to randomly sample for *B. houstonensis* juveniles throughout the adjacent upland habitat surrounding Pond 2. PVC pipes were cut to form

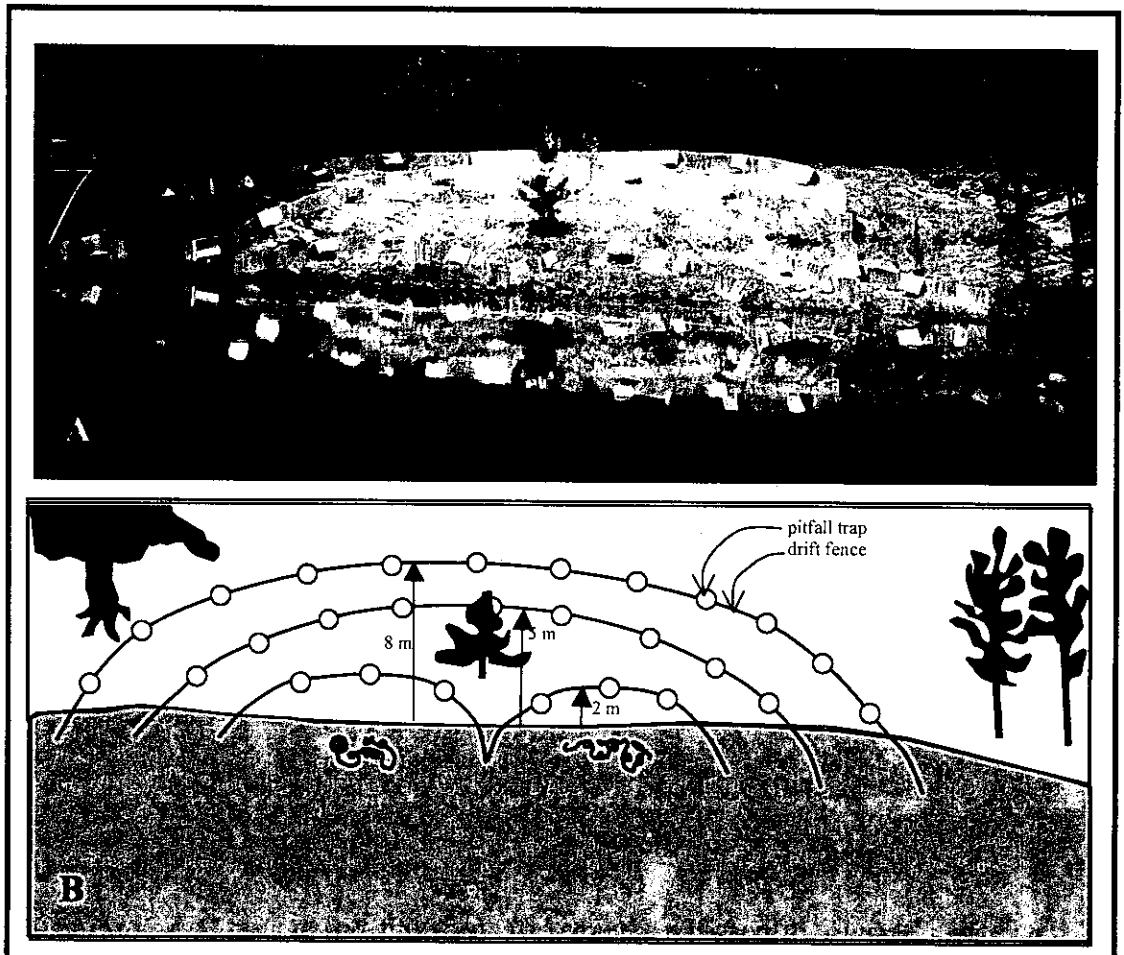


Figure 1. Elliptical aluminum arrays in (A) 2002 where the arrays curve around two *B.* houstonensis egg strands, and (B) an illustration of these arrays. The semicircles represent the elliptical aluminum arrays around an egg strand (shown in black). The arrays were placed at 2 m, 5 m, and 8 m away from the water's edge and in 3 m intervals away from the egg strand on each side. Distances are shown in red.

a 5-m^2 plot. The plots were randomly sampled in a flagged grid encircling the pond and extending 50 m into the surrounding upland habitat. A 1-m^2 plot was used to sample only the pond's immediate edge in 2002.

Five small moist refuges were constructed in 2002 at random distances around the natural pond. Figure 2 illustrates the 3 stages of construction of local refugia around the natural habitat. These refugia were dug 15 cm into the ground, lined with 3m x 3m rubber liner (65 ml EPDM—Anjon Building Products), and filled with leaf litter, water, and sand. They were flush with the ground surface to simulate the juveniles' environment. Every 3 days the refugia were sprayed with 7.5 l of water to ensure moisture. They were checked every 3 days to determine distances juveniles moved from the pond beyond ellipses and the abundance of juveniles at a distance.

In 2003, a combination of quadrat plots and refugia was used. Fifty 1-m^2 refugia were randomly dug into the flagged grid, which now extended 250 m into the upland habitat. Because refugia were randomly placed, they took the place of the previous quadrat plots and large refugia.

Sampling for juveniles covered a variety of habitat conditions. Five categories were used to determine habitat preference: (1) habitat type: forest vs. pasture; (2) vegetation type: loblolly pine vs. mixed hardwood-juniper vegetation; (3) soil type: carrizo sand vs. red clay subsoil; (4) soil moisture: moist vs. dry; and (5) sun exposure: shade vs. sunlight. At the time of capture, habitat condition was recorded.

Figure 2. Three stages of construction of local refugia found around the natural habitat. They are made from (A) pond liner, then covered with (B) moist, carrizo sand, and then layered with (C) pine needles. The refugia were checked every 3 days and sprayed with water to determine if juveniles tend to gather only near moistened areas.

Research Site — Artificial Ponds

Twenty-four artificial ponds were constructed in February 2002 in a large, cleared field for a separate project. These ponds were in a pattern consisting of 4 rows with 6 ponds per row. The ponds were used to rear cohorts of Houston toads, thus providing data for comparison with results from natural ponds.

For all 3 years, pitfall traps positioned around artificial ponds captured juveniles as they emerged from ponds. I marked juveniles and followed their movement throughout the habitat. There were 2 sets of traps: (1) around each individual pond, and (2) around the perimeter of the entire 24-pond array.

Aluminum flashing was placed around each pond as a 1-m border. Two 2.5-quart paint buckets were positioned as pitfall traps around each pond's border. The outer perimeter of pitfall traps was placed 10 m on the north and south sides and 20 m on the east and west sides away from the outer ponds. These traps consisted of two 30.5 m and two 45.7 m linear pitfall arrays. Additional traps were 5-gallon buckets. The buckets were buried in the ground so they were flush with the surface. Once a juvenile was captured in a trap, it was released outside of the 24-pond array to determine the time of arrival at the outer perimeter. All juvenile Houston toads were marked with the method used at the natural pond site.

Additional Pond Sampling

Ponds in Bastrop County were sampled in 2003 in addition to the main research sites. Two privately owned ponds with dispersing juveniles were surveyed. Pond 11 on

the GLR had juveniles dispersing in early summer and was counted as a third additional pond. At least 15 measurements and tissue samples were taken from each of the 3 locations. These samples were collected to compare juvenile mass from the natural pond site to those in the surrounding area and to determine if there was a significant difference in growth patterns.

Statistical Analysis

I used an analysis of variance (ANOVA) to compare the 3 years (2001-2003) to determine if there was a difference in juvenile mass. ANOVA was also used to compare the initial capture masses of all five ponds (2 research sites and 3 additional ponds in 2003). A 2-factor ANOVA was used to compare initial and recapture data with the different years (2002 and 2003 only). T-tests were performed on comparisons between previous data and this study's data, between initial and recapture data, and between the 2 research sites (natural vs. artificial ponds). Pearson and Spearman Correlation Coefficients were used to determine a correlation between mass and time.

Molecular Identification

Bufo sp. juveniles are phenotypically indistinguishable at the species level. Several sympatric species of *Bufo* inhabit the GLR, including *B. valliceps, B. woodhouseii,* and *B. houstonensis*. A polymerase chain reaction (PCR) marker system was used to distinguish the different species. This test positively identified juvenile *B. houstonensis* by using species-specific primers designed in our laboratory. A positive band identified Houston toads to the exclusivity of other taxa. A sample of the cohort

was tissue sequenced. DNA was extracted from tissue obtained by clipping toes of captured juveniles using the DNeasy protocol for Animal Tissues (Quiagen #69506). The DNA was amplified by PCR using the primers BHCB primers sequence and BHDLR2 primers sequence. The PCR product was analyzed using agarose gel electrophoresis to determine the species of the tissue donor. This identified species by the presence and relative size of the resolved PCR product on the electrophoresed gel.

RESULTS

GROWTH & DEVELOPMENT

Evaluation of 2001, 2002, & 2003 Data

General information about all ponds sampled, the abundance of initial juvenile captures, and the number of days sampled can be found in Table 1. The number of pond sites increased over the 3-year period. Sites were not consistent in the number of juveniles caught and number of days of observation. A t-test indicated a significant difference in abundance and days observed ($P = 0.040$), confirming that multiple juveniles were captured some days and few on others. For a detailed description of the mark/recapture data, refer to APPENDICES 3 - 5.

Molecular identification confirmed that juveniles used in this research were indeed *B. houstonensis*. There were several juvenile tissue samples taken later in the summer when cohort marking was already completed that were not expected to be *B. houstonensis*. These specimens were confirmed to be the genus *Bufo*. These juveniles were most likely *B. valliceps* as this species' breeding season is later than *B. houstonensis*. *B. valliceps* juveniles emerged later but grew more quickly than *B.*

houstonensis juveniles and could look similar to *B. houstonensis* juveniles. Figure 3 shows PCR results of *Bufo* tissue samples taken. For a complete record of PCR results from *Bufo* tissue, refer to APPENDIX 1. For a detailed description of each sample taken, refer to APPENDIX 2. Two PCR analyses were performed on the inconclusive samples (white labels).

Initial *Bufo* juvenile mass for all 3 years is illustrated in Figure 4 where mass gradually increased more rapidly in 2003 than in preceding years. This analysis excluded recapture data for 2001. There was no significant difference (Table 2) in juvenile mass ($P = 0.663$) for the three years. This was surprising as collection numbers and patterns were variable throughout the 3-year period.

Evaluation of 2002 & 2003 Data

I compared juvenile mass data for this study to the mass data reported in the captive study of Quinn and Mengden (1984). The 2003 data was consistent with Quinn and Mengden's (1984) captive SUL data because Quinn and Mengden's (1984) regression line ($R^2 = 0.99$) was not significantly different ($P = 0.36$) from this study's 2003 SUL data regression line ($R^2 = 0.81$) (Figure 5). The regression lines explained > 80% of the variation in both datasets.

However, when the regression line for 2002 ($R^2 = 0.34$) was compared to the 2 other years (1984 and 2003), a significant difference was found ($P = 0.040$). When comparing the raw data among all 3 years (1984, 2002, & 2003), a significant difference was also found ($P = 2.51E-05$). A post hoc test, Tukey's procedure, was performed and the

Table 1. Description of pond site abundance of initially captured bufonid juveniles and number of days observed at each pond per year. Pond 2, the artificial arrays, pond 11, various sites on the Griffith League Ranch (GLR), and 2 privately owned (PO) ponds from Bastrop County were sampled.

Year	Site	Abundance	Days Observed
2001*	Pond 2	15	7
2002	Pond 2	325	37
	Artificial Arrays	118	13
2003	Pond 2	332	43
	Artificial Arrays	57	29
	GLR	14	7
	Pond 11	62	6
	PO #1	67	5
	PO #2	16	1

*2001 data were used from a previous collection

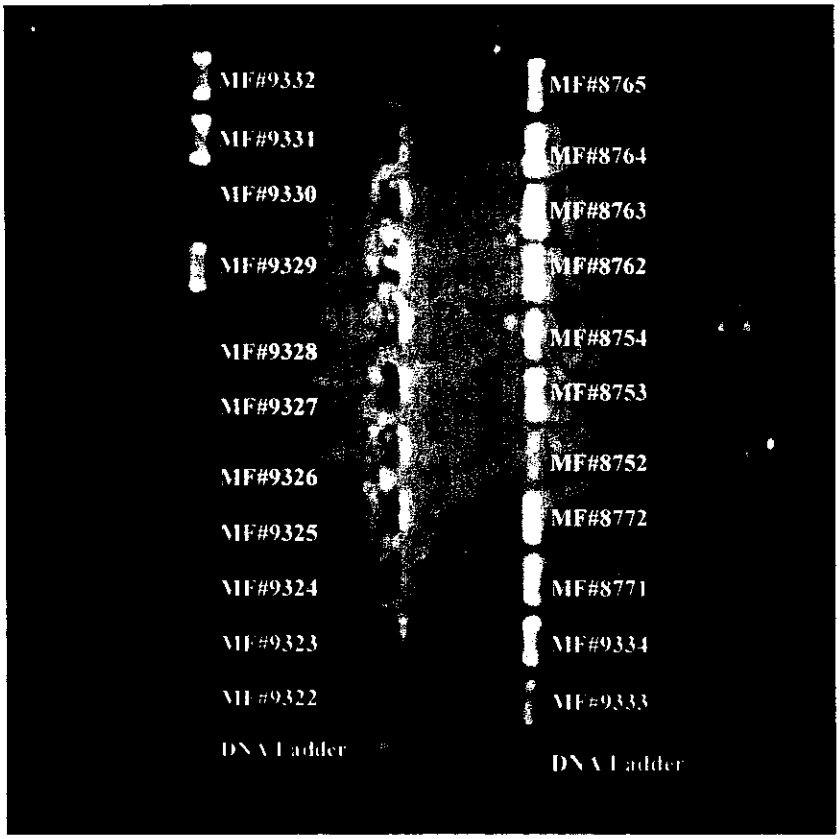


Figure 3. Example of PCR Molecular identification results of juvenile *Bufo* tissue samples from MF#9322 - MF#9334 and from MF#8752– MF#8772 using a 1% Agarose gel and *Bufo houstonensis* specific primers, BHCB and BHDLR2. Samples in yellow are *B. houstonensis* positive. Samples in white are inconclusive. These samples were run again and were attempted to be identified a second time.

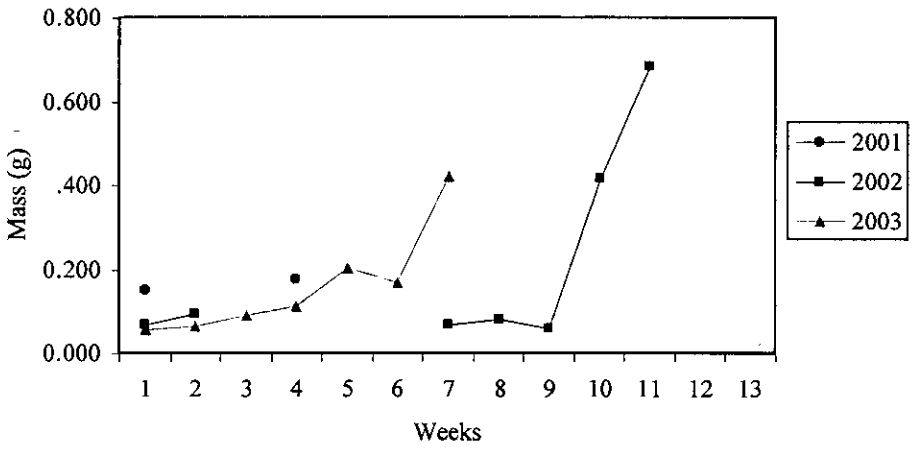


Figure 4. Initial mass of *B.houstonensis* metamorphs for 13 weeks during spring of 2001, 2002, and 2003 after metamorphosis. The 13-week interval that began the last week of April and ended the second to last week of July was the same for all three years. Initial mass was used for all three years in this analysis due to the lack of recapture data in 2001. The blue circle represents data collected in 2001, the red square represents data collected in 2002, and the green triangle represents data collected in 2003.

Table 2. Summary of ANOVA comparison among years 2001, 2002, and 2003 using weekly juvenile mass averages. The variance for 2001 = 0.000313, for 2002 = 0.03011, and for 2003 = 0.241656. The P = 0.663659 indicating no significant difference among the three compared years.

Anova: Single Factor Comparison Among Years 2001, 2002, 2003

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
2001	2	0.325	0.1625	0.000313
2002	7	1.474138	0.210591	0.06011
2003	11	4.006474	0.364225	0.241656

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.137254	2	0.068627	0.420035	0.663659	3.591538
Within Groups	2.777529	17	0.163384			
Total	2.914783	19				

significant difference was found within the 2002 SUL data (P = 0.031) confirming the regression line analysis conclusion.

There was a significant correlation between mass and snout-to-urostyle length as was expected in both 2002 and 2003. This 2003 correlation is shown by the Pearson Correlation Coefficient (r = 0.93) and the Spearman Correlation Coefficient (r_s = 0.66). The Spearman coefficient is more conservative than the Pearson coefficient, but it still indicates a positive correlation. Figure 6 depicts the correlation of SUL and mass in 2003. The linear equation is y=9.7027x=8.2814.

Figures 7 and 8 both depict weekly mass averages of juveniles captured in the natural pond (Pond 2) for the 11-week period of emergence in 2002 and 2003. The t-test was used for comparison. For the 2002 data shown in Figure 7, there was no significant difference between initial and recapture data (P=0.41). For the 2003 data shown in Figure 8, there was no significant difference between initial and recapture data (P=0.45).

A two-factor ANOVA was used to compare differences between initial/recapture data and years (2002 & 2003). There was no significant difference between 2002 and 2003 (P = 0.43), initial and recapture data (P = 0.26), or the interactions among the four variables (P = 0.08).

Two sites, the natural and artificial settings, were compared for 2002 and 2003. No juveniles were recaptured in the artificial pond setting in both years. In 2002, there was not a significant difference (P = 0.12) between Pond 2 and the artificial pond (Figure 9) initial masses. However, in 2003, there was a significant difference (P = 0.03) in the data between Pond 2 and the artificial pond (Figure 10) initial masses. In 2003, ponds were covered by a mesh lining that lowered water temperature and caused a very

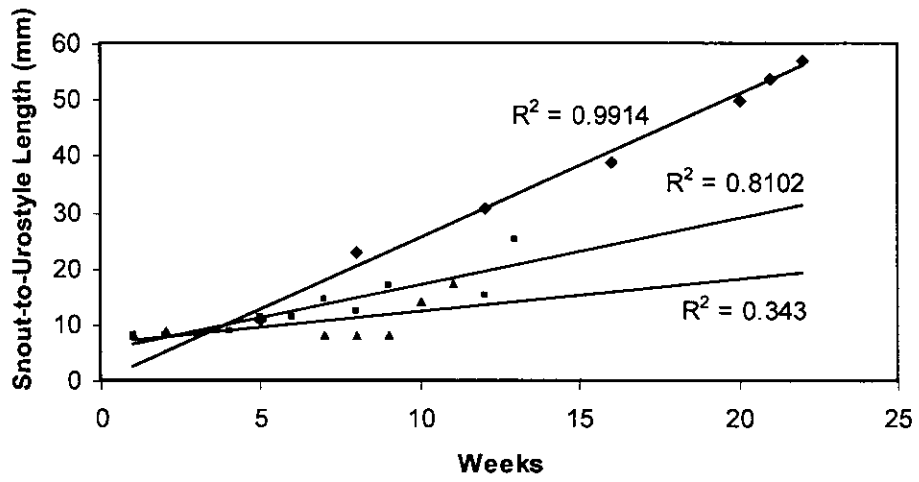


Figure 5. Snout-to-urostyle length comparison between 1984 captive data and this study's 2002 & 2003 wild data. Comparison was categorized by weeks, from the last week of April to the first week in October. The blue triangles represent data collected in 1984 from Quinn and Mengden with an R^2 value of 0.99. The black triangles represent the data collected from this study in 2002 with an R^2 value of 0.34. The red squares represent the data collected from this research study in 2003 with an R^2 value of 0.81.

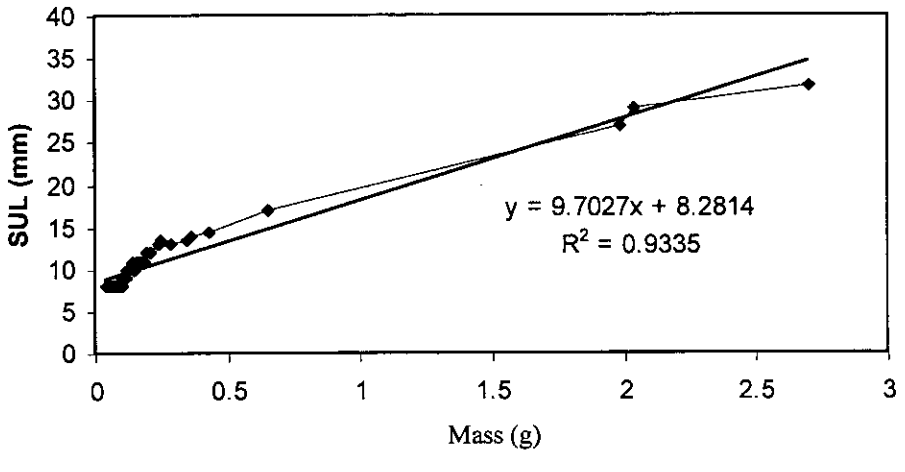


Figure 6. 2003 correlation of mass (g) to snout-to-vent length (mm). R^2=0.93 indicating that 93% of the variation in the dataset is explained by this line (y=9.7027x+8.2814).

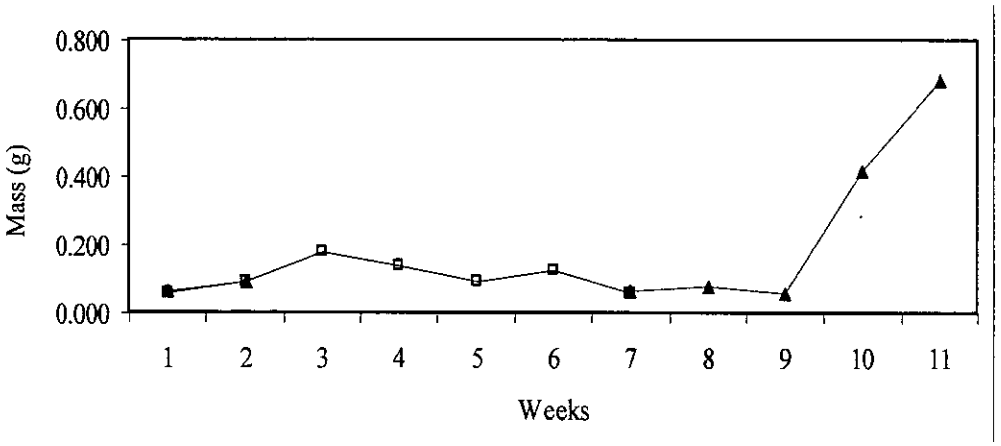


Figure 7. Initial and recapture mass of postmetamorphic *B. houstonensis* juveniles at Pond 2 for 11 weeks in 2002. The 11-week interval that began the last week of April and ended the second week of July was the same for all three years. The blue triangle represents the initial average mass per week and the red open square represents the recapture average mass per week. No significant difference was found between initial and recapture data (P = 0.41)

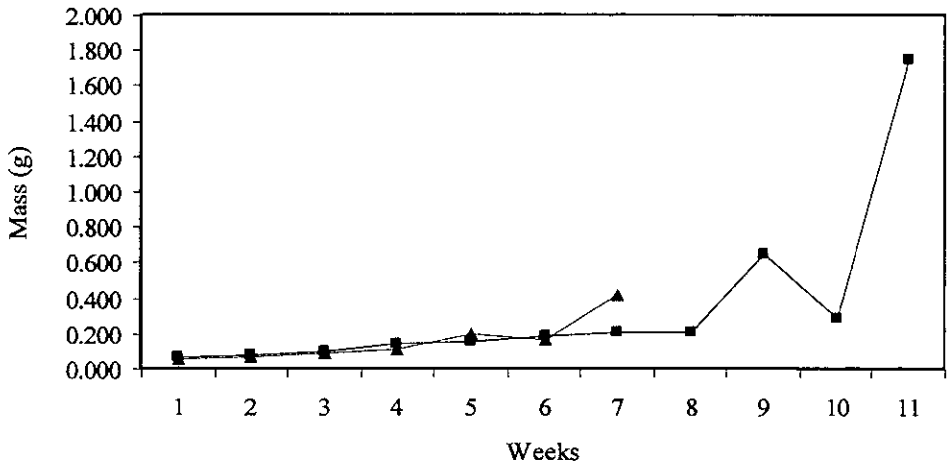


Figure 8. Initial and recapture mass of postmetamorphic *B. houstonensis* juveniles at Pond 2 for 11 weeks in 2003. The 11-week interval that began the last week of April and ended the second week of July was the same for all three years. The blue triangle represents the initial average mass per week and the red square represents the recapture average mass per week. No significant difference was found between initial and recapture data (P = 0.45).

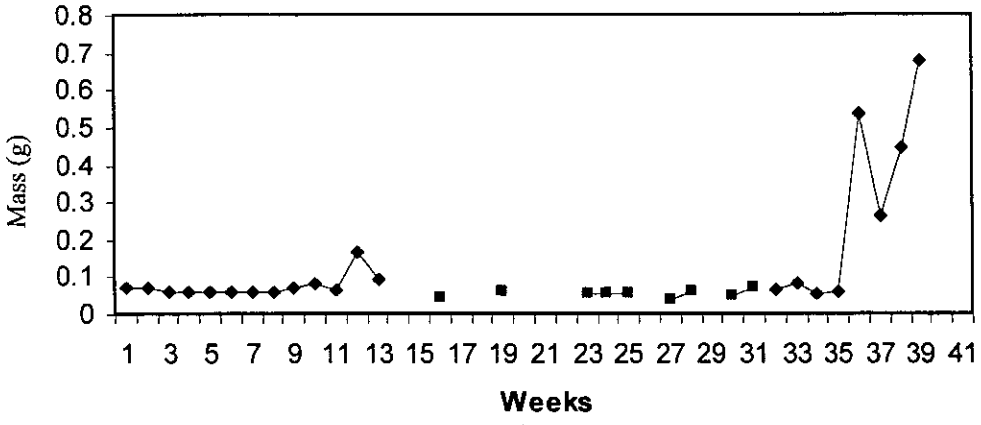


Figure 9. Initial mass of postmetamorphic *B. houstonensis* juveniles from one cohort in 2002. Data shown is average mass/day from 18 April 2002 to 25 June 2002. Half of the cohort was reared in a natural pond setting and the other half was reared in 24 artificial ponds. The solid blue diamond represents data collected from Pond 2, the natural pond selected for this research. The solid red square represents data collected from the 24 artificial ponds.

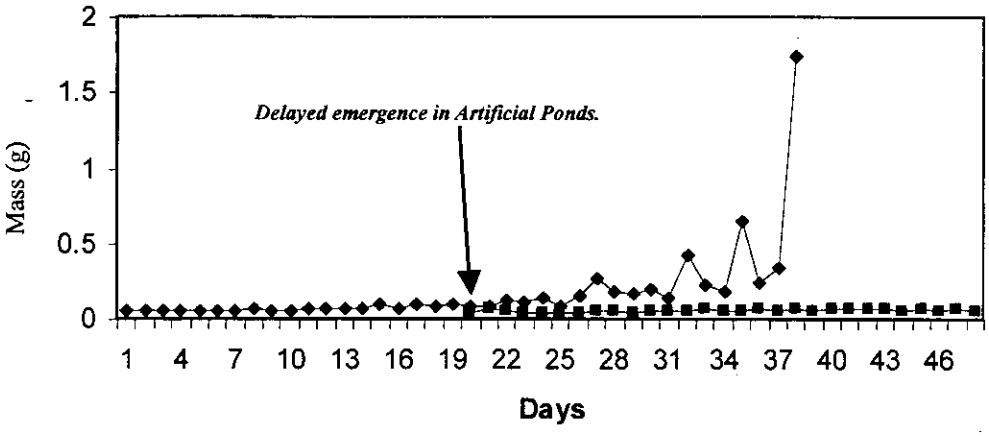


Figure 10. Initial mass of postmetamorphic *B. houstonensis* juveniles from one cohort in 2003. Data shown is average mass/day from 23 April 2003 to 20 July 2003. Half of the cohort was reared in a natural pond setting and the other half was reared in 24 artificial ponds. Juveniles were still found emerging by week 11 in the artificial pond; however, growth was stagnant. The solid blue diamond represents data collected from Pond 2, the natural pond selected for this research. The solid red square represents data collected from the 24 artificial ponds.

different effect. Emergence out of the artificial array did not begin until almost 3 weeks after those in the natural pond in 2003. In 2002, juveniles emerged from artificial ponds almost a week and a half after Pond 2 juveniles ceased to be found.

The difference in mass between a juvenile from the natural pond and a juvenile from the artificial pond is illustrated in Figure 11. At the time the picture was taken, both juveniles were 99 days old (from egg to postmetamorph) and weighed 0.450 g (juvenile on left) and 0.079 g, respectively. The juveniles had the same age, but different emerging times. The juvenile that emerged from the natural pond was almost five and a half times larger, which was a significant difference ($P < 0.01$).

Evaluation of 2003 Data

Various ponds were surveyed for *B. houstonensis* juveniles in addition to the 2 main research sites (natural and artificial ponds). One privately owned pond (Ponderosa Dr., Bastrop County) had over 500 juveniles dispersing from a recently excavated pond. Sixty-nine measurements and tissue samples were taken from this site. Another privately owned pond near HWY 290 in Bastrop County was inspected. This pond yielded 18 juvenile tissue samples and measurements. Pond 11 on the GLR also had dispersing juveniles. Sixteen samples were taken from this site. The samples were determined to be *B. houstonensis* tissue (APPENDIX 1) and a detailed description of each sample can be found in APPENDIX 2.

Figure 11. Differences in mass between a juvenile from the natural pond setting (left) and a juvenile from the artificial pond setting (right). Both are 99 days old (from egg to postmetamorph). The masses were 0.450 g (left) and 0.079 g (right).

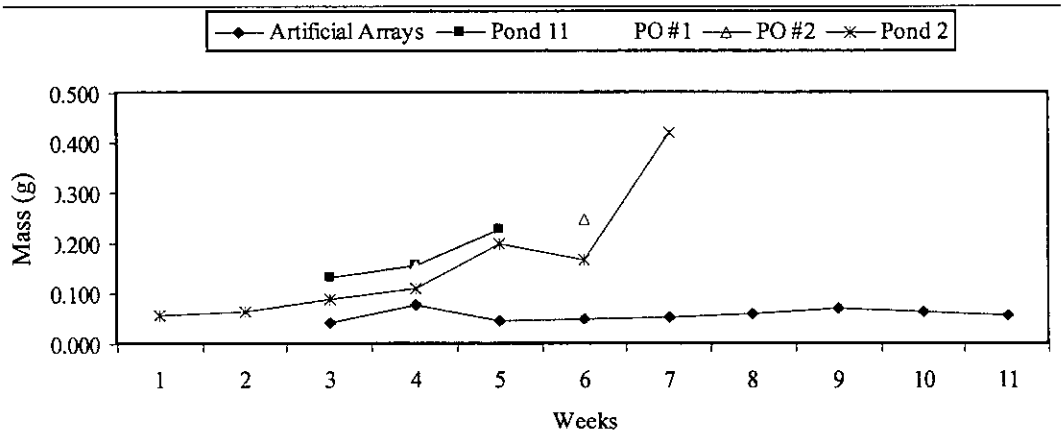


Figure 12. Initial mass of postmetamorphic *B. houstonensis* juveniles for 11 weeks in five ponds sites in Bastrop County. All pond sites used the same 11-week interval that began the last week of April and ended the second week of July. The solid blue diamond represents data collected from the artificial arrays. The solid red square represents data collected from pond 11 on the Griffith League Ranch. The solid yellow triangle represents data collected from a privately owned (PO) pond in Bastrop County. The open green triangle represents data collected from another privately owned (PO) pond in Bastrop County. The purple star represents data collected from Pond 2, the breeding pond selected for this research.

Table 3. Summary of ANOVA comparison among five Bastrop County pond sites from 2003. The variance for the artificial arrays=0.000121, for Pond 11=0.002547, for one privately owned pond=0.002082, and for Pond 2=0.016371. PO #2 was eliminated from this analysis because it only had a single data point and was skewing the results. P=0.06 which does not indicate a significant difference.

Anova: Single Factor Comparison Among Five Pond Sites in Bastrop County

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Artificial Arrays	9	0.522911	0.058101	0.000121
Pond 11	3	0.518468	0.172823	0.002547
PO #1	3	0.332202	0.110734	0.002082
Pond 2	7	1.113974	0.159139	0.016371

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.052778	3	0.017593	2.919893	0.062238	3.159911
Within Groups	0.108452	18	0.006025			
Total	0.16123	21				

The 5 different pond sites in Bastrop County for 2003 are compared in Figure 12 and their statistics are described in Table 3. Growth from all but 1 pond shows a similar pattern. Growth for the artificial ponds was slow and inconsistent with the other 4 pond sites. Pond 2 had the highest average growth for each week. There was no significant difference among the 5 ponds (P = 0.36). The 2 privately owned ponds were compared and had no significant difference (P = 0.12).

MORPHOLOGICAL IDENTIFICATION

Assumptions have been made that morphological differentiation cannot be identified until adulthood (Blair, 1972). However, morphological differences of *B. houstonensis* and sympatric *B. valliceps* could be determined after a certain age. Figure 10 illustrates morphological distinction in *B. houstonensis* and *B. valliceps*. These photos were taken on 20 July 2003 in week 13 of the *B. houstonensis* post metamorphosis.

Figure 13 illustrates a *B. houstonensis* juvenile alone (Figure 13-A). The same *B. houstonensis* juvenile is compared to a *B. valliceps* juvenile in Figure 13-B. Note the smaller size of *B. valliceps* indicating a possibly later emergence date. Head and side views of 3 *B. houstonensis* juveniles are shown in Figure 13-C. The *B. valliceps* juvenile is shown with *B. houstonensis* juveniles in Figure 13-D. The dorsal side of the same four juveniles is shown.

Morphological characteristics that distinguish *B. houstonensis* from *B. valliceps* were: continuous dorsal spots of light color and blotchiness, inconspicuous mid-dorsal line, and a lack of dark lateral coloration. These characteristics can be identified in the pictures (Figure 10). Reddish spots and light coloration are highlighted in Figure 10-B.

B. valliceps (right) is absent of these colorations. In Figure 10-C, an arrow is pointing to a dark, solid lateral coloration on the *B. valliceps* juvenile. The 3 other juveniles (all *B. houstonensis*) do not have this dark coloration. In Figure 10-D, an arrow is pointing to a conspicuous mid-dorsal line on the *B. valliceps* juvenile which is either absent from the 3 *B. houstonensis* or is present in only a pale form (as the red arrow indicates). All juveniles from all locations were compared consistently for the 13 weeks post emergence. The 13th week was the first week that differentiation could be recognized.

PCR positive bands for *B. houstonensis* only primers and genus *Bufo* primers confirmed these morphological differentiation hypotheses. Figure 14 illustrates the confirmation of what was thought to be *B. houstonensis* tissue. All thought to be *B. houstonensis* were confirmed. All those thought to be *B. valliceps* were negative on the gel that used *B. houstonensis*-specific primers and were positive on *Bufo* genus-specific primers.

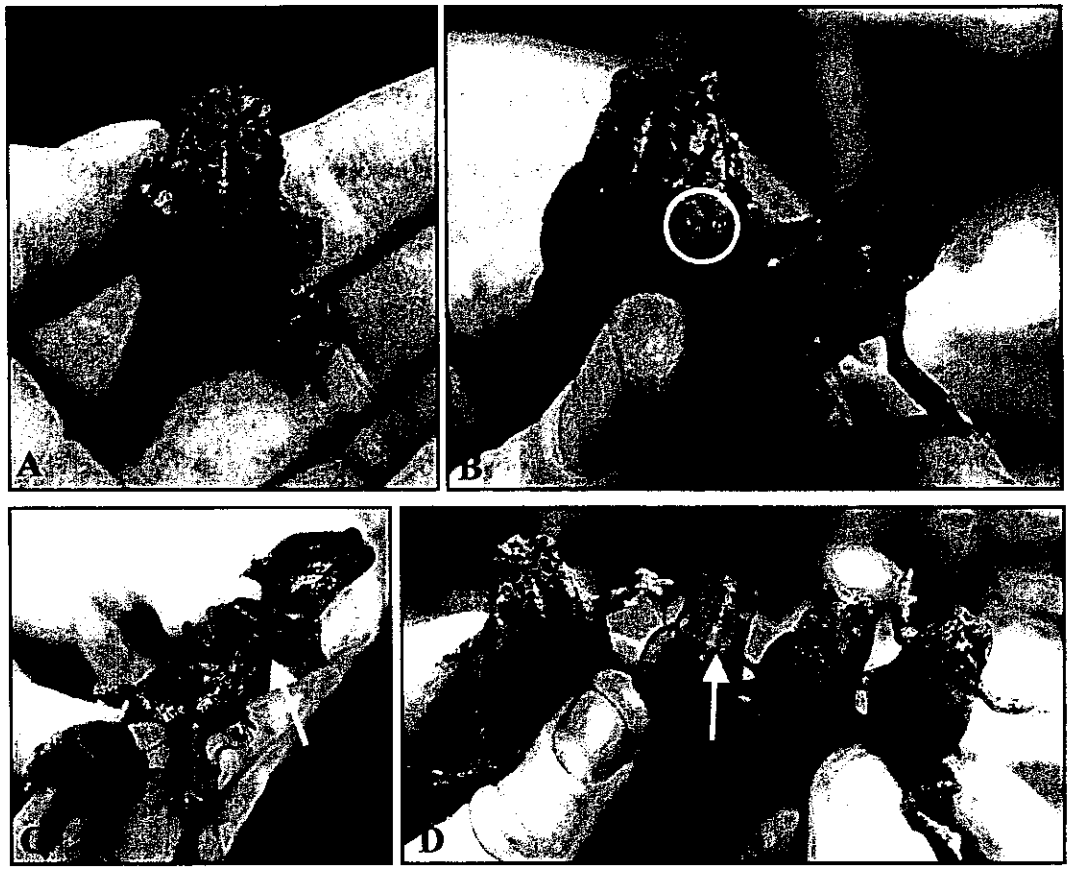


Figure 13. Four illustrations of morphological distinction. These photos were taken on 20 July 2003 that is week 13 of the 2003 *B. houstonensis* postmetamorphic emergence. *Bufo* juvenile illustrations depict (A) a *B. houstonensis* juvenile alone (wt=2.696 g), (B) the same *B. houstonensis* juvenile from (A) along with a *B. valliceps* juvenile, (C) head and side views of 3 *B. houstonensis* juveniles and only one *B. valliceps* juvenile (2nd from the top), and (D) dorsal side of the same four juveniles. *B.* valliceps is the 2nd one from the left. The masss of the juveniles are from left to right as shown in (D): wt=2.696 g, 1.627 g, 2.035 g, and 1.980 g.

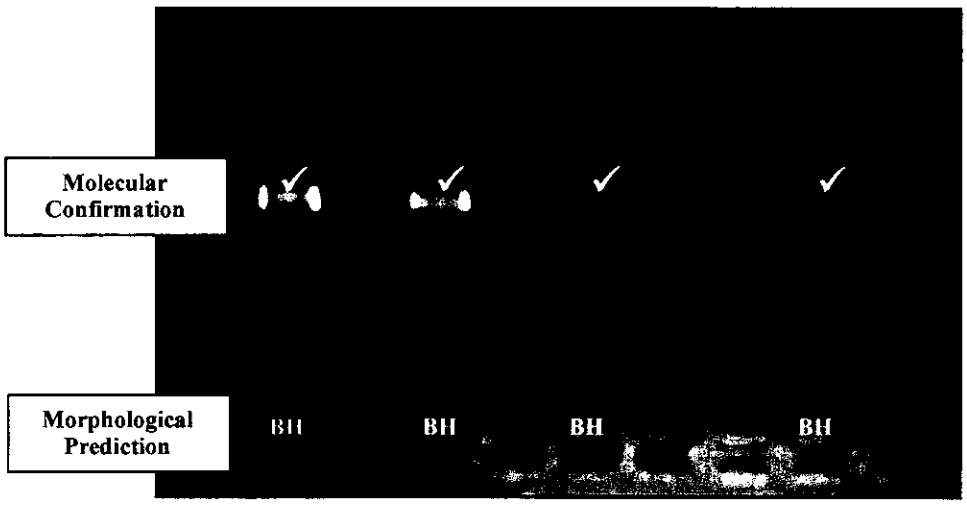


Figure 14. PCR results on a 1% Agarose gel using primers BHCB and BHDLR2. The row of morphological predictions represents the field assessment of morphological differences and the educated deduction as to which species the juvenile was. The molecular confirmation represents which tissue donor was *B. houstonensis* (BH). Those that were thought to be *B. houstonensis* were indeed confirmed to be. Those that were thought to be *B. valliceps* (BV) were negative on this gel. These five *Bufo* tissue donors were tested with the Bufo genus primers and those came out positive, indicating that these samples were not degraded, just not *B. houstonensis*.

DISPERSAL PATTERNS

Dispersal was observed only at the natural pond. No recapture data was collected in the habitat surrounding the artificial arrays in 2002 and 2003. During late April through mid July, many young, recently metamorphosed Houston toads left the breeding pond and invaded Pond 2's upland habitat a week or more elapsing after emergence.

Using the elliptical arrays at Pond 2, juveniles reached the 8 m aluminum barrier by week 2 in both years. In 2002, juveniles reached the last elliptical array on day 19 post emergence. In 2003, juveniles reached the last array on day 13 post emergence. Juveniles stayed close to the water's immediate edge for the first 3 weeks and then gradually started migrating towards the upland habitat adjacent to the pond.

Five *Bufo* juveniles were found using the large refugia 20 m - 35 m away from the pond in 2002. Four juveniles were found using the small refugia up to 50 m away from Pond 2 in 2003. Eighteen *Bufo* juveniles were found throughout the GLR in 2003, but only after precipitation occurred.

A correlation was found when comparing distance dispersed to juvenile mass. A greater correlation was recognized in 2002 ($r = 0.67$) than in 2003 ($r = 0.51$). However, both years indicated a positive correlation. This positive relationship signified that larger juveniles disperse farther away from breeding ponds.

Because all sampling covered a large array of habitat, moist vs. dry, shade vs. sunlit, sand vs. clay, and pine vs. mixed oak-juniper, habitat preference could be deduced.

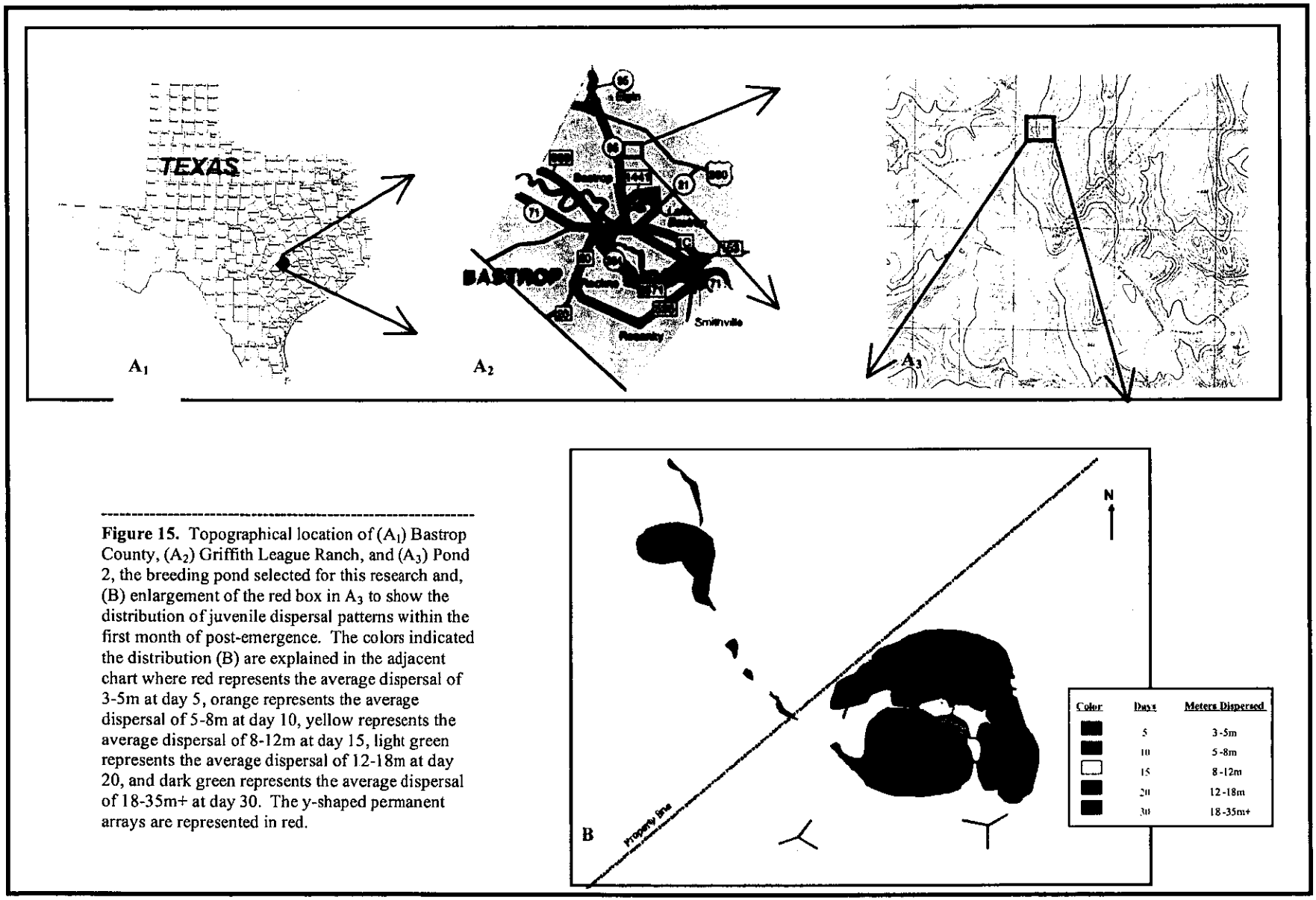


Figure 15. Topographical location of (A_1) Bastrop County, (A_2) Griffith League Ranch, and (A_3) Pond 2, the breeding pond selected for this research and, (B) enlargement of the red box in A_3 to show the distribution of juvenile dispersal patterns within the first month of post-emergence. The colors indicated the distribution (B) are explained in the adjacent chart where red represents the average dispersal of 3-5m at day 5, orange represents the average dispersal of 5-8m at day 10, yellow represents the average dispersal of 8-12m at day 15, light green represents the average dispersal of 12-18m at day 20, and dark green represents the average dispersal of 18-35m+ at day 30. The y-shaped permanent arrays are represented in red.

Juveniles were only found in moist, shady areas. Juveniles were found in both soils and vegetation types. Therefore, soil and vegetation type were not necessarily factors in habitat choice.

Figure 15 provides a topographical location of Pond 2, the breeding pond selected for this research and, the distribution of juvenile dispersal patterns within the first month of post emergence. Color is used to demonstrate correlation between time and the maximum scale of dispersal distance over which *Bufo* juveniles were found.

DISCUSSION

GROWTH & DEVELOPMENT

Bufo houstonensis postmetamorphic development was inconsistent, at least in part, with previously documented juvenile growth data (Quinn and Mengden, 1984). The significant difference found among the 2002 and 2003 research SUL data and Quinn and Mengden's SUL data (1984) was not surprising as captive studies tend to rear healthier cohorts than those found in the wild where consumption, predatorial, and desiccation factors are a constant threat. However, it was important to note the relationships within each dataset, especially the high R^2 value in Quinn and Mengden where mass/time was almost 100% correlated. Perhaps juveniles in the lab, with consistent food availability and absence of survivorship stresses, are more uniform in development. Another advantage of captive-raising toads is that toads can be measured for a longer time than in the wild because wild-caught juveniles tend to disappear after 13-15 weeks and will not appear until the following breeding season.

As expected, there was a high significant correlation between mass and snout-to-urostyle length in both 2002 and 2003. This correlation was important because the relationship indicates proportionality in growth. In some cases, when only mass or SUL can be measured, this high correlation allows assumption for the missing measurement by using the simple linear equation, $y = 9.7x+8.3$, where x = mass (g) and y = SUL (mm). This equation may vary from year to year and has only been applied to juveniles. Therefore, adult correlations would need development.

In 2003, collections at 5 different ponds in Bastrop County showed a consistent pattern up until week 5 (late May) when either collections ceased or juveniles became rare. It is important to note the lack of a significant difference in the timing of juvenile abundance and elevated densities among the Bastrop County pond cohorts. Juvenile bufonid growth was consistent throughout the county for at least the first 6 weeks post emergence.

The juveniles from Pond 11 on the GLR were larger in size than those found at pond 2 in 2003. Juveniles in Pond 2 were larger in size than those found at artificial ponds. Pond 11 juveniles emerged earlier than those in Pond 2 just as juveniles in Pond 2 emerged and were larger than those found at artificial arrays.

The comparison of initial and recapture measurements for 2002 and 2003 did not indicate a significant difference. This result should not be surprising as these data are from the same individuals of a cohort. The 2 figures (Figure 6, Figure 7) show that there were small inconsistencies with these data collections indicating precision in measurements.

Growth was rapid in the first weeks of life. Development can be inconsistent at this time, even among individuals from the same cohort. When 2 halves of the same cohort were divided into natural and artificial pond settings, results were not as expected (Figure 8). In 2002, mass from juveniles emerging from the artificial ponds was comparable to the natural pond and showed no significant difference ($P = 0.12$). However, 2003 was unpredictable as there was a significant difference in the mass of juveniles emerging from artificial ponds to the natural pond ($P = 0.03$). In 2003, black mesh screens were made to cover ponds and prevent non-experimental individuals from breeding. The temperature was reduced from these dark screens and development of larval *B. houstonensis* was delayed. This delay persisted for 3 weeks after juveniles emerged from the natural pond. Emergence was slow; only 1 or 2 juveniles were captured a day. In fact, emergence continued for at least 5 weeks after most individuals finished metamorphosis in the natural pond. This slow process was most likely caused by the decreased temperature and light, deregulating normal metamorphosis. Another factor involved in delayed metamorphosis could be the lack of required resources (i.e., food and protection).

Individuals from these artificial ponds not only encountered delayed metamorphosis, but were never recaptured again. This absence was most likely related to unfavorable habitat conditions. The artificial ponds were in an open field and received no shade, allowing no protection for juveniles against the sunlight. This lack of protection increased chances for dessication. In addition, *Solenopsis invicta*, the imported fire ant, was a known predator present in large numbers in this pasture. Ant mounds were observed daily around artificial arrays and ants were observed surrounding the water's

edge when juveniles were metamorphosing and emerging. This absence is supported by the 2002 and 2003 natural pond site data where no juvenile was found in a sunlit and /or dry environment. This is extremely important because the 100% mortality of juveniles in the artificial arrays and the 100% absence of capture indicate *B. houstonensis* postmetamorphs will not likely be found in open fields or pastures. This is consistent with the fact that adult Houston toads have yet to be caught in the middle of an open field on the GLR. Adults were caught along the perimeter of several open fields, but none were found in the center of an unshaded open field (Forstner, M.R.J. and Swannack, T., pers. comm.).

Pastures or grasslands could act as populations sinks because of decreased juvenile survival during emigration (Rothermel, in press). The implication that open fields are injurious to *B. houstonensis* juveniles and adults could lend support to conservation efforts, perhaps in the conservation/restoration of Houston toad corridors. Habitat alteration resulting in continuous open grassy areas with no canopy cover should be considered detrimental to Houston toad juvenile dispersal and ultimately, its survival.

Morphological Identification

Bufo juvenile species can be correctly identified using morphology at 13 weeks of age. However, this distinction has only been observed in 2 sympatric species, *B. houstonensis* and *B. valliceps.* Comparisons of *B. houstonensis* with other juveniles of congeners, such as *B. woodhousei* and *B. americanus,* should be explored due to their potentially less distinct juvenile morphological appearances. Therefore, more information must be gathered before this early differentiation can become reliable.

However, these data are immediately useful in monitoring in Bastrop County and can be supplemented by DNA identification.

To distinguish *Bufo* juveniles at the earliest age possible (aside from molecular analyses) would be extremely valuable for individuals with properties containing Houston toad habitat. Proper implementation of Houston toad regulations can occur at an earlier date for Bastrop County landowners and an effective conservation plan can be established or sustained more quickly. In addition, distinguishing the species morphologically at such an early age would aid surveyors of multiple Houston toad populations. This early detection, in addition to typical surveying procedures, might increase the accuracy in estimating populations and improve yearly survival estimates.

Earlier detection of juvenile Houston toads could improve population surveys. Emerging juveniles have been determined to linger near the pond's edge for the first 3 weeks. Therefore, there are 3 weeks to determine whether a pond has compatible Houston toad habitat and if adults return in subsequent breeding seasons. Surveyors performing night surveys may not get the chance to detect Houston toad chorusing for a pond due to a variety of circumstances (weather conditions, time of night, the presence of a predator near a pond), but finding emerging juveniles documents the toad's presence. By 13 weeks, species differences can be determined and can aid in proper identification of Houston toad, and viable habitats.

DISPERSAL PATTERNS

Results from monitoring dispersal and movement patterns are demonstrative of the positive correlation to the presence of moisture. Juveniles immediately sought shade

and cover and were most abundant in thick leaf litter. Either juveniles had a preference towards shadier, moist areas or they simply died in areas lacking in these qualities. Soil and vegetation type were neither preferred nor avoided as juveniles occurred in all 4 types (carrizo sand, red clay subsoil, pine, or oak-juniper forests). A preference for forest habitat (Semlitsch 2000a) is found in many amphibians and thus, supports these observations.

After metamorphosis, juveniles remained by the immediate water's edge for 3 weeks. It was observed that juveniles did not move nearly as quickly the first few days of metamorphosis as they did weeks later. Therefore, initial movement was gradual as juveniles were particularly dependent on the moisture from the saturated sands immediately bordering the water's edge. Clarke (1974) observed this dependency in bufonid juveniles where the postmetamorphs lived in wet litter on the shore of the pond for approximately a week.

Juveniles remained in the adjacent upland habitat for at least 13 weeks, where juveniles were still caught within a 50 m radius surrounding the pond of emergence. Refugia were useful in demonstrating that juveniles could be found in moist habitats. This environment may also be a suitable habitat for a more permanent refuge throughout the year and could be used as a potential habitat supplement for the conservation of isolated subpopulations.

The small numbers of individuals that were found in these refugia may seem inconsequential, but compared to the absence of juveniles found when doing the quadrat surveys, these data are invaluable. Less than 1% of bufonid juveniles survive to adulthood. The majority fall victim to predators or desiccation. Therefore, it is of the

utmost importance when juveniles are found utilizing moisture or a safe haven, which could possibly increase their chance for survival. These data show that the few survivors found and used damp, protected areas.

The correlation of mass and distance is vital in understanding dispersal patterns of *B. houstonensis* juveniles. This information provides support for the contention that juveniles disperse widely yet stay within at least a 50 m range of the pond of emergence for approximately 11 weeks.

Knowing the estimated distance from a breeding pond that the majority of juveniles stay within can be valuable information (Bellis, 1965) for the conservation of Houston toad populations. This known radius (30 m) around the breeding pond can aid in the implementation of a buffer zone, where little to no human interaction in that zone is permitted. The buffer zone should be enforced while *B. houstonensis* juveniles are still highly concentrated around the pond and have yet to disperse fully. Buffer zones are preferred to have little to no human interaction including hunting, fishing, or allowing cattle near Houston toad breeding ponds. Reduction in these interactions this would reduce exploitation of habitat juveniles' use as shelter and protection.

By using juvenile dispersal to define buffer zone requirements, a layer of protection will hopefully be afforded to future generations. Consequently, juvenile growth, development, and morphological differentiation can then be given a better chance at functioning properly in an ever-threatening environment.

Conservation Implications of Juvenile Ecology and Survivorship Techniques in the Houston Toad *Bufo houstonensis* (Anura: Bufonidae)

Kensley L. Greuter and Michael R. J. Forstner

To assist the recovery of an endangered species, conservation management options attempt to increase the size of populations and achieve a self-sustaining level in the wild (Maxwell and Jamieson 1997). In accomplishing these requires consideration of several factors in any management plan; one of which includes local population dynamics or in the case of the Houston toad, the number/density of individuals dispersing from individual wetlands.

For local population dynamics in the toad, metamorphosis from the aquatic habitat to the terrestrial environment is the critical step by which individuals are potentially recruited into the breeding population (Semlitsch 2000a). When migrating toward other ponds, juveniles tend to travel greater distances than adults (Breden 1987), sometimes up to 200 m (Semlitsch 1998). This extensive travel makes the metamorphosing juveniles the primary dispersal stage (Gill 1978) and hence, better indicators of required habitat. If success rates of metamorphosis are high, juveniles can help maintain local populations, and will likely supply dispersers to new or extirpated populations. Therefore, management plans that include actions to ensure a high probability of juvenile survival (e.g., protection of critical habitat adjacent to the pond or

wetland), will help maintain local species populations and provide dispersers for recolonization (Semlitsch 2000b).

Unfortunately, current management of the Houston toad, *Bufo houstonensis*, seldom includes aspects of juvenile ecology. As stated in Chapter 1, early PHVA management recommendations included minimizing the disturbance of soil, pesticide use, and habitat fragmentation. Maximizing the restoration of corridors and potential habitat (Lacy and Seal 1994) were also included. However, in this PHVA, juvenile ecology failed to be incorporated in recommendations.

Hatfield et al. (2002) conducted several Population Viability Analysis (PVA) computer simulations for *B. houstonensis* in 2002 to estimate survival rates under variable circumstances. This study acknowledged that upon simulating multiple scenarios, juvenile survival is obviously important. If juvenile survival is 1%, single populations usually have a high probability of extinction. If juvenile survival is 2%, single populations usually have a low probability of extinction. Hatfield et al. recommended that to determine whether the simulations performed were closer to low or high juvenile survival scenarios, actual estimation of the number of eggs laid, estimation of the number of metamorphs, and marking/recapturing juveniles should be done. With these recommendations, the significance of juvenile data is finally recognized.

The estimation techniques used to determine survivorship data are a vital addition in censusing Houston toad populations. The Wire Section model is the technique recommended for realistic application. This technique allows for quick maneuverability and is simple in concept. The model uses wires that are cut to the average known length of 50 eggs to estimate the number of eggs in a cohort. Estimation time is 40 to 60

minutes. Conservationists and/or endangered species surveyors can use this method to estimate egg numbers without displacement or manipulation that could cause detrimental effects, such as abnormalities or even mortality.

From Chapter 2, *B. houstonensis* survival from egg to metamorph was 4.7%. This survivorship percentage was based on recently emerged metamorphs, not juveniles surviving beyond a few weeks in the terrestrial environment. There were 15% fewer juveniles estimated to be living by week 13 (4.0% survivorship). It is estimated that by the first year of adulthood, 1 out of every 200 (0.05%) Houston toads survive (Forstner, M.R.J. and Swannack, T., pers. comm.). This percentage is greatly reduced from the estimated 1% survival rate where populations have a high probability of extinction (Hatfield et al. 2002) indicating an even higher extinction probability. If 15% of juveniles die every 13 weeks, juveniles from the 2003 egg strand will be almost completely eradicated by year 2 with an estimated survivorship of 0.0001%. This paired with the fact that juvenile survivorship at 13 weeks (4.0%) is overestimated, it is concluded that the population is serious risk of extinction. Survivorship data from older juvenile (> 13 weeks) to adult is greatly needed to fill the gap in survivorship estimation. With this study's juvenile data, a more detailed indication of the Houston toad's survival rate can be considered and the immanency of the situation can be addressed. With the knowledge that this endangered species is quickly moving towards extinction, thorough management efforts should continue, if not increase in intensity.

The multiple aspects of juvenile ecology explained in Chapter 3 will certainly change the way we look at managing the Houston toad. Analysis of juvenile growth and developmental patterns are essential because it can contribute to an understanding of the

population processes of the species (Clarke, 1974). Juvenile growth in the Houston toad was found to be rapid and variable. When comparing this study's wild-caught data to that of previously published captive-bred data, it was not surprising that the wild-caught data would show lower growth rates. However, what could be beneficial to Houston toad management is not that growth rates were lower; it is that wild-caught data 2003 was not significantly different from that of captive-bred data. Therefore, wild-caught juveniles, at least the ones that survive, are growing as well as can be expected considering multiple stressors involved.

It is important to note the correlation between SUL and mass because with such a high correlation, measurement assumptions can be made when there are constraints on time in the field. By using the simple linear equation, $y = 9.7x+8.3$, where y = SUL (mm) and x = mass (g), field biologists can then use just 1 measurement and calculate the other.

Juveniles were not found in areas without shade and leaf litter. Therefore, it is recommended that conservation efforts should focus on preventing large areas of open fields near and/or around Houston toad habitat or breeding ponds. If open areas are already present near and/or around Houston toad habitat or breeding ponds, then vegetative reconstruction of native flora for canopy cover should be implemented when possible.

The presence of shade in the terrestrial habitat is a necessity for juvenile survival. However, tadpoles require the exactly the opposite. In 2003, delayed growth of a cohort was discovered when tarps were placed over artificial ponds and juveniles emerging from these ponds took 3 weeks longer to metamorphose. The constant shade decreased the

light exposure and pond temperature and, consequently, delayed emergence. The lack of required resources such as food or shelter could also influence the delay of metamorphosis. This along with temperature decrease created an inhospitable environment which ultimately caused metamorphosis to slow.

Due to the relative density of water and the presence of aquatic vegetation, a longer amount of time is needed to heat up a pond than a terrestrial environment. Tadpoles need an optimum aquatic environment for normal metamorphosis to occur (Breven and Chadra 1988). Sun exposure above a pond will allow the pond to warm and speed up metamorphosis. Once tadpoles metamorphose into the harsh terrestrial environment, shade is required for protection. Therefore, there should be little to no shade above the pond, but rather around the pond. Relatively dense ground vegetation should either be planted or retained around the pond to prevent desiccation upon emergence.

Morphology, although thought to be slow in development (Blair 1972), can be distinguishable down to the species level at 13 weeks post emergence for *B. houstonensis* using these characteristics: continuous dorsal spots of light color and blotchiness, inconspicuous mid-dorsal line, and a lack of dark lateral coloration. Molecular identification can also be used to distinguish bufonid juveniles from one another using the PCR method. A polymerase chain reaction (PCR) marker system was used to distinguish the different species. This test positively identified juvenile *B. houstonensis* by using species-specific primers designed in our laboratory. A positive band identified Houston toads to the exclusivity of other taxa. Both identification methods have their advantages and disadvantages. Table 1 explains the pros and cons of each method.

This study focused on morphological identification as it was lacking in research. To distinguish *Bufo* juveniles at the earliest age possible (save molecular analyses) would be extremely valuable for those parties whose properties are concerned with Houston toad habitat. Proper implementation can then occur at an earlier date for landowners and an effective conservation plan can be established or sustained more efficiently. In addition, morphological distinction between species at such an early age would aid surveyors in distinguishing *B. houstonensis* from other bufonid species. This early detection, in addition to typical surveying procedures, might increase the accuracy of estimating population numbers and the estimate of yearly survival.

Juvenile dispersal is one of the key factors in determining species range in the critical metamorphosing period. By establishing the radius around a breeding pond which most juveniles (> 75%) inhabit, one can apply this known distance to management practices. The radius around a breeding pond can act as a guide for the implementation of a buffer zone, where little to no human interaction is permitted. This implementation does not have to be permanent. However, it is preferable that this buffer zone is enforced throughout the duration of the time juveniles are concentrated around the breeding pond. This would significantly decrease anthropogenic activities such as hunting and fishing. The reduction of cattle around breeding ponds would also be a major improvement as Houston toad breeding ponds are associated with water tanks used for cattle, more so than ponds used for anthropogenic recreation (pers. obs.). The decrease of human interaction with Houston toad breeding ponds during the time of juvenile

Table 1. Positives and Negatives of Morphological identification vs. Molecular Identification.

	Morphological ID	Molecular ID
Positives	• Easy • Quick • Cheap • Minimal Training	• Accurate • Begin Immediately • Reliable
Negatives	• Inaccurate • Unreliable • Begin at 13 wks	• Extensive Training • Expensive • Time Consuming

Table 2. Recommendations and helpful suggestions for the improvement of field techniques and management of the endangered Houston toad, *Bufo houstonensis*.

Recommendations For Management Improvements of the Houston toad, *Bufo houstonensis*
1. Prevent large, open fields in near Houston toad habitat or breeding ponds.
2. If open fields exist, then conduct vegetative reconstruction for corridor passage.
3. Buffer zone implementation in 50 m radius around Houston toad breeding ponds in optimal habitat.
4. If optimal habitat bordering breeding ponds is unavailable, the biologist's judgement must be used to determine the best scenario possible.
5. Buffer zone implementation for the duration of April through July.
6. For future construction of ponds, at least half of the pond should be unshaded with canopy cover. To compensate for excessive sunlight exposure, low ground vegetation or structures should be either planted or retained.
Suggestions in Improving Field Techniques
1. For egg number estimation used in survivorship calculations, use the Wire Section Model as it is efficient, simple in concept, and disturbance-free.
2. When in the field, morphological identification will be efficient as long as characteristics have developed.
3. If time constraints prevent complete measurement of juvenile, either measure mass (g) or SUL (mm) and use equation to determine missing measurement: $y=9.7x+8.3$.

emergence and early dispersal would prevent habitat damage and simply prevent unnecessary mortality. Therefore, based on the results of this study, it is recommended that a buffer zone be implemented around known Houston toad breeding ponds at least 50 m in radius when in suitable Houston toad habitat between March and July. When incompatible habitat happens to border Houston toad breeding ponds, the biologist's judgement must be used to determine appropriate measures for the best scenario possible. The length of time and distance in radius are conservative in being less than ideal in both size and duration, but allow for variation among breeding seasons.

The results of this research demonstrate the significance of the juvenile ecology in proper management techniques. Aspects of the juvenile ecology such as growth and development, morphological and molecular identification, and dispersal patterns are all equally important and ultimately help define population survivorship. These recommendations and helpful tips described in Table 2 will enhance Houston toad conservation and management practices in efficiency and productivity. However, more information can and should always be gathered to improve these statements.

BUFO HOUSTONENSIS (Houston Toad) **JUVENILE DISPERSAL.** *Bufo houstonensis* is an endangered anuran endemic to Central-East Texas. While its breeding behavior has been well documented (Hillis et al 1984. J. Herp. 18:56-72), very little published information exists concerning the juvenile life stages, and those studies focused on predation (Freed and Neitman 1988. Tex. J. Sci. 40:454-456), coloration (Mays and Freed 1985. Herpetol. Rev. 16:108-109), and growth (Greuter and Forstner. 2003. Herpetol. Rev. 34:355-356, Quinn and Mengden 1984 Southwest. Nat. 29:189-195).

A Houston toad egg string was surrounded with an aluminum flashing enclosure during the spring of 2004 at the Griffith League Ranch (GLR) in Bastrop County, Texas, in order to monitor post-metamorphic behavior. Upon emergence, 993 individuals were captured and toe-clipped to identify the cohort. The flashing was carefully removed and 100 individuals were dusted with inert fluorescent powder (Radiant Color, T1 pigment) and released at the point of emergence as a single group. Fluorescent pigment was used with success to track *Pelobates fuscus* (Eggert 2002. Herpetol. J. 12:69-74) and the same technique was applied here. Toadlets were monitored immediately following release in order to determine if the pigment caused any malaise. Metamorphs were located with a UV light for two consecutive nights and observed during the early morning hours of the day following their pigment marked release. Metamorph locations were marked with marking flags; the area was left as undisturbed as possible, and the dispersal pattern was not analyzed until after the metamorphs left the pond's edge. It was our intention to follow the juveniles for a longer period, but in the afternoon of the third day, the GLR received over 25 mm of rain. We believe this resulted in the pigment powder washing off of the toads, as well as, washing away all previous trackways.

Within two days after emergence, at least a few (n=4) of the *B. houstonensis* metamorphs had dispersed from the pond's edge up to 4m. We released the pigment marked individuals in one location causing a large amount of powder to be deposited in a small (~0.5m) area. One consequence of this was the tracks from individual metamorphs were not distinguishable within 0.5 m of the release site, but could be easily distinguished beyond that initial confused area of powder marks. When dispersing from the pond edge metamorphs did not move in a straight line, but a seeming random pattern may have been foraging or shade seeking behavior. The furthest distance an individual moved was 4 meters from the pond's edge. However, the vast majority of individuals stayed between 2 – 3 meters from the edge, buried under grass or sedge tussocks. The dispersal pattern did not increase in diameter from 24 – 48 hours after marking. After the rainfall event, no metamorphs with pigments were relocated; however, toe-clipped individuals were found. Based on this information we assume the pigment washed off the pigment marked metamorphs' bodies.

During this study, metamorphs did not show any ill effects due to the powder. Using this method was an easy, efficient and cost effective way to track post-metamorphic juveniles. Nighttime observations of the movements of juveniles marked with the pigment were easily monitored using UV light. Metamorphs were also observable during the day as the powder is highly visible, allowing us to observe toadlets without disrupting the point of emergence. We could visually track the individuals during day or night using either naked eye or binoculars from a distance of 3m without difficulty. This is particularly relevant given the concentration of individuals at the pond's edge during the emergence period and the consequent care required to prevent

accidental mortalities when trying to observe the behavior of these juveniles. We did not observe direct foraging during our observation of these individuals during day or night surveys. When moving individuals appeared to be simply moving between shaded cover (daylight) or moving from one resting area to the next (night time). The technique does have an inherent weakness in very wet or rainy areas. Rainfall, in particular, appears to be detrimental to this type of study, as it washes the powder off of the body, limiting the observation time to periods between pigment marking and the first rain.

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Chapter 3:
LARVAL HOUSTON TOAD ECOLOGY

EFFECTS OF VARIOUS LEVELS OF PREDATION AND ABIOTIC FACTORS ON THE SURVIVORSHIP OF BUFONID TADPOLES

JACOB T. JACKSON, SUSANNAH R. MORRIS, AND MICHAEL R. J. FORSTNER

Predation is an important factor affecting the survival of larval anurans (Manteifel and Reshetnikov 2002). Toads breed in both permanent and temporary water sources, which have differentially structured predator assemblages (Werner and McPeek 1994). Hydroperiod has significant effects on the structure of amphibian populations (Skelly et al 1999). It is not clear in which case predation has the greatest effect on *Bufo* tadpoles, or if the effect is significantly different among different habitat types. It has been found that the presence of fish is beneficial to bullfrog tadpoles, while detrimental to green frog tadpoles (Werner and McPeek 1994). It has also been observed that fish and other predators show preference for some tadpoles over others as prey items (Werner and McPeek 1994). Manteifel and Reshetnikov (2002) found fish not only preferred to consume *Rana* tadpoles over *Bufo*, but that fish would actually reject a *Bufo* tadpole if they took one into their mouth. The authors also observed that the defense of the *Bufo* tadpoles was not as effective against invertebrate predators.

Both the endangered Houston Toad (*Bufo houstonensis)* and Gulf Coast toad (*Bufo valliceps*)are R selected species, or species whose reproductive strategy consists of the production of large quantities of offspring. Very few of these offspring will survive to maturity. This produces a node in the life history of these organisms that might be an opportune one from a management perspective. One potential aspect of that management would be to understand whether or not ephemeral ponds (which by default could not have fish as predators) actually represent more suitable habitats for Houston toads than do

permanent ponds. The environment of the Bastrop Co., Texas area has experienced radical change since its settlement. Agriculture and development have resulted in a shift from ephemeral ponds to permanent ponds constructed for livestock and other agricultural uses. Many of these ponds have been stocked with introduced gamefish species, which are potential tadpole predators. These species were not present in ephemeral water sources. A counter argument can also be made that the presence of the fish may be beneficial for the tadpoles due to the ability of the piscine vertebrate predators to lower aquatic invertebrate predator densities. We conducted experiments to empirically determine in which type of habitat toad tadpole survival was greatest.

MATERIALS AND METHODS

Experiments were conducted over three consecutive years in an experimental pond array on the Griffith League Ranch in Bastrop Co., TX to investigate the relative effects of various abiotic and predator combinations on tadpole survival. To accomplish this required the collection of tadpoles for the Houston toad. Obviously reproduction in this endangered species is not common, hence relatively few total eggs could be safely collected for use in any given year. Consequently, *Bufo houstonensis* tadpoles were used in 2002 and 2003. However, 2004 experiment was conducted to determine the effects of the three different predation regimes on *Bufo valliceps* tadpoles, a species sympatric with the endangered *Bufo houstonensis* and, as a congener, an effective substitute for the endangered species. Tadpoles were introduced into artificial ponds that contained vertebrate and invertebrate predators, established invertebrate populations, or

invertebrate populations in the process of establishing through migration as would occur in ephemeral pond habitats.

On the Griffith League Ranch in Bastrop County, Texas, 24 artificial ponds were constructed in 2001. These ponds measure 1.5 m x 1.8 m x 76 cm, and have a volume of approximately 1703 liters. Twelve of the ponds had a bank slope of 10 degrees, the other twelve had a slope of 45 degrees. Each pond is surrounded by an aluminum flashing enclosure to keep amphibians from escaping. Three treatments were originally distributed randomly among the ponds. Fire ants were treated with Amdro inside the array beginning in early April of each year. As mentioned previously, the final year of the study differed from the first two years the experiment was conducted.

2002/2003

Twenty-four man-made experimental ponds on the Griffith League Ranch in Bastrop County, TX were monitored in 2002 and 2003 from approximately March 23 through July 19. Prior to the spring breeding season, in late winter, the water in each pond was drained, filtered to remove macroinvertebrates and then used to refill the pond. Pond sediments were dredged to the banks and dried to facilitate removal of any remaining macro-organisms. The ponds were subsequently stocked with 100 non-*Bufo* tadpoles; these were primarily *Rana* tadpoles although some *Hyla* tadpoles were also included. Predators were stocked according to Figure 1. Predator treatments were assigned randomly to ponds within the two slope treatments. All ponds that required fish were stocked with one bass and one perch, or one bass and four juvenile perch approximately 3 cm in length. The insect treatment ponds each received 24 Odonate

larvae. All fish, insects, and tadpoles were obtained from other ponds on the Griffith League Ranch, as was the case for all biological materials used in these experiments.

In 2003 screens were constructed and used to cover the ponds throughout the season. They were in use soon after the ponds were stocked and used to prevent invertebrate colonization of the experimental pond array. On March 23, each pond was stocked with 183 *Bufo houstonensis* tadpoles from 1.5 egg strands (one half of each of three egg strands) that were taken from Pond 2. The tadpoles from each egg strand were divided equally among the 24 ponds; they were counted out in groups of 30 using small buckets and a plastic eyedropper. The experimental pond array was treated as necessary with Amdro throughout the summer to kill fire ants within the flashing.

2004

In a modification of the previous two years of treatments, bank slope was ignored. Three treatments were then conceived in order to evaluate tadpole survival under different predation regimes. The first treatment was designed to simulate emphemerality. The ponds assigned to this treatment were drained on 8 April 2004. The intake on the pump was fitted with a paint-straining screen, to ensure removal of all vertebrate and invertebrate organisms, and the water was transferred to another pond. Each pond was drained to a level of approximately six inches, and then fine mesh nets were repeatedly swept through the water until no more invertebrates were found. All aquatic vegetation and sediment were rinsed in a net to remove any predators hiding in them. Sediment and vegetation were removed from the rubber pond liners with a shovel, and allowed to dry on the bank for two weeks before being returned to the water. All invertebrate predators

removed from these ponds were kept in alcohol, quantified, and taxonomically identified. All ponds were left uncovered to allow insects to migrate among ponds, facilitating natural invertebrate colonization. This was the only treatment in which the ponds were drained.

The second treatment was designed to simulate a permanent habitat with an established invertebrate community. Invertebrate communities that had become established over the previous years were therefore left intact.

The third treatment included vertebrate predators. One *Micropterus salmoides* (Largemouth bass) mean length 27.53 cm and three *Lepomis megalotis* (longeared sunfish) mean length 7.93 cm were added to each of these ponds on April 28th, before any *Bufo* tadpoles were added. The established invertebrate communities were left intact as with treatment two.

Difficulties were encountered globally in obtaining sufficient numbers of tadpoles in all three years. The use of *B. valliceps* in the third year was in a direct attempt to enable placing many more tadpoles into each pond. Unfortunately even with the substitution of *B. valliceps*, in 2004 a total of 200 *Bufo* tadpoles per pond was established.

The ponds were checked daily for emergent toads from May through July 2004. Dissolved oxygen and pH were checked weekly in the morning. 2004 protocol changes included diurnal pH and DO measurements were recorded on 01 and 07 at 11 pm and 11 am the next morning. A data logger was placed in one pond receiving the second treatment to record temperature through June 28th. Invertebrate surveys were conducted on 17 April, 15 June, and 8 July using a six-inch diameter pipe thrust into the sediment on the bottom of the pond (Werner and McPeek 1994). The depth was recorded using

graduations on the side of the pipe, and all invertebrates were netted out of the pipe with a small aquarium net. The volume of water contained by the pipe was calculated for each sample, allowing the density of invertebrates to be calculated, and population size of each pond to be estimated.

RESULTS

2002

Twenty-seven post-emergent metamorphs were recovered from the 1200 (50 per pond) initially stocked. Of those twenty-seven, all but one came from a pond containing only fish and tadpoles. The other emerged from a pond only containing tadpoles.

2003

The first Houston toadlet emerged on May 12 from Experimental Pond (EP) 3, and the last toadlet emerged on July 4 from EP-3. In total, 57 toadlets emerged from EP-3. The ponds where emergence occurred were EP 3, 5, 12,13, and 18. These ponds represent all treatments with the exception of that with only insect predators.

The low number of toadlets emerging may be attributed to several factors. The toadlets emerged much later than their cohort that was left in the natural pond, probably due to lower water temperature attributed to shading by the new screens. In 2002, the pond temperatures varied from 81.6- 96.6 degrees F; in 2003, the temperature varied from 79-80 degrees F on July 9. The lower temperature prolonged the larval stage thus increasing the opportunity for predation by fish and insects. The *Rana* stocked in the ponds could have been predators; since *Rana* were stocked early in the season, the tadpoles were in their second year of development and therefore quite large. Although checking ponds every 24 hours should ensure that toadlets remain near the water after

emerging, increased vegetation around the ponds may hide toadlets. Additionally, this vegetation harbors potential predators such as wolf spiders, fishing spiders, and lizards.

Statistical analysis shows that there is no difference between 10 and 45-degree angle ponds ($F = 0.92$, $p = 0.35$), no difference among predator treatments ($F = 1.02$, $p = 0.41$), and no interaction between factors ($F = 0.93$, $p = 0.45$). However, the amount of zeros (ponds with no emergence) in the data set could skew results; the majority of emergence occurred from one pond, EP-3, which was not stocked with fish (tadpoles only). Additional analyses may extract more meaningful results from the data.

2004

On June 15th, two juveniles (metamorphs) were recovered from pond 21. On June 16th, another was recovered from pond 21, as well as three from pond one. These six were the only metamorphs recovered.

Shannon-Weiner indices were calculated from the invertebrate data collected from the eight ponds drained at the beginning of the experiment are shown in Table 1. Table 2 shows the average densities and population sizes for the four most common predatory invertebrates, from both the drained ponds and the pipe samples conducted over the course of the experiment.

The diurnal pH and DO data from both sampling events for all ponds included in the experiment showed some fluctuation (Fig. 2). Mean a.m. pH ranged from 6.58 to 7.22, while p.m. readings had a range of 7.18 to 7.52. Mean DO had and a.m. range of 5.77 to 6.89, and a p.m. range of 7.08 to 7.25 for the experimental ponds in the array. The pH and DO data recorded weekly in the mornings was averaged over the course of the experiment. Figure three illustrates the pond/treatment layout.

Discussion

Few toads were recovered in any of the three years. Due to the fact that only six emergent metamorphs were recovered in 2004, the survivorship of the toads could not be calculated due to low sample size. Treatment types two (permanent pond) and three (permanent pond with fish) each produced three of the recovered toads. Estimated survivorship to metamorphosis, based on the results of chapter two, for the 2004 stocking rate would be 112 metamorphs (see chapter 2).

The low survival rate of the toads may be due to a variety of factors, not the least of which is interspecific competition. Significant numbers of *Hyla, Acris*, and *Rana* tadpoles and emergents were observed within each enclosure throughout the experiment. It has been found, for example, that *Bufo woodhouseii* have a significant effect on *Hyla crucifer* when they are given a temporal advantage, yet *Hyla* do not have the same effect on *Bufo* (Lamler and Morin 1993). Terrestrial predation may also have been a factor. Raccoon tracks were observed within several of the enclosures. Also, predatory birds were not observed at these ponds but had been seen often on ponds nearby, suggesting that they may have visited the experimental ponds. Fish scales were found within the enclosure around pond 21, suggesting that the bass was removed from that particular pond by a predator. Fire ant control was difficult due to the greater than normal rainfall experienced during the experiment. A snake was observed hiding under the pond liner of pond 19, but could not be captured and was not seen again. This suggests that snakes may be able to negotiate the enclosure fences.

Pond chemistry was monitored in 2004. Fluctuations in dissolved oxygen and pH should not have affected the tadpoles, given the success of the other anuran species.

Rosenberg and Pierce (1995), noted that while acidic conditions resulted in considerable mortality in *Pseudacris clarkii*, it did not cause significant mortality in *B. valliceps*. The authors did note, however that growth of *B. valliceps* tadpoles at pH 4 was significantly less than at a neutral pH. The success or survivorship of the other species is hard to determine, since the numbers of eggs or tadpoles that they originated from could not be quantified. Other anuran species were not controlled in the experiment because they were considered to be a standard factor present in all natural toad habitats, emphemeral or permanent. It can be concluded that the number of tadpoles added was not enough to produce sufficient power in the analyses, suggesting that *Bufo* tadpoles naturally experience a very high rate of mortality.

The Shannon-Weiner indices suggest that the invertebrate predator communities are not very diverse (Table 1). This index reveals nothing about the size of any given population, however, only how evenly the number of individuals is distributed across all species. The average estimated population sizes calculated from the pipe sampling data are much larger than the average population sizes from the actual count data from the drained ponds. There are two possible interpretations of this difference. The first would be that the pipe sampling method applied here was susceptible to underestimation. This is particularly notable, in the case of the Notonectidae and Dytiscidae, which are much more agile swimmers than the Odonate larvae. This may have given them an advantage in avoiding capture by avoiding the sampling apparatus itself. In a few cases, many of them were observed, but none were captured. However, enough were captured for sampling to be useful qualitatively if not quantitatively. The second, and more likely explanation for the differences is that the invertebrate communities were maturing in both

number and density. The first samples were taken in when the weather was cooler and the photoperiod shorter. It could be expected, then, that results would change as the days became longer and warmer. Many of the Odonate larvae emerged during the experiment, evidenced by their nymphal shucks being found along the pond banks.

This was the third attempt of an experiment of this type in this pond array. To date, none of the experiments attempted have produced statistically significant results due to low sample size resulting from difficulties encountered obtaining the quantity of tadpoles required. Qualitatively, no differences were found among the treatments with or without the fish as predators in the ponds.

Table 1: Shannon -Weiner indices calculated from the invertebrate specimens collected from eight ponds that were drained in preparation for the 2004 experimental pond investigation on the Griffith League Ranch in Bastrop Co. Texas.

Pond	4	5	9	11	14	17	19	20
S-W	.37	.38	.26	.33	0	.075	.18	.31

Table 2: Average invertebrate densities and population estimates calculated from invertebrate sampling data collected over the course of the experiment conducted in the experimental pond array on the Griffith League Ranch in Bastrop Co. Texas.

	Libellubidae	Coenagrionidae	Notonectidae	Dytiscidae
Drained ponds:				
Avg. Density	0.047	0.0073	0.00022	0.00051
Avg. Population	80	1.25	0.375	0.875
Samples:				
Avg. Density	0.144	0.063	0.0064	0.102
Est. Population	244	107	11	173

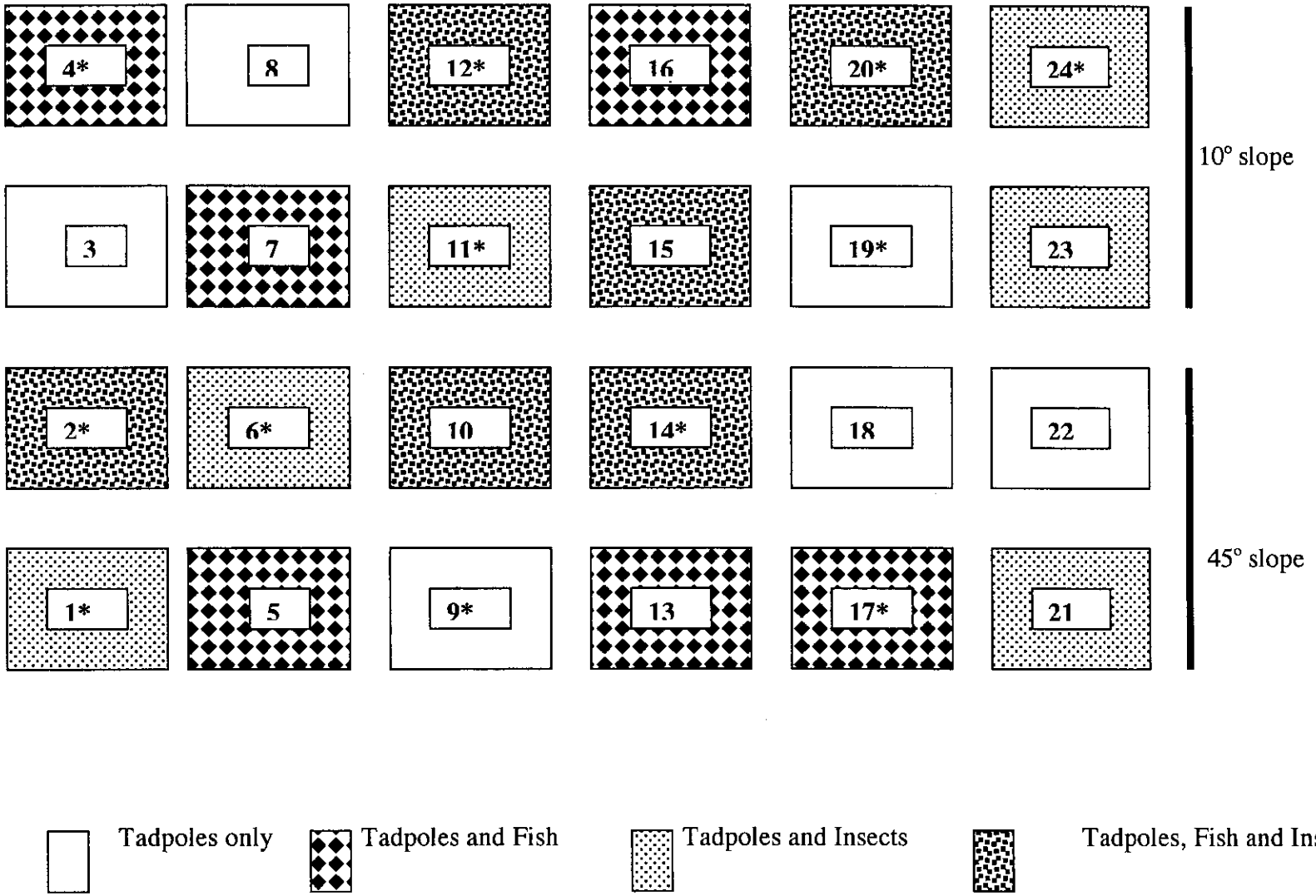

Figure 1. The 24 experimental ponds located on the Griffith League Ranch with respective numbers and treatments (as indicated in key) for 2003. * = pond used in 2004 experiment. Treatments were redistributed each year.

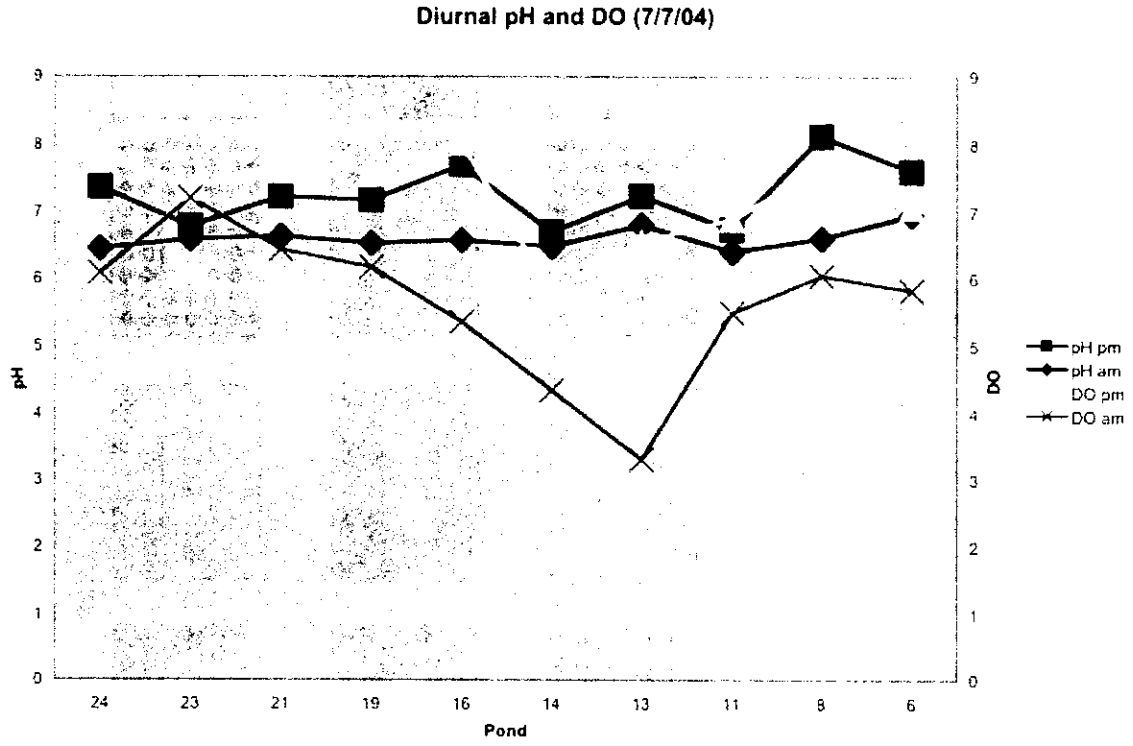

Figure 2: Diurnal fluctuations in pH and dissolved oxygen for the ponds used in the 2004 experiment. Day and night readings were taken 12 hours apart (11 p.m. and 11 a.m.) in the experimental pond array on the Griffith League Ranch.

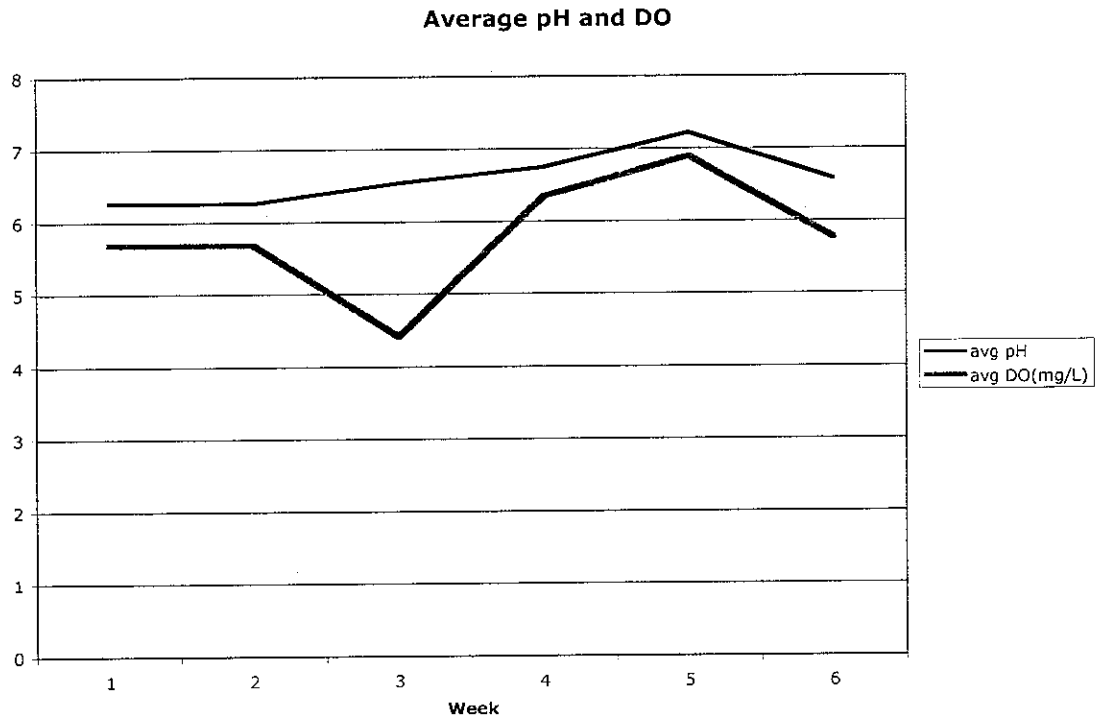

Figure 3: Fluctuation in pH and dissolved oxygen over the course of the experiment. Lines on the graph represent the average of weekly measurements across all ponds included in the 2004 study conducted in the artificial pond array on the Griffith League Ranch in Bastrop Co. Texas. The top line represents the average pH and the bottom line represents the average DO (mg/L).

Chapter 4:
VERTEBRATE PREDATION ON ENDANGERED SPECIES

Active Predation of Pitfall Traps at a Study Site for the Endangered Houston toad (*Bufo houstonensis*)

Adam W. Ferguson and Michael R. J. Forstner

Pitfall traps with or without associated terrestrial drift fences remain a commonly applied technique in the sampling of small vertebrate animals (Jenkins et al., 2003; Shoop, 1965; Stenhouse, 1985; Sutton et al., 1999). This field technique is utilized throughout the world and several studies have evaluated their effectiveness and reported on problems with the methods (Brown, 1997; Crosswhite et al., 1999). Drawbacks of pitfall traps with terrestrial drift fences have been covered in the literature and include topics such as the escape of captured animals (Mazerolle, 2003), pitfall avoidance by certain species such as terrestrial turtles (Christiansen and Vandewalle, 2000), and the ability of animals to trespass or circumvent the fences themselves (Dodd, 1991). Another problem in the use of drift fence arrays is the indirect mortality of trapped animals (Enge, 2001; Yunger et al., 1992).

Several other studies have addressed some of the many mortality factors associated with pitfall trapping (Karraker, 2001; Kogut and Padley, 1997; Padget-Flohr and Jennings, 2001). Those factors include desiccation (Jenkins et al., 2003), drowning (Aubry and Stringer, 2000), starvation (Yunger et al., 1992), exposure (Padget-Flohr and Jennings, 2001), and predation among species within the pitfall buckets (Dodd and Scott, 1994). However, the act of direct predation upon drift fence arrays by foraging vertebrate predators is rarely mentioned and most studies have attempted to correct or calculate the

effects of only the aforementioned suite of problems. In fact, no previously published study to our knowledge directly addresses the effects potential vertebrate predators might have on drift fence sampling. Most direct predation events mentioned in the literature are anecdotal and deal mainly with predation within the buckets themselves especially by trapped mammals such as shrews (Jenkins et al., 2003) or minor disturbances to pitfall covers by meso-mammals such as the raccoon (Sutton et al., 1999). Predation of drift fence arrays by larger carnivores or other potential vertebrate predators is rarely discussed and has not been studied quantitatively in any of the publications we located.

Our objective was to quantify the amount, rates, and variety of predators visiting and potentially predating upon the animals captured in a grid of terrestrial drift fence arrays established to monitor the dynamics of the endangered Houston toad (*Bufo houstonensis*). In addressing such predatory activities we hope to provide insight into the consequences vertebrate predators might engender in drift fence sampling and provide solutions to curtail the problems associated with such behaviors.

Materials and Methods

This study was conducted on the Boy Scouts of America's Griffith League Ranch – a 2012 hectare ranch located in Bastrop County within the Lost Pines ecological region of Texas. Vegetation communities consisted of mixed conifer hardwoods made up of loblolly pine (*Pinus taeda*), blackjack oak (*Quercus marilandica*) and post oak (*Quercus stellata*), mature pine stands of loblolly pines, several open pasture lands and mixed deciduous hardwoods made up of oaks (*Quercus spp.*) with an under story of yaupon

(*Ilex vomitoria*), American beauty berry (*Callicarpa americana*), and farkleberry (*Vaccinium arboreum*). Soil is composed of 91% sandy loam as illustrated by the enclosed soil map.

Eighteen drift fence arrays were constructed to begin monitoring the local herptofauna including the endangered Houston toad (*Bufo houstonensis*). Five treatment groups were installed. Treatment group one consisted of four Y-shaped drift fence arrays surrounding a known Houston toad breeding pond set various distances from the pond edge. Three other Y-shaped drift fence arrays were placed in a deciduous-evergreen mixed forest intercepting another known breeding pond, these three arrays were designated treatment group two. Treatment group three also possessed three Y-shaped drift fence arrays set to intercept a third known breeding pond found in a similar habitat as treatment group two but more dominated by loblolly pines. Treatment group four contained three Y-shaped arrays set in a line that began near a two ha lake and extended outward east to west through a mixed deciduous-evergreen forest. Five straight line drift fence arrays, two with four pitfall buckets and three with five pitfall buckets, made up treatment group five. Y-shaped arrays were made up of three radiating arms of 18 cm by 15 m aluminum flashing buried five cm into the ground with four buckets; three terminal and one central. Straight lined drift fences found in treatment group five were built of the same aluminum flashing in the same manner as the Y-shaped arrays.

Each of the Y-shaped arrays had 6 funnel traps (2 on each arm) supplementing the pitfall traps. Traps were checked daily from March 2001 through August 2004 for trapped taxa which were subsequently measured, marked either by toe clippings, PIT tags, with or ventral scale clips, gendered and then released >20 m from the array.

The predation study was initiated in 2003 in response to a perceived increase in potential predator activity around the arrays (scat, tracks, disturbances, etc.). The study involved a two-pronged approach using both track monitoring stations and motion sensor cameras to document predator activity around the arrays.

Track Monitoring Stations

Track monitoring stations consisted of a two m diameter circle of cleared earth with the bucket as the circles center point. Y-shaped arrays had two buckets, one central and one randomly chosen terminal bucket, surrounded by the two m diameter circles of tracking sand taken from the ranch. In areas where the substrate was inadequate as a tracking media sand was imported from suitable sands on the ranch. The pasture lines in treatment group five had alternating buckets fitted with the track monitoring stations. To prevent grass re-growth, a 13 cm deep hole of two m diameter was dug out around the bucket and then lined with artificial pond liner and filled with appropriate sand substrate from the pasture. Once completed a total of 38 track monitoring stations were set in place in addition to five controls of two m circles placed >100 m from the nearest track monitoring station in each treatment group totaling 43 track monitoring stations.

Each track monitoring station was raked four times a month to clear the trap of any previous tracks and prepare the sand for new track detection. Each was checked the following morning for the occurrence, pattern, and kinds of animal tracks present. Tracks were identified to species when possible and recorded as unknowns when this was not possible. The presence or absence of animals in the pitfall buckets was also recorded.

CAMERA TRAPS

Ten Deer Cam® Model DC-100 cameras were set up in addition to the track monitoring stations with two cameras in each treatment group. The buckets with the cameras were randomly chosen; cameras were either placed near a terminal bucket or over a central bucket. Central cameras were found on the Y-shaped arrays and were supported 2.43 m over the central bucket by t-posts made into a H. Date and time were recorded by the cameras to capture multiple visitors versus repeat visitors.

Results

Track monitoring stations

The track monitoring stations were operational from the 21 of October 2003 through 17 of June 2004. A total of 1236 observations were recorded. Of those 1236 observations, 443 of them had tracks present when checked (35.8%). Fifteen categories of visitors were documented with 10 species identified and 5 general categories noted. Predatory species observed included the raccoon (*Procyon lotor*), gray fox (*Urocyon cinereoargentius*), Virginia opossum (*Didelphis virginiana*), striped skunk (*Mephitis mephitis*), coyote (*Canis latrans*), domestic dog (*Canis familiaris*), and the American crow (*Corvus brachyrhynchos*). Non-predatory tracks present were the white-tailed deer (*Odocoileus virginianus*), wild turkey (*Meleagaris gallopovo*), and the armadillo (*Dasypus novemcinctus*). General categories documented included unknown tracks, snake drags, multiple visitors (tracks of more than one animal in the same trap night), bird

tracks, and rodent tracks. Such tracks were unidentifiable to the species specific level. The distribution of visits by each species is depicted in Figure 1.

Each bucket affixed with a track monitoring station was visited at least once by potential predators during the study. On 14 separate occasions, a combination of potential predators visited the same station in a single night. There were combinations of nocturnal (gray fox, opossum, raccoon) and diurnal predators (American crow), as well as combinations of strictly nocturnal meso-mammals i.e., gray fox and opossum.

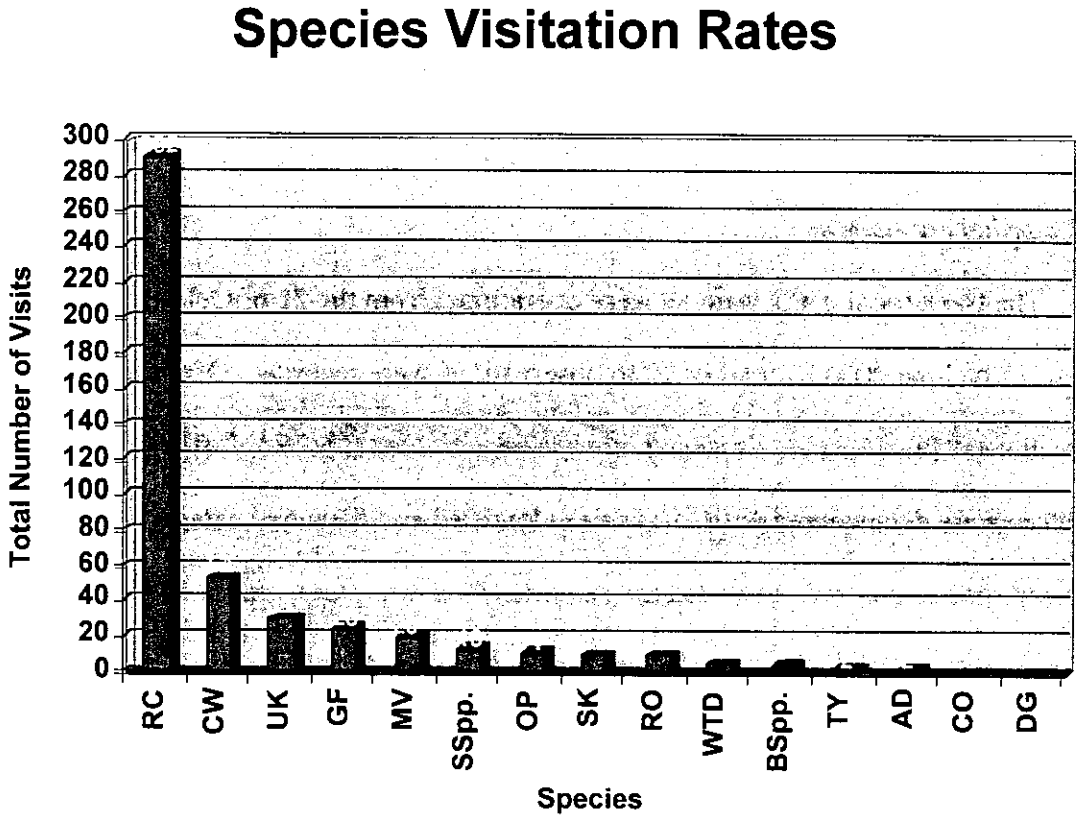

Figure. 1. Species specific visitation rates out of the 443 track observations recorded at a series of 43 track monitoring stations on drift fence/pitfall arrays in Bastrop County, Texas. The yellow numbers represent the individual times that the species was observed entering the track monitoring station area after a raking event. RC=raccoon, CW=American crow, UK=Unknown, GF=gray fox, MV=multiple visitors, SSpp.=snake species, OP=Virginia opossum, SK=striped skunk, RO=rodent spp., WTD=white-tailed deer, BSpp.=bird species, TY=wild turkey, AD=armadillo, CO=coyote, DG=domestic dog.

The most frequent visitor to the track monitoring stations was the raccoon (65.9%) with 292 visits out of the 443 total visits documented. American crows (12.2%) were the second most common predatory visitor to the track monitoring stations with 54 visitations followed by the gray fox (5.6%), snake spp. (2.9%), opossum (2.5%), striped skunk (2.3%), and domestic dog (0.2%) and coyote (0.2%).

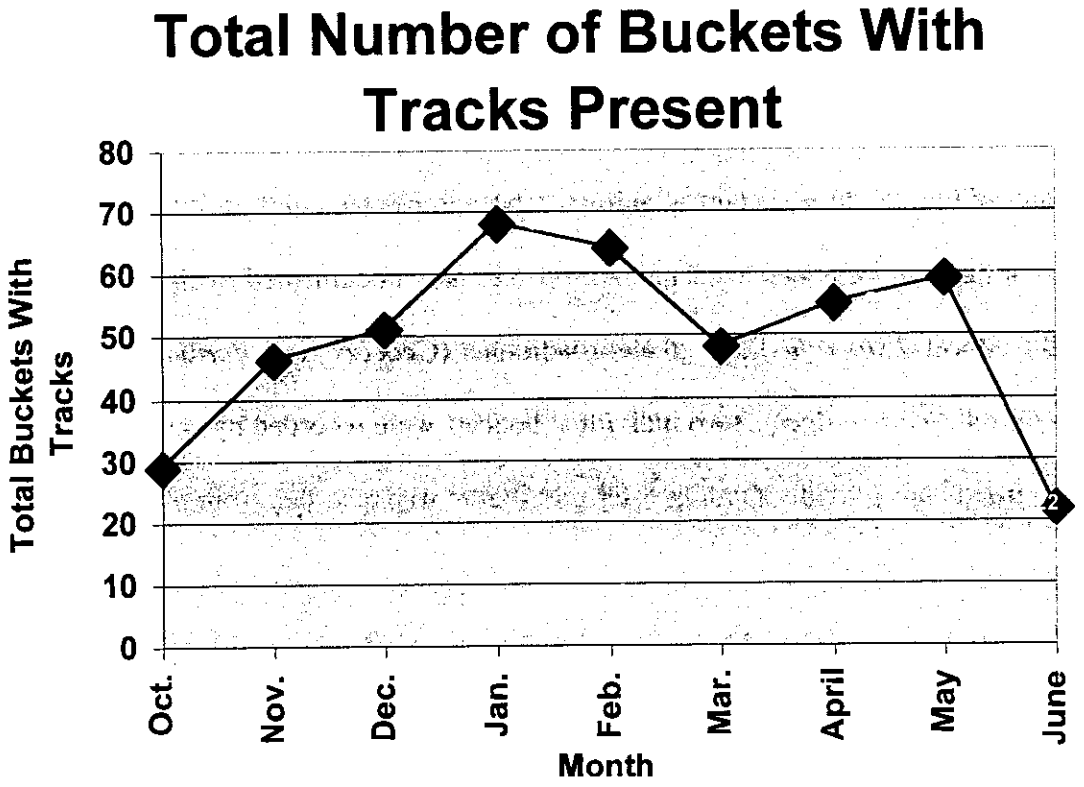

Figure 2. Total number of pitfall buckets on drift fence arrays at a study site in Bastrop County, TX, visited by potential vertebrate predators from the 21 October 2003 through 17 June 2004. A visit constitutes when a track or tracks are detectable in the station after the station has been raked. In this chart, visits were not separated out by species.

The total number of buckets was lowest with 29 visits in the month of October and peaked at 68 in the month of January. Visits declined in the months of February and March and then increased in April and May. June had the lowest visitation rate out of all the months in the study with only 22 buckets visited.

Camera Results

The 10 motion sensor Deer Cam® cameras were operational for 168 days beginning 11 of February 2004 through 29 July 2004. Since their installation the cameras have detected all of the predator species whose tracks appeared in the track monitoring stations and additional three species of animals whose tracks were not positively identified or had not been detected. The three species only documented by the cameras include the bobcat (*Lynx rufus*), the greater roadrunner (*Geococcyx californianus*), and the fox squirrel (*Sciurus niger*). Two individual bobcats were recorded by cameras in treatment group 2 and 3 and a single greater roadrunner was recorded from treatment group 3.

The cameras were also able to document the actual behavior of the putative predators at the buckets themselves. Pictures were taken of raccoons entering the buckets as well as removing bucket lids from temporarily closed buckets. One of the fox squirrel pictures was of an adult leaning over the edge of the bucket and looking into the center. In addition to verifying the predators present, the cameras also captured times when more than one carnivore was present. Several pictures were taken of multiple raccoons visiting the same bucket at the same time, or of multiple visitors occurring at different times during the night.

Discussion

The variety, abundance, and frequency of potential predators recorded at the track monitoring stations obviously poses a threat to animals captured in drift fence arrays. The high visitation rates of the raccoon, 292 out of 443, is a particular concern given the dexterity of raccoons and their ability to procure as well as consume a wide variety of prey items. Consistent visits by such carnivores (Figure 1) indicate that predation on drift fence arrays is a serious concern often overlooked from the literature addressing problems with drift fence sampling. In our research, in which an endangered species is being monitored through drift fence sampling, concern about predation is particularly significant. The presence of such known toad predators as the raccoons (Jones et. al, 1999; Schaaf and Garton, 1970) and skunks (Groves, 1980) could potentially pose a serious threat to Houston toads captured in the buckets. Although no direct Houston toad predation events were recorded from drift fence arrays during this study, there was anecdotal evidence of other amphibians experiencing predation along the fences. Six Hurter's Spadefoot toads (*Scaphiopus hurteri*) and one Gulf Coast toad (*Bufo valliceps*) were found partially consumed alongside the buckets. The partial consumption of the toads is consistent with other studies documenting toad predation, in which the toxins of the toads eventually deter or force the predator to selectively consume the prey item (Groves, 1980; Jones et al., 1999). The Gulf Coast toad was found in a track monitoring station that had raccoon prints all around the carcass; this was the same case for two of the Hurter's spadefoot toads. The presence of the fresh tracks alongside these carcasses

indicates that a raccoon was the predator involved. Another instance involved the disappearance of a dead northern pygmy mouse (*Baiomys taylori*) from one of our buckets. While raking one evening I found a dead pygmy mouse in one of our traps which I subsequently moved to another trap with known visitation rates. Arriving early the next morning to check the track monitoring stations I found the mouse was missing. There was no sign of disturbance except for the presence of clearly visible gray fox tracks up to and across the bucket. The pattern of the tracks would have forced the fox to go over the top of the flashing and the bucket itself after venturing to the very edge of the bucket itself.

Very few previous publications mention the problem of direct predation on drift fence arrays. Often the concept of predation on captured animals is completely omitted when considering the use of drift fence arrays for monitoring animal populations. Our results disclose regular visitation of drift fence arrays by known predators such as raccoons and gray fox. Both of these animals are more than capable of entering and removing animals from a 19 L bucket. Regional considerations, such as the taxa targeted by the drift fence arrays with pitfall traps as well as the specific predators found in the study area need to be taken into account before implementing a drift fence array study. For example, areas such as the Pacific Northwest where drift fences are commonly used (Aubry and Stringer, 2000), agile predators such as pine martens (*Martes americana*) and minks (*Mustela vison*) might pose additional threats to captured species. The results of this paper illustrate the need to consider predation as a problem when using drift fences to sample animals, especially in the case of rare or endangered animals such as the Houston toad. Measures need to be taken to prevent known predators from entering and obtaining

animals from buckets used in drift fence array surveys in order to reduce unwanted mortalities.

As illustrated by the continued visitation of predators throughout the study period, some form of predator prevention might be necessary when implementing drift fence sampling. In order to reduce the risk of predation of Houston toads, we implemented predatory exclusion devices, or PEDs made out of plywood and built similarly to the shade covers often used in drift fence studies. We are in the process of determining if these devices successfully reduce the risk of predation to animals confined in the buckets. Future studies on the success of predators in procuring prey items from drift fence arrays as well as the initiation, habituation periods, and correlation with trapping frequency, along drift fences by such carnivores need are now being addressed by our continuing work.

Chapter 5:
WILDLIFE DYNAMICS AND VEGETATIVE ANALYSIS OF THE LOST PINES

HABITAT AFFINITY FOR WHITE-TAILED DEER AND RIO GRANDE WILD TURKEY AT THE GRIFFITH LEAGUE RANCH, BASTROP COUNTY, TEXAS

SHANE J. KIEFER AND *JOHN T. BACCUS*

Game animal research throughout the range of southern pine forests is extensive, but the reasons for low populations of white-tailed deer and Rio Grande wild turkey in the isolated Lost Pines region of Bastrop County, Texas were not well understood. I characterized the vegetative communities on a 2012 ha ranch in the Lost Pines and related white-tailed deer and wild turkey abundance and distribution to available habitats. I measured woody plant cover and density in summer 2002 and assessed herbaceous cover, horizontal obscurity, duff depth, and percent canopy cover once per calendar season from summer 2002 to spring 2003. I estimated white-tailed deer abundance using a non-linear spotlight transect method and used GPS locations of sightings of both deer and wild turkeys to assess distribution and habitat affinity within a geographic information system. I found three major habitat types: pine forest, oak-juniper woodland, and grassland (improved pasture). Loblolly pine with an understory of yaupon dominated pine forests, which had higher woody plant density (7257 plants/ha), greater duff accumulation (60.2 mm), higher canopy coverage (87.1%), and lower visibility based on VPB measurements than oak-juniper woodlands (4419 plants/ha, 40.2 mm duff, 74.4% canopy). Post oak and blackjack oak mixed with eastern red cedar characterized oak-juniper woodlands. Pine and oak habitats showed sparse herbaceous plant cover (1.2% and 5.0%, respectively). Distance analysis estimated deer density at 0.010 deer/ha (20 deer on the ranch). Wild turkey abundance was approximately 20-30 individuals based on incidental sightings. Both species were associated with grassland habitat more than expected based on availability. Wild turkeys also appeared to favor oak-juniper woodlands over pine forests, while deer showed no preference for either. Long-term lack of management on the ranch has produced forest habitat that produces little food available to white-tailed deer and wild turkeys so they must utilize the grasslands for foraging. A combination of thinning and prescribed fire is recommended to increase forage quantity and quality. Future research should address the effectiveness of management practices in the Lost Pines compared to more mesic southern pine forests to the east.

Management of white-tailed deer (*Odocoileus virginianus*) and wild turkey (*Meleagris gallopavo*) in southern pine forests has been studied extensively (Lay 1956, Lay 1967a, Halls 1973, Blair et al. 1977, Melchiors et al. 1985, Thill et al. 1987, Bidwell et al. 1989, Campo et al. 1989, Miller et al. 2000). Generally these studies addressed game management within the silvicultural practices of pine forests for production of lumber in the southeastern United States. Collectively, they suggested integrating

management practices that initiate early stage succession and encourage plant diversity, particularly enhancing palatable herbaceous growth. Prescribed burning (Hodgkins 1958, Moore 1982, Thill et al. 1987, Miller et al. 2000), thinning (Patton and McGinnes 1964, Halls 1970, 1973), grazing (Thill 1984), and clear-cutting to produce openings (Campo et al. 1989, Thill et al. 1990, Johnson et al. 1995) are common suggestions for habitat improvement.

White-tailed deer prefer diverse habitat with a variety of forage sources and cover (Allen et al. 1996). Many studies consider browse the principal food source and stress increasing browse production, availability, and quality, while noting the importance of herbaceous growth (Wolters and Schmidtling 1975, Blair and Feduccia 1977, Blair et al. 1977, Thill et al. 1990, Johnson et al. 1995). However, Lay (1964) suggests browse may not be as dominant in the diet as indicated by the literature. Fungi may also be a highly used resource when available (Johnson et al. 1995).

Wild turkeys prefer older, mixed pine-hardwood stands with an open understory and scattered clearings (Bidwell et al. 1989, Campo et al. 1989, Allen et al. 1996, Miller et al. 2000). Turkeys need herbaceous cover for nesting (Allen et al. 1996), and well-forested riparian corridors for travel lanes (Miller et al. 2000).

Density estimates for deer in Bastrop County range from 0.016-0.042 deer/ha, depending on year and location in the county (Len Polasek, Texas Parks and Wildlife Department, personal communication). County estimates from 1998-2002 were lowest in 1999 (0.023 deer/ha) and highest in 2002 (0.037 deer/ha) (Wolf 2003). Reasons for low deer populations are not well understood, because there are very little data for the species in Bastrop County. The Post Oak Savannah ecoregion harvest data for wild turkey

indicate lower hunter participation and success than in other ecoregions with higher wild turkey populations (Texas Parks and Wildlife Department 2003). Information is needed about this unique and isolated ecoregion to elucidate why it does not support higher populations of game species.

No research exists regarding management for game species in the Lost Pines, where important differences from typical southern pine forests exist. Consequently, current management guidelines applied to eastern Texas and United States forests may be unsuitable.

The objectives of my study were to describe the composition and distribution of habitats on Griffith League Ranch including horizontal and vertical coverage of herbaceous and woody plants and woody plant density, and to provide baseline data about white-tailed deer and Rio Grande wild turkey populations and corresponding habitat affinities on the ranch. In addition, management recommendations for the ranch were developed in accordance with my findings.

STUDY AREA

The Lost Pines in Central Texas are the westernmost extension of southern pine forest in the United States. Separated from the Pineywoods of East Texas by about 130 km, the Lost Pines region receives substantially less rainfall than other southern forests. There is disagreement about the Lost Pines ecological status, both as an island and a remnant of a larger contiguous forest to the east. Descriptions range from island or archipelago (because separate stands occur in 5 counties) to fractured peninsula (Taber

and Fleenor 2003). However, pollen records indicate no change in the distribution of pines in the last 10-12,000 years (Larson et al. 1972).

The Lost Pines are maintained naturally by local adaptations by vegetation for water conservation and coarse, sandy soils that act as water reservoirs (Taber and Fleenor 2003). Annual evapotranspiration is ≤ 25 mm higher in the Lost Pines than in similar communities endemic to the western edge of the Pineywoods (Schultz 1997). The amount of water available to plants after surface evaporation declines by ≤ 381 mm from the Pineywoods to the Lost Pines (Taber and Fleenor 2003). The climate is humid subtropical receiving about 889 mm of annual precipitation with peaks in spring and early fall (Taber and Fleenor 2003).

The Griffith League Ranch (GLR) owned by the Capitol Area Council of the Boy Scouts of America is located about 13 km north of Bastrop, Texas in Bastrop County. It has a total area of 2012 ha composed of mixed pine-oak forest (1778 ha) that is typical of the Lost Pines and improved pastures (234 ha). Principal overstory vegetation is loblolly pine (*Pinus taeda*), blackjack oak (*Quercus marilandica*), post oak (*Q. stellata*), and eastern red cedar (*Juniperus virginiana*) with an understory of yaupon (*Ilex vomitoria*), farkleberry (*Vaccinum arboreum*) and beautyberry (*Callicarpa americana*) (Koepp 2001, Taber and Fleenor 2003). Improved pastures are dominated by coastal Bermudagrass (*Cynodon dactylon*) and bahia grass (*Paspalum notatum*).

The topography is rolling, with elevations ranging from 136 m above sea level in the western part of the ranch to 202 m in the east. Contrary to the eastern portion of Texas where bottomlands are occupied by hardwood species, this unique area supports pines along creeks and drainages (Taber and Fleenor 2003). Soils are sandy, with Patilo

(63%), Silstid (22%), and Demona (6%) loamy fine sands dominating the ranch (Baker 1979, Koepp 2001). At least 20 ponds occur on the property. Ponds range in surface area from about 0.5 ha to 1.5 ha. Nine ponds hold water intermittently (Koepp 2001). Alum Creek flows through the extreme eastern part of the ranch.

METHODS

Vegetation Analysis

I initially determined habitat types using 1-m Digital Orthophoto Quarter Quadrangles (DOQQ). I chose and ground-truthed 25 points to confirm the initial selection of 4 different habitat types (Fig. 1). The 4 habitat types were designated as grasslands composed of open areas of improved pasture, oak-juniper woodlands dominated by post and blackjack oak with eastern red cedar, pine forests dominated by loblolly pine, and pond habitats containing a permanent water source. I performed vegetational analysis in conjunction with another study (White 2003), but excluded pond habitats in my study, because water was not considered a limiting factor for highly mobile game animals on the ranch. Pond locations were assigned to grassland, oak-juniper woodland, or pine forest based on the surrounding habitat type, which was confirmed by PCA analysis (White 2003).

I assessed woody cover during summer 2002 using line intercept methodology (Higgins et al. 1996). Using each of the 25 sample points as a focus, I spaced 3 100-m transects at 120 degree angles. I used a random compass heading for placement of the initial transect at each point. I measured the vertical projection for all woody vegetation crossing the tape, summed distances by species, and calculated the mean across all 3

transects at each point to determine percent cover. I measured density of woody vegetation using 3 100-m^2 quadrats at each point. I spaced quadrats 120 degrees apart and placed them 15 m from the sample point to avoid overlapping measurements. I counted the number of individuals of each species within the quadrat boundary and calculated a mean across all 3 quadrats at each point. I discarded quadrats intersecting water at pond habitat sites to avoid biasing density estimates of the surrounding habitat. I measured both line intercepts and woody density only once at each site, because I presumed that conditions during summer were representative of the year. I used a total of 75 100-m line intercept transects and 75 100-m^2 quadrats.

I determined herbaceous cover at each of the same 25 points using 0.1-m^2 quadrats and 5 cover classes (Daubenmire 1959). I identified plants to species and later grouped them into forbs, grasses, and sedges, as well as native and introduced species. At each sample point I placed 10 quadrats randomly within 10-m intervals at random distances from the line along a single 100-m transect placed at a random compass heading. I measured litter or duff depth in the center of each quadrat. I measured percent canopy cover at 5 quadrats on each line using a spherical densiometer (Higgins et al. 1996). I took 5 measurements of horizontal obscurity on each line using a 2.5-m vegetation profile board (VPB) divided into 5 0.5-m segments placed 15 m from the transect (Nudds 1977). Measurements represented percent cover (obscurity) at each segment grouped in 5 equal classes from 0-100%. I used the midpoint of each class to calculate means. I took herbaceous cover, duff depth, densiometer readings, and VPB measures once per calendar season. I used a total of 250 0.1-m^2 quadrats and took 125

canopy and horizontal obscurity measurements per season (1000 quadrats and 500 canopy and obscurity readings over the course of one year).

I analyzed differences in vegetative variables by habitat and season. Because of an absence of woody vegetation in grasslands, I compared only oak-juniper and pine habitats. I transformed variables if necessary to meet assumptions of homoscedasticity and normality. I used t-tests to compare coverage of pine, oak, eastern red cedar, woody plant density, and square-root transformed yaupon between oak-juniper and pine habitats. I compared forb cover, grass cover, and total herbaceous cover (all log transformed) between all habitats and seasons using analysis of variance (ANOVA) and Tukey's honestly significant differenced (HSD) test (Quinn and Keough 2002). I analyzed duff by habitat, and duff and percent canopy by season and seasonally within habitats using ANOVA. I compared only the lowest VPB level (0.0 - 0.5 m) across all 3 habitat types because of heteroscedasticity resulting from absence of variance in the upper levels of grassland VPB data, which I anticipated because I found no coverage in any season above about 1 m and the majority below 0.5 m. All measurements above 1 m received the lowest value, resulting in minimal variance at low obscurity readings. I analyzed changes in obscurity by height within habitats, and among both habitats and seasons within each level. All statistical analyses were performed in S-PLUS 6.1 (Insightful Corporation, Seattle, Washington).

Wildlife Abundance and Distribution

Sighting data have been used to assess habitat use and predict distributions of species in relation to habitat (Agee *et al.* 1989, Stoms *et al.* 1993, Knick and Dyer 1997, Ortega-Huerta and Medley 1999). These analyses covered large geographic areas and

used existing data in creating regression models. However, the same technique should be applicable to smaller areas using fine-scale data.

I conducted 12 spotlight surveys totaling > 192 km traveled during late summer and early fall of 2002 (4) and 2003 (8) and estimated white-tailed deer density using a non-linear spotlight transect method (Pierce 2000). I conducted surveys on nonconsecutive nights within about 30 days. Line transect methodology eliminated the need to conduct an unfeasible complete census (Buckland *et al.* 2001) and bias associated with calculating visibility necessary for traditional strip transect methods, which makes it appropriate in areas with dense vegetation and clumped animal distributions (Pierce 2000). I used Global Positioning System (GPS) units (Garmin 12CX, Garmin International, Olathe, Kansas) to map transects (Fig. 1) and establish my location at each sighting of an individual(s).

I chose a survey route representative of all 3 habitat types, while confining observations to areas near sample points to allow definitive conclusions from sighting differences. I measured distances to animals using a laser range finder (Bushnell Yardage Pro 1000, Bushnell Corporation, Overland Park, Kansas), and calculated perpendicular distances from each sighting to the transect using a spatial join within ArcMap 8.3 (Environmental Systems Research Institute, Redlands, California). I used the resulting distance data to estimate density and abundance in Program Distance 4.1 (Thomas *et al.* 2003).

In addition to spotlight surveys, I used incidental sightings and directed searches throughout the year to locate both wild turkeys and white-tailed deer and recorded these locations via GPS. The regular occurrence of individuals and flocks of wild turkey at the

same locations allowed an estimate of abundance. I analyzed all GPS locations using ArcMap. I digitized records of additional sighting locations made by other persons directly into the GIS database if GPS locations were not available. The extremely low densities of both species made it necessary to use all available locations for analysis.

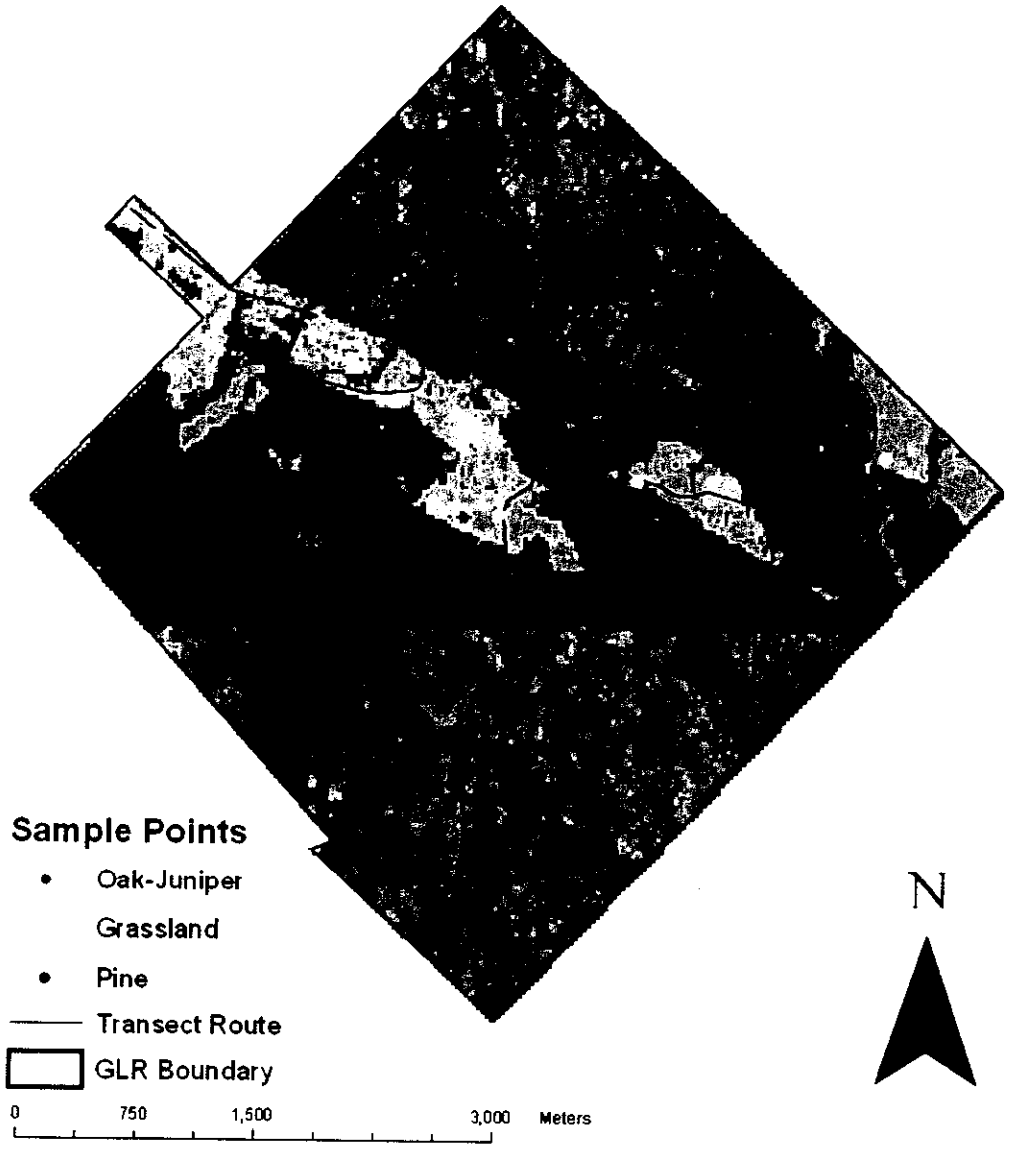

Figure 1. Vegetation sample points and spotlight survey transect route on Griffith League Ranch, Bastrop County, Texas.

Habitat Affinity

I created three buffers (0.5, 1.0, and 1.5 km) around each animal sighting within ArcMap (Knick and Dyer 1997) to allow analysis at multiple range sizes (Fig. 2). This technique allows an assessment of association without identifying individuals (Johnson 1980). I used a map of habitat type digitized at 1:10,000 scale in ArcMap 8.3 generated by the Texas Forest Service (Bastrop, Texas) (Fig. 3). I used the intersect function in the geoprocessing wizard to combine the buffer layer with the habitat layer to produce a table that identified the size and habitat type of polygons within the buffer around each animal. I summarized these data by habitat type to determine the percent area of each habitat type within the buffers. I used the same method on 43 random points within GLR to determine the expected habitat distribution if sightings were random.

To test for differences in habitat affinity from a random model, I used a ^{2} goodness-of-fit test to compare percentages from non-overlapping sighting buffers to non-overlapping random buffers within GLR. Because I used only non-overlapping buffers to maintain independence in the ^{2} goodness-of-fit test, some differences in habitat percentages exist from the analysis including all sightings and random points.

Figure 2. White-tailed deer sightings with a 0.5 km buffer around each location at Griffith League Ranch in 2002 and 2003.

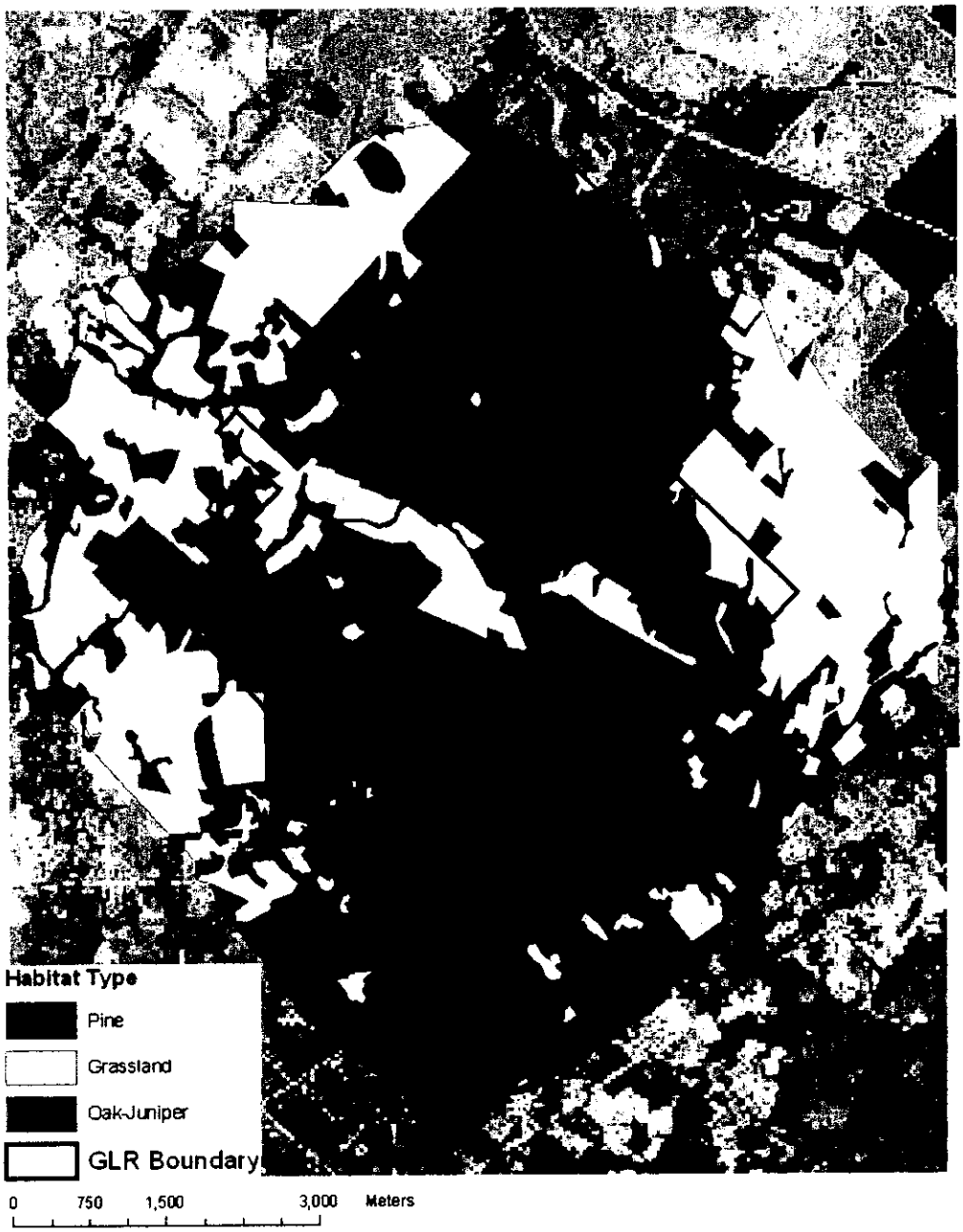

Figure 3. Digitized habitat types on Griffith League Ranch and surrounding properties.

RESULTS

Vegetation

Pine habitats had significantly greater coverage of loblolly pine (t = -7.43, P < 0.01) and yaupon (t = -3.12, P < 0.01), and oak-juniper habitats had greater coverage of oak species (t = 5.03, P < 0.01) and eastern red cedar (t = 4.23, P < 0.01) (Table 1). The only other woody plant with > 1% of cover in both habitats was farkleberry, which was not different between habitats (t = -0.470, P = 0.64). American beautyberry was relatively prominent, although variable in distribution, in pine forest habitat with a mean (± SE) coverage of 4.03 m/100m ± 1.15, but made only a minor appearance in oak-juniper woodlands with a total coverage of only 1.9 m from all 7 points. Seventeen other woody species were encountered, but none constituted more than 20 m across all 25 sites (Table 2).

Table 1. Mean coverage (m/100 m of intercept length) of 5 dominant woody species and mean density (plants/ha) of all woody plants at 25 sampling sites in oak-juniper woodland, pine forest, and grassland habitat types on Griffith League Ranch (Summer 2002).

Habitat	Loblolly Pine	Post Oak	Blackjack Oak	Yaupon	E. Red Cedar	Density
Oak-Juniper	28.0	43.7	19.9	12.2	30.8	4419 (1467-6834)
Pine	64.1	26.8	7.6	30.7	13.8	7257 (3600-10450)
Grassland	5.5	0.9	1.5	0.7	0.4	n/a

Table 2. Total Coverage (m) from 25 sample points of the most commonly encountered woody species on Griffith League Ranch (Summer 2002).

Species	Total Coverage (m)
Ampelopsis arborea	16.9
Bumelia lanuginosa	10.0
Callicarpa americana	50.2
Carya texana	19.9
Ilex vomitoria	457.8
Juniperus virginiana	384.1
Myrica cerifera	1.5
Pinus taeda	998.0
Prosopis glandulosa	2.6
Quercus incana	4.3
Quercus marilandica	239.6
Quercus nigra	2.7
Quercus stellata	632.4
Rhus aromatica	3.4
Rubus trivialis	1.4
Ulmus rubra	5.5
Vaccinum arboreum	72.2
Vitis mustangensis	3.1

Density of woody plants ranged from 1,467 plants/ha to over 10,000 plants/ha, with a significant difference between pine forest and oak-juniper habitats (t = -2.46, P = 0.02) (Table 1). Yaupon had the greatest individual density overall (3,446 plants/ha ±

519) across all 25 sites and within each forest habitat with 4,328 plants/ha ± 674 and 1,933 plants/ha ± 404 in pine forest and oak-juniper woodlands, respectively. It was followed by post oak with 720 plants/ha ± 136 in both habitats, 854 plants/ha ± 186 in the pine forest, and 490 plants/ha ± 165 in oak-juniper woodland. Eastern red cedar (587 plants/ha ± 105) and farkleberry (259 plants/ha ± 78) were also prominent with respect to overall density across both habitats.

Forb cover did not differ by season when blocking by habitat type (F (3, 88) = 1.42, $P = 0.242$) however, it was different between habitats (F (2, 88) = 99.5, $P < 0.01$) with grasslands having the highest coverage of 14.3% ± 1.54 (Table 3). Both grass cover and total herbaceous cover showed habitat by season interactions (F (6, 88) = 3.59, $P <$ 0.01 and F (6, 88) = 4.10, $P < 0.01$, respectively). When analyzed only by season, there was no difference for grass cover (F (3, 96) = 0.938, $P = 0.426$) or total herbaceous cover (F (3, 96) = 2.03, $P = 0.113$).

Forest duff depths showed differences by both season (F (3, 68) = 6.03, $P < 0.01$) and habitat type (F (1, 68) = 32.7, $P < 0.01$). Summer duff levels (65.9 mm ± 5.24) were significantly higher than both winter and spring levels (45.9 mm ± 3.70 and 47.1 mm ± 3.28, respectively). Duff depth did not differ between all other seasons. Pine forests showed more duff accumulation (60.2 mm ± 2.53) than oak-juniper woodlands (40.2 mm ± 2.67). Duff depths changed seasonally within the pine forest (F (3, 44) = 6.29, $P <$ 0.01), but not in oak-juniper woodlands (F (3, 24) = 1.62, $P = 0.211$). Pine forest summer duff levels (75.9 mm ± 5.79) were higher than in all other seasons. Percent canopy cover in forest habitats averaged 74.4 % ± 3.26 and 87.1 % ± 1.27 in oak-juniper and pine habitats, respectively. Canopy cover changed seasonally within the oak-juniper

woodlands (F (3, 24) = 3.24, P = 0.040) with a difference between summer (83% ± 3.47) and winter (59% ± 7.03).

Table 3. Mean coverage (%) across all seasons of herbaceous vegetation in oak-juniper woodland, pine forest, and grassland habitat types on Griffith League Ranch for 2002-2003.

Habitat	Grasses	Forbs	Sedges	Total Herb Cover
Oak-Juniper	3.1	1.4	0.4	5.0
Pine	0.5	0.7	0.0	1.2
Grassland	45.2	14.3	0.5	60.0

Horizontal obscurity was dependent upon plant height in oak-juniper woodlands (F (4, 135) = 5.92, P < 0.01) and grasslands (F (4, 115) = 64.5, P < 0.01). In oak-juniper woodlands the highest two levels (1.5 – 2.5 m) showed the greatest obscurity, while the lowest levels were most occluded in grasslands (Table 4). Pine forests showed no change in visual obstruction by plant height (F (4, 235) = 0.589, P = 0.67). Pine habitats had significantly less visibility than oak-juniper habitats at the lowest three VPB levels, 1.0-1.5 m (t = -2.23, P = 0.04), 0.5-1.0 m (t = -2.85, P = 0.01), and 0.0-0.5 m (t = -2.40, P = 0.03).

Table 4. Height variation in mean horizontal obscurity (%) for all seasons within oak-juniper woodland, pine forest, and grassland habitat types on Griffith League Ranch for 2002-2003.

Habitat	VPB Layer (m)				
	0.0-0.5	0.5-1.0	1.0-1.5	1.5-2.0	2.0-2.5
Oak-Juniper	50.4	45.3	53.6	55.1	61.1
Pine	64.3	61.3	66.0	63.2	65.3
Grassland	45.0	16.7	12.2	11.2	11.3

Wildlife Abundance and Habitat Affinity

I recorded a total of 41 sightings of 73 deer (11 bucks, 34 does, 1 fawn, 27 unidentified) over the course of 2 years. Eighteen sightings occurred during spotlight surveys with sufficient information for calculating density. Density estimates based on distance analysis suggested a density of 0.010 deer/ha (20 deer on GLR). The lowest Akaike's Information Criterion (AIC) resulted from a uniform sighting distribution with no adjustments (AIC = 179.90), where animals were equally likely to be seen at any distance (Fig. 4). Using this distribution resulted in the lowest density estimate of 0.009 deer/ha. I adjusted the uniform key distribution two different ways to generate a more realistic sighting distribution (one where sighting probability declines with distance) to

compare to the uniform model. A cosine adjustment yielded a density estimate of 0.012 deer/ha (AIC = 181.04). A polynomial adjustment yielded an intermediate density estimate of 0.011 deer/ha, and gave a slightly smaller bootstrap confidence interval (0.006 – 0.018 deer/ha) than the cosine adjusted model (0.006 – 0.021 deer/ha). The data fit both the uniform/cosine and the uniform/polynomial models better than the unadjusted uniform model based on quantile-quantile plots (Fig. 5).

The percentage of grassland habitat within the sighting buffers showed a decrease from 41.4% to 31.2% as distance increased (Table 5). As random buffer size increased the percentage of grassland habitat only increased from 13.6% to 16.7%. Pine forest made up the largest percentage of habitat within the sighting buffers at all distances except 1.5 km, where oak-juniper woodlands were slightly higher. Within the random buffers, pine forest made up the highest percentage of habitat at all distances.

The ^{2} values indicate that habitat around white-tailed deer sightings at 0.5 km was not in proportion to that available at random ($^2 = 42.3$, $P < 0.01$). If deer were using habitat at levels available, the ^{2} values would be near null. Pine forest (35%) and oak-juniper woodland (37%) were equally represented within 8 non-overlapping, 0.5 km buffers around deer sightings. Grassland constituted 28% of habitat within non-overlapping sighting buffers. Within 13 random, non-overlapping, 0.5 km buffers grassland only accounted for 9% of habitat, while percentages of pine forest (47%) and oak-juniper woodland (44%) both increased while remaining similar to each other.

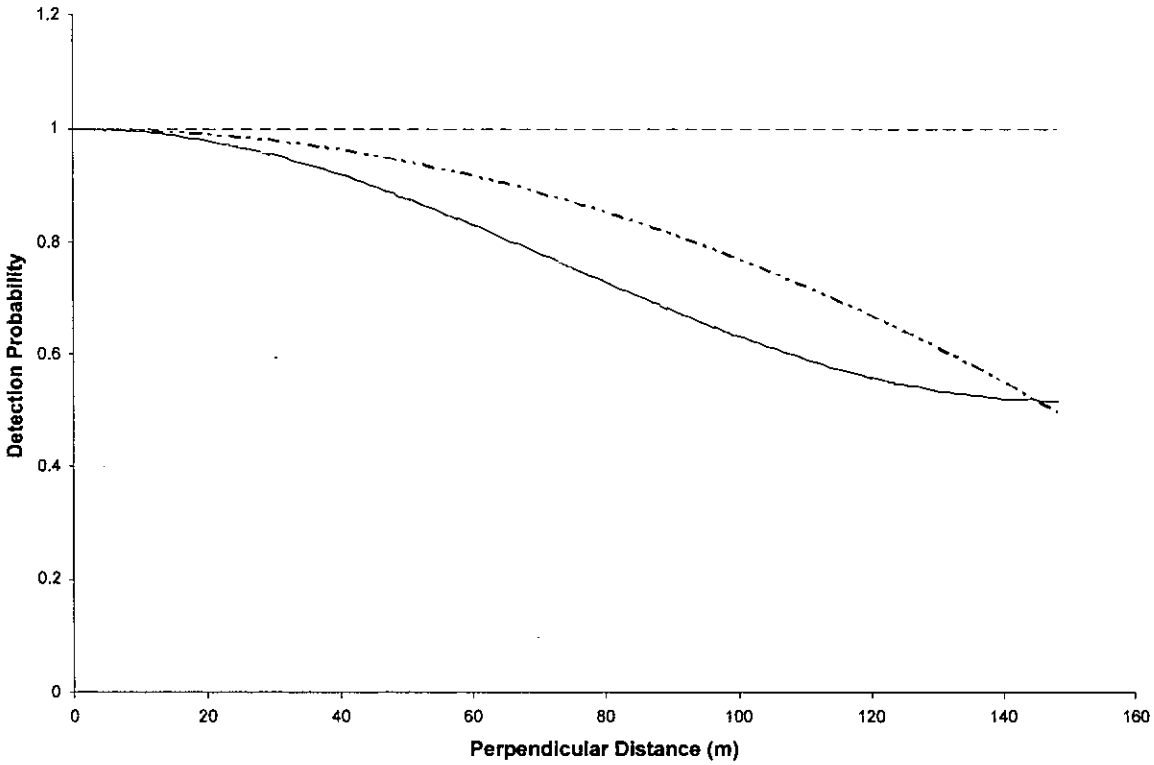

Figure 4. Detection functions used to estimate density of white-tailed deer on Griffith League Ranch. ——— Uniform distribution. — -- — Uniform distribution with a 2nd order polynomial adjustment term. —— Uniform distribution with a cosine adjustment term. The adjustment terms create a decline in sighting probability with increasing distance.

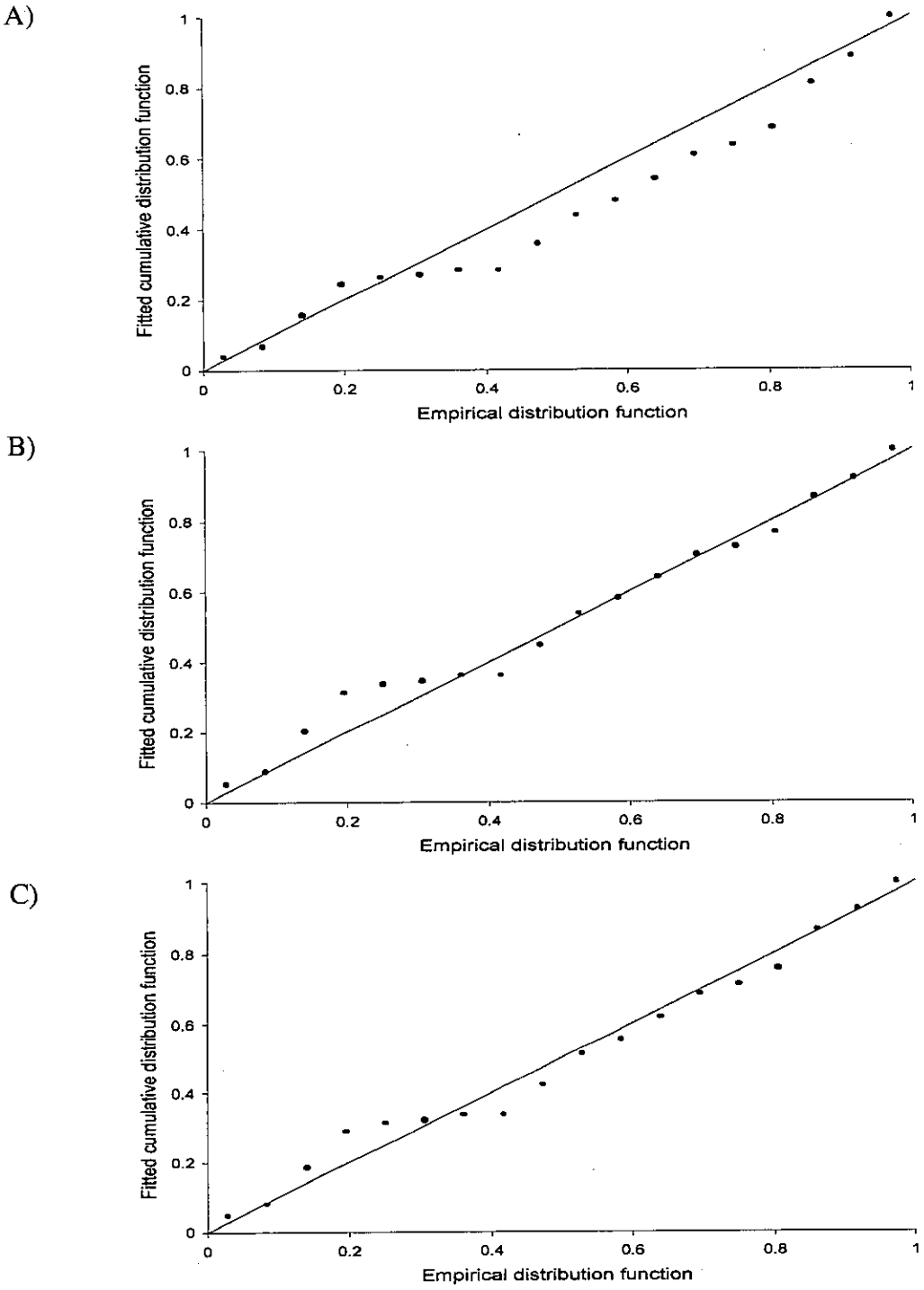

Figure 5. Quantile-quantile (qq) plots of fitted distribution function versus distribution of data used to assess how well the data fit the model. A) Qq plot for unadjusted uniform model showing deviation at medium distances. B) Qq plot for cosine adjusted model. C) Qq plot for polynomial adjusted model. Both B and C show only minor spikes just beyond zero distance.

Table 5. Comparison of habitat coverage within buffers around sightings of white-tailed deer and around random locations on Griffith League Ranch.

Buffer Distance (km)	Habitat	Percent Area* (Sightings)	Percent Area* (Random Points)
1.5	Pine	33.3	44.2
	Grassland	31.2	16.7
	Oak-Juniper	35.5	39.1
1.0	Pine	34.9	45.2
	Grassland	33.1	14.6
	Oak-Juniper	32.0	40.2
0.5	Pine	32.2	45.1
	Grassland	41.4	13.6
	Oak-Juniper	26.4	41.3

*All sightings and random locations are included in calculation of percent area. Only non-overlapping buffers were used to calculate percent area for goodness-of-fit tests and differences do exist.

I recorded 24 sightings of 119 Rio Grande turkeys (8 males, 63 hens, 25 poults, 23 unidentified) during the study. Maximum group size was 16 with a mean of 5. Turkeys showed more affinity for forested habitats than deer (Table 6). Oak-juniper habitat was most prominent at all distances around sightings. Percentage of grassland habitat within sighting buffers decreased from 33.6% to 21.1% and only exceeded pine habitat at 0.5 km. Pine forests accounted for about 45% of habitat within random buffers but did not exceed 35% at any distance around sightings. Oak-juniper woodlands were present in similar quantities around both sightings and random locations. Based on

sightings, wild turkey abundance was estimated at between 20-30 individuals. A single large flock concentrated on a main drainage in the central portion of the ranch.

The goodness-of-fit test showed strong deviation from expected percentages in 6 non-overlapping sighting buffers ($^2 = 28.2$, $P < 0.01$). Once again, a ^{2} value near null indicates similarity in habitat around sightings and at random. Oak-juniper woodland represented 44% of habitat at 0.5 km around both sightings and random points. Pine forest was underrepresented around sightings (33%) compared to random points (47%), while grassland was more common around sightings (24%) than random locations (9%).

Table 6. Comparison of habitat coverage within buffers around sightings of Rio Grande wild turkey and around random locations on Griffith League Ranch.

Buffer Distance (km)	Habitat	Percent Area* (Sightings)	Percent Area* (Random Points)
1.5	Pine	35.0	44.2
	Grassland	21.1	16.7
	Oak-Juniper	44.0	39.1
1.0	Pine	33.8	45.2
	Grassland	25.1	14.6
	Oak-Juniper	41.1	40.2
0.5	Pine	25.9	45.1
	Grassland	33.6	13.6
	Oak-Juniper	40.6	41.3

*All sightings and random locations are included in calculation of percent area. Only non-overlapping buffers were used to calculate percent area for goodness-of-fit tests and differences do exist.

DISCUSSION

Prior observations from GLR indicated that white-tailed deer and wild turkey favored areas with greater hardwood abundance. The extremely low density of deer and their affinity for grassland areas (the only areas to produce any quantity of herbaceous forage) suggested that deer were using the small amount of edge habitat available, regardless of surrounding habitat type. Habitats occurring within 0.5 km, 1.0 km, and 1.5 km of deer sightings showed more grassland area than either pine forest or oak-juniper. Grassland habitat was least available and changed little with distance in random buffers (Table 5). The 1.0 km and 1.5 km sighting buffers showed an almost even distribution of habitats, despite the uneven distribution seen in random buffers. Non-overlapping buffers used for the goodness-of-fit tests showed a similar pattern of high grassland use.

Unmanaged pine forests slowly decrease in quality and quantity of forage because fire suppression allows litter to accumulate while browse grows out of reach and density and basal area increase (Halls 1973, Conroy *et al.* 1982, Scanlon and Sharik 1986, Thill *et al.* 1990). However, they may eventually become oak dominated communities (Allen *et al.* 1996) important for mast and fungi production (Johnson *et al.* 1995). On intensively managed plantations, deer may not favor edge areas in stands up to 276 ha if there is sufficient production of forage in the forest (Melchiors *et al.* 1985). High yaupon production in GLR pine forests (30.7 % canopy cover and 4,328 plants/ha) should provide plenty of favored forage (Lay 1956, 1967a, 1967b). Some of this may be out of reach, or the deer may not be willing to utilize large tracts of forest for a single food item. The forest on GLR is largely unbroken by openings except for a line of central

pastures. The large stand sizes, lack of management, and low forage production on GLR may force deer to use edges to subsist.

The decreasing percentage of grassland habitat with increasing buffer distance indicates that Rio Grande wild turkeys on GLR were more associated with forest habitat than deer. I observed them using openings to forage on insects and seeds and for reproductive displays on several occasions. They primarily roosted in the center of the ranch at the head of a large drainage. Miller *et al.* (2000) suggested that riparian corridors are important for female movement and reproduction. Improvement of habitat surrounding other drainages on the ranch and creation of small openings for herbaceous forage production may increase use of the ranch by wild turkeys.

Vegetative density (Halls 1973), canopy cover (Conroy *et al.* 1982), and duff (Thill *et al.* 1987) negatively impact forage production. Despite lower levels of litter accumulation in oak-juniper woodlands (40.2 mm annually) than pine forests (60.1 mm annually), canopy cover remained high (74% annually) and forb production remained low (1.4%), making both pine and hardwood habitats marginal wildlife habitat. One pine forest sample point (Point 12) averaged 116 mm of duff in summer, and is likely responsible for the apparent seasonal change in duff depth. The unusually high measurement probably resulted from random placement of quadrats near tree bases where duff can build up substantially. Scanlon and Sharik (1986) found that forage production in hardwood stands in a low mast year was similar to that of 17-year-old pine stands with < 30 kg/ha, so low production of both on GLR is predictable. Oak mast may make up large portions of white-tailed deer diets (Harlow *et al.* 1975), but almost no mast

production by oaks was observed on GLR, negating its influence on deer distribution on the ranch.

Similar to white-tailed deer, wild turkeys tend to favor pine-hardwood mixtures with low shrub densities, an open understory, and high herbaceous production (Bidwell et al. 1989, Campo *et al.* 1989). The high woody plant density and decreased visibility at low VPB levels in the pine forest on GLR may explain the apparent lack of affinity for this habitat by wild turkeys (Allen et al. 1996). Campo *et al.* (1989) found that turkeys in eastern Texas tended to inhabit stands associated with openings. The results of my study supported that finding, because I recorded only 2 locations > 400 m from open areas and 18 of 24 sightings were < 100 m. The combination of lower woody plant density, decreased low-level visual obscurity in the surrounding oak-juniper woodlands, and access to the central pastures probably accounted for observed centralized locations of most turkey sightings.

Some of the apparent selection for grassland habitats by both species may result from differential sighting ability. However, wide scale use of the forested areas is unlikely since there was a lack of sightings and sign (tracks, droppings) in forested areas. The concentration of many deer sightings near the eastern and western boundaries of the ranch, where surrounding ranches are mostly pasture, suggested selection for open areas and edge despite differences in sighting probability.

Woody vegetation in forested areas was dense and dominated by few species in both the overstory and understory. Herbaceous production was essentially nonexistent. Unusually low measurements for herbaceous coverage in the fall for both oak-juniper and pine habitats, which may be a result of the patchy nature of herbaceous cover in the

forest, explains the habitat by season interactions seen in grass and total herbaceous cover measures. This complicates analysis of these data, but it is doubtful that any biologically significant differences exist.

Bidwell *et al.* (1989) reported green herbaceous cover > 20% in 5 to 7-year-old pine plantations, and even the oldest stands (11 to 13-years-old) had around 10% coverage. Loblolly forests tend to have lower herbaceous species richness and higher midstory and overstory densities than other southern pine forest types, but they can contain herbaceous communities similar to longleaf forests, which are known for high levels of herbaceous production (Hedman et al. 2000). The forest on GLR had a long time for litter accumulation, midstory development, and canopy closure, all of which inhibited forage production. In older stands, midstory browse tends to grow out of reach of white-tailed deer (Halls 1973, Thill et al. 1990). In oak-juniper woodlands on GLR horizontal obscurity was highest at higher VPB levels (Table 4), suggesting an elevated midstory.

I found no differences in visual obscurity at the highest VPB levels between oak and pine habitats. Spotlighting occurs from an elevated position and is probably influenced most by these upper levels, but differences in sighting angle can change visibility and an animal's distance from the vehicle and position (bedded or standing) may make lower levels important in sighting bias as well. Visibility in oak-juniper woodlands was higher along the survey route but did not result in a substantial increase in sightings. Only one spotlight sighting of a deer occurred in forest habitat. Increased sampling of forest habitats would most likely increase numbers of deer sighted; however, restrictions on available access precluded this option.

About 36% (5.9 km) of the transect route was in grassland habitat, and much of this ran along edges, where two habitats were sampled simultaneously. The better precision and lower AIC of the uniform sighting distribution probably resulted from the high number of observations from grassland habitats where a uniform distribution is more likely. All models pointed to an extremely low number of white-tailed deer on GLR. Based on the confidence limits, the maximum density estimated is no more than the lowest countywide estimates of 0.023 deer/ha.

Management Implications

This study provided baseline data for two important game species in an area largely ignored in the published literature. The current focal species in the Lost Pines is the Houston toad (*Bufo houstonensis*), a federally listed endangered species. Its presence means that management activities on GLR have to consider potentially negative impacts to this species (US Code, Title 16, Ch. 35; State of Texas Parks and Wildlife Code, Title 5, Ch. 68). Greuter (2004) recommended a 75-m buffer around ponds from March to July to protect juvenile toads. Even if a more generous 500-m buffer is used year-round and all known ponds are protected, 64% (1,295 ha) of the ranch area is still available for other wildlife management activities. Much of this area is pine forest habitat, which is in the most serious need of management.

Fire is an important tool for maintaining loblolly pine forests. Prescribed burning reduces and lowers the woody understory and encourages herbaceous growth by improving light penetration and removing litter (Hodgkins 1958, Moore 1982, Thill et al. 1987, Miller et al. 2000) as well as reducing the possibility of destructive fires (Hunter 1990). Burning too frequently can negatively impact hardwoods, but longer cycles

increase the risk of hotter fires which destroy old growth individuals (Schultz 1997). Burning on 3 to 5-year intervals is recommended (Lay 1967a, Halls 1973, Miller et al. 2000). The large litter accumulations in some areas, patchy fuel loads, and lack of fire breaks may make burning difficult on GLR. Caution must also be used to avoid destroying large snags (Hunter 1990). An initial tree thinning followed by an early winter burn to avoid active toads should promote increased forage production and diversity on the ranch.

Tree thinning stimulates sprouting by woody browse plants and reduces competition for herbaceous plants (Patton and McGinnes 1964; Halls 1970, 1973). Old, unthinned pine forests support fewer plant species and produce less forage for herbivores (Hunter 1990). Patton and McGinnes (1964) reported a 200% increase in browse production following a 30% thinning in a Virginia forest, and Patton (1974) noted that harvest of an Arizona forest increased forage and use of the area by deer. Blair and Feduccia (1977) found an 81% increase in browse after burning in Louisiana. Removing the midstory increased herb production as well. White (2003) made similar recommendations for GLR to improve avian habitat. An increase in forage production and diversity should benefit all wildlife on the ranch by reinstating previously naturally occurring cycles and enhancing productivity.

To further improve forage production and use of GLR by white-tailed deer and Rio Grande wild turkeys, small openings or clear-cuts can be made throughout the forest. Clear-cutting produces openings to increase foraging opportunities and edge (Campo et al. 1989, Thill et al. 1990) and may increase use because of increases in quality of forage available (Johnson et al. 1995). Sweeney et al. (1984) found similar use of all distances

from the edge in young, open cuts < 25 ha in size. Seeding these openings could help speed up forage production in these often poor soils. Many ponds on the ranch are located close to existing roads. Careful placement of long, narrow clearings may allow their simultaneous use as firebreaks when widening roads is not an option because of nearby ponds.

The large size and relatively undeveloped conditions which exist at GLR hold potential for quality habitat enhancement for game and nongame communities in the Lost Pines. The presence of the Houston toad and several endemic and newly discovered species of insects (Taber and Fleenor 2003) make the Lost Pines a unique and important ecosystem. Future research should concentrate on specific needs of game animals that are or are not being met in largely unmanaged pine forests in the area. Current management should focus on immediate increases in forage availability. While most southern pine forests suffer from a lack of quality forage because of high rainfall levels (Lay 1956), animals on GLR are likely more heavily impacted by a lack of quantity. Future changes can be compared to the current research to assess the effectiveness of traditional pine forest management activities in improving habitat and abundance of species in the Lost Pines. Increased game populations in Bastrop County may provide more recreational opportunities for residents as well as increasing the awareness of proper management and conservation of the Lost Pines. This is contingent on the prudent use of applied ecology in maintaining the health of the ecosystem and the communities it supports.

Avian Habitat Affinity in the Lost Pines Region of Texas

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Avian diversity and habitat association has been investigated by many workers (MacArthur and MacArthur 1961, MacArthur 1964, Rice et al. 1983, McCollin 1998). Rice et al. (1983) found that avian communities can be used as an indicator of habitat type, while Todt (1989) showed that avian diversity corresponds to differences observed in the habitat. Diversity may also be used as an indicator of habitat quality. High quality habitat is assumed to support a more diverse avian community which could include both specialists and generalists (Gabbe et al. 2002). This could also indicate that low species richness or the absence of common bird species may indicate poor habitat quality. Among terrestrial vertebrate groups, birds are the most numerous and easiest to detect making them the logical choice for the evaluation of habitat quality.

Single species or specific group studies focusing on habitat selection and habitat attributes are also common, (Bertin 1977, Conner and Adkisson 1977, Martinez and Jaksic 1996, Ritter and Savidge 1999). McClelland and McClelland (1999) reported that Pileated Woodpeckers (*Dryocopus pileatus*) indicate a healthy old growth forest. Single species studies are useful, but somewhat limited in overall conclusions that can be drawn for an avian community. While these studies are necessary, especially when dealing with endangered species, community based approaches must be used to conduct sound ecosystem management. A community based approach would consider all species present within the community, and any potential interactions among those species.

Avian Habitat Use

Habitat use by avifauna varies among species, seasons, and possibly broad-scale location. Use of habitat is a well studied field with many proposed hypotheses on how and why birds choose one or more habitat types. Boulinier et al. (2001) suggests that landscape structure may influence forest bird communities at regional scales through its effects on the total number of species and also on the temporal rates of change in community composition. Reduction of nest predation pressure (Sieving and Wilson 1998), foliage profile characteristics (MacArthur and MacArthur 1961), latitude (Tramer 1969), heterogeneity and patchiness (Franzreb and Ohmart 1978), edge density (Howell et al. 2000), plant species (Tomoff 1974), guild structure (Rice et al. 1983), interaction and competition avoidance, microclimate modification, and vegetation structure (McCollin 1998) all have been suggested as possible factors influencing habitat use. Not only do these factors vary among species, but also among other taxonomic groupings and seasonal groups (breeders, winter residents, permanent residents) as well. Flather and Sauer (1996) reported Neotropical migrants show a more sensitive response than temperate migrants or permanent residents to changes in landscape structure and utilize large contiguous habitats with continuous canopy cover. Permanent residents in the same study showed less affinity with landscape structure while temperate migrants were correlated with habitat diversity and edge attributes rather than with the amount, size, and dispersion of forest habitats.

Prior to any understanding of potential relationships between avian communities and habitat, thorough descriptions of all possible factors influencing that relationship must be investigated. Vegetation is the major component of most terrestrial habitats and

quantifying measurements of vegetation are useful in identifying any relationship between avian diversity and habitat. Vegetation does not account for other influential factors (avoidance of nest predation, guild structure, competition avoidance) that may also affect avian diversity associations with habitat types. In addition to vegetation, avian communities must be surveyed to estimate current populations. This data may also be used to establish trends and evaluate progress following application of management techniques.

The Lost Pines is a well known part of Texas, but little research has been conducted that quantify the plant and animal communities associated with the region (Taber and Fleenor 2003). Many scientists propose that this isolated pocket of pines is a remnant of a greater forest that once covered the eastern half of the state. Correll (1966) described the Lost Pines as a fractured, western peninsula that currently is a distant island or archipelago of pine. Tabor and Fleenor (2003) proposed that the pines possibly arose independently of the pines to the east but offered no support for this hypothesis. Pollen analysis has suggested that loblolly pines have been in the area for nearly twenty thousand years (Bryant 1977) and no significant change or expansion of the Lost Pines has occurred during the last sixteen thousand years (Larson et al. 1972). Regardless of origin, habitat fragmentation from recent urban sprawl has made its impact on the land and will continue to do so over time. The best chance for conservation of the Lost Pines is its strong hold for the endangered Houston Toad (*Bufo houstonensis*). Research on the larger unfragmented areas of forest is necessary in order to identify habitats that support the greatest diversity and the factors within those habitats that are most critical. In

addition to conservations efforts, baseline plant and animal populations will establish a starting point for analyzing the effects of forest fragmentation in the Lost Pines Region.

Population decline among Neotropical migrants is a well documented occurrence (Robbins et al. 1989). With this current trend, identification of diverse habitats and key habitat components must be found within the Lost Pines for proper management of bird populations. Among the problems contributing to the decline are habitat fragmentation, urban sprawl, loss of old growth forest, loss of large scale contiguous forest, nest parasitism and others. Habitat fragmentation coupled with Brown-headed Cowbird (*Molothrus ater*) nest parasitism may multiply the detrimental effects on declining populations. Fragments of habitat create more edge, which is utilized by the cowbirds, and offer more hosts to parasitize (Robinson et al. 1995). Other combinations of causes likely are having similar affects. With modern progress (urbanization, forest fragmentation) and declining bird populations, application of sound ecosystem management practices must focus on habitat types and factors that are most influential across all seasons for the avian communities present.

Like many studies dealing with habitat use, components of the habitat that best describe avian diversity may be site specific. Factors influencing a species may not differ greatly by site but factors affecting diversity could and probably do vary by site. This potential variation may be explained by vegetation, guild structure and microclimate differences found within each habitat.

Research Objectives

In this paper I will present findings which 1) establish a baseline inventory of the avian community found on Griffith League Ranch, 2) establish a vegetation profile of the plant community including canopy coverage, vertical structure, woody species density, herbaceous plant coverage, and duff depths, 3) identify habitats of higher diversity by season and 4) identify primary vegetative components that are associated with avian community diversity by season.

MATERIALS AND METHODS

Study Site

The Griffith League Ranch (hereafter GLR) occupies 1,961 ha of the Lost Pines Region of south central Texas (Fig.1). GLR lies in an isolated loblolly pine (*Pinus taeda*) forest geographically separated from the Piney Woods region of east Texas by approximately 160 km. GLR is approximately thirteen km northeast o f Bastrop, Texas in Bastrop County.

Forested areas (1,728 ha) of GLR are a mix of loblolly pine, post oak (*Quercus stellata*), blackjack oak (*Q. marilandica*) and eastern red cedar (*Juniperus virginianus*). Cleared pasture lands (233 ha) consisting of costal Bermuda (*Cynodon dactylon*) and Bahia grass (*Paspalum notatum*) formerly were grazed by livestock. Within the forested areas, the understory includes American beautyberry (*Callicarpa americana*), yaupon (*Ilex vomitoria*) and farkleberry (*Vaccinium arboreum*). Herbaceous vegetation under the forest canopy is sparse, but does include Texas bull-nettle (*Cnidoscolus texanus*), panic grasses (*Dicanthelium spp*,) and flowering spurge (*Euphorbia corolata*).

Rolling hills of sandy soils make up the topography of GLR. Demona loamy fine sand, Patilo and Silstid loamy fine sand of the sand range site cover more than 90% of

GLR (USDA Soil Conservation Service 1979). Elevations range from 137 m to 198 m with Alum Creek on the eastern edge of the ranch and Piney Creek and Spicer Creek drainages to the west and southwest, respectively. Nineteen known ponds exist on the ranch varying in size from less than 0.5 ha to just under 1.5 ha, eleven of which hold water permanently (Koepp 2001).

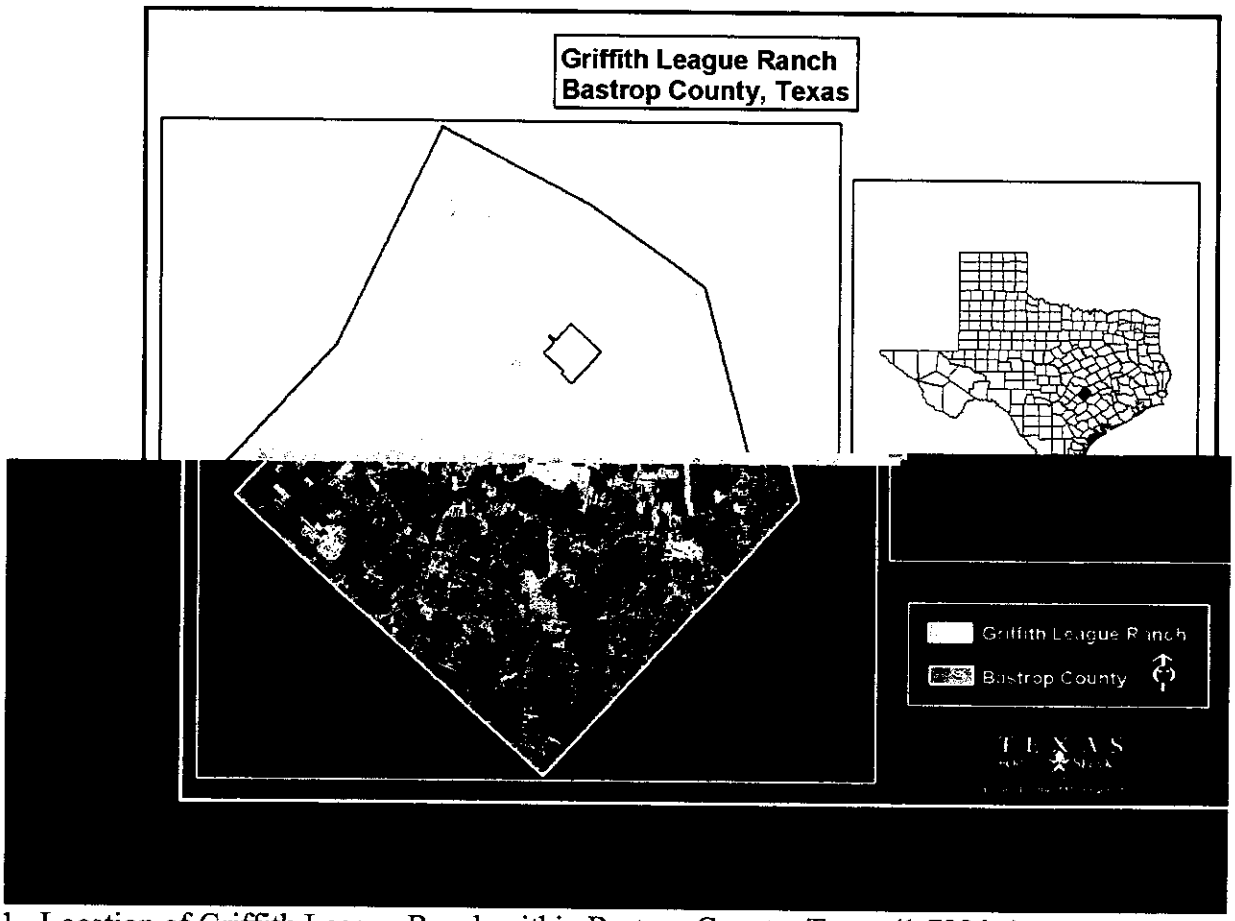

Figure 1. Location of Griffith League Ranch within Bastrop County, Texas (1,728 ha).

Habitats Associated with Griffith League Ranch

Delineation of habitats was determined by the use of digital orthophoto quarter quadrangles (DOQQ). Points within selected habitat types were selected and ground truthed to verify habitat type identification. At each point one t-post was driven into the ground to serve as a center point of that habitat. Twenty-five points within four habitat types were chosen and measured to quantify habitat type assignments (Fig. 2). Habitat types were grasslands with reduced woody species canopy cover, oak/cedar habitats with greatest amounts of post and blackjack oaks and eastern red cedar, pine habitats with a dominant overstory of loblolly pine, and pond habitats containing a permanent pond within a 100 m radius of the point center (Fig. 2). Points were treated as independent samples and spaced > 250 m apart to prevent violation of independence by overlapping points.

Sampling Methods

Avian Surveys – Point counts were used to identify avifaunal communities at each point within habitat types. Point counts are used to monitor trends of bird populations over time, but are also useful in bird-habitat relationship studies (Dettmers et al. 1999) and less time consuming than line-transect surveys (Robel et al. 2000). Detection of birds varies among species (Mayfield 1981, Lynch 1995), seasons (Best 1981, Best and Peterson 1985), habitat types (Reynolds et al. 1980, Schiek 1997), and time of day (Fuller and Langslow 1984, Gates 1995).

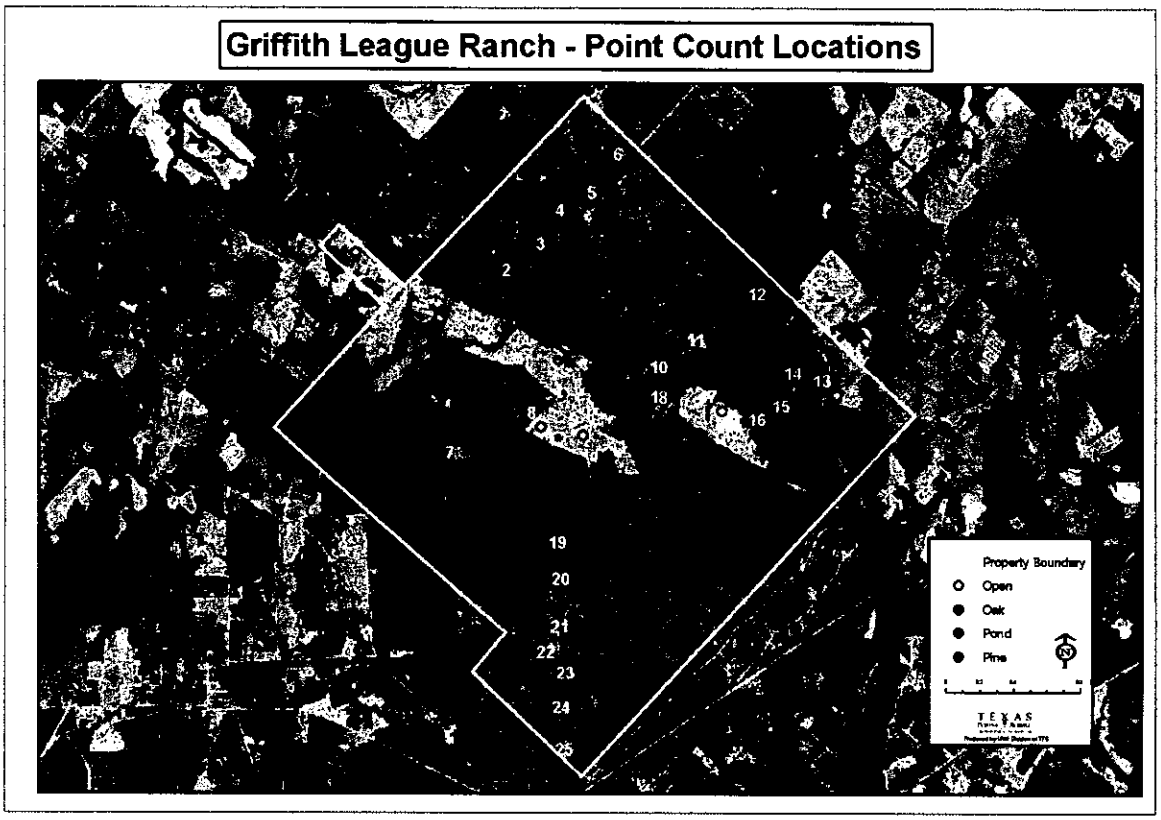

Figure 2. Point count locations in the four habitat types surveyed on Griffith League Ranch, Bastrop County, Texas.

To reduce potential bias from samples, each point count site was surveyed three times in each season, starting in the summer of 2002, with sampling occurring in four assigned habitat types. Calendar dates were used to estimate seasonal changes (Winter, Dec. 22 – Mar. 20; Spring, Mar. 21 – Jun. 20; Summer, Jun. 21 – Sept. 22; Fall Sept. 23 – Dec. 22). Dettmers et al. (1999) reported that sampling twice is sufficient with little to no improvement on the third sample. However, when studying bird-habitat associations, more counts may be necessary (Petit et al. 1995, Thompson and Schwalbach 1995).

Point counts lasted for ten minutes (Ralph et al. 1993, Brooks et al. 2001) and all birds counted were confined within a 100 m radius of the point count site center. All point counts were conducted from 6:00 am to 9:00 am (Lynch 1995) with the earliest count occurring no more than thirty minutes before sunrise.

In addition to point counts, mist netting results and incidental observations were recorded to supplement a list of birds recorded on GLR. These observations were not used in the statistical analysis of point count data. Mist netting was conducted under authority of Texas State University IACUC permit (N2E772) as well as a federal bird marking and salvage permit (22280-P).

Vegetation Sampling – To understand avian diversity-habitat associations, vegetation variables were measured at each point count site. Percent canopy cover of each tree species sampled, density of trees sampled, duff depth (decaying leaves and branches), vertical obscurity and herbaceous vegetation cover were measured to identify potential associations between avian diversity and habitats. Many of the variables measured at each point count site were discarded prior to analysis of the data due to an infrequency of measurements among sites. These variables included uncommon woody species present in small amounts at only a few locations. Woody vegetation variables were measured only one time assuming that no change among seasons within a year would occur while all other variables (herbaceous and structure composition) were measured each season.

Line-intercepts method was used to measure the canopy cover of each woody species. Three 100 m transects separated 120 degrees were stretched from center point to perimeter of the point count circle. Woody vegetation crossing the tape was recorded by

site. Line-intercept data was summed by species to yield a percent canopy cover for each species observed. In addition to line-intercepts, woody stems/ha were measured for each species using a 10 X 10 m quadrat. Three quadrat measurements were taken within each point count site to determine the density of each woody species. This technique also measured the number of standing dead trees/ha. Woody species/ha were calculated from the pooled quadrat samples measured at each point count site. Both line-intercept and woody stems/ha were measured only once per site.

Horizontal visual obscurity below 2.5 m was measured using a vegetation profile board (VPB) (Nudds 1977). Five VPB measures were taken at all points in each season. Canopy cover of woody structure was measured using a spherical densiometer (model A) (Lemmon 1957). Five readings using the densiometer were recorded per point in each season. Means by point count sites were calculated for VPB and canopy cover measures for each season.

Daubenmire frames (25 cm X 100 cm) were used in quantifying the herbaceous vegetation at each site (Daubenmire 1959). Ten frame samples were used at each point count site to determine herbaceous vegetation composition by season. Each plant was identified and then classified as grass, forb or sedge. Plants again were classified into groups of native and introduced herbaceous vegetation. Total percent cover by point for each season was then determined for each of the five classifications.

Duff measures were recorded within each Daubenmire frame sample. Ten duff measures were averaged and used to assess duff depth by point each season.

Canopy coverage, woody species density, VPB, herbaceous vegetation coverage and duff measures are only representative of point count sites and may not adequately describe the entire ranch on a broader scale.

Statistical Methods and Diversity Indices

Habitat assignments delineated through DOQQ's and ground truthing were verified using the percent canopy cover measures of the common woody species in a Principal Component Analysis using the covariance matrix (S-Plus). Principal component loadings were then graphed on a biplot to examine the predetermined habitat assignments. The biplot is a graphical representation of the first two component scores in relation to the data set with the arrows representing the loadings of the two components.

Avian diversity was calculated using the observations from three point counts performed at each point count site, within each season. This resulted in 100 diversity values for the 25 point count sites. Diversity for habitats within each season was then determined by taking the mean of diversity values for points in their respective habitat types. Avian diversity was measured using Brillouin's Index (H') (Exeter Software 2000). This index is more appropriate because the total number of species within the point count area is unknown (Krebs 1989). This conservative index is prone to underestimate the diversity however, with sample sizes often exceeding thirty observations the potential bias is reduced (Zar 1996).

A single factor Analysis of Variance (ANOVA) was performed on the diversity indices to evaluate possible differences among habitats within seasons. Contrasts (3, -1,-1,-1) were performed on the seasons showing differences among habitats to identify those differences (S-Plus).

A fully factorial ANOVA with three fixed factors was used to identify differences among seasons, habitats and half meter height increments for VBP measures. Line-Intercept data was summed by species to yield a percent canopy cover for each species observed.

To identify possible factors that influence avian diversity all possible subsets regression was used (Montgomery et al. 2001). Data transformation (ln(1+value)) and variable reduction procedures preceded the multiple linear regression analysis. Variables that contained more that five zero values were first excluded from the data set leaving only the variables with ample observations. Correlation matrices of all independent variables that may be related were then constructed to reduce variable multicollinearity in the model. The remaining variables were examined choosing the best three to five variables to use in the complete model. All possible subsets multiple linear regression (MLR) was then used to identify the best model from all possible combinations of the selected variables. Selection of the best model was based on a combination of lowest Mallow's Cp, highest r^2 value and lowest residual standard error (Montgomery et al. 2001). Mallow's Cp is a measure of bias within each subset model, assuming that the complete model has no bias. The p value represents the results from an ANOVA test on the selected model.

RESULTS

Habitat Identification

Habitat classification based on visual interpretation of DOQQ and ground truthing first produced five habitat types (grassland, oak, cedar, pine, and pond) with five point count sites in each habitat. Principal Component Analysis (PCA) was selected to verify

habitat classification of the 25 point count sites. Principal Component I explained 60.4% of the variation among variables and Principal component II explained 20.9% of the variation. Pine was highly correlated (0.81) with Principal Component I while both post oak and eastern red cedar were moderately correlated with Component II (0.61, 0.54 respectively).

From the principal component loadings (Table 1) and biplot (Fig. 3) five grassland habitats (point count sites 1, 8, 9, 13, 17), six oak/cedar habitats (2, 3, 4, 5, 6, 23), and nine pine habitats (10, 11, 12, 15, 16, 19, 20, 21, 24) were identified (Fig. 2), reducing the prior habitat designations to three habitat types. PCA designations for pond habitats were disregarded based on the previous designation of permanent water, which was not a variable considered in this analysis. Pond habitats, the fourth designated habitat, occurred in both grassland (18) and oak/cedar (22) habitats one time each and pine habitats (7, 14, 25) three times.

Table 1. Principal component loadings of yaupon, eastern red cedar, loblolly pine, blackjack oak and post oak canopy coverage of PC I and II for 25 point count sites on Griffith League Ranch, Bastrop Co. Texas.

	Principal Component I	Principal Component II
Yaupon	0.4623	-0.3413
Cedar	0.1794	0.5418
Pine	0.8060	-0.1879
Blackjack	0.0350	0.4213
Post	0.3213	0.6114

Avian Diversity

3,487 detections of 75 avian species were recorded from 300 point counts on the Griffith League Ranch. Mist netting and incidental observations increased the total number of species to 110. One hundred hours of mist netting accounted for five of the species not detected in point counts.

Fall had the highest number of observations with 1,156 and the fewest number of species at 39. Summer had the fewest observations with 749 and spring had the highest number of species recorded totaling 74 (Table 2). Fall also had the lowest mean diversity ($H' = 2.02$), while spring had the highest mean diversity ($H' = 2.43$). Pond habitats for combined seasons had the highest mean diversity ($H' = 2.59$) and open habitats had the lowest mean diversity ($H' = 1.64$). ANOVA and contrast results showed diversity by habitat within each season to be fairly consistent yielding similar results for winter, spring and summer. For each of these three seasons the ANOVA resulted in a significant difference ($p < 0.001$) and the contrast identified pond habitats, oak habitats and pine habitats as similar groups. Grassland habitats were dissimilar from all others in winter, spring and summer. Fall diversity values had no significant differences among habitats (Table 3).

Table 2. Mean number of observations, number of species and mean Brillouin's Index (H') of diversity for birds counted on the Griffith League Ranch from 300 point counts by season and habitat type.

	Habitat type	n	Mean Number of Observations	Number of Species	Mean H'
Winter					
	Grassland	5	17.4	17	1.43
	Pond	5	49.0	30	2.77
	Oak/Cedar	6	31.2	23	2.67
	Pine	9	26.3	25	2.41
Spring					
	Grassland	5	12.2	18	1.44
	Pond	5	41.2	30	2.76
	Oak/Cedar	6	37.2	22	2.64
	Pine	9	37.2	28	2.63
Summer	Grassland	5	17.0	16	1.63
	Pond	5	39.2	29	2.42
	Oak/Cedar	6	29.7	18	2.63
	Pine	9	32.2	24	2.46
Fall	Grassland	5	26.2	20	1.81
	Pond	5	40.8	19	2.48
	Oak/Cedar	6	81.5	16	1.84
	Pine	9	37.9	18	1.99

Table 3. ANOVA results comparing Brillouin's Index of diversity values for grassland, oak/cedar, pond and pine habitats within seasons.

Source	$df_{numerator}$	$df_{denominator}$	F	p
Winter	3	21	12.32	< 0.001
Spring	3	21	10.23	< 0.001
Summer	3	21	13.74	< 0.001
Fall	3	21	1.59	> 0.5

Vegetation Inventory

Thirty woody vegetation species were identified among the 25 point count sites. The dominant trees across the property were loblolly pine (40% canopy cover), post oak (25%), yaupon (18%), eastern red cedar (15%) and blackjack oak (10%). Measurements of woody stem density suggest yaupon to be the most dense (2,620 individuals/ha) followed by post oak (547/ha), eastern red cedar (446/ha) and loblolly pine (435/ha).

The herbaceous vegetation inventory identified 21 species of winter plants, 45 species of spring plants, 40 species of summer plants and 30 species of fall plants. Grasses were the dominant herbaceous plants comprising 60% to 80% of the overall herbaceous vegetation when viewed by season (Table 4). Dominant grasses in the open areas included Bahia grass and costal Bermuda grass, both introduced species. Within the forested areas, panic grasses (*Dichanthelium spp.*) were more common. Sedges were present and identified as a third group which covered less than 1% of the point count sites in each season. Pooled seasonal data revealed 70% of the identified herbaceous cover to be introduced and 30% native. Mean duff depth for pooled points and seasons was 44.5 mm.

Table 4. Percent herbaceous cover including forbs and grasses of 25 point count sites measured using Daubenmire frames for Griffith League Ranch, Bastrop Co. Texas.

	Winter	Spring	Summer	Fall
Forb Cover	4.7	4.5	5.0	2.5
Grass Cover	10.2	6.9	16.8	13.9
Total herb cover	14.9	11.7	22.4	16.5

VPB measures had a grand mean of 57.83% horizontal obscurity with means for GLR reported in Table 5. No interaction was found among the main effects ($p > 0.05$) and habitat was the only main effect to show a significant difference.

Table 5. Mean Vegetation Profile Board measures of horizontal obscurity for five half meter height increments by habitat type within seasons for 25 points on Griffith League Ranch, Bastrop Co, Texas.

	Habitat	VPB1 (2-2.5 m)	VPB2 (1.5-2 m)	VPB3 (1-1.5 m)	VPB4 (0.5-1m)	VPB5 (0-0.5m)
Winter						
	Grassland	20.0	20.0	20.0	20.8	41.6
	Oak/Cedar	60.7	58.0	60.0	53.3	58.0
	Pond	66.8	64.6	67.4	77.6	75.6
	Pine	72.0	74.7	74.7	73.8	72.9
Spring						
	Grassland	20.0	20.0	23.2	24.8	62.4
	Oak/Cedar	72.7	67.3	62.0	55.3	59.3
	Pond	65.6	65.6	64.8	64.8	71.2
	Pine	71.1	68.0	76.9	71.6	77.3
Summer						
	Grassland	23.2	22.4	22.4	29.6	68.0
	Oak/Cedar	82.7	69.3	69.3	56.0	66.0
	Pond	72.8	68.0	64.8	60.0	73.6
	Pine	72.9	66.7	74.7	62.2	70.2
Fall						
	Grassland	20.0	20.0	20.8	28.0	48.8
	Oak/Cedar	72.0	68.0	63.3	51.3	50.7
	Pond	69.8	69.0	66.0	63.4	73.0
	Pine	72.9	70.7	72.4	66.7	68.4

Factors Influencing Diversity

Factors affecting diversity within seasons varied greatly when compared across all habitats. Multiple linear regression (MLR) models each season showed diversity correlated with one to four variables. The MLR model for winter was represented by a Mallow's Cp of 4.579, $r^2 = 0.61$ and $p < 0.001$. For the winter model, positive correlations

were found between diversity and both yaupon and post oak canopy covers. An inverse correlation existed with duff depth.

H' = 2.651 + 0.203 (yaupon canopy cover)

+ 0.233 (post oak canopy cover)

– 0.402 (duff depth)

The MLR model for spring had a Mallow's Cp of 4.904, $r^2 = 0.646$ and $p < 0.001$. Like the winter model, the spring model shows a positive correlation between diversity and yaupon canopy cover. A positive correlation with pine canopy coverage is also present in the spring. The spring model also had an inverse correlation with horizontal obscurity measures from 0.0 – 0.5 m.

H' = 2.995 + 0.176 (yaupon canopy cover)

+ 0.274 (pine canopy cover)

– 1.273 (VPB 0.0 – 0.5 m height increment)

The model for summer was represented by a Mallow's Cp of 1.7, $r^2 = 0.699$ and $p < 0.001$. This model was the simplest with only one factor needed to describe diversity, post oak canopy cover with which a positive correlation was found.

H' = 1.629 + 0.258 (post oak canopy cover)

The MLR model for fall was represented by a Mallow's Cp of 8.244, $r^2 = 0.323$ and $p = 0.086$. Of the four seasons, fall had the lowest desirable selection criteria (Cp, r^2, p). This model shows a positive correlation between diversity and both yaupon and eastern red cedar canopy cover. Inverse correlations were shown with vertical obscurity measures at 0.0 – 0.5 m and post oak canopy cover.

H' = 3.538 + 0.512 (yaupon canopy cover)

+ 0.481 (eastern red cedar canopy cover)

– 1.340 (VPB 0.0 – 0.5 m height increment)

– 0.702 (post oak canopy)

I. DISCUSSION

Avian Diversity

I documented the presence of 110 species of birds representing fifteen orders on the Griffith League Ranch. Order Passeriformes, as expected, dominated the total number of species detected with 62 detections through point counts, incidental observation and mist netting. Other orders with modest representations include both Orders Ciconiiformes and Piciformes, each with eight species. Two members of the Order Ciconiiformes, White Ibis (*Eudocimus albus*) and White-faced Ibis (*Plegadis chihi*), were unexpected birds for GLR due to the lack of suitable habitat. The White-faced Ibis is currently listed as Threatened by the State of Texas.

Most species observed were expected, however, some common birds never were found or were present in low numbers. One such group was ducks. Freeman (1996) notes 23 possible species for the area; only five species were recorded on GLR. Also missing were Eastern Screech Owl (*Megascops asio*), Ruby-throated Hummingbird (*Archilochus colubris*), Dickcissel (*Spiza americana*), Red-winged Blackbird (*Ageliaus phoeniceus*) and Pine Siskin (*Carduelis pinus*). The only species of woodpecker (Piciformes) not observed that could potentially occur on GLR was the Red- headed Woodpecker (*Melanerpes erthrocephalus*). With an abundance of snags (75/ha) on GLR this diversity of woodpeckers was expected, since woodpeckers show a positive correlation with snag

abundance (Lohr et al. 2002, Showalter and Whitmore 2002). While the species richness for GLR is moderate at best, no introduced birds were observed.

Diversity values did not vary significantly among seasons but were different among habitat types within three of the four seasons. Because diversity values did not change across seasons, avian community variation may best explain the differences seen among seasons, habitat use, and factors influencing avian diversity.

Birds common during all seasons (permanent residents) through point count detections were Red-shouldered Hawk (*Buteo lineatus*), Red-bellied Woodpecker (*Melanerpes carolinus*), Tufted-titmouse (*Baeolophus bicolor*), Carolina Wren (*Thryothorus ludovicianus*), Pine Warbler (*Dendroica pinus*), and Northern Cardinal (*Cardinalis cardinalis*). Winter birds common to GLR were Ruby-crowned Kinglet (*Regulus calendula*), American Robin (*Turdus migratorius*), Cedar Waxwing (*Bombycilla cedrorum*), Yellow-rumped Warbler (*Dendroica* coronata), American Goldfinch (*Carduelis psaltria*) and Chipping Sparrow (*Spizella passerine*). Birds common in the summer season were Yellow-billed Cuckoo (*Coccyzus americanus*), White-eyed Vireo (*Vireo griseus*), Summer Tanager (*Piranga flava*) and Painted Bunting (*Passerina ciris*).

Winter and summer had a similar number of species, 49 and 51 respectively. Twenty-six species were present both seasons and can be classified as permanent residents. Spring observations totaled 74 species, the most of any season. Twenty-six species were seen only in the spring season, seventeen of these were migrants. Fall had the fewest number of species, 39, with thirteen percent of the avian community being sparrows of the Family Emberizidae. The low number of species in the fall may be a

reflection of the point count method as birds did not appear to vocalize as often or as late into the morning when compared to the spring or summer. Fall did have the highest number of observations but they commonly were visual detections of large groups of American Robins and Cedar Waxwings.

From the results of the ANOVA and contrasts comparing habitats within season, winter had similar diversity values for oak/cedar habitats, pond habitats and pine habitats. Grassland habitats were less diverse and dissimilar from the other habitat types. Eastern Phoebe (*Sayornis phoebe*), Vesper Sparrow (*Poecetes gramineus*) and Lincoln Sparrow (*Melospiza lincolnii*) were common in winter grassland habitats. Mallard (*Anas platyrhynchos*), Ring-necked Duck (*Aythya collaris*) and Purple Gallinule (*Porphyrio* martinica) were found in pond habitats. Oak/cedar habitats and Pine habitats were similar in avian composition.

Spring had similar results as winter for diversity among habitats. Mourning Dove (*Zenaida* macroura), Scissor-tailed Flycatcher (*Tyrannus forficatus*) and Northern Mockingbird (*Mimus polyglottos*) were commonly found in grassland habitats during the spring. All spring migrants, excluding the Common Snipe (*Gallinago gallinago*) and Northern Harrier (*Ciris cyaneus*), were found in the three other habitat types but appeared more common (13 of 17) in the pond habitats.

Summer also had habitat diversities similar to winter and spring. Summer breeders were commonly found in the three similar habitat types, but the late summer migrant, Blue-gray Gnatcatcher (*Polioptila caerulea*), was most common in the oak/cedar habitat. This may contribute to the slightly higher, though not significant, diversity value found in the oak/cedar habitat.

Fall had the fewest number of species and no difference in diversity among habitat types. The avian communities differed with sparrows common in the grasslands while both kinglets, American Robins and Carolina Chickadees (*Parus bicolor*) were common in the other habitats.

Vegetation Inventory

The USDA Soil Conservation Service, SCS (1979) lists an historical, stable plant community most like that of a post oak savannah habitat with a mix of perennial grasses and deciduous oaks. Little bluestem (*Schizachyrium scoparium*), indiangrass (Sorghastrum nutans), brownseed paspalum (*Paspalum plicatulum*) and switchgrass (*Panicum virgatum*) should be the dominant grass species and post oak, blackjack oak, elm (*Ulmus sp.*), hackberry (*Celtis sp.*) and yaupon are listed as the dominant trees. Interestingly, loblolly pine and eastern red cedar are not part of the historical plant community according to the SCS but pollen records from nearby bogs document their presence in the region for the last twenty-thousand years (Bryant 1977) and should be included in any description of the vegetation.

Current vegetation conditions for GLR yield different results with the absence of almost all dominant native grasses. In the northern corner of GLR little bluestem appears to be holding strong, however brownseed paspalum and switchgrass are rare and Indiangrass was never observed on GLR. Increased forest canopy, grazing history of the property, and introduction of non-native grasses are possible explanations for the reduction in these native grasses.

Dominant trees have increased from their historical proportion and contributed to the lack of herbaceous vegetation by shading and excess deposits of duff. Post oak and

blackjack oak were the dominant woody species and have continued to increase along with other tree species. Hackberry was not a common tree on the property, nor were elm species except in the riparian habitats. One tree species quickly becoming abundant in some parts of GLR is honey mesquite (*Prosopus glandulosa*). Chinaberry (*Melia azedarach*) also is becoming abundant in some areas, but this tree was not noted at any of the 25 point count sites. Fire suppression and grazing are the most likely causes for the change in woody vegetation composition (Smeins and Diamond 1984).

VPB measures suggest that grassland habitats consistently have lower percent horizontal obscurity in four or five height levels for the grassland habitat. All other habitats are similar. This shows possible correlations with avian diversity and should influence habitat use.

According to measures of herbaceous vegetation using Daubenmire frames 70% of the herbaceous plant cover is composed of introduced species. The majority of this is attributed to the cleared pastures of Bahia grass and costal Bermuda found across the property. Habitat restoration of the grasslands as well as the woodlands must be considered and are further discussed in the Management Implications section.

Factors Influencing Diversity

Avian communities may vary greatly among seasons at a given location (Rice et al. 1980). If those communities vary, factors that best describe avian communities also may vary from season to season. Rotenberry et al. (1979) found this variation of factors among seasons, but also found that some factors were common in multiple seasons. Factors of the habitat that best describe the association found between avian diversity and habitats for my study are similar to that of Rotenberry et al., except post oak showed both

positive and inverse correlations to bird diversity among seasons. Also as previously noted, there were similar results from ANOVA of VPB measures and avian diversity. Both tests showed no difference among seasons, but were different among habitats. Vertical structure has been reported in other studies as a factor relating to avian diversity (MacArthur and MacArthur 1961, MacArthur 1964, Recher 1969). These factors associated with avian diversity are simply the first step in understanding how diversity relates to habitat components. How and why birds use these components were not investigated in this study but possible explanations are offered.

Winter – American Robins and Cedar Waxwings, both known frugivores, were abundant in the winter. Fruit produced by yaupon in the fall that persists throughout much of the winter probably account for much of the positive

Chapter 6:
TAXONOMY OF AN ENDEMIC LOST PINES' SHREW

SYSTEMATICS OF LOCALLY ENDEMIC POPULATIONS OF SHORT-TAILED SHREWS, BLARINA (INSECTIVORA: SORICIDAE), IN BASTROP AND ARANSAS COUNTIES, TEXAS

SUSANNAH R. MORRIS AND MICHAEL R. J. FORSTNER

Two isolated populations of short-tailed shrews exist in Texas; one in the Lost Pines region including Bastrop County and one at Aransas National Wildlife Refuge on the Gulf Coast. Fossil evidence suggests that two species of short-tailed shrew once were widespread in central Texas; *Blarina hylophaga* now inhabits Nebraska, Kansas, Oklahoma and Montague County, Texas, and *Blarina carolinensis* inhabits the Southeastern United States through the eastern third of Texas. Molecular and morphological methods were used to determine the systematic status of the two disjunct Texas populations. In morphological analyses, nine cranial measurements were analyzed using principal components analysis (PCA), and it was determined that Texas specimens were intermediate between the smaller *B. carolinensis* and larger *B. hylophaga*. Multivariate analysis of variance (MANOVA) determined that the nine cranial measures could be used to differentiate among three groups: *B. carolinensis*, *B. hylophaga*, and Texas *Blarina* (Pillai's Trace= 1.23, $P< 0.001$). Phylogenetic analyses of the mitochondrial cytochrome *b* gene for three specimens from Aransas County and 20 specimens from Bastrop County revealed that the Texas groups are sister to *B. hylophaga* from Kansas and Nebraska. Based on the available evidence, subspecies status is warranted for the Texas short-tailed shrews. Because the Aransas population previously had been designated *Blarina hylophaga plumbea*, it is recommended that this subspecies now include the Bastrop County population as well. Biogeographic hypotheses are examined with respect to Texas as a Pleistocene refugium and these disjunct populations as relictual isolates. Areas in Texas inhabited by short-tailed shrews may harbor other locally endemic taxa; these areas should be examined closely as they may represent high value habitat for conservation efforts.

During the Late Pleistocene glacial maximum, approximately 18,000 years ago, glaciers covered northern North America (Pielou 1991). Fossil evidence suggests that the southern United States were much colder at that time and supported boreal flora and fauna (e.g. Larson et al. 1972). The southern states provided refugia from the extreme cold of periglacial environments, but as the climate warmed and the ice sheets in northern North America receded, many animals and plants expanded their ranges northward.

These former refugia still harbor relictual populations of species that have shifted their ranges northward. For example, *Neotoma floridana smalli* in the Florida Keys (Hayes and Harrison 1992) and *Microtus pinetorum* in Texas (Lundelius 1967) are considered to be relictual populations because they are small isolated populations that remain in the Pleistocene refugium that is no longer a primary part of the species' range. Both of these states also harbor unique populations of short-tailed shrews (*Blarina*); in Florida, the now extirpated subspecies *Blarina carolinensis shermani* is hypothesized to have been a relictual isolate of the northernmost species of short-tailed shrew, *B. brevicauda* (Genoways and Choate 1998). In Texas, one isolated population of short-tailed shrew in Aransas County has been classified as *B. hylophaga plumbea*, also belonging to a species whose distribution is further north.

Short-tailed shrews in the genus *Blarina* are endemic to eastern North America. Three species have been described using morphological and molecular characters: *Blarina brevicauda* is the northern short-tailed shrew, *Blarina hylophaga* is Elliot's short-tailed shrew, and *Blarina carolinensis* is the southern short-tailed shrew (Nowak 1999). *Blarina brevicauda* is the largest of the short-tailed shrews, and can be found from the Plains States through New England and southeastern Canada. *Blarina carolinensis* inhabits the southeastern United States. *Blarina hylophaga* is found in the south-central section of the country in Oklahoma, Nebraska, Missouri, and Arkansas and on the Oklahoma border in Montague County in Texas (Figure 1). These shrews are known for their voracious appetites and venomous saliva; *Blarina* is one of four genera of mammals known to be venomous, three of which are members of Order Insectivora (Vaughan et al. 2000). These small mammals may be found in diverse habitats, including grasslands,

bottomland forest, and upland woods; their diet includes invertebrates, small vertebrates, and some plant matter (Schmidly 1983).

Systematics and taxonomy of *Blarina* has been primarily based on morphology. Initially, only one species was recognized in the genus, *Blarina brevicauda*, with the subspecies *B. b. carolinensis* and *B. b. hylophaga* elevated to specific status in the early 1970s (Genoways and Choate 1972; Handley 1971) and 1980s (George et al. 1981), respectively. Karyotypes (George et al. 1982) and molecular analyses have redefined the systematics of this taxon (Brant and Ortí 2002, 2003). However, questions remain regarding the isolated populations of *Blarina* found in central and coastal Texas.

In 1941, two short-tailed shrews were discovered at the Aransas National Wildlife Refuge, Aransas County, Texas. The shrews were 400 kilometers southwest of the known range of any congeners, and morphologically unique enough to warrant recognition as a new subspecies, *Blarina hylophaga plumbea* (Davis 1941). A later study using seven shrews collected at Aransas NWR reported that these individuals were morphologically distinct from *Blarina carolinensis* in East Texas, but no comparison was made to *B. hylophaga* (Schmidly and Brown 1979). Later it was found that shrews from Aransas NWR were more similar to *B. hylophaga* from Oklahoma than *B. carolinensis* from East Texas (George et al. 1981). In 1994, *B. hylophaga plumbea* was evaluated as a candidate for listing as a federally endangered subspecies, but it subsequently was rejected due in part to a lack of taxonomic clarity (Beattie 1994).

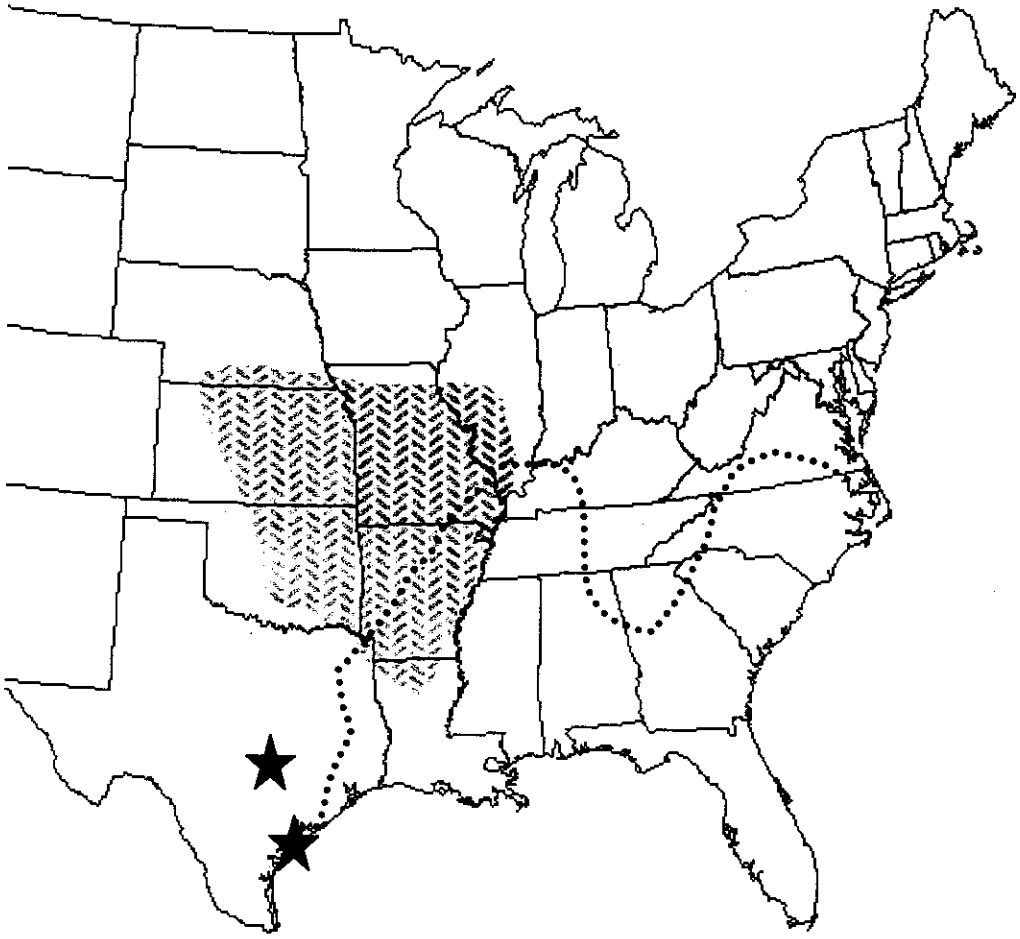

FIGURE 1. Distribution of three species of short-tailed shrew, *Blarina*, in the eastern United States (modified from George et al. 1982). Stars represent two isolated populations of *Blarina* in Texas. *Blarina brevicauda* is in grey, *B. hylophaga* is patterned, and the range of *B. carolinensis* is below the dotted line.

In 1989, another isolated population of *Blarina* was discovered at Bastrop State Park, Bastrop County, Texas (Dixon et al. 1989; Dixon et al. 1990). Of four specimens collected, three were identified as *B. hylophaga* and one as *B. carolinensis* based on a suite of cranial measurements (Baumgardner et al. 1992). This population exists within the Lost Pines region, approximately 130 kilometers west of the range of *B. carolinensis*. This region is characterized by stands of loblolly pine (*Pinus taeda*) on sandy soils, and is the westernmost outpost of these pines in Texas. Although Bastrop County is located in the Blackland Prairie region of Texas (Diamond et al. 1987), it bears more resemblance to the Piney Woods of East Texas.

The two Texas populations are separated by large geographic distances. There are no museum records that would indicate any continuity between them, nor connecting either to other populations of *Blarina*. It is difficult to assess the factors that limit species ranges, and short-tailed shrews present a particularly challenging case because they are found in a variety of habitats. Although they are habitat generalists, *Blarina* do prefer areas that are mesic but not saturated (Genoways and Choate 1998). These shrews are semi-fossorial and inhabit areas with pliable soil or ground cover, allowing construction of burrows and runways (Genoways and Choate 1998). Bastrop and Aransas counties both have sandy soils and considerable ground cover in unburned areas. While mammal-trapping efforts are largely undocumented, major universities in Texas consistently engage in widespread trapping that would likely have uncovered evidence of a continuous, contemporary Texas *Blarina* population.

The Bastrop and Aransas populations of *Blarina* are limited to small areas of a once extensive range in Texas. Based on fossil evidence, short-tailed shrews were

widespread in Texas for several thousand years. In Travis County, central Texas, the most recent record is a specimen dated at approximately 1,015 years before present and tentatively identified as *B. carolinensis* (Table 1 and Figure 2) (Jones et al. 1984; Lundelius 1967). Lundelius (1986) also noted a possible *B. carolinensis* from Mac's Cave in Travis County dated at 600 years before present. The changes in habitat associated with post-Pleistocene warming may have caused the short-tailed shrews to shift their ranges to the east and north to stay within more mesic habitats (Graham 1987; Lundelius 1967). The two disjunct populations that remain in Texas may be relictual isolates, although it also has been hypothesized that shrews may have arrived at Aransas via dispersal rather than being Pleistocene inhabitants of the area (Schmidly and Brown 1979).

Based on the fossil evidence (Table 1), it is possible that the isolation of these populations occurred between 1,000 and 5,000 years ago. Species-level identification based on morphological characters is not necessarily straightforward. These two species are remarkably similar, and in fact clinal variation in size may result in overlap between small *B. hylophaga* from the southern portion of their range with large *B. carolinensis* from their northern range; where the ranges of the two species overlap, however, they are morphologically distinct (George et al. 1981). In fact, Schmidly and Brown (1979) stated that shrews from what is now delineated as *B. carolinensis* were "a southward extension of the cline {*B. hylophaga*}," and Stangl and Carr (1997) stated that range limits established by previous studies enabled "workers in Texas and Oklahoma to assign their specimens of *Blarina* to one species or the other based solely on geographic grounds." Thus, identification of *Blarina* within their respective ranges is possible, but isolated

populations present a problem in that they may overlap morphologically with both *B. hylophaga* and *B. carolinensis*. Additionally, due to clinal variation in size, disjunct populations may be smaller or larger than the typical population. Because morphological characters used in previous studies of *Blarina* are size-related (e.g., Choate 1972), this overlap in size is a confounding factor when identifying isolated populations of either taxon.

TABLE 1. Localities from which fossils of *Blarina hylophaga* and *B. carolinensis* have been reported in Texas; species identification based on 26 dental and dentary measurements in comparison to modern specimens (Jones *et al.* 1984). All dates are noted in years before present.

Location	County	Date or Period	Species
Barton Springs	Travis	1,015 +/- 150	*B. carolinensis*
Cave Without a Name	Kendall	10,900 +/- 190	*B. carolinensis*
Felton Cave	Sutton	7,770 +/- 130	*B. carolinensis*
Friesenhahn	Bexar	Wisconsinan	*B. carolinensis*
Hall's Cave	Kerr	Holocene	*B. carolinensis*
Klein Cave	Kerr	7,683 +/- 643	*B. carolinensis* and *B. hylophaga*
Longhorn Cavern	Burnet	Late Wisconsinan (~10,500)	*B. carolinensis*
Schulze Cave	Edwards	9,680 +/- 700	*B. hylophaga*
Miller's Cave	Llano	3,008 +/- 410 and 7,200 +/-300	*B. carolinensis* and *B. hylophaga*

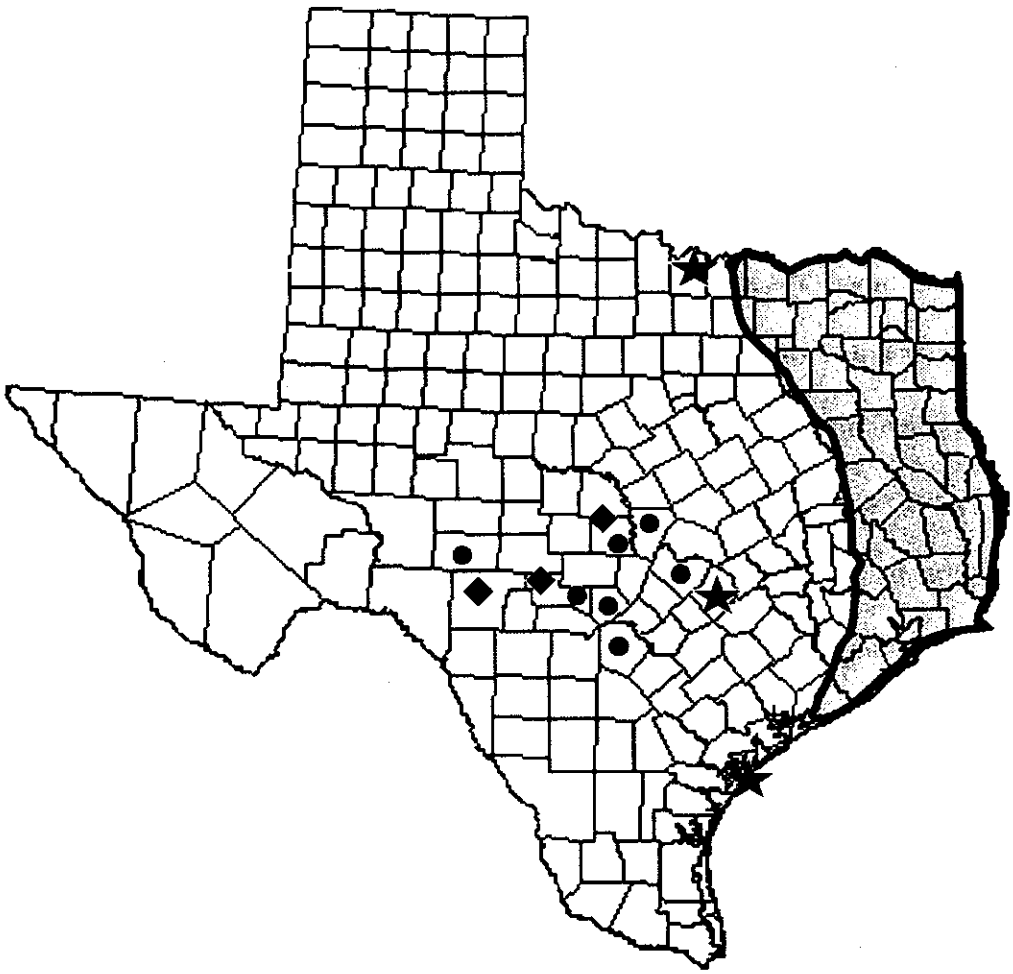

FIGURE 2. Distribution of fossil and extant short-tailed shrews (*Blarina*) in Texas. Shaded region in East Texas represents current range of *B. carolinensis*; stars represent extant populations of *B. hylophaga*. Solid circles represent fossil *B. carolinensis* and diamonds represent fossil *B. hylophaga*.

A technique that offered a potential solution to the shrew identification problem was karyotyping. *Blarina* exhibit considerable polymorphism with respect to chromosome fusions, and evidence for Robertsonian fans has been noted in *B. carolinensis* (Qumsiyeh et al. 1999; Qumsiyeh et al. 1997). Fundamental numbers (FN) for each species are distinct, with the largest variation within *B. carolinensis* (FN= 44, 45, or 52); *B. hylophaga* has a fundamental number of 60 or 61 for the one locality sampled (George et al. 1982). Another technique useful in identifying morphologically indistinct organisms is DNA sequencing, which is becoming more readily available. Whereas karyotyping requires tissue from live specimens, DNA sequencing can be performed on stored tissue or even museum skins. DNA also provides more information for phylogenetic analyses as well as population genetics and individual identification, obviating the need for karyotyping for identification purposes. Studies contrasting chromosomal and mitochondrial DNA evolution in the *Sorex araneus* group, which exhibits considerable intraspecific variation in chromosome arrangements, have shown that homoplasy in karyologic data can obscure evolutionary relationships (Taberlet et al. 1994).

Taxonomic revisions of the genus *Blarina* and particularly *B. hylophaga*, which was recognized as a species only recently (George et al. 1981), have caused confusion in the literature over the identity of Texas short-tailed shrews. Studies performed to date on short-tailed shrews in Bastrop and Aransas counties, Texas, have utilized cranial and external morphological characters that are ambiguous due to size overlap between *B. hylophaga* and *B. carolinensis*. The study by George et al. (1981) reaffirmed the

taxonomic status of the Aransas subspecies *Blarina hylophaga plumbea*, but the tentative identification of both *B. hylophaga* and *B. carolinensis* in Bastrop County by Baumgardner et al. (1992) cast doubt on the classification of Texas *Blarina*. It seems that in this case, additional characters are necessary to support or refute morphological data. Brant and Ortí (2002) used mitochondrial DNA sequences to resolve evolutionary relationships among the three species of *Blarina*. The sequences from their study were available on the GenBank database, providing baseline *Blarina* sequences for comparison of novel Texas *Blarina* sequences.

Phylogenetic analyses of mitochondrial DNA and quantitative morphological techniques were chosen to resolve this taxonomic problem. Both Davis (1941) and Dixon et al. (1989) recognized that the respective isolated populations differed from *B. carolinensis* in East Texas. However, the taxonomic status remains unresolved for these populations. With the availability of museum specimens for morphological analyses and the recent publication of a molecular phylogeny for *Blarina*, the data were available to help resolve questions regarding the two disjunct Texas populations.

The objective of this study was to resolve the systematic status of the two isolated populations of short-tailed shrew in Texas using morphological and molecular techniques. Additionally, biogeographical hypotheses were explored in an attempt to explain the current distribution of short-tailed shrews in Texas.

MATERIALS AND METHODS

SPECIMEN COLLECTION

Specimens were handled according to directives of TSU IACUC permit #KUMJTX_02.

Trapping for the Bastrop County population occurred in conjunction with concurrent herpetofaunal survey work on the Griffith League Ranch, which comprises approximately 5,000 acres, under Texas Parks and Wildlife permit SPR-0102-191 and U.S. Fish and Wildlife Service permit TE039544-1. Over 100 pitfall traps were placed along 23 drift fence arrays in grasslands, pine-oak woodlands, and oak-juniper woodlands and checked daily from 2001 through 2003. Thirteen Y-shaped arrays were created using three lines of drift fence 50 feet in length radiating from a central point, with 5-gallon buckets buried at the center and each terminus. Five linear arrays were set in grassland with buckets every 100 feet; two lines were 400 feet and three lines were 500 feet in length. One linear array in a pine forest was 100 feet in length with two terminal buckets and two internal buckets spaced 25 feet apart. Four linear arrays were placed in a grassland in a rectangular formation, two 100 feet in length with two terminal buckets and one central bucket, and two 150 feet in length with four evenly spaced buckets. Six arrays were added in the spring of 2003 that lacked drift fence; each consisted of four buckets arranged linearly along a 150 foot transect. These traps were checked daily from June 26 through July 31, 2003. Trapping in Aransas County took place at the Aransas National Wildlife Refuge (ANWR), comprising approximately 54,000 acres, under permit number 03-013. Twelve pitfall traps were placed along three 100-foot drift fence arrays located in or near oak mottes. Four 50-meter lines, each with two terminal pitfall

traps, in use for an ANWR herpetofauna survey were monitored for shrews ten days of every month during 2003.

Voucher specimens were taken as necessary, prepared by Richard W. Manning and deposited at the Texas Tech University collection. Skeletal muscle, organs, and/or blood were catalogued and stored at –80° C.

Specimens collected and prepared by previous researchers also were utilized in this analysis. Jim Yantis collected several specimens in Houston and Anderson counties in east Texas. Texas Cooperative Wildlife Collection (TCWC) housed several specimens and allowed us access to them (Appendix I), including the removal of skin samples from museum study skins. Samples from museum skins were taken with utmost care to preserve the integrity of the skin and to prevent contamination among specimens. Skin samples were approximately 5 mm^2 taken from the area around the incision made during the preparation of the skin. Scissors were flame-sterilized and gloves were changed before each skin clip.

Morphological Analyses

External measurements were taken when the condition of the specimen allowed; these included total length, tail length, hind foot length, and ear length. Cranial measurements, as established by Choate (1972) included, as seen in Figure 3:

- Occipitopremaxillary length
- Length P4-M3
- Cranial breadth
- Breadth of zygomatic plate
- Maxillary breadth
- Interorbital breadth

- Length of mandible
- Height of mandible
- Articular breadth

Principal Components Analysis (PCA) (Quinn and Keough 2002) using a covariance matrix was used to determine whether statistical differences exist between these populations of *Blarina* and specimens from other parts of the United States. Multivariate analysis of variance (MANOVA) (Quinn and Keough 2002) was performed using species as the independent variable and the nine cranial characters as response variables to determine if there were differences between species. After performing MANOVA, individual analysis of variance (ANOVA) tests (Quinn and Keough 2002) were performed for each cranial measure using the same *a priori* groups as in the MANOVA. Because of the increase in Type I error associated with multiple tests, the Bonferroni procedure was used to adjust the significance level (Quinn and Keough 2002). The resulting alpha level, adjusted for nine tests, was 0.0055.

S-Plus 6.1™ software was used for all morphological analyses.

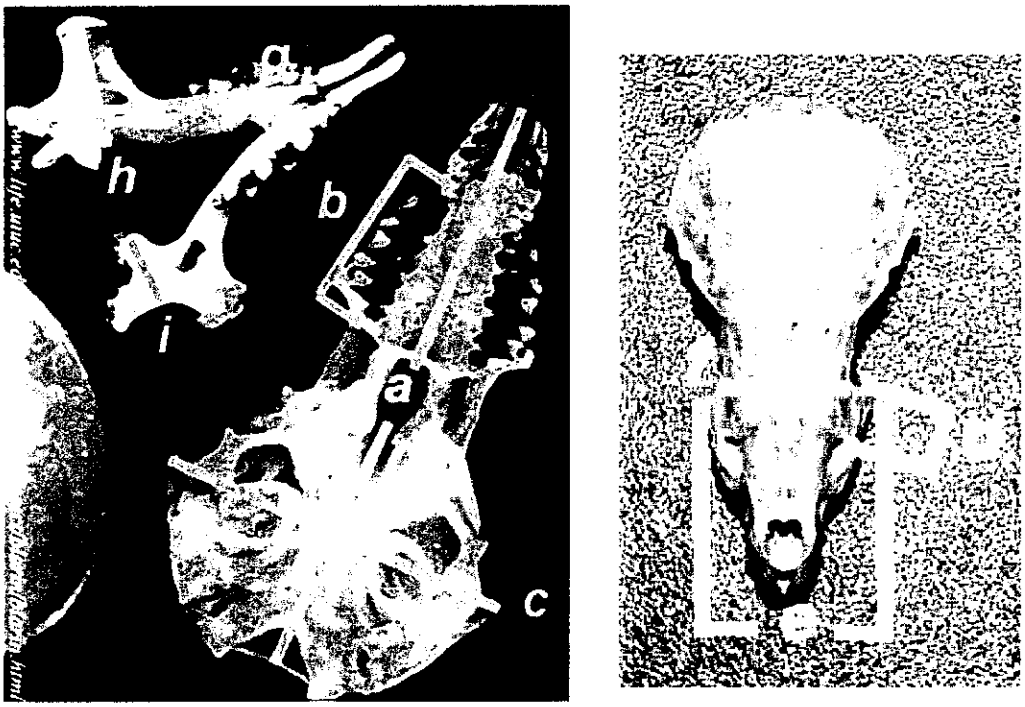

FIGURE 3. NINE CRANIAL MEASUREMENTS USED IN THIS STUDY, AS IN CHOATE (1972). OCCIPITO-PREMAXILLARY LENGTH (A), P4-M3 (B), CRANIAL BREADTH (C), BREADTH OF ZYGOMATIC PLATE (D), MAXILLARY BREADTH (E), INTERORBITAL BREADTH (F), LENGTH OF MANDIBLE (G), HEIGHT OF MANDIBLE (H), AND ARTICULAR BREADTH (I).

DNA SEQUENCING

Cytochrome *b* from the mitochondrial genome was used in all analyses because baseline data for large-scale sampling of United States *Blarina* exclusive of Texas were readily available from GenBank. Although 500 bases from the 16S gene also were available for *Blarina*, that gene was excluded from these analyses because of perceived ambiguities regarding its alignment and phylogenetic analysis (Springer and Douzery 1996). The cytochrome *b* gene encodes a protein which spans the inner matrix, inner membrane, and outer intermembrane area in the mitochondrion, and acts as a component of complex III of the mitochondrial oxidative phosphorylation system (Griffiths 1997; Irwin et al. 1991). This gene has been widely utilized in systematic studies (e.g. Bradley and Baker 2001; Johns and Avise 1998; Voelker and Edwards 1998; Yoder et al. 1996). Although cytochrome *b* is useful in assessing phylogenies, potential pitfalls include differential evolutionary pressures based on location in the membrane (Griffiths 1997), insertions of the gene into the nuclear genome (Mirol et al. 2000; Mundy et al. 2000), and rate heterogeneity among taxa (Spradling et al. 2001).

The Qiagen™ DNeasy kit was used to extract genomic DNA from skeletal muscle samples. The polymerase chain reaction (PCR) was used to amplify fragments of the mitochondrial genome. Amplification of the cytochrome *b* gene was in a 50 μl reaction using 10 μl *Taq* buffer (0.3 M TRIS, 0.0175 M $MgCl_2$, and 0.075 M $(NH_4)_2SO_4$, pH 8.5), 0.5 μl DMSO, 0.5 μl dNTP's (2.5 mM dATP, dCTP, dGTP, and dTTP), 0.5 μl (10 mM) of each primer, 0.25 μl *Taq* polymerase, and 0.5 μl tDNA. The cytochrome *b* gene was sequenced for all samples. The following primers were used in PCR and sequencing: cytochrome *b*, L14724: 5'-CGAAGCTTGATAGAAAAACCATCGTTG-3' and H15915: 5'-AACTGCAGTCATCTCCGGTTTACAAGAC-3' (Irwin et al. 1991); plus an internal

sequencing primer, cytbR1: 5'-GCTTCGTTGTTTGGAGGT-3' (Brant and Ortí 2002), and novel internal sequencing primers shrewCBF1: 5'-YTATTTTCTCCAGACTTACTAGGAGACCC-3' (where Y is C or T), and shrewCBR3: 5'-CCTCATGGAAGGACATACCCTATAAAGGCAGT-3'. The GeneAmp ® PCR System 9700 performed denaturation at 94° C for 1 min followed by 35 cycles of 94° C (for 1 min), 50° C (for 30 s), and 72° C (for 1 min), and then a final extension of 72° C for 5 minutes. Results of PCR were visualized on a 1% agarose gel. The Marligen™ Rapid PCR Purification System was used for PCR cleanup. Clean PCR product was cycle sequenced in a 9 μl reaction using 0.5 μl primer, 3 μl Big Dye version 3.0, 2- 4 μl clean PCR product (depending on concentration) and 1.5- 3.5 μl water (depending on concentration of PCR product). GeneAmp® PCR System 9700 was used to perform 25 cycles of 96° C for 30 s, 50° C for 1 min, and 60° C for 4 min. Cycle sequence products were cleaned using 700 μl Sephadex solution (0.0625 g/ml) in a Centri-sep column (Princeton Separations). The ABI Prism 377 XL DNA sequencer assayed clean cycle sequence products using a 6% polyacrylamide gel.

Precautions were taken to avoid contamination when extracting, amplifying, and sequencing DNA from TCWC museum skins. Protocols for ancient DNA extraction included bleaching all laboratory bench surfaces, flame sterilizing metal utensils, UV sterilizing consumables such as tubes and micropipettor tips as well as pipettors, and including a negative control reaction for the extraction and subsequent PCR reactions. Extraction was performed using standard phenol-chloroform protocols rather than a kit. Tissue was incubated for 48 hours at 55 °C after adding 500 μl STE buffer, 25 μl 20%SDS, and 25 μl of 20 mg/ml proteinase K. After two purifications in 500 μl of PCI

solution (25 phenol: 24 chloroform: 1 isoamyl alcohol) and two purifications in 500 μl chloroform, tDNA was precipitated in 0.1 volume of 2M NaCl and 2.5 volumes of 99% ethanol. The tDNA pellet was dried 24 hours later and re-suspended in ddH_2O. Amplification was performed using primers cytbL as the forward primer and shrewCBR3 as the reverse primer for a total of 395 bases. Cycle sequencing was performed as previously described with ancient DNA protocols enforced.

Sequences were aligned using Sequencher™ 4.1. Sequences were compared with 38 previously aligned sequences obtained from GenBank, as published in Brant and Ortí (2002).

PHYLOGENETIC ANALYSES

Thirty-four *Blarina*, two *Cryptotis parva* and two *Sorex cinereus* cytochrome *b* sequences from GenBank (accession numbers AF395449-86) were aligned with 23 Bastrop/Aransas sequences and exported as a NEXUS file to PAUP* 4.0b10 (Swofford 1999). *Sorex cinereus* and *Cryptotis parva* were designated as paraphyletic outgroups. Individuals were coded according to geographic location and GenBank accession number (Figure 4, Table 2). Morphological characters were excluded from phylogenetic analyses because of continuous variation in cranial measurements that could not be coded for analysis in an objective manner; see Poe and Wiens (2000) and Zelditch et al. (2000) for a full review.

Saturation was estimated by graphing the uncorrected-p distance on the x-axis as a measure of time since divergence against absolute number of transitions and

TABLE 2. Collection localities and abbreviations for *Blarina* specimens from Brant and Ortí (2002). Map codes correspond to locations in Figure 4. Individual abbreviations are noted as used in all phylogenetic analyses. Two-letter abbreviations used for all U.S. states. B.bre= *Blarina brevicauda*, B.car= *Blarina carolinensis*, B.hyl= *Blarina hylophaga*.

Species	County and State	Map Code	Individual Number
B. BREVICAUDA	Lancaster, NB	8	64.B.bre.NB
	Dixon, NB	9	65.B.bre.NB
	Valley, NB	1	61.B.bre.NB
	Wooster, OH	10	69.B.bre.OH
	Wooster, OH	10	72.B.bre.OH
	Manitoba, Canada	12	62.B.bre.Manitoba
	Manitoba, Canada	12	63.B.bre.Manitoba
	Allamakee, IA	11	66.B.bre.IA
	Trigg, KY	14	67.B.bre.KY
	Trigg, KY	14	68.B.bre.KY
	Grafton, NH	13	70.B.bre.NH
	Grafton, NH	13	71.B.bre.NH
	James City, VA	15	73.B.bre.VA
	James City, VA	15	74.B.bre.VA
B. HYLOPHAGA	Nuckolls, NB	3	75.B.hyl.NB
	Nuckolls, NB	3	80.B.hyl.NB
	Lincoln, NB	2	81.B.hyl.NB
	Lincoln, NB	2	78.B.hyl.NB
	Richardson, NB	4	79.B.hyl.NB
	Richardson, NB	4	76.B.hyl.NB
	McPherson, KS	5	77.B.hyl.KS
	Montgomery, KS	6	82.B.hyl.KS
B. CAROLINENSIS	GA	22	50.B.car.GA
	GA	22	49.B.car.GA
	Vernon, LA	18	57.B.car.LA
	Webster, LA	17	55.B.car.LA
	Polk, AR	19	59.B.car.AR
	Polk, AR	19	56.B.car.AR
	Highlands, FL	20	53.B.car.FL
	Highlands, FL	20	54.B.car.FL
	Lancaster, VA	23	51.B.car.VA
	Lancaster, VA	23	52.B.car.VA
	Jackson, IL	21	60.B.car.IL
	Jackson, IL	21	58.B.car.IL

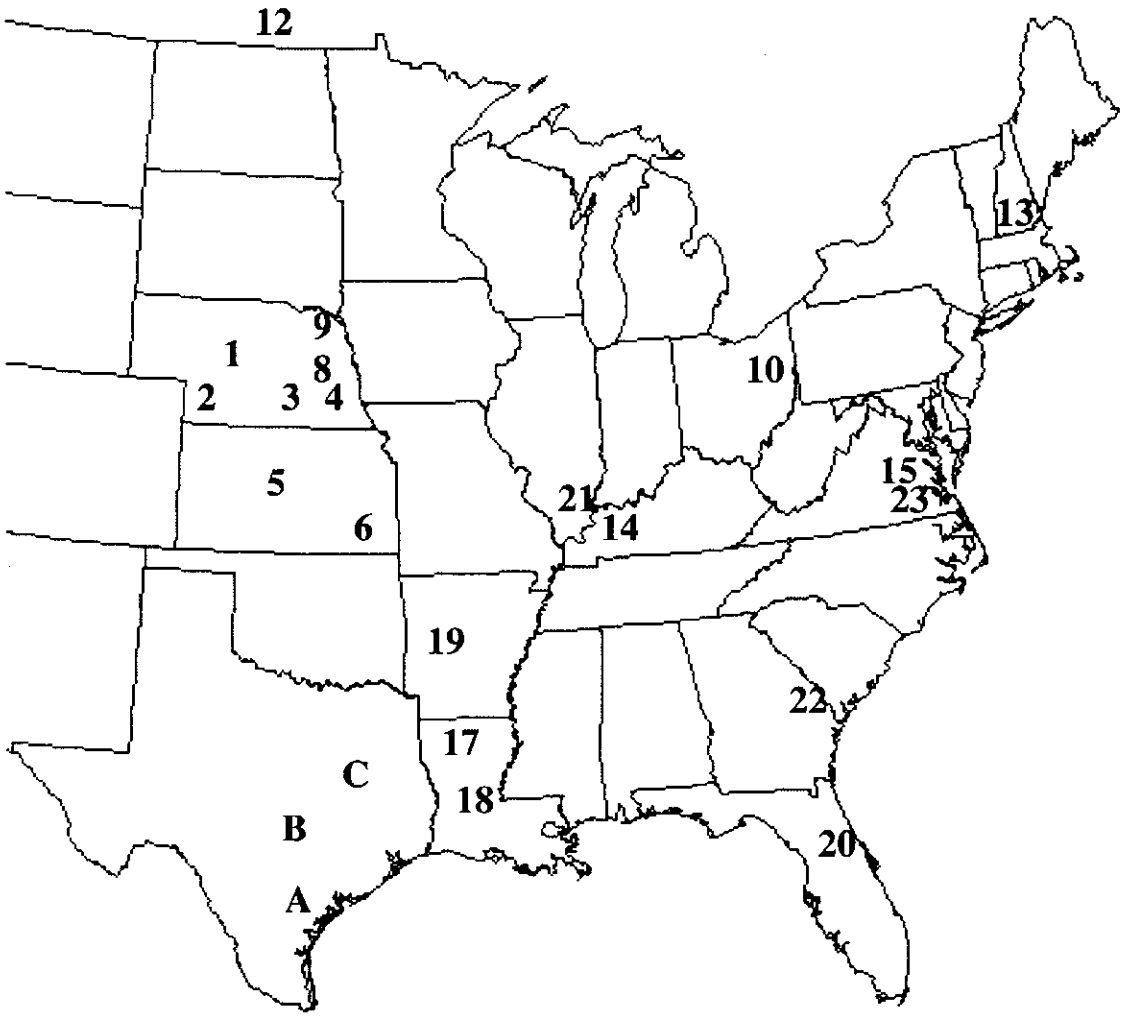

FIGURE 4. Sampling locations for this study and Brant and Ortí (2002). Numbers represent trapping locations by county as in Table 2. In Texas, A is Aransas County, B is Bastrop County, and C approximates Houston and Anderson counties where trapping occurred for this study (Appendix I).

transversions separately on the y-axis (Griffiths 1997). Saturation curves were created for the entire gene as well as for each codon position within the intermembrane, matrix, and transmembrane regions as in Griffiths (1997). Additionally, a partition homogeneity test with 100 repetitions was conducted to ensure that the three regions were suitable for uniform analyses. Neighbor joining (NJ) analysis was performed using HKY distances and bootstrapped using 1,000 pseudoreplicates. Maximum parsimony (MP) analysis was performed using a heuristic search with tree-bisection reconnection (TBR) branch swapping; gaps were treated as missing. The dataset was bootstrapped using 1,000 pseudoreplicates each with 5 random addition replicates. Weighted parsimony was performed similarly, but with transversions (TV) and transitions (TI) weighted based on estimation of the TI/TV ratio using maximum likelihood and codon positions weighted based on number of changes at each position. A bootstrap similar to that of MP analysis was performed on the weighted dataset.

Modeltest 3.06 (Posada and Crandall 1998) was used to determine the nucleotide substitution model that best fit the data. This model with the parameters given by Modeltest was used in a maximum likelihood (ML) analysis using a full heuristic search. Bayesian analyses were performed using MrBayes (v. 3.0, Huelsenbeck and Ronquist 2001) with 1,000,000 generations of four Monte Carlo Markov chains (MCMC) sampled every 1,000 generations. Only one outgroup is allowed by the software, so *Sorex cinereus* AF395485 was specified as the outgroup; however, *Cryptotis parva* was retained in the analysis.

The "Evaluate Random Trees" option in PAUP* (Swofford 1999) was used to create a frequency distribution of random trees and generate the g_1 statistic. This statistic is a measure of skewness that indicates the amount of phylogenetic signal in a dataset (Huelsenbeck 1991). A random dataset would have a normal distribution of random trees and a positive g_1 statistic, whereas a dataset with signal will produce a left-skewed distribution of random trees with a negative value for g_1 (Huelsenbeck 1991).

Each novel sequence and the compiled data set was examined thoroughly, including translation to amino acid sequence and substitution pattern, to ensure the integrity of subsequent analyses. Likewise such scrutiny ensured that any erroneous sequence contaminant whether exogenous (PCR contamination) or endogenous (nuclear introns) was exposed prior to the final analyses.

RESULTS

SPECIMEN COLLECTION

Three fresh specimens were collected from traps placed on the Aransas Wildlife Refuge (Appendix I). Twenty-seven specimens were collected on Griffith League Ranch in Bastrop County, and one (MF8057) was collected at Schulz Ranch in southern Bastrop County (Appendix I). Additional morphological and molecular data were obtained from preserved specimens at the Texas Cooperative Wildlife Collection (Appendix I).

Morphological Analyses

Most cranial measures were linearly correlated, with Pearson's correlation coefficient (r) ranging from 0.29 to 0.84 (Table 3). PCA (n= 52) recovered nine principal

components, the first of which (PC1) explained 77.7% of the variance and had an eigenvalue of 0.97. The second component (PC2) was retained for use in the ordinal plot (Figure 5) but only explained 7.9% of the variation and had an eigenvalue of 0.31. All cranial characters had positive loadings on PC1, the largest being occipito-premaxillary length which had a loading of 0.708. Remaining principal components were not included in further analyses; all had eigenvalues less than 0.25 and explained less than 5% of the variation. On PC1, *B. carolinensis* and *B. hylophaga* were distinguishable with *B. carolinensis* having lower scores on that component. Specimens from Texas were intermediate between the two species on PC1, although there was overlap between two Bastrop specimens and *B. carolinensis*.

MANOVA was used to differentiate among three groups: TCWC *B. carolinensis* and TCWC 51797 (previously identified as *B. carolinensis*), *B. hylophaga* from Kansas, and Texas *Blarina*; the Pillai's trace test statistic was 1.22837 ($P<0.001$). In univariate tests of the nine cranial measures, two were non-significant: zygomatic plate breadth ($P= 0.792$) and interorbital breadth ($P= 0.009$).

TABLE 3. Pearson's correlation (*r*) between cranial measurements for 48 specimens of *Blarina*. Cranial measurements abbreviated as follows: OPM occipito-premaxillary length, P4-M3 length from anterior edge of P4 to posterior edge of M3, CB cranial breadth, ZPB breadth of zygomatic plate, MB maxillary breadth, IOB interorbital breadth, LM length of mandible, HM height of mandible, AB articular breadth.

	OPM	P4-M3	CB	ZPB	MB	IOB	LM	HM	AB
OPM	-	0.670	0.811	0.493	0.821	0.736	0.781	0.820	0.735
P4-M3		-	0.580	0.275	0.637	0.616	0.745	0.646	0.498
CB			-	0.353	0.809	0.699	0.703	0.710	0.635
ZPB				-	0.290	0.526	0.511	0.354	0.380
MB					-	0.740	0.696	0.840	0.807
IOB						-	0.741	0.706	0.646
LM							-	0.725	0.589
HM								-	0.709
AB									-

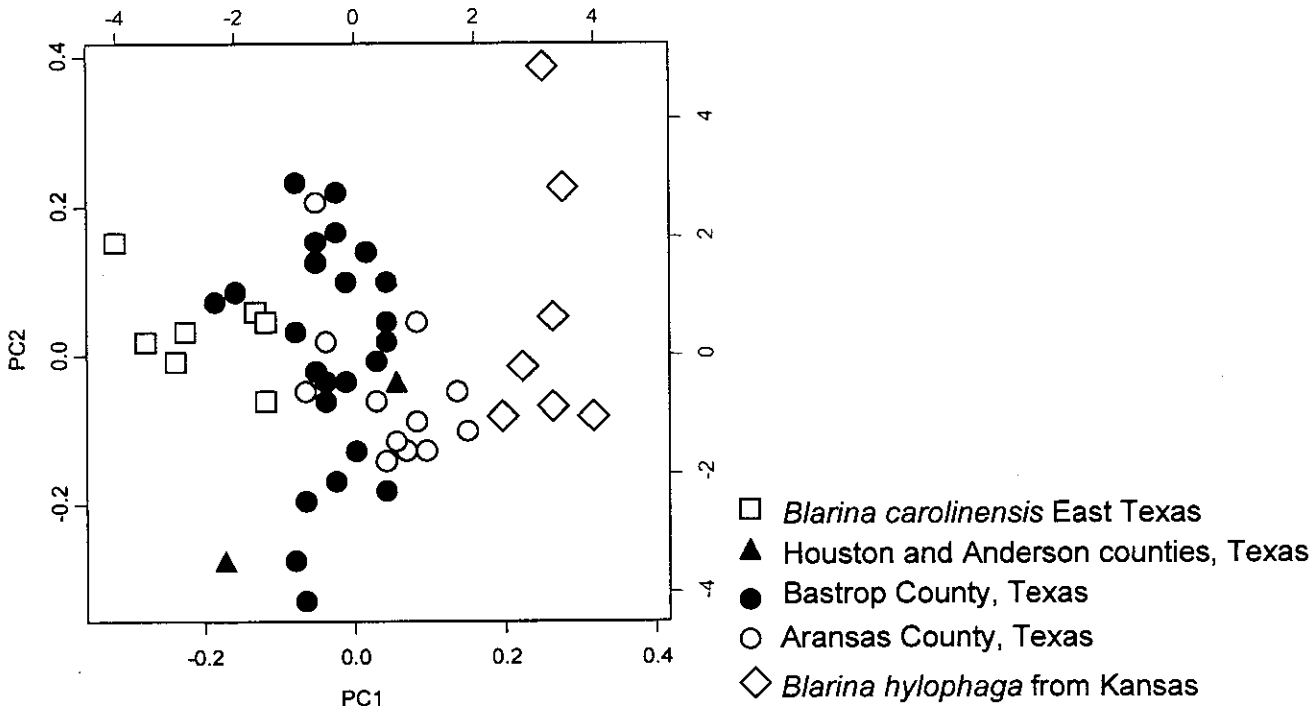

FIGURE 5. Ordinal plot of the first two principal components in the analysis of nine cranial measurements for *Blarina* (n= 52). All nine cranial characters, as used in Choate (1972), were positively correlated with PC1. Symbols differ for geographic regions as indicated in key.

DNA SEQUENCING

Cytochrome *b* sequences 1140 nucleotides in length were obtained for three *Blarina* specimens from Aransas NWR and 20 specimens from Bastrop County. The Texas populations do not share any haplotypes with *Blarina hylophaga* from Kansas and Nebraska (Brant and Ortí 2002). Four distinct haplotypes were present, one unique to Aransas NWR (Haplotype A) and three in the Bastrop population (Haplotypes B1, B2, and B3). Haplotype A was unique to the three Aransas individuals. Haplotype B1 was the predominant haplotype in Bastrop County (n= 14) and Haplotype B2 was less common (n= 4). Haplotype B3 was present in only one individual, MF 9158, and differed from Haplotype B2 by only one nucleotide (see Appendix 2). Non-synonymous mutations resulted in differing proteins, with changes at amino acid positions 23, 25, 189, and 327 (Table 4). Amino acid 25 is located in the matrix domain of the cytochrome *b* protein, which normally includes mostly polar amino acids; amino acid 189 is located in the transmembrane domain in Helix D, which usually contains hydrophobic amino acids (Griffiths 1997). Partial cytochrome *b* sequence was obtained for the paratype of *B. hylophaga plumbea*, TCWC#1542; the first 400 bases of cytochrome *b* were sequenced successfully and found to be identical to Haplotype B1.

The sequences obtained are assumed to be mtDNA rather than nuclear pseudogenes from mtDNA sequence (also known as numts) based on the following characteristics as outlined by Zhang and Hewitt (1996): 1) PCR amplification consistently produced only one band, 2) No sequence ambiguities or background bands persisted, 3) No unexpected insertions, deletions, or stop codons occurred, 4) Nucleotide

sequences were not radically different from those expected, and 5) Phylogenetic analyses did not yield an unusual or contradictory tree topology.

Phylogenetic Analyses

The complete alignment consisted of 1140 nucleotides of cytochrome *b* for 38 taxa from GenBank, three Aransas specimens, and 20 Bastrop specimens. The treelength distribution for 10,000 randomly generated trees was skewed left (Figure 6) with a g_1 of -0.389, therefore these data are significantly more structured than random data ($P<0.01$) (Hillis and Huelsenbeck 1992). There was no significant difference found among the intermembrane, matrix, and transmembrane regions of the gene in the partition homogeneity test ($P=0.50$). Base frequencies were equivalent at first positions ($\chi^2= 1.5$, $P>0.5$) but did not conform to a 1:1:1:1 ratio for position 2 ($\chi^2 =65.64$, $P<0.01$) or position 3 ($\chi^2 = 240.6$, $P<0.01$) (Table 5).

Saturation of base substitutions does occur in the cytochrome *b* data set between ingroup and outgroup (Figure 7). Saturation curves produced for each codon position within the three domains of the membrane protein showed that saturation does not occur at any codon position in any protein region within ingroup taxa, but ingroup-outgroup saturation is present in third position transitions of all three regions of the protein as well as first codon position transitions of the transmembrane region (Appendix 3). There is 0.5-0.6% divergence in cytochrome *b* between the two Texas lineages and 1.2-2.2% between Texas and other populations of *B. hylophaga* (Table 6).

TABLE 4. Amino acids for five haplotypes in two isolated populations of *Blarina* in Texas compared to GenBank sequences from all three species of *Blarina* (see Table 2) from Brant and Ortí (2002). Haplotype B1 was the predominant haplotype in samples from Bastrop County (*n*= 14), with Haplotype B2 (n= 4) less common, and Haplotype B3 found in only one individual. Haplotype A is the only haplotype found in the Aransas County samples. Haplotype S was only present in the individual from southern Bastrop County.

	A.A. 23	A.A. 25	A.A. 189	A.A. 327
Haplotype B1	Alanine	Serine	Isoleucine	Isoleucine
Haplotypes B2, B3	Alanine	Alanine	Isoleucine	Isoleucine
Haplotype A	Alanine	Serine	Valine	Isoleucine
Haplotype S	Threonine	Serine	Isoleucine	Valine
BLARINA HYLOPHAGA	Alanine	Serine	Isoleucine	Isoleucine
BLARINA CAROLINENSIS	Alanine	Serine	Isoleucine	Isoleucine
BLARINA BREVICAUDA	Alanine	Serine	Isoleucine	Isoleucine

TABLE 5. Mean base frequencies for all *Blarina* individuals in this study as well as outgroups for each codon position over the entire cytochrome *b* gene.

	A	C	G	T
Codon Position 1	0.26596	0.26096	0.22433	0.24875
Codon Position 2	0.19741	0.23756	0.14467	0.42036
Codon Position 3	0.38871	0.35490	0.04418	0.21221

TABLE 6. Percent difference between and within species of *Blarina*, calculated over all 1140 bases of cytochrome *b*. *Blarina carolinensis*, *B. hylophaga*, and *B. brevicauda* sequences from Brant and Ortí (2002), Texas *Blarina* sequences from Bastrop and Aransas counties.

	B. carolinensis	*B. hylophaga*	Texas *Blarina*	*B. brevicauda*
B. carolinensis	0.09- 3.9%			
B. hylophaga	6.1- 11.1%	0.1- 0.97%		
Texas *Blarina*	5.7- 6.6%	1.2-2.5%	0.1- 0.6%	
B. brevicauda	6.5- 8.2%	8.6- 10%	8.3-9.0%	0.1- 2.9%

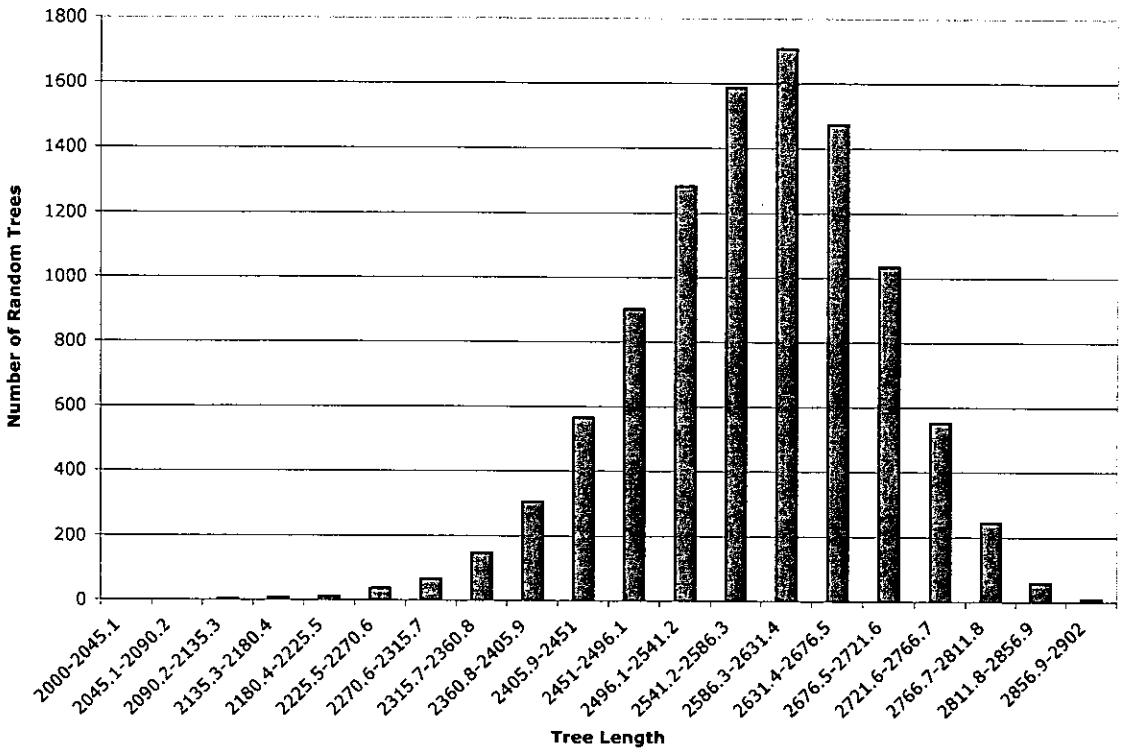

FIGURE 6. Distribution of 10,000 randomly generated trees from the *Blarina* data set of 1140 nucleotides of cytochrome *b* for 61 taxa. Trees were generated using parsimony criteria. The g_1 statistic, a measure of skewness and phylogenetic signal, was -0.389.

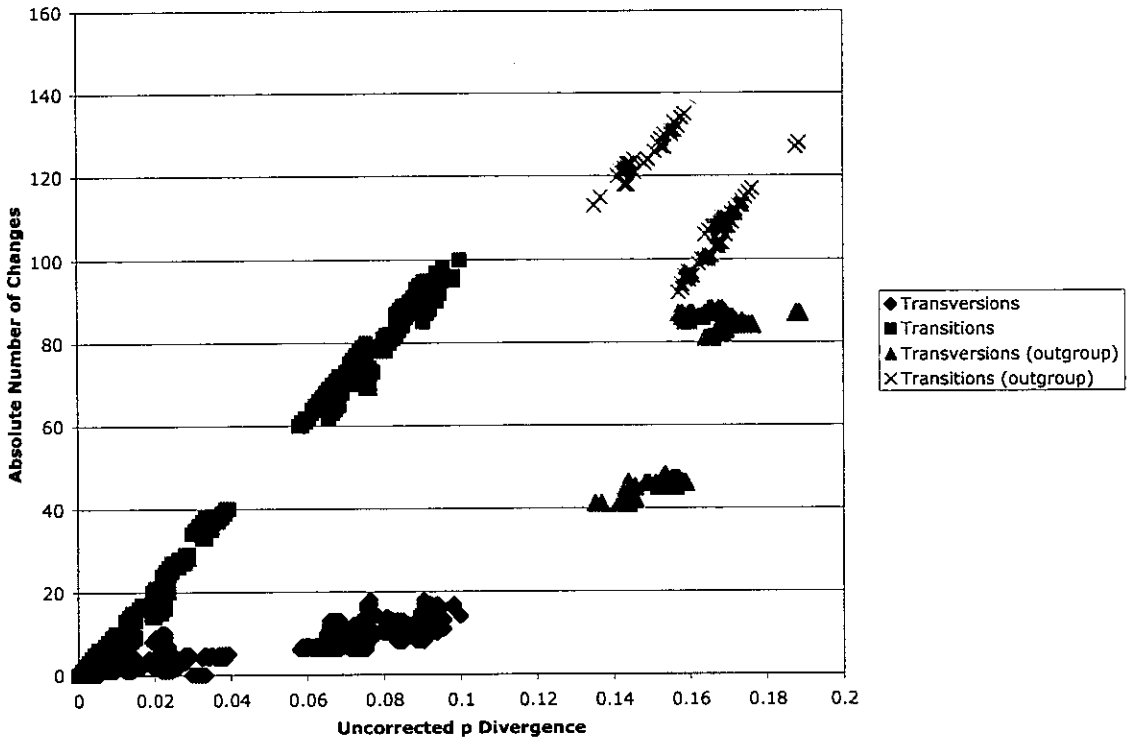

FIGURE 7. Saturation curve for the complete cytochrome *b* gene for the *Blarina* data set, constructed using uncorrected p distance as a measure of time on the X-axis and absolute number of transitions and transversion on the Y-axis. Transitions and transversions labeled separately for ingroup and outgroup. Saturation does not occur within the ingroup but does occur between ingroup and outgroup transitions.

Parsimony analyses of the full data set with all characters equally weighted resulted in 18 equally parsimonious trees with tree length 565, retention index (RI) of 0.941 and consistency index (CI) of 0.724 (Figure 8). Of the molecular characters, 801 were constant and 322 of the variable sites were parsimony informative. The MP topology supports three monophyletic species of *Blarina* with *B. brevicauda* basal within the genus, and Texas *Blarina* sister to *B. hylophaga*. The NJ bootstrap topology agrees with MP at the interspecific level; the Texas clade is sister to Kansas/Nebraska *B. hylophaga* (Figure 9). Weighted parsimony was performed with differential weights for each codon position based on the number of changes for each position in the unweighted MP topology (Figure 8): codon position 1= 0.1125, position 2= 1, and position 3= 0.019, transversions were weighted at 5 times the transversion weight based on the TI/TV ratio from the ModelTest 3.06 analysis. The resulting topology had a polytomy at the interspecific level and a sister relationship between Texas *Blarina* and Kansas/Nebraska *B. hylophaga* (Figure 10).

Maximum likelihood analysis was performed using the parameters given by Modeltest 3.06, which were nucleotide frequencies of A=0.3118, C= 0.2912, T= 0.2779, and G= 0.1191; TI/TV ratio of 5.2241; proportion of invariable sites equal to zero; and a gamma distribution parameter of 0.1813, which cumulatively indicated a high number of practically invariable sites (Nei and Kumar 2000). The resulting topology supported a monophyletic *B. hylophaga* within the Texas *Blarina*, and *B. hylophaga* basal within the genus (Figure 11).

The 50% majority-rule consensus tree for Bayesian analysis was calculated using 500 trees (Figure 12), and the resulting topology supported a sister relationship between

B. brevicauda and *B. carolinensis*. Also, the Texas clade was sister to *B. hylophaga* with 97% posterior probability. The proportion of trees supporting a particular clade is considered to be the Bayesian posterior probability for that clade (Wilcox et al. 2002). Bayesian support values are considered less conservative than bootstrap and possibly closer estimates of phylogenetic accuracy, given that the correct model of evolution is in use (Wilcox et al. 2002).

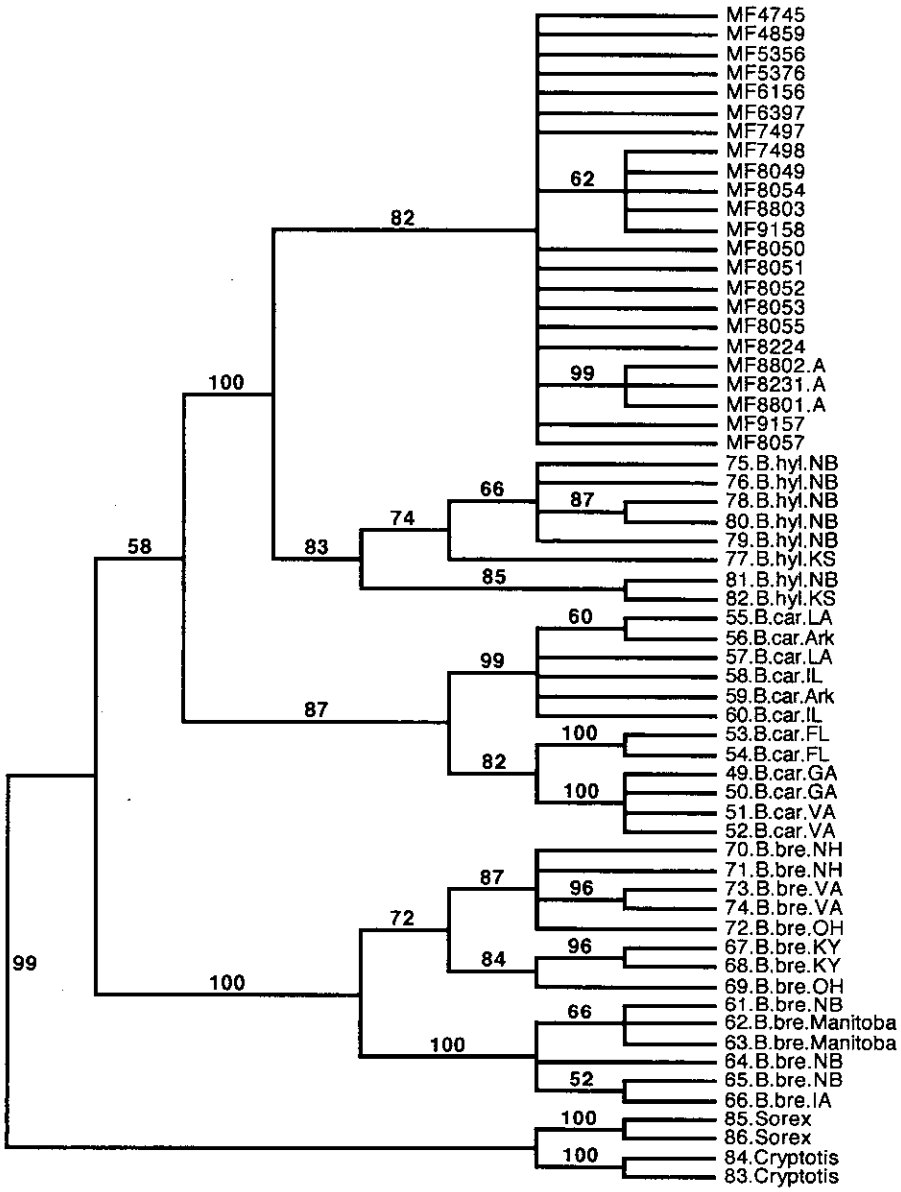

FIGURE 8. Bootstrap consensus tree for cytochrome *b* in three species of *Blarina* and Texas specimens using unweighted parsimony with bootstrap support values on respective branches. Three species of *Blarina* abbreviated as in Table 2, MF numbers are assigned to all Bastrop and Aransas county specimens, the latter with an "A" appended. *Cryptotis* and *Sorex* were used as outgroups.

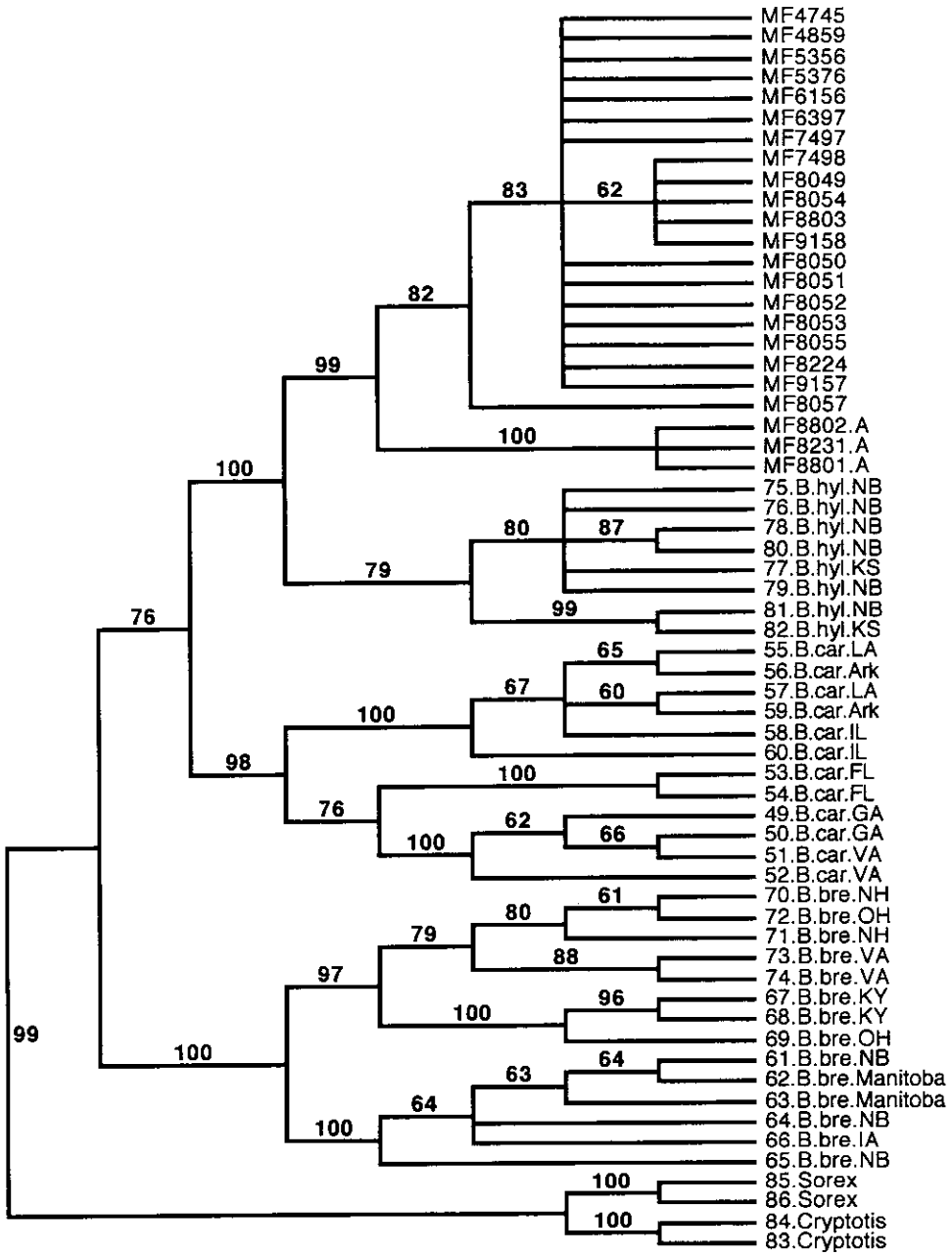

FIGURE 9. Neighbor joining consensus tree with bootstrap values on respective branches created using HKY nucleotide substitution model. *Sorex* and *Cryptotis* are outgroups, three species of *Blarina* labeled as in Table 2; MF numbers for Texas specimens as in Appendix I, all from Bastrop except for three with an "A" appended. Tree created using 1140 bases of cytochrome *b*.

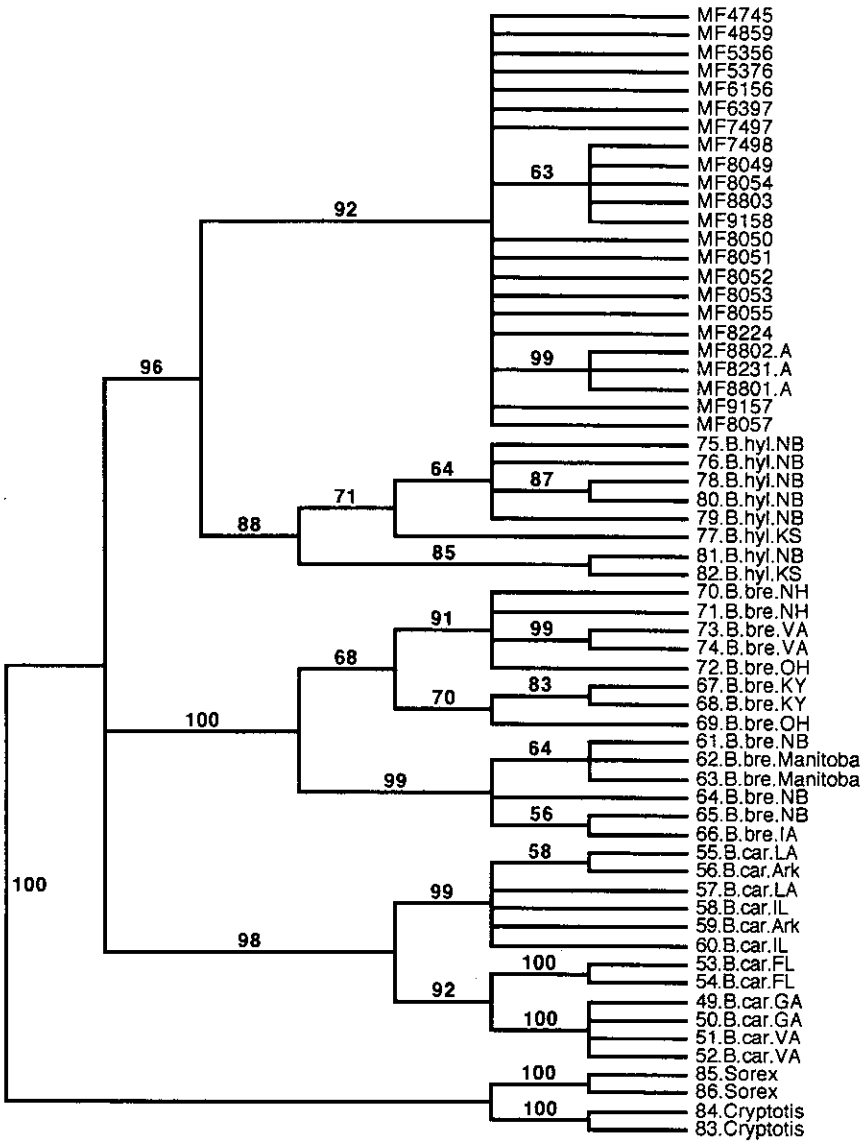

FIGURE 10. Consensus tree with bootstrap values on respective branches for weighted parsimony in which codon position 1 was weighted 0.1125, position 2 at 1, and position 3 at 0.019. Transversions weighted at five times the weight of transitions. *Sorex* and *Cryptotis* are outgroups, three species of *Blarina* labeled as in Table 2; MF numbers for Texas specimens as in Appendix I, all from Bastrop except for three with an "A" appended. Tree created using 1140 bases of cytochrome *b*.

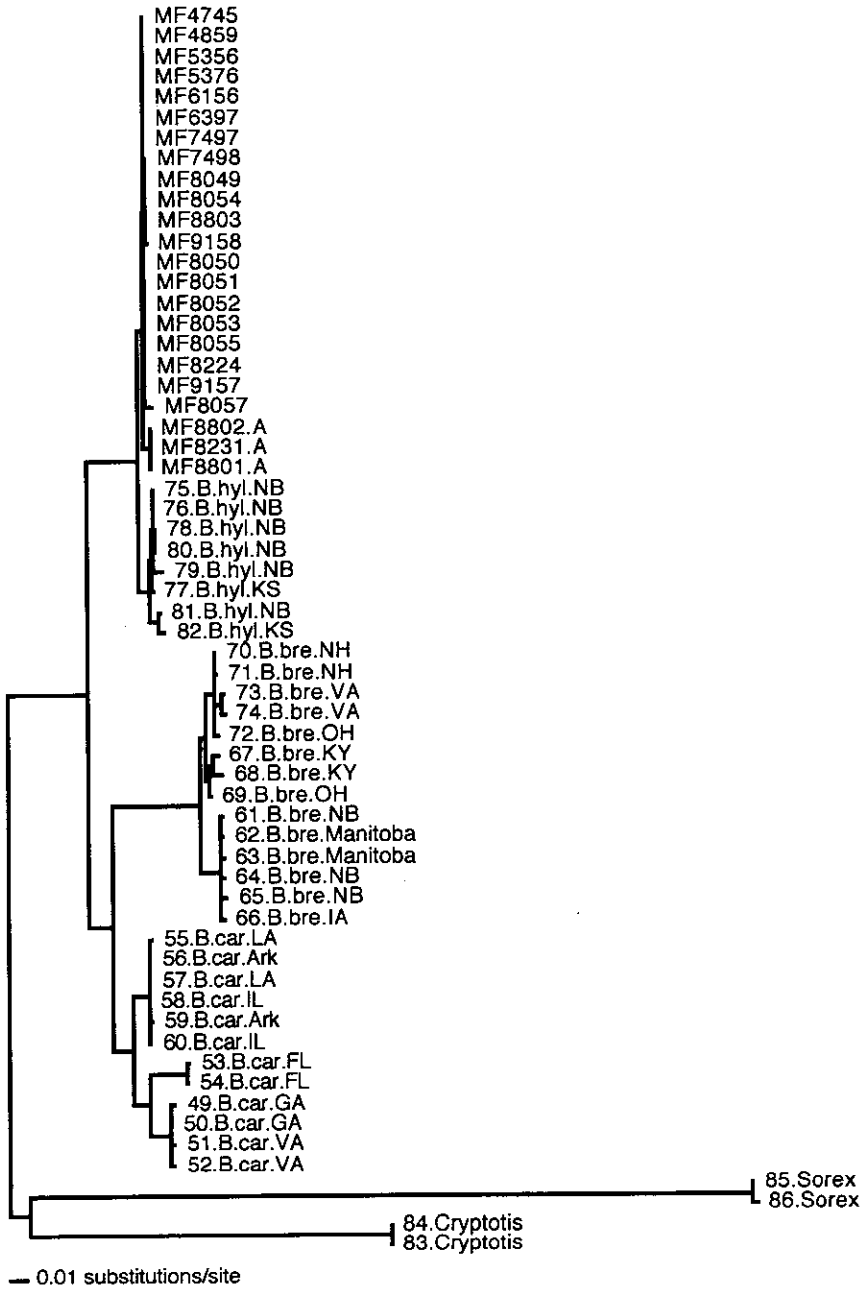

FIGURE 11. Maximum likelihood topology created from the *Blarina* cytochrome *b* data set of 1140 bases for 55 individuals using the HKY+G model of nucleotide substitution with gamma of 0.1813, Ti/Tv ratio of 5.2241, proportion of invariate sites equal to zero, and base frequencies of A=0.3118, C=0.2912, G=0.1191, and T=0.2779. Branch lengths proportional to number of changes; see key directly below figure.

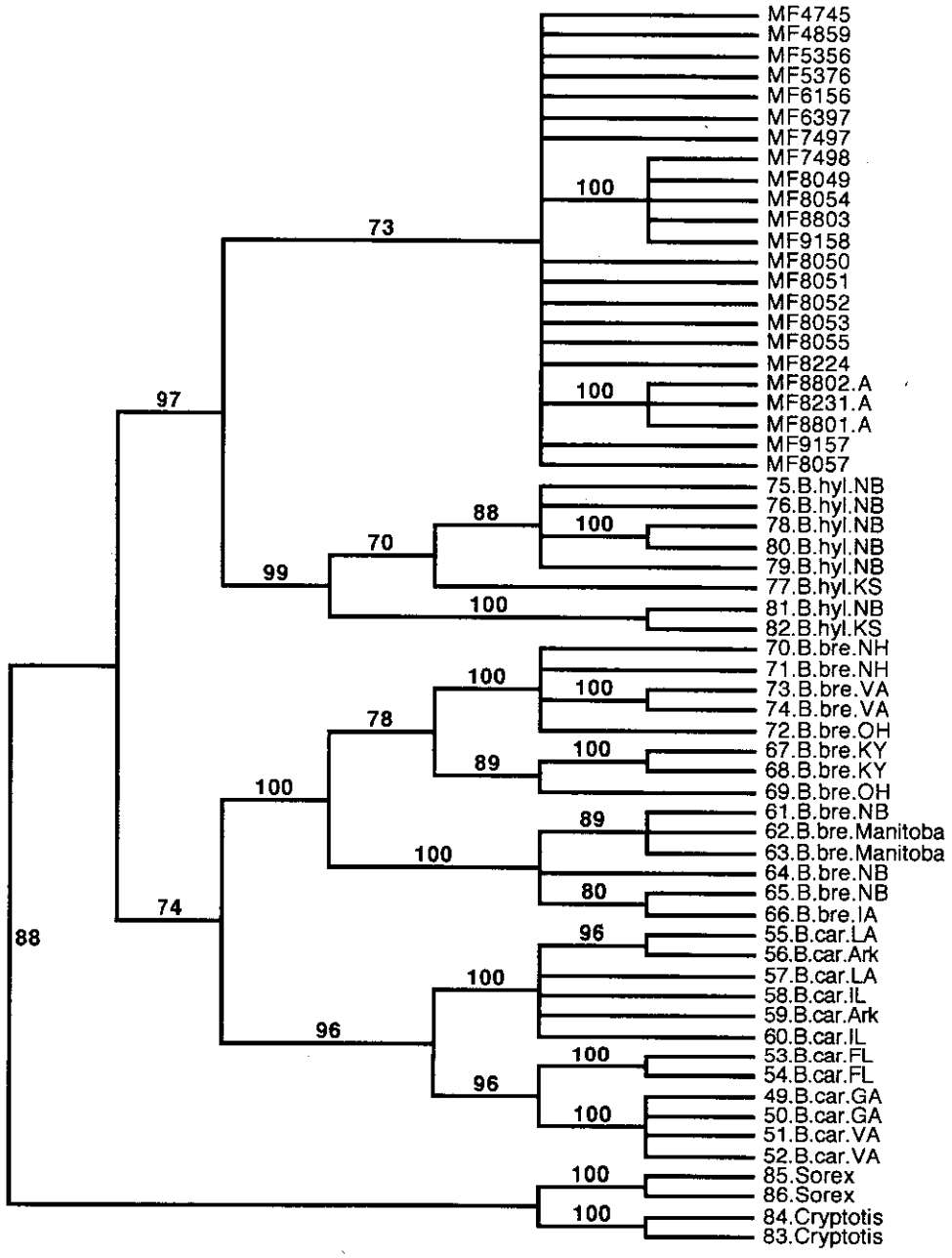

FIGURE 12. 50% Majority-rule consensus tree from 500 trees produced after 1,000,000 generations of four Monte Carlo Markov Chains in MrBayes v.3.0. Bayesian posterior probabilities are noted on respective branches. *Sorex* and *Cryptotis* are outgroups; abbreviations for individuals follows Table 2 except for Texas specimens designated by MF numbers, Aransas specimens with an "A" appended.

DISCUSSION

MORPHOLOGY

Analyses performed here support the two Texas populations being intermediate in size between *B. hylophaga* and *B. carolinensis*. Because all of the cranial measures were positively correlated, PC1 can be considered a size component in the PCA (Flessa and Bray 1977; Rohlf and Bookstein 1987), and PC 2 and subsequent principal components can be considered size-free shape components (Humphries et al. 1981). Thus, these shrews vary in size with *B. carolinensis* being the smallest, Texas *B. hylophaga* intermediate and *B. hylophaga* the largest of the groups in the analysis. The shape component PC2 accounts for little variation (7.9%) in cranial morphology and the three groups in this comparison have considerable overlap on that axis (Figure 5). This is congruent with previous studies using PCA to differentiate species of *Blarina* (Genoways and Choate 1972).

One individual short-tailed shrew (TCWC# 51209) captured in Bastrop County previously had been identified as *B. carolinensis*, and indeed fit with that species in the morphological analyses. However, as previously mentioned, the characters used to identify these shrews are purely size-based, and this individual most likely was a young adult based on degree of ankylosis and lack of tooth wear. This example illustrates the pitfalls of using size-based characters to identify animals; individuals that are not fully grown will be classified as the smaller of the species. Another example can be found in the East Texas specimens from Houston and Anderson counties, one of which fits in with the Texas *B. hylophaga* on the ordinal plot (Figure 5). Although *B. hylophaga* has not

previously been reported from these counties, it is difficult to say if these specimens are unusually large *B. carolinensis* or an additional population of *B. hylophaga.*

Clinal variation in size has been documented in previous studies of *Blarina* (e.g. George et al. 1981; Jones and Findley 1954), and the Texas shrews being smaller than northern populations of *B. hylophaga* supports those studies. This clinal variation correlates with Bergmann's Rule, which states that within a taxon, animals at higher latitudes are larger than those closer to the equator (Brown and Lomolino 1998). However, factors other than latitude also affect body size. Because the *Blarina* in Central Texas inhabit an island-like fragment of pine forest and the coastal population is equally insular by analogy, it is possible that their body sizes have changed due to differences in predators, competitors, or other niche factors from the main population. Vertebrate species that colonize islands often exhibit size differences from their mainland ancestors (Case 1978). There is no consistent trend for insectivores to be larger or smaller on islands (Lomolino 1985), so there is no prediction for these shrews to be larger or smaller than the analogous "mainland" shrew population means.

Because morphological differences among species of *Blarina* are primarily size-based, however, island effects should be a consideration when observing trends in body size. Additionally, as there is no size-free shape component to differentiate between species of *Blarina*, morphology is not useful to identify shrews in geographically isolated populations. For example, one key to the identification of soricids differentiates between *B. hylophaga* and *B. carolinensis* based on size, stating that animals less than 100 millimeters (mm) in total length, with condylobasal length of less than 20 mm and weighing less than 10 grams are *B. carolinensis* (Choate et al. 1994). Few of the Texas

B. hylophaga would be correctly identified using this key, as they are small enough to be incorrectly identified as *B. carolinensis*.

MOLECULAR PHYLOGENETICS

The use of mitochondrial DNA in phylogenetic studies is widespread and, despite controversy, been well established as an effective tool to examine evolutionary histories of taxa. Because the mitochondrial genome is uniparentally inherited and haploid, its effective population size (N_e) is 1/4 that of nuclear markers (Moore 1995). While this may enable early detection of speciation, ancestral polymorphisms in incipient species may result in misleading patterns in gene trees (Neigel and Avise 1986). Conversely, speciation may be detected early based on phenotypic differences between incipient species in which mtDNA divergence cannot be detected (Nice and Shapiro 1999). It is essential to be aware of these possibilities and to be cautious when making conclusions based primarily on mtDNA.

Cytochrome *b* frequently is used in studies of molecular phylogenetics of animals (Adachi and Hasegawa 1996). Evolutionary pressures differ within this gene by membrane region, codon position, and amino acid. Based on the saturation curves produced for the codon positions within each transmembrane region (Appendix 3), down-weighting third positions throughout the gene will result in a loss of significant amounts of unsaturated positions that could otherwise be informative. Hence, weighted parsimony may result in a loss of phylogenetic signal in this gene. Additionally, when creating saturation curves it is essential to consider saturation within the ingroup and between the ingroup and outgroup separately.

DNA was successfully extracted and sequenced from two museum skins, one of which was the paratype for the subspecies *B. hylophaga plumbea.* This individual was collected at the Aransas NWR in 1941 by Davis (1941). The 400 bases of cytochrome *b* sequenced for this individual are identical to Haplotype B1, the predominant haplotype in Bastrop County. Phylogenetic analyses using cytochrome *b* unambiguously place the paratype of *Blarina hylophaga plumbea* in the *B. hylophaga* clade. These analyses should allay any doubts regarding the phylogenetic placement of this isolated population.

The Texas populations of short-tailed shrews form a monophyletic sister group to other U.S. populations of *Blarina hylophaga*; within Texas, Bastrop and Aransas harbor unique clades. Many of the molecular analyses were in agreement in the placement of the Texas clade as a sister group to *B. hylophaga.* Maximum likelihood supported an alternate hypothesis of a sister relationship between Aransas and the Kansas/Nebraska *B. hylophaga*, with the remainder of Texas *Blarina* sister to that group; this topology is probably an artifact of anomalies in the Kansas/Nebraska GenBank sequences, and is in fact unsupported by any synapomorphies. This can be assessed by observing the length of the terminal branches of *B. hylophaga* in comparison to *B. carolinensis.* There was no overlap in haplotypes between Texas and Kansas/Nebraska *B. hylophaga.* Because sampling was limited at Aransas NWR, it is possible that other haplotypes are present in the population but were not found in this study.

Results differed among analyses with respect to the relationships of the three species of *Blarina.* Previous morphological studies have supported the (*B. brevicauda* (*B. carolinensis, B. hylophaga*)) topology (Jones et al. 1984; Stangl and Carr 1997), but the recent molecular phylogeny by Brant and Ortí (2002) supports a (*B. hylophaga* (*B.*

brevicauda, *B. carolinensis*)) topology. In this study, the former relationship was supported in neighbor joining and parsimony analysis, and the latter in Bayesian and ML analyses. Weighted parsimony did not resolve the interspecific relationship. Support for either of the resolved relationships was generally low and consisted of synonymous third and first codon position changes, many of which had low CI values for the topology in question, indicating homoplasy. Other genes may be successful in recovering any interspecific structure, or morphology can be used as additional evidence to confirm *B. brevicauda* as a basal species within *Blarina*.

Biogeography and Areas of Endemism

These shrews may represent relictual isolates of *B. hylophaga's* Pleistocene range in Texas. Aransas and Bastrop counties do not have similar floras, but must share some characteristics that make them hospitable to short-tailed shrews. Similarities between Aransas and Bastrop counties that could make both suitable as shrew habitat include deep sandy soils, ancient trees, deep leaf litter, abundance of prey, and suitable thermal climate. While other areas of sandy soil exist in Texas, such as the coastal sand plain in South Texas, lack of leaf litter or other factors may prohibit shrews from inhabiting the area. It also is possible that *Blarina* do not occupy all suitable habitats in Texas, and other circumstances have narrowed their distribution. Examining patterns of distribution in other species may aid in understanding the pattern of short-tailed shrew distributions in Texas.

Many small mammals inhabited Texas in the Pleistocene and may aid in understanding the pattern of distribution in *Blarina hylophaga* in Texas. Other small mammals with ranges extending southward from the midwest include the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) and the woodland vole (*Microtus pinetorum*), both of which were reported at Aransas NWR by Davis (1941). Small mammals having Pleistocene-era fossils in Texas whose current ranges no longer include Texas or include only relictual populations include two additional species of vole (*Microtus pennsylvanicus* and *M. ochrogaster*), the southern bog lemming *Synaptomys cooperi*, the ermine *Mustela erminea*, and the cinereus shrew *Sorex cinereus* (Lundelius 1967). The common pattern among these animals is a northward-shifting range, probably a consequence of warming temperatures over time. Some of these taxa, such as *Microtus*, may be more tolerant of warm weather and therefore able to maintain isolated populations in Texas, especially in favorable habitats.

The Lost Pines area in Central Texas is an area characterized by stands of loblolly pine (*Pinus taeda*) on sandy soils. This area is superficially similar to the Piney Woods of East Texas, also an area containing loblolly pines on sandy soils. The Lost Pines hosts the westernmost distribution of these pines in Texas, as well as the fauna associated with them. Animals such as flying squirrels (*Glaucomys volans*), pileated woodpecker (*Dryocopus pileatus*), and pine warbler (*Dendroica pinus*) have presumably disjunct populations in the Lost Pines. The endangered Houston toad (*Bufo houstonensis*) now is effectively restricted to the Lost Pines, although its distribution once included a much larger area. An endemic insect, the Texas long-lipped beetle (*Telegeusis texensis*),

recently was described from specimens collected in the Lost Pines (Taber and Fleenor 2003).

These animals inhabit a unique ecosystem that may be a relictual isolate or an outpost of pines created by dispersal. Fossil pollen evidence confirms that Bastrop County was the westernmost limit of the range of pine forests in Texas in late glacial and postglacial times (Larson et al. 1972), and that pines have been present for nearly 20,000 years (Bryant 1977). This evidence strongly suggests that the Lost Pines region is a remnant of a more widespread pine forest.

Aransas National Wildlife Refuge also harbors unique fauna; it is most well known as the wintering grounds for the endangered whooping crane (*Grus americana*). The rare Texas scarlet snake, *Cemophora lineri*, also has been found on the refuge. Both the Lost Pines and Aransas NWR may represent areas of local endemism for many taxa. Both areas have been scrutinized as endangered species habitat, but also should be examined at the ecosystem level because of their unique nature.

TAXONOMY

While the debate over species concepts continues, some model must be applied in order to define species taxonomically. Often a combinatorial approach is necessary, using several data types to confirm the status of a taxon; for example, Wiens and Penkrot (2002) proposed an approach using DNA and morphological data to delimit species. In the case of these isolated populations of *Blarina*, however, morphological characters can be nebulous or even misleading. Characters such as nuclear DNA or a karyotype may be useful to support mtDNA phylogenies in this case. In an instance involving isolated

populations of pocket gophers which only had mtDNA evidence available, a "molecular-phenetics species concept" was implemented, using known differences of closely related taxa to create a standard to identify potential species-level differences (Demastes et al. 2002). This model is similar to the "cytochrome-*b* species concept" (Bradley and Baker 2001), but with the caveat that other types of evidence should be applied as well.

The Biological Species Concept (BSC) (Mayr 1942), Genealogical Species Concept (GSC) (Mishler and Donoghue 1982), and Phylogenetic Species Concept (PSC) (Cracraft 1983; Nelson and Platnick 1981) have been discussed philosophically to a tremendous extent. It is difficult to decide which concept is appropriate to apply, so it is necessary to consider the applicability of each concept to this research, which involves two allopatric populations of morphologically indistinguishable animals with differences in molecular traits.

The BSC defines species as "groups of interbreeding natural populations that are reproductively isolated from other such groups" (Mayr 1969). Observations of interbreeding are difficult to obtain for many if not most species, and for allopatric populations these observations could only take place in captive organisms. For Texas *Blarina*, it is unknown whether breeding with Oklahoma, Kansas, or Nebraska *Blarina* would be successful; therefore, no evidence is available to support or refute classification of Texas *Blarina* under this concept.

Employment of the phenetic measure of percent divergence as a criterion to define species may be misleading when considering the differential rate of evolution among taxa (Spradling et al. 2001) that may be affected by body size, thermal habit, and metabolic rate (Martin and Palumbi 1993; Rand 1994). Oxygen radicals cause damage to

mtDNA, increasing rate of evolution in the mitochondrial genome (Richter et al. 1988); shrews exhibit extremely high oxygen consumption per gram body mass, *Blarina brevicauda* basal metabolic rates being over 150% of the expected value (Churchfield 1990). Therefore intra- and inter-species sequence divergences cannot be standardized among taxa; however, it remains reasonable to use a within-taxon standard to gauge differences in that taxon. Both *B. carolinensis* and *B. brevicauda* have intraspecific divisions that are attributable to the Mississippi River; distances between those east-west clades average 3.3% and 2.5%, respectively (Table 6). The genetic distance between Texas *B. hylophaga* and the northern population of that species ranges from 1.2-2.2%, and the genetic distance between the Aransas and Bastrop populations is 0.5%. This evidence along with the monophyly of this group is sufficient evidence to classify the Texas *B. hylophaga* as a subspecies, *Blarina hylophaga plumbea* (Davis 1941) under the GSC.

The PSC defines species as "the smallest aggregation of ... populations... diagnosable by a unique combination of character states" (Wheeler and Platnick 2000). Under the strictest interpretation of this concept, every haplotype in this study would constitute a separate species; there is no room for intraspecific classification under the PSC. Certainly both Aransas and Bastrop populations would warrant species status, since each population does have a unique combination of character states. Widespread use of the PSC by taxonomists would undoubtedly lead to significant increase in the number of recognized species, if only by elevating subspecies to species.

The designation of subspecies is a taxonomic tradition that has been brought into question under several species concepts. A subspecies can be defined as "a recognizably

different population...that occupies a different geographic area from other populations of the same species" (Futuyma 1998); however, problems arise when attempting to differentiate between a subspecies and a population, which is "a group of conspecific organisms that occupy a...well defined geographic region and exhibit reproductive continuity from generation to generation" (Futuyma 1998). Many subspecies are recognized as morphologically different ecotypes; this use is perpetuated in field guides. It has been noted that recognition of a subspecies requires as much evidence as for a species, with the additional assumptions that the lineage may reconnect and interbreed with the main lineage; although it is convenient to designate subspecies in collections and field guides, it is difficult to justify a subspecies concept (Frost et al. 1992). One potential utility of subspecies lies in conservation of locally endemic subspecies which may be protected under the Endangered Species Act; such geographic variants of a species are important components of biodiversity (O'Brien and Mayr 1991). From the perspective of traditional recognition of subspecies as geographic variants or "a genetically distinct set of populations with a discrete range" (Brown and Lomolino 1998), the short-tailed shrews in Aransas and Bastrop counties can be recognized as a subspecies.

The two isolated populations of *Blarina* in Texas certainly warrant subspecies recognition, and possibly species recognition under strict interpretation of the PSC (Cracraft 1983). The Texas populations appear to be monophyletic (*sensu* Hennig 1966) in that they are more closely related to each other than to other lineages and they appear to have descended from a common ancestor. Therefore, based on geographic isolation and genetic similarity, it is justifiable to separate them into a single Texas endemic subspecies: *Blarina hylophaga plumbea.*

Parallels can be drawn between the Texas and Florida subspecies of short-tailed shrews. Two subspecies inhabit Florida: *Blarina carolinensis peninsulae* and *B. carolinensis shermani*. Despite several concerted efforts, the latter has not been captured at the type locality in Ft. Myers since the initial type series was collected in 1955 (Layne 1992). Specimens collected in the 1980s are smaller than those in the series collected by Hamilton (1955), and are postulated intergrades between *B. c. shermani* and *B. c. peninsulae*. Again, because size-based morphological characters are the basis of classification for these subspecies, clinal variation in size as well as other factors make it difficult to determine if this subspecies is a relictual isolate of *B. brevicauda* or a population of large *B. carolinensis*. Additionally, because material available for *B. c. shermani* is limited to museum specimens, karyotyping is not possible and DNA analyses have not yet been attempted. This subspecies was extirpated before it could be described; whether it was a relictual isolate of *B. brevicauda* or a unique population of *B. carolinensis* may be determined using DNA, but the opportunity to conserve this unique population was lost.

The description of the endemic Texas subspecies *Blarina hylophaga plumbea* should be followed with population estimates and characterization of the ecology of these populations. The isolated populations in Aransas and Bastrop counties, Texas, should be monitored so that they do not meet the same fate as *B. c. shermani*.

APPENDIX 1

Tissue sample and voucher information for individuals of the genus *Blarina* collected for this study or used in morphological analyses. All individuals in the Michael Forstner (MF) tissue catalog are identified with their unique catalog number. Location, collector, and external measurements may be noted; if cytochrome *b* was successfully sequenced for that individual, haplotype is noted (B1, B2, and B3 from Bastrop County, A from Aransas County, and S for one individual from southern Bastrop County—see Results). For individuals belonging to the Texas Cooperative Wildlife Collection (TCWC) and used only for morphological analyses, TCWC number, collection date, sex, and location are noted.

MF#	Collector #	Haplotype	Voucher	Sex
MF# 4745	GL 101; RWM 3686	B1	Skin, skull	
MF# 4859	GL 75; RWM 3683	B1	Skin, skull	
MF# 5356	RWM 3691 GL275	B1	Skin, skull	M
MF# 5375	RWM3693 G374		Skin, skull	F
MF# 5376	RWM3692 G373	B1	Skin, skull	F
MF# 6156	RWM 3694	B1	Skeleton only	U
MF# 6397	RWM 3689; GL300		Skin, skull	M
MF# 7497	RWM 3700	B1	Skin, skull	M
MF# 7498	RWM 3701	B2	Skin, skull	F
MF# 8049	RWM 3711	B2	Skin, skull	F
MF# 8050	RWM 3712	B1	Skin, skull	F
MF# 8051	RWM 3713	B1	Skin, skull	F
MF# 8052	RWM 3714	B1	Skin, skull	M
MF# 8053	RWM 3716	B1	Skin, skull	M
MF# 8054	RWM 3717	B2	Skin, skull	F
MF# 8055	RWM 3718	B1	Skin, skull	F
MF# 8057	RWM 3715		Skin, skull	M
MF# 8224	RWM 3720	B1	Skin, skull	F
MF# 8225	RWM 3721		Skin, skull	M
MF# 8226	RWM 3722		Skin, skull	F
MF# 8227	RWM 3723		Skeleton	
MF# 8228	RWM 3724		Skeleton	
MF# 8230	RWM 3675		Skin, skull	
MF# 8231	RWM 3725	A		F
MF# 8741			Released alive	U
MF# 8801	RWM 3726	A	Skin, skull	U
MF# 8802	RWM 3727	A	Skin, skull	M
MF# 8803	RWM 3728	B2	Skin, skull	M
MF#9156	RWM#3729		Skel. only	F
MF#9157	RWM#3730	B1	Skin, skull	Juv M
MF#9158	RWM#3731	B3	Skin, skull	M
MF#9159	RWM#3732		Skel. only	U
MF#9238	Yantis			
MF#9239	Yantis			
MF#9240	Yantis			
MF#	Collector #	Haplotype	Voucher	Sex
MF#9241	Yantis			
MF#9242	Yantis			
MF#9243	Yantis			

MF#9268	TCWC#1541		Skin, skull	F
MF#9269	TCWC#1542	B1	Skin, skull	M
MF#9270	TCWC#30395		Skin, skull	F
MF#9271	TCWC#30396		Skin, skull	F
MF#9272	TCWC#31833		Skin, skeleton	F
MF#9273	TCWC#31834		Skin, skeleton	F
MF#9274	TCWC#31835		Skin, skeleton	F
MF#9275	TCWC#31836		Skin, skeleton	F
MF#9276	TCWC#31837		Skin, skull	F
	TCWC#51207		Skull, alcohol specimen	U
	TCWC#51208		Skin, Skull	M
	TCWC#51209		Skeleton	U
	TCWC#51797		Skin, skeleton	F
	TCWC#27628			F
	TCWC#33360			F
	TCWC#34970			F
	TCWC#34971			F
	TCWC#33359			F
	TCWC#33361			F
	TCWC#50138			M
	TCWC#50143			F
	TCWC#50131			F
	TCWC#50132			M
	TCWC#50133			M
	TCWC#50134			F
	TCWC#33351			M
	TCWC#34952			M
	TCWC#34956			F
	TCWC#33337			F
	TCWC#33355			F

MF#	Location
MF# 4745	Griffith League Ranch
MF# 4859	Griffith League Ranch
MF# 5356	Griffith League Ranch
MF# 5375	Griffith League Ranch
MF# 5376	Griffith League Ranch
MF# 6156	Griffith League Ranch
MF# 6397	Griffith League Ranch
MF# 7497	Griffith League Ranch
MF# 7498	Griffith League Ranch 10-2
MF# 8049	Line 14, Griffith League
MF# 8050	Bucket 5-1, Griffith League
MF# 8051	Line 13, Griffith League
MF# 8052	Bucket 12-2, Griffith League
MF# 8053	Griffith League
MF# 8054	Griffith League
MF# 8055	Griffith League
MF# 8057	10 miles west of Smithville
MF# 8224	Griffith League Ranch; 16-1
MF# 8225	Griffith League Ranch; 16-E
MF# 8226	Griffith League Ranch
MF# 8227	# 8
MF# 8228	Griffith League Ranch
MF# 8230	Aransas National Wildlife Refuge
MF# 8231	Aransas National Wildlife Refuge
MF# 8741	Griffith League bucket B-S
MF# 8801	Aransas NWR, Stinson's trap in U-20
MF# 8802	Aransas NWR, trap in open motte by rocky spot
MF# 8803	Griffith League Ranch trap 14-1
MF#9156	Griffith League Ranch 12-3
MF#9157	Griffith League Ranch A-W
MF#9158	Griffith League Ranch V20 bucket 1
MF#9159	Griffith League Ranch
MF#9238	
MF#9239	14 mi. E. center of Palestine. 31 deg. 41' N, 95 deg. 23' W
MF#9240	
MF#	Location
MF#9241	12 mi. NNW center of Ratcliff, 31deg.33' N, 95deg 9' W
MF#9242	12 mi NE center of Crockett, 31 deg 24' N, 95 deg 17' W
MF#9243	20 mi SE center of Palestine 31 deg 39' N, 95 deg 19' W

MF#9268	Aransas National Wildlife Refuge
MF#9269	Aransas Refuge, near Dagger Point
MF#9270	Aransas National Wildlife Refuge
MF#9271	Aransas National Wildlife Refuge
MF#9272	Aransas National Wildlife Refuge
MF#9273	Aransas National Wildlife Refuge
MF#9274	Aransas National Wildlife Refuge
MF#9275	Aransas National Wildlife Refuge
MF#9276	Aransas National Wildlife Refuge
TCWC#51207	ca 2 mi. E Bastrop (county seat), 30deg7'N, 97deg16'W
TCWC#51208	ca 2 mi. E Bastrop (county seat), 30deg7'N, 97deg16'W
TCWC#51209	ca 2 mi. E Bastrop (county seat), 30deg7'N, 97deg16'W
TCWC#51797	ca 2 mi. E Bastrop (county seat), 30deg7'N, 97deg16'W
TCWC#27628	
TCWC#33360	
TCWC#34970	
TCWC#34971	
TCWC#33359	
TCWC#33361	
TCWC#50138	
TCWC#50143	
TCWC#50131	
TCWC#50132	
TCWC#50133	
TCWC#50134	
TCWC#33351	
TCWC#34952	
TCWC#34956	
TCWC#33337	
TCWC#33355	

MF#	County	State	Collection date	Tissue type
MF# 4745	Bastrop	TX	4/22/01	skeletal muscle
MF# 4859	Bastrop	TX	4/13/01	skeletal muscle
MF# 5356	Bastrop	TX	5/25/01	skeletal muscle
MF# 5375	Bastrop	TX	7/2/01	skeletal muscle
MF# 5376	Bastrop	TX	7/2/01	skeletal muscle
MF# 6156	Bastrop	TX	7/10/01	skeletal muscle
MF# 6397	Bastrop	TX	5/7/01	skeletal muscle
MF# 7497	Bastrop	TX	7/10/02	Heart and skeletal muscle
MF# 7498	Bastrop	TX	7/10/02	Heart and skeletal muscle
MF# 8049	Bastrop	TX	1/11/03	Skeletal muscle and heart
MF# 8050	Bastrop	TX	1/27/03	Skeletal muscle and heart
MF# 8051	Bastrop	TX	1/11/03	Skeletal muscle and heart
MF# 8052	Bastrop	TX	1/26/03	Skeletal muscle and heart
MF# 8053	Bastrop	TX	unknown	Skeletal muscle and heart
MF# 8054	Bastrop	TX	unknown	Skeletal muscle and heart
MF# 8055	Bastrop	TX	unknown	Skeletal muscle and heart
MF# 8057	Bastrop	TX	2/2/03	no eyelids; skel muscle, heart
MF# 8224	Bastrop	TX	5/12/03	heart and sk. muscle
MF# 8225	Bastrop	TX	6/4/03	heart and sk. muscle
MF# 8226	Bastrop	TX	6/4/03	heart and sk. muscle
MF# 8227	?	?	?	heart and sk. muscle
MF# 8228	Bastrop	TX	5/18/03	heart and sk. muscle
MF# 8230	Aransas	TX	12/3/00	Vertebrae off study specimen
MF# 8231	Aransas	TX	6/12/03	Heart and skel muscle; 1st array
MF# 8741	Bastrop	TX	6/18/03	blood
MF# 8801	Aransas	TX	6/13/03	Muscle
MF# 8802	Aransas	TX	6/25/03	Muscle, heart, liver
MF# 8803	Bastrop	TX	7/4/03	Muscle, heart, liver
MF#9156	Bastrop	TX	5/21/03	Muscle, heart, liver
MF#9157	Bastrop	TX	6/14/03	Muscle, heart, liver
MF#9158	Bastrop	TX	6/21/03	Muscle, heart, liver
MF#9159	Bastrop	TX		Muscle, heart, liver
MF#9238				tooth
MF#9239	Anderson	TX	6/13/02	Dried skin from skull
MF#9240				Alcoholic specimen skin/ muscle sample
MF#9241	Houston	TX	6/5/02	Alcoholic specimen skin/ muscle sample
MF#9242	Houston	TX	5/19/02	Alcoholic specimen skin/

				muscle sample
MF#9243	Anderson	TX	6/6/02	
MF#9268	Aransas	TX	1/13/41	Museum skin clip
MF#9269	Aransas	TX	3/15/41	Museum skin clip
MF#9270	Aransas	TX	1/23/76	Museum skin clip
MF#9271	Aransas	TX	1/24/76	Museum skin clip
MF#9272	Aransas	TX	5/24/76	Museum skin clip
MF#9273	Aransas	TX	5/22/76	Museum skin clip
MF#9274	Aransas	TX	7/25/76	Museum skin clip
MF#9275	Aransas	TX	6/18/76	Museum skin clip
MF#9276	Aransas	TX	6/18/76	Museum skin clip
TCWC#51207	Bastrop	TX	3/25/89	
TCWC#51208	Bastrop	TX	3/27/90	
TCWC#51209	Bastrop	TX	7/25/90	
TCWC#51797	Bastrop	TX	Jun-91	
TCWC#27628	Newton	TX	4/10/74	
TCWC#33360	Tyler	TX	1/12/78	
TCWC#34970	Tyler	TX	1/6/79	
TCWC#34971	Tyler	TX	1/7/79	
TCWC#33359	Tyler	TX	1/12/78	
TCWC#33361	Tyler	TX	2/11/78	
TCWC#50138	Geary	KS	6/24/86	
TCWC#50143	Geary	KS	6/30/86	
TCWC#50131	Geary	KS	6/23/86	
TCWC#50132	Geary	KS	6/23/86	
TCWC#50133	Geary	KS	6/23/86	
TCWC#50134	Geary	KS	6/23/86	
TCWC#33351	Tyler	TX	1/5/78	
TCWC#34952	Hardin	TX	1/28/79	
TCWC#34956	Hardin	TX	1/28/79	
TCWC#33337	Hardin	TX	1/12/78	
TCWC#33355	Tyler	TX	1/7/78	

MF#	Collector	Total L.	Tail L.	HF	Weight
MF# 4745	unknown				
MF# 4859	unknown				
MF# 5356	unknown	94	20	12	8.2
MF# 5375	unknown	80	20	11	6.1
MF# 5376	unknown	92	21	12	7.4
MF# 6156	unknown	90	21	12	6.2
MF# 6397	unknown	90	20	12	8
MF# 7497	unknown	88	18	11	8
MF# 7498	unknown	94	21	11	7.5
MF# 8049	unknown				
MF# 8050	unknown				6.9 g
MF# 8051	unknown				5.7 g
MF# 8052	unknown				10 g
MF# 8053	unknown				
MF# 8054	unknown				
MF# 8055	unknown				
MF# 8057	TR Simpson				
MF# 8224	Todd Swannack	84	17	12	
MF# 8225	Todd Swannack	91	18	12	
MF# 8226	Todd Swannack	80	17	12	
MF# 8227	Todd Swannack	90	21	13	
MF# 8228	Todd Swannack				
MF# 8230	Richard W, Manning				
MF# 8231	S. Morris	88	18	13	
MF# 8741	S. Morris				
MF# 8801	S.Morris				
MF# 8802	S. Morris	83	18	12	
MF# 8803	T.Swannack	83	18	11	
MF#9156	S. Morris	86	19	13	6.2
MF#9157	S. Morris	80	17	12	5.4
MF#9158	S. Morris	89	18	12	5.6
MF#9159	S. Morris				
MF#9238	Jim Yantis				
MF#9239	Jim Yantis				
MF#	Collector	Total L.	Tail L.	HF	Weight
MF#9240	Jim Yantis				
MF#9241	Jim Yantis				
MF#9242	Jim Yantis				

MF#9243	Jim Yantis				
MF#9268	Stevenson, J.				
MF#9269	Davis, W.B.				
MF#9270	R.A. Sparks				
MF#9271	R.A. Sparks				
MF#9272	W. Brown				
MF#9273	W. Brown				
MF#9274	W. Brown				
MF#9275	W. Brown				
MF#9276	R.A. Sparks				
TCWC#51207	J.R. Dixon and J. Godwin				
TCWC#51208	N. Dronen and Scarbrough				
TCWC#51209	N. Dronen and Scarbrough				
TCWC#51797	G. Baumgardner				
TCWC#27628					
TCWC#33360					
TCWC#34970					
TCWC#34971					
TCWC#33359					
TCWC#33361					
TCWC#50138					
TCWC#50143					
TCWC#50131					
TCWC#50132					
TCWC#50133					
TCWC#50134					
TCWC#33351					
TCWC#34952					
TCWC#34956					
TCWC#33337					
TCWC#33355					

APPENDIX 2

Final alignment of 1140 nucleotides of cytochrome *b* for 61 individuals used in phylogenetic analyses in this study. Rows are taxa, columns are nucleotide positions as numbered in column headings. Specimens from Texas have unique numbers as catalogued in the Michael Forstner (MF) tissue catalog (Appendix 1). Thirty-eight sequences downloaded from GenBank are designated using the last two digits of the GenBank accession number, an abbreviated species name (B.hyl= *Blarina hylophaga*, B.car= *B. carolinensis*, B.bre= *B. brevicauda*, Cryptotis= *Cryptotis parva*, Sorex= *Sorex cinereus*), and either a two-letter state code for U.S. specimens or a complete province name for Canadian specimens.

```
                          10        20        30        40        50
                          .         .         .         .         .
MF4745                    ATGACAAATATCCGAAAAACTCACCCACTAATAAAAATCATCAACAGCTC
MF4859                    ..................................................
MF5356                    ..................................................
MF5376                    ..................................................
MF6156                    ..................................................
MF6397                    ..................................................
MF7497                    ..................................................
MF7498                    ..................................................
MF8049                    ..................................................
MF8050                    ..................................................
MF8051                    ..................................................
MF8052                    ..................................................
MF8053                    ..................................................
MF8054                    ..................................................
MF8055                    ..................................................
MF8224                    ..................................................
MF8802.A                  ..................................................
MF8231.A                  ..................................................
MF8801.A                  ..................................................
MF8803                    ..................................................
MF9157                    ..................................................
MF8057                    ..................................................
MF9158                    ..................................................
75.B.hyl.NB               ..........................C.......................
76.B.hyl.NB               ..........................C.......................
78.B.hyl.NB               ..........................C.......................
80.B.hyl.NB               ..........................C.......................
81.B.hyl.NB               ..........................C.......................
82.B.hyl.KS               ..........................C.......................
77.B.hyl.KS               ..........................C.......................
79.B.hyl.NB               ..........................C...................TG..
70.B.bre.NH               ........C.................C...........T...........
71.B.bre.NH               ........C.................C...........T...........
73.B.bre.VA               ........C.................C...........T...........
74.B.bre.VA               ........C.................C...........T...........
67.B.bre.KY               .....T..C...........C.....C...........T..?........
69.B.bre.OH               ........C.................C...........T...........
68.B.bre.KY               .....T..C.................C...........T...........
72.B.bre.OH               ........C.................C...........T...........
61.B.bre.NB               ..................G........C...........T...........
62.B.bre.Manitoba         ..................G........C...........T...........
63.B.bre.Manitoba         ..................G........C...........T...........
64.B.bre.NB               ..................G........C...........T...........
65.B.bre.NB               ..................G........C...........T...........
66.B.bre.IA               ..................G........C...........T...........
55.B.car.LA               ..........................C.......................
56.B.car.Ark              ..........................C.......................
57.B.car.LA               ..........................C.......................
58.B.car.IL               ..........................C.......................
53.B.car.FL               ..........................C.......................
54.B.car.FL               ..........................C.......................
59.B.car.Ark              ..........................C.......................
60.B.car.IL               ..........................C.......................
49.B.car.GA               ..........................C.......................
50.B.car.GA               ..........................C.......................
51.B.car.VA               ..........................C.......................
52.B.car.VA               ..........................C.......................
85.Sorex                  ........CC.A........C.....CT..........T..T........
86.Sorex                  ........CC.A........C.....CT..........T..T........
84.Cryptotis              ........C............C..T.....T.....................
83.Cryptotis              ........C............C..T.....T.....................
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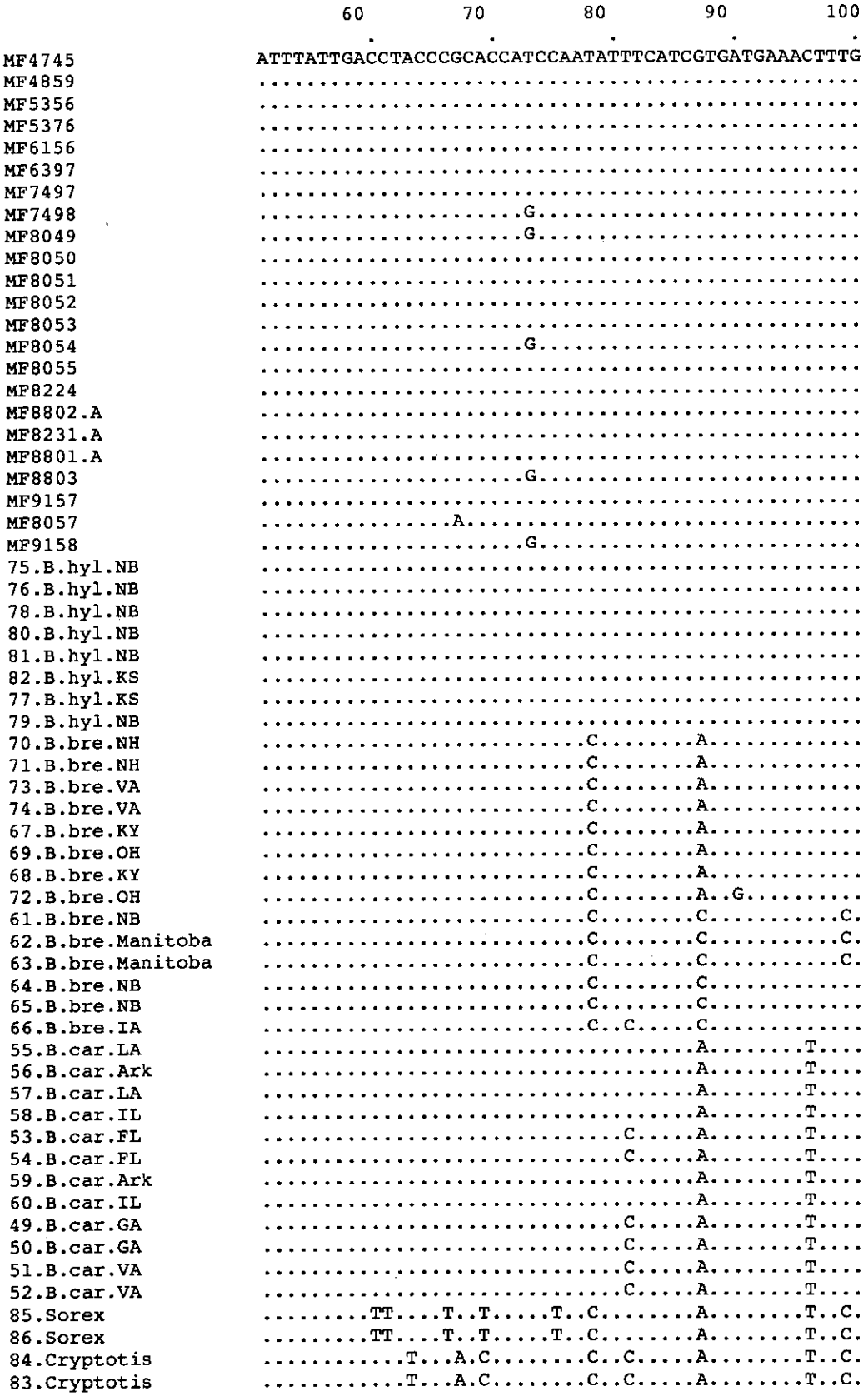
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MF5376
MF6156
MF6397
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MF7498 G
MF8049 G
MF8050
MF8051
MF8052
MF8053
MF8054 G
MF8055
MF8224
MF8802.A
MF8231.A
MF8801.A
MF8803 G
MF9157
MF8057 A
MF9158 G
75.B.hyl.NB
76.B.hyl.NB
78.B.hyl.NB
80.B.hyl.NB
81.B.hyl.NB
82.B.hyl.KS
77.B.hyl.KS
79.B.hyl.NB
70.B.bre.NH C A
71.B.bre.NH C A
73.B.bre.VA C A
74.B.bre.VA C A
67.B.bre.KY C A
69.B.bre.OH C A
68.B.bre.KY C A
72.B.bre.OH C A G
61.B.bre.NB C C C
62.B.bre.Manitoba C C C
63.B.bre.Manitoba C C C
64.B.bre.NB C C
65.B.bre.NB C C
66.B.bre.IA C C C
55.B.car.LA A T
56.B.car.Ark A T
57.B.car.LA A T
58.B.car.IL A T
53.B.car.FL C A T
54.B.car.FL C A T
59.B.car.Ark A T
60.B.car.IL A T
49.B.car.GA C A T
50.B.car.GA C A T
51.B.car.VA C A T
52.B.car.VA C A T
85.Sorex TT T T T C A T C
86.Sorex TT T T T C A T C
84.Cryptotis T A C C C A T C
83.Cryptotis T A C C C A T C

```
                          110         120         130         140         150
                           .           .           .           .           .
MF4745                    GCTCCCTATTAGGCATTTGCCTAATCATTCAAATCCTAACAGGCCTATTC
MF4859                    .............................................................
MF5356                    .............................................................
MF5376                    .............................................................
MF6156                    .............................................................
MF6397                    .............................................................
MF7497                    .............................................................
MF7498                    .............................................................
MF8049                    .............................................................
MF8050                    .............................................................
MF8051                    .............................................................
MF8052                    .............................................................
MF8053                    .............................................................
MF8054                    .............................................................
MF8055                    .............................................................
MF8224                    .............................................................
MF8802.A                  .............................................................
MF8231.A                  .............................................................
MF8801.A                  .............................................................
MF8803                    .............................................................
MF9157                    .............................................................
MF8057                    .............................................................
MF9158                    .............................................................
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76.B.hyl.NB               ........C....................................................
78.B.hyl.NB               ........C....................................................
80.B.hyl.NB               ........C....................................................
81.B.hyl.NB               ........C....................................................
82.B.hyl.KS               ........C....................................................
77.B.hyl.KS               ........C....................................................
79.B.hyl.NB               ........C....................................................
70.B.bre.NH               .......GC.................T..C..G...T.............
71.B.bre.NH               .......GC.................T..C..G...T.............
73.B.bre.VA               .......GC.................T..C..G...T.............
74.B.bre.VA               .......GC.................T..C..G...T.............
67.B.bre.KY               .......GC.................T.........T.............
69.B.bre.OH               .......GC.................T.........T.............
68.B.bre.KY               .......GC.................T.........T.............
72.B.bre.OH               .......GC.................T..C..G...T.............
61.B.bre.NB               .......GC...............G.T.........T.............
62.B.bre.Manitoba         .......GC...............G.T.........T.............
63.B.bre.Manitoba         .......GC...............G.T.........T.............
64.B.bre.NB               ........C...............G.T.........T.............
65.B.bre.NB               .......GC...............G.T.........T.............
66.B.bre.IA               .......GC...............G.T.........T.............
55.B.car.LA               ........C.................T.........T.G...........
56.B.car.Ark              ........C.................T.........T.G...........
57.B.car.LA               ........C.................T.........T.G...........
58.B.car.IL               ........C.................T.........T.G...........
53.B.car.FL               ............T.....................T.............
54.B.car.FL               ............T.....................T.............
59.B.car.Ark              ........C.................T.........T.G...........
60.B.car.IL               ........C.................T.........T.G...........
49.B.car.GA               ........................T.....G...T.............
50.B.car.GA               ........................T.........T.............
51.B.car.VA               ........................T.........T.............
52.B.car.VA               ........................T.........T.............
85.Sorex                  .T.....TC....TG.....T.....G.A.....T..T.....A......
86.Sorex                  .T.....TC....TG.....T.....G.A.....T..T.....A......
84.Cryptotis              ....TT..C.......C...T....T..C......T.......T.....T
83.Cryptotis              ....TT..C.......C...T....T..C......T.......T.....T
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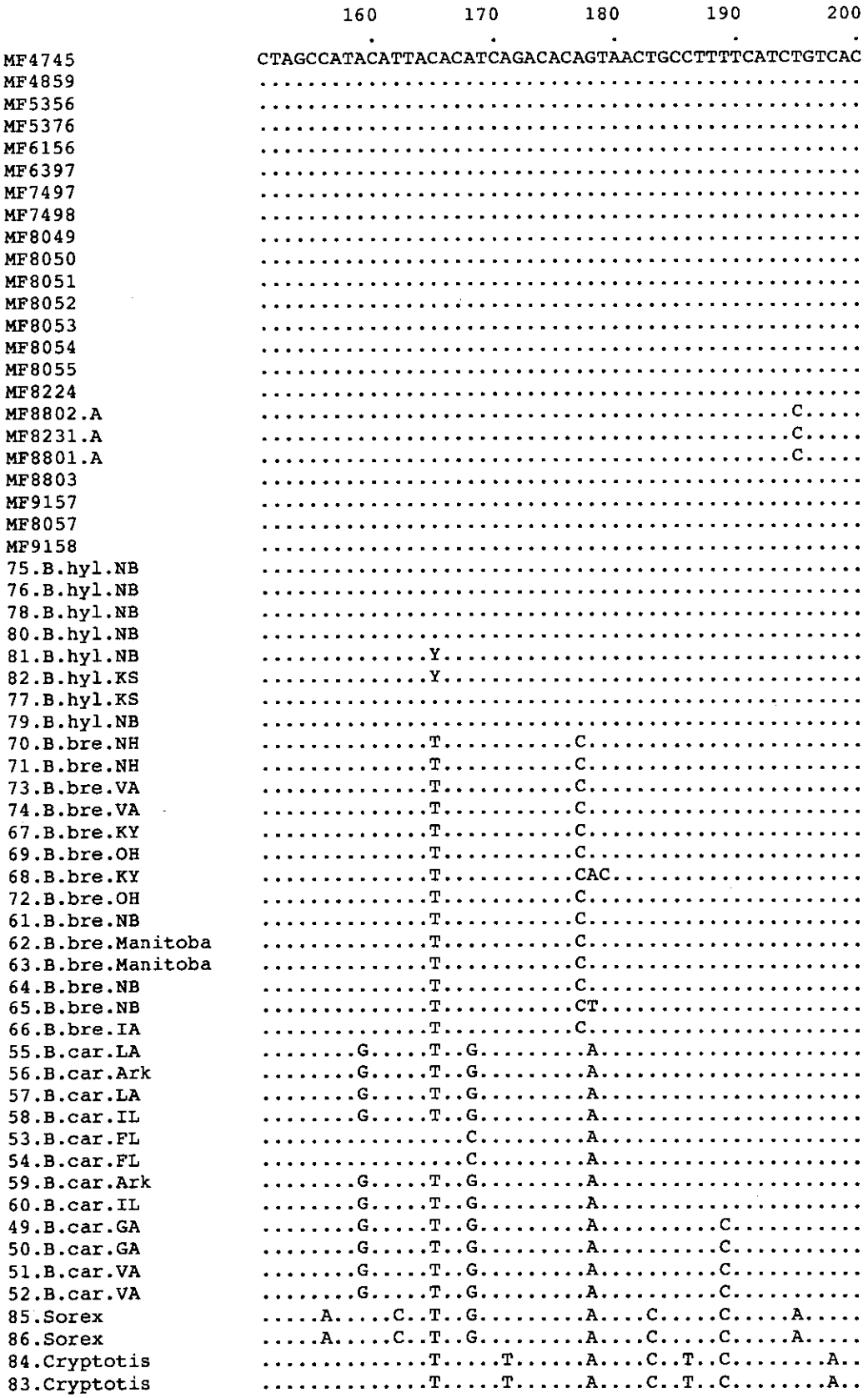
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MF4859
MF5356
MF5376
MF6156
MF6397
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MF8050
MF8051
MF8052
MF8053
MF8054
MF8055
MF8224
MF8802.A
MF8231.A
MF8801.A
MF8803
MF9157
MF8057
MF9158
75.B.hyl.NB
76.B.hyl.NB
78.B.hyl.NB
80.B.hyl.NB
81.B.hyl.NB
82.B.hyl.KS
77.B.hyl.KS
79.B.hyl.NB
70.B.bre.NH
71.B.bre.NH
73.B.bre.VA
74.B.bre.VA
67.B.bre.KY
69.B.bre.OH
68.B.bre.KY
72.B.bre.OH
61.B.bre.NB
62.B.bre.Manitoba
63.B.bre.Manitoba
64.B.bre.NB
65.B.bre.NB
66.B.bre.IA
55.B.car.LA
56.B.car.Ark
57.B.car.LA
58.B.car.IL
53.B.car.FL
54.B.car.FL
59.B.car.Ark
60.B.car.IL
49.B.car.GA
50.B.car.GA
51.B.car.VA
52.B.car.VA
85.Sorex
86.Sorex
84.Cryptotis
83.Cryptotis

```
                        210        220        230        240        250
                         .          .          .          .          .
MF4745                  CCATATTTGCCGAGACGTTAATTACGGTTGACTGATCCGCTATCTACACG
MF4859                  ..................................................
MF5356                  ..................................................
MF5376                  ..................................................
MF6156                  ..................................................
MF6397                  ..................................................
MF7497                  ..................................................
MF7498                  ..................................................
MF8049                  ..................................................
MF8050                  ..................................................
MF8051                  ..................................................
MF8052                  ..................................................
MF8053                  ..................................................
MF8054                  ..................................................
MF8055                  ..................................................
MF8224                  ..................................................
MF8802.A                ......C.............C.............................
MF8231.A                ......C.............C.............................
MF8801.A                ......C.............C.............................
MF8803                  ..................................................
MF9157                  ..................................................
MF8057                  ..................................................
MF9158                  ..................................................
75.B.hyl.NB             ....................C.............................
76.B.hyl.NB             ....................C.............................
78.B.hyl.NB             ....................C.............................
80.B.hyl.NB             ....................C.............................
81.B.hyl.NB             ..................................................
82.B.hyl.KS             ....................C.............................
77.B.hyl.KS             ....................C.............................
79.B.hyl.NB             ....................C.............................
70.B.bre.NH             ...C........G.....C.............A.................
71.B.bre.NH             ...C........G.....C.............A.................
73.B.bre.VA             ...C........G.....C.............A.................
74.B.bre.VA             ...C........G.....C.............A.................
67.B.bre.KY             ...C..............C.............A.................
69.B.bre.OH             ...C..............C.............A.................
68.B.bre.KY             T..C..............C.............A...........T.....
72.B.bre.OH             ...C........G.....C.............A.................
61.B.bre.NB             ...C..............C.........C.....A.................
62.B.bre.Manitoba       ...C..............C.........C.....A.................
63.B.bre.Manitoba       ...C..............C.........C.....A.................
64.B.bre.NB             ...C..............C.........C.....A.................
65.B.bre.NB             ...C..............C.........C.....A.................
66.B.bre.IA             ...C..............C.........C.....A.................
55.B.car.LA             ...C........................C..G...................
56.B.car.Ark            ...C........................C..G...................
57.B.car.LA             ...C........................C..G...................
58.B.car.IL             ...C........................C..G...................
53.B.car.FL             ............................C..G.............G.....
54.B.car.FL             ............................C..G.............G.....
59.B.car.Ark            ...C........................C..G...................
60.B.car.IL             ...C........................C..G...................
49.B.car.GA             ............................C.................G.....
50.B.car.GA             ............................C.................G.....
51.B.car.VA             ............................C.................G.....
52.B.car.VA             ............................C.................G.....
85.Sorex                A..C.....T........A..C.....A.....A........C..T..T.
86.Sorex                A..C.....T........A..C.....A.....A........C..T..T.
84.Cryptotis            ......C...........A.....T........A.....A...T.......
83.Cryptotis            ......C...........A.....T........A.....A...T.......
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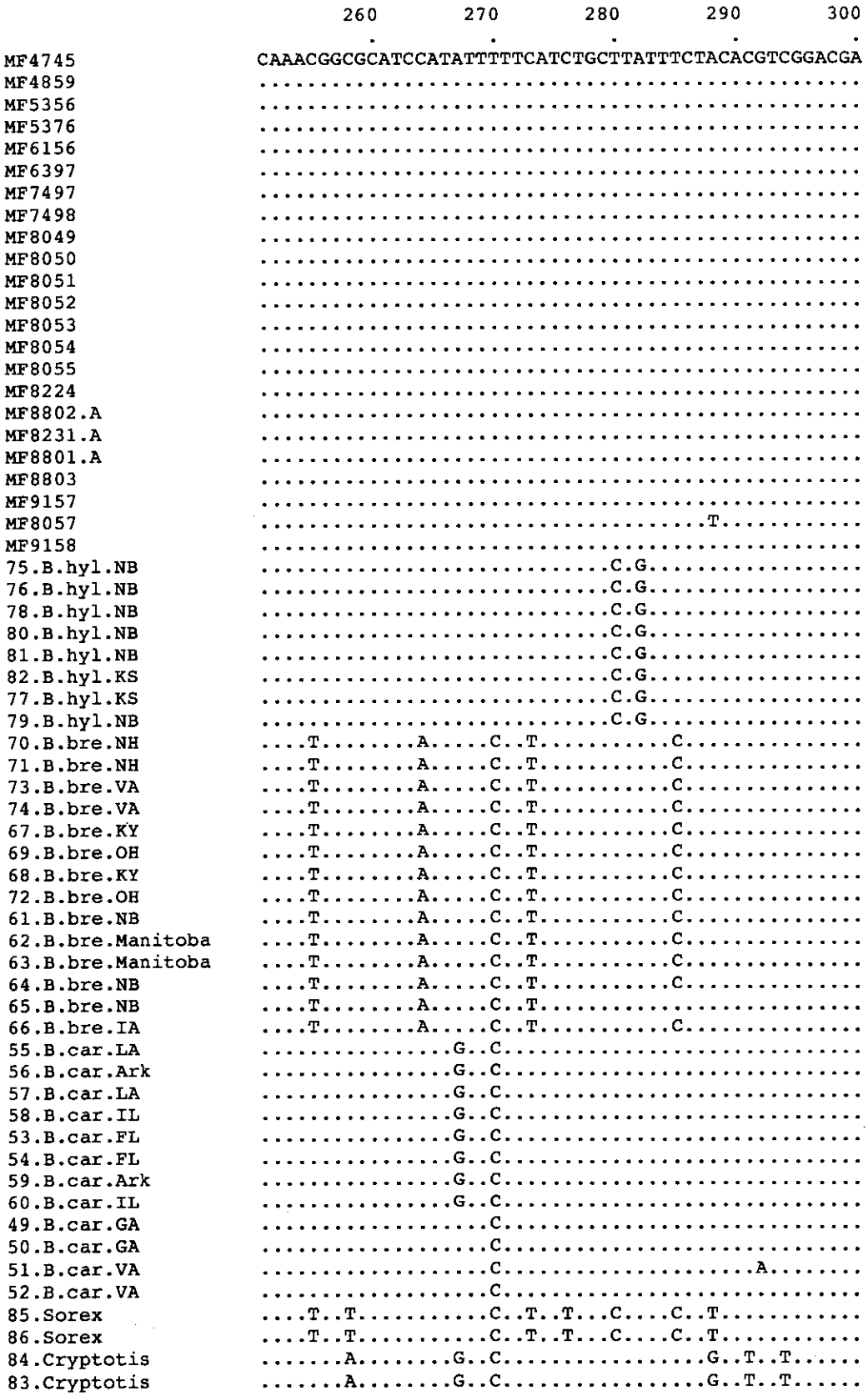
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MF8224
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MF8231.A
MF8801.A
MF8803
MF9157
MF8057 T
MF9158
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76.B.hyl.NB C.G
78.B.hyl.NB C.G
80.B.hyl.NB C.G
81.B.hyl.NB C.G
82.B.hyl.KS C.G
77.B.hyl.KS C.G
79.B.hyl.NB C.G
70.B.bre.NH T A C T C
71.B.bre.NH T A C T C
73.B.bre.VA T A C T C
74.B.bre.VA T A C T C
67.B.bre.KY T A C T C
69.B.bre.OH T A C T C
68.B.bre.KY T A C T C
72.B.bre.OH T A C T C
61.B.bre.NB T A C T C
62.B.bre.Manitoba T A C T C
63.B.bre.Manitoba T A C T C
64.B.bre.NB T A C T C
65.B.bre.NB T A C T
66.B.bre.IA T A C T C
55.B.car.LA G C
56.B.car.Ark G C
57.B.car.LA G C
58.B.car.IL G C
53.B.car.FL G C
54.B.car.FL G C
59.B.car.Ark G C
60.B.car.IL G C
49.B.car.GA C
50.B.car.GA C
51.B.car.VA C A
52.B.car.VA C
85.Sorex T T C T T C C T
86.Sorex T T C T T C C T
84.Cryptotis A G C G T T
83.Cryptotis A G C G T T

```
                          310        320        330        340        350
                           .          .          .          .          .
MF4745                    GGTCTTTACTACGGATCCTATATATTTCTAGAGACATGAAACATTGGTGT
MF4859                    ...........................................
MF5356                    ...........................................
MF5376                    ...........................................
MF6156                    ...........................................
MF6397                    ...........................................
MF7497                    ...........................................
MF7498                    ...........................................
MF8049                    ...........................................
MF8050                    ...........................................
MF8051                    ...........................................
MF8052                    ...........................................
MF8053                    ...........................................
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MF8224                    ...........................................
MF8802.A                  ...........................................
MF8231.A                  ...........................................
MF8801.A                  ...........................................
MF8803                    ...........................................
MF9157                    ...........................................
MF8057                    ...........................................
MF9158                    ...........................................
75.B.hyl.NB               ................A..........................
76.B.hyl.NB               ................A..........................
78.B.hyl.NB               ............G..A..........................
80.B.hyl.NB               ............G..A..........................
81.B.hyl.NB               ................A.........T................
82.B.hyl.KS               ................A.........T................
77.B.hyl.KS               ................A..........................
79.B.hyl.NB               ................A..........................
70.B.bre.NH               ..C..........G.....C.....CT....A...........
71.B.bre.NH               ..C..........G.....C.....CT....A...........
73.B.bre.VA               ..C..........G.....C.....CT....A...........
74.B.bre.VA               ..C..........G.....C.....CT....A...........
67.B.bre.KY               ..C..........G.....C.....CT....A...........
69.B.bre.OH               ..C..........G.....C.....CT....A...........
68.B.bre.KY               ..C..........G.....C.....CT....A...........
72.B.bre.OH               ..C..........G.....C.....CT....A...........
61.B.bre.NB               ..C..........G.....C.....CA....A...........
62.B.bre.Manitoba         ..C..........G.....C.....CT....A...........
63.B.bre.Manitoba         ..C..........G.....C.....CT....A...........
64.B.bre.NB               ..C..........G.....C.....CT....A...........
65.B.bre.NB               ..C..........G.....C.....CT....A...........
66.B.bre.IA               ..C..........G.....C.....CT....A...........
55.B.car.LA               ..C..........G.....C.....CT................
56.B.car.Ark              ..C..........G.....C.....CT................
57.B.car.LA               ..C..........G.....C.....CTC...............
58.B.car.IL               ..C..........G.....C.....CT................
53.B.car.FL               ..C..........G.....C......T.G..............
54.B.car.FL               ..C..........G.....C......T.G..............
59.B.car.Ark              ..C..........G.....C.....CT................
60.B.car.IL               ..C..........G.....C.....CT................
49.B.car.GA               ..C..............C.....CT.G................
50.B.car.GA               ..C..............C.....CT.G................
51.B.car.VA               ..C..............C.....CT.G................
52.B.car.VA               ..C..............C.....CT.G................
85.Sorex                  .....C..T........A........C.....A...........C..A..
86.Sorex                  .....C..T........A........C.....A...........C..A..
84.Cryptotis              ..C..A...........A.........A.............T.....G..
83.Cryptotis              ..C..A...........A.........A.............T.....G..
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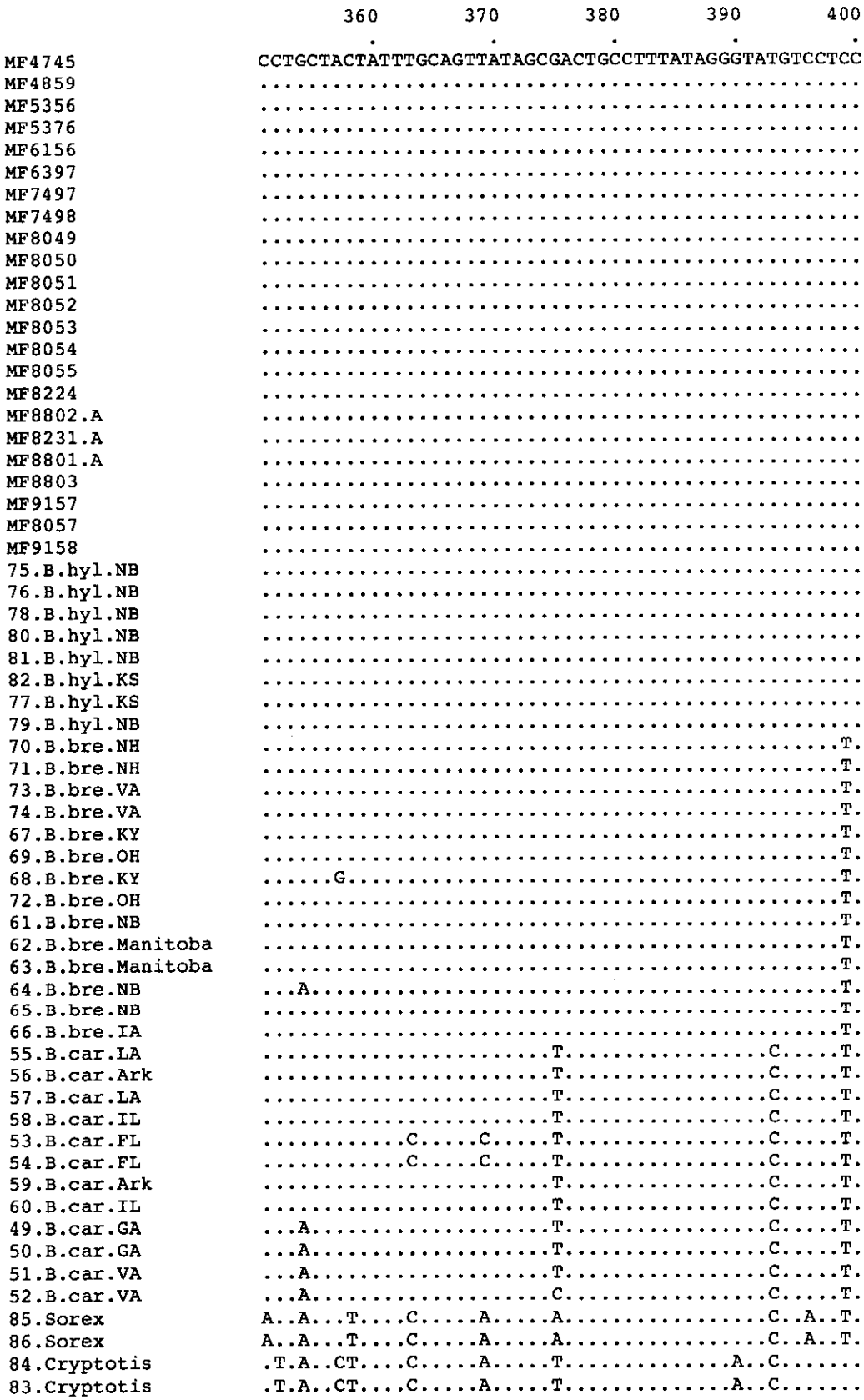
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MF8224
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MF8231.A
MF8801.A
MF8803
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MF8057
MF9158
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78.B.hyl.NB
80.B.hyl.NB
81.B.hyl.NB
82.B.hyl.KS
77.B.hyl.KS
79.B.hyl.NB
70.B.bre.NH
71.B.bre.NH
73.B.bre.VA
74.B.bre.VA
67.B.bre.KY
69.B.bre.OH
68.B.bre.KY
72.B.bre.OH
61.B.bre.NB
62.B.bre.Manitoba
63.B.bre.Manitoba
64.B.bre.NB
65.B.bre.NB
66.B.bre.IA
55.B.car.LA
56.B.car.Ark
57.B.car.LA
58.B.car.IL
53.B.car.FL
54.B.car.FL
59.B.car.Ark
60.B.car.IL
49.B.car.GA
50.B.car.GA
51.B.car.VA
52.B.car.VA
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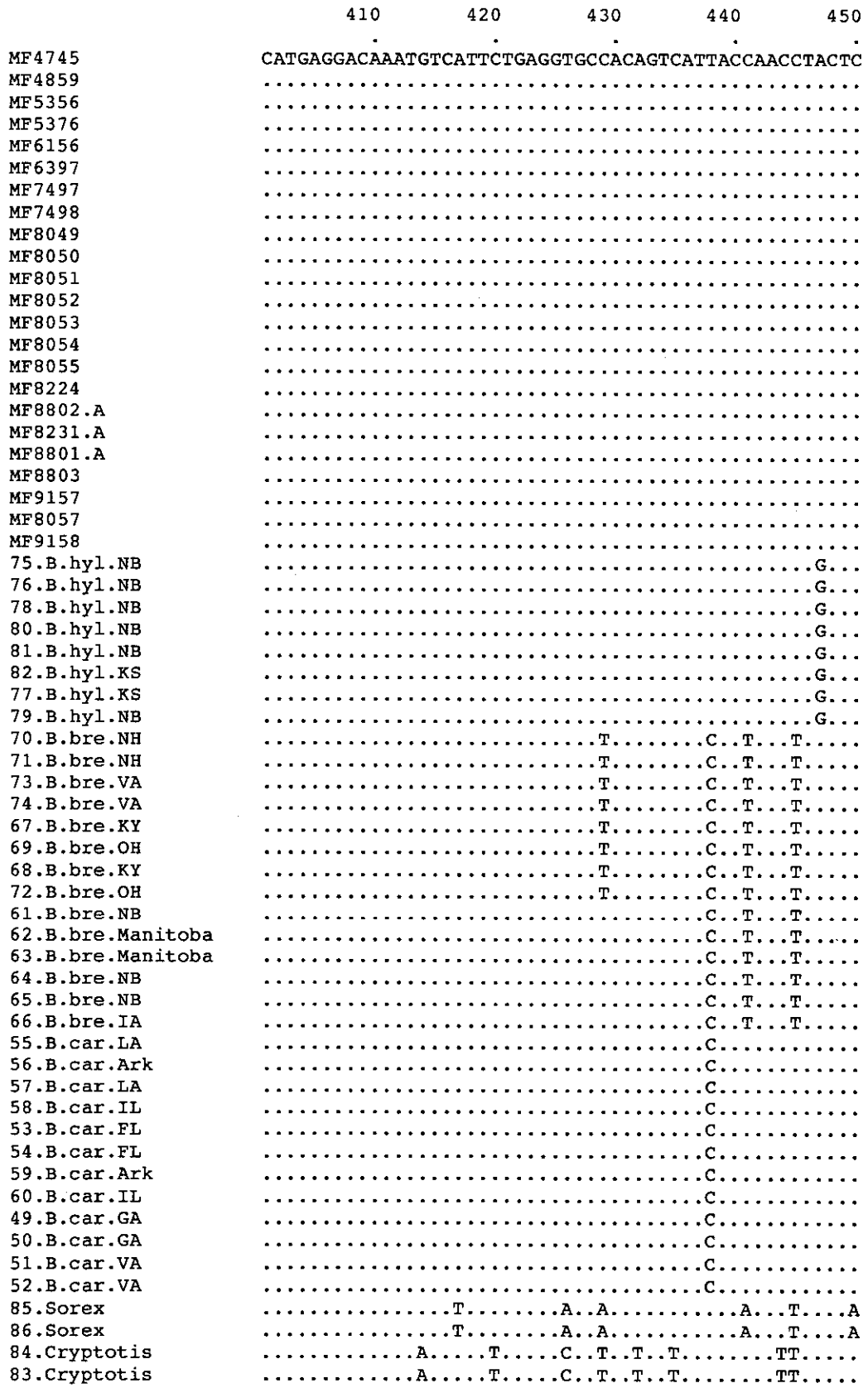
86.Sorex
84.Cryptotis
83.Cryptotis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75.B.hyl.NB
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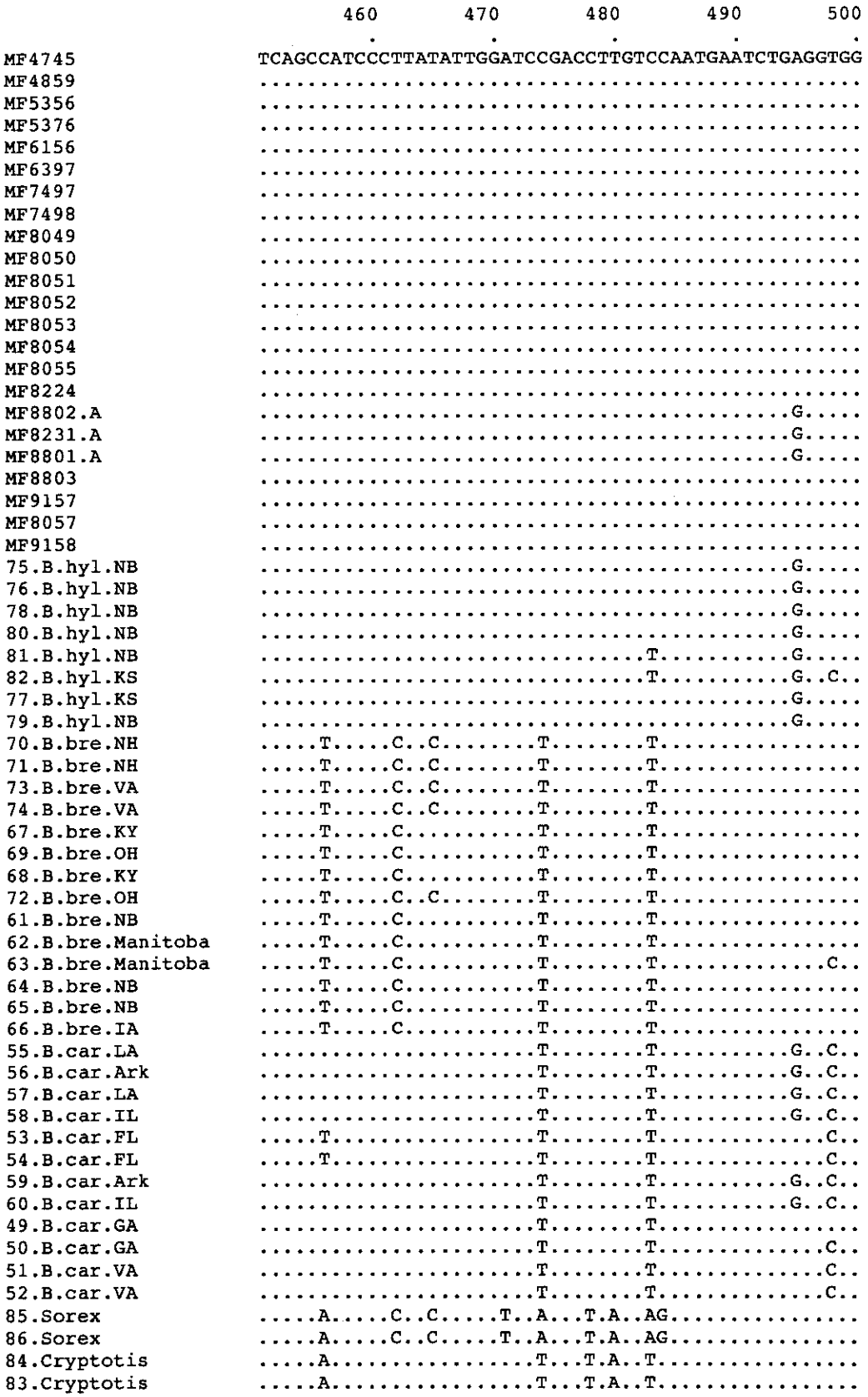
80.B.hyl.NB
81.B.hyl.NB
82.B.hyl.KS
77.B.hyl.KS
79.B.hyl.NB
70.B.bre.NH
71.B.bre.NH
73.B.bre.VA
74.B.bre.VA
67.B.bre.KY
69.B.bre.OH
68.B.bre.KY
72.B.bre.OH
61.B.bre.NB
62.B.bre.Manitoba
63.B.bre.Manitoba
64.B.bre.NB
65.B.bre.NB
66.B.bre.IA
55.B.car.LA
56.B.car.Ark
57.B.car.LA
58.B.car.IL
53.B.car.FL
54.B.car.FL
59.B.car.Ark
60.B.car.IL
49.B.car.GA
50.B.car.GA
51.B.car.VA
52.B.car.VA
85.Sorex
86.Sorex
84.Cryptotis
83.Cryptotis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MF5356
MF5376
MF6156
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MF8052
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MF8054
MF8055
MF8224
MF8802.A
MF8231.A
MF8801.A
MF8803
MF9157
MF8057
MF9158
75.B.hyl.NB
76.B.hyl.NB
78.B.hyl.NB
80.B.hyl.NB
81.B.hyl.NB
82.B.hyl.KS
77.B.hyl.KS
79.B.hyl.NB
70.B.bre.NH
71.B.bre.NH
73.B.bre.VA
74.B.bre.VA
67.B.bre.KY
69.B.bre.OH
68.B.bre.KY
72.B.bre.OH
61.B.bre.NB
62.B.bre.Manitoba
63.B.bre.Manitoba
64.B.bre.NB
65.B.bre.NB
66.B.bre.IA
55.B.car.LA
56.B.car.Ark
57.B.car.LA
58.B.car.IL
53.B.car.FL
54.B.car.FL
59.B.car.Ark
60.B.car.IL
49.B.car.GA
50.B.car.GA
51.B.car.VA
52.B.car.VA
85.Sorex
86.Sorex
84.Cryptotis
83.Cryptotis

```
                          510        520        530        540        550
                           .          .          .          .          .
MF4745                    ATTCTCAGTTGACAAAGCAACTCTTACCCGATTCTTCGCCTTCCACTTCA
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MF5356                    ..................................................
MF5376                    ..................................................
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MF6397                    ..................................................
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MF8055                    ..................................................
MF8224                    ..................................................
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MF8231.A                  ..................................T...............
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MF9157                    ..................................................
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81.B.hyl.NB               ............T.....................................T.
82.B.hyl.KS               ............T.....................................T.
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79.B.hyl.NB               ..................................................
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71.B.bre.NH               ...T..............C..C............................
73.B.bre.VA               ...T..............C..C............................
74.B.bre.VA               ...T..............C..C............................
67.B.bre.KY               G..T..............C..C..............T...........
69.B.bre.OH               ...T..............C..C..............T...........
68.B.bre.KY               G..T..............C..C..............T...........
72.B.bre.OH               ...T..............C..C............................
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63.B.bre.Manitoba         ...T.....C..........C..............................
64.B.bre.NB               ...T.....C..........C..............................
65.B.bre.NB               ...T.....C..........C..............................
66.B.bre.IA               ...T.....C..........C..............................
55.B.car.LA               G..................................T...............
56.B.car.Ark              G..................................................
57.B.car.LA               G..................................................
58.B.car.IL               G..................................................
53.B.car.FL               G.........................T..........T.....T....
54.B.car.FL               G.........................T..........T.....T....
59.B.car.Ark              G..................................................
60.B.car.IL               G..................................................
49.B.car.GA               G........C..................T..........T........T.
50.B.car.GA               G........C..................T..........T........T.
51.B.car.VA               G........C..................T..........T........T.
52.B.car.VA               G........C..................T..........T........T.
85.Sorex                  ......T..A........C..C..A..T..........T........T.
86.Sorex                  ......T..A........C..C..A..T..........T........T.
84.Cryptotis              C..T.....A..........C.....T..........T........T.
83.Cryptotis              C..T.....A..........C.....T..........T........T.
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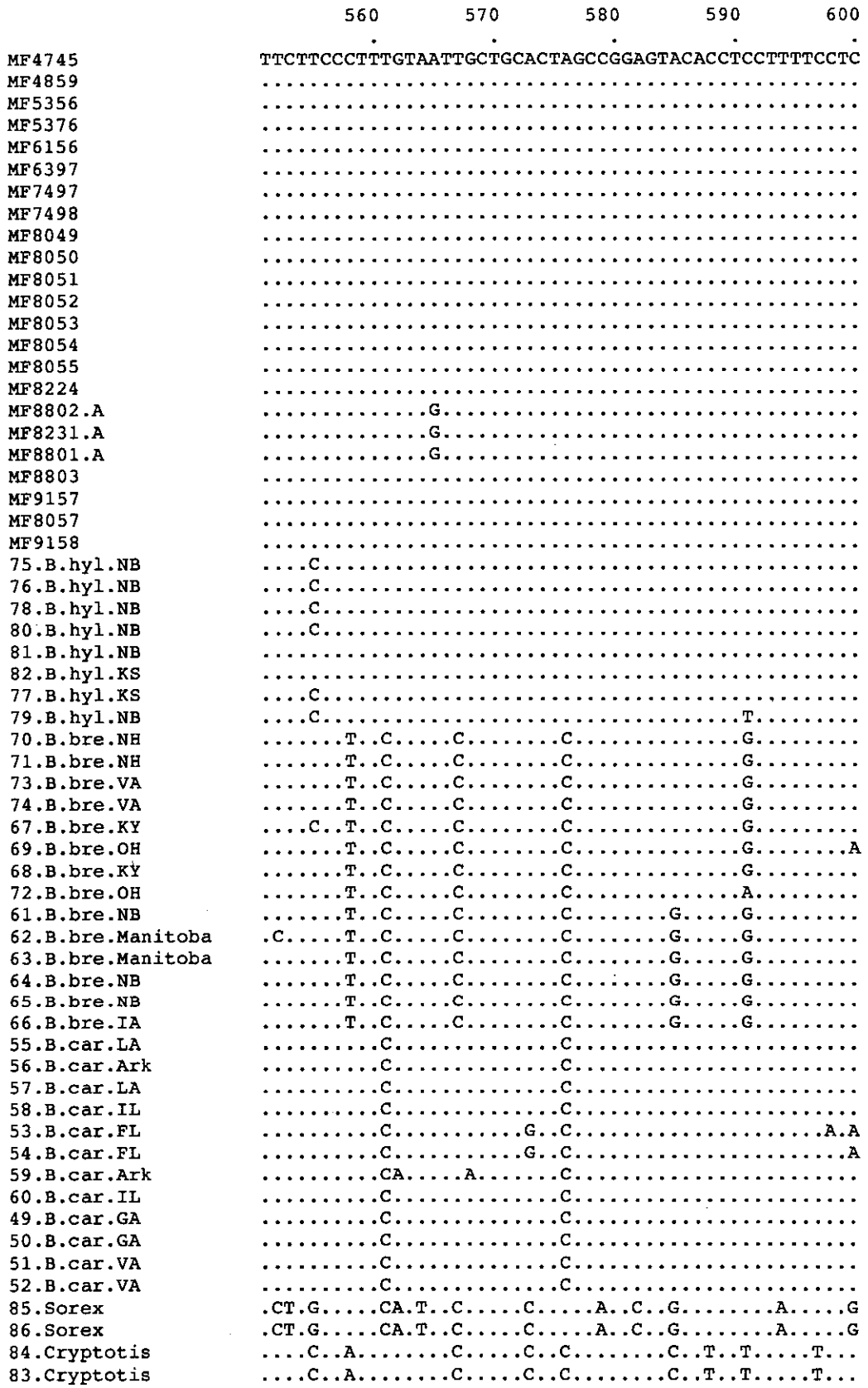
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MF8054
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MF8224
MF8802.A
MF8231.A
MF8801.A
MF8803
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71.B.bre.NH
73.B.bre.VA
74.B.bre.VA
67.B.bre.KY
69.B.bre.OH
68.B.bre.KY
72.B.bre.OH
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63.B.bre.Manitoba
64.B.bre.NB
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56.B.car.Ark
57.B.car.LA
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54.B.car.FL
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60.B.car.IL
49.B.car.GA
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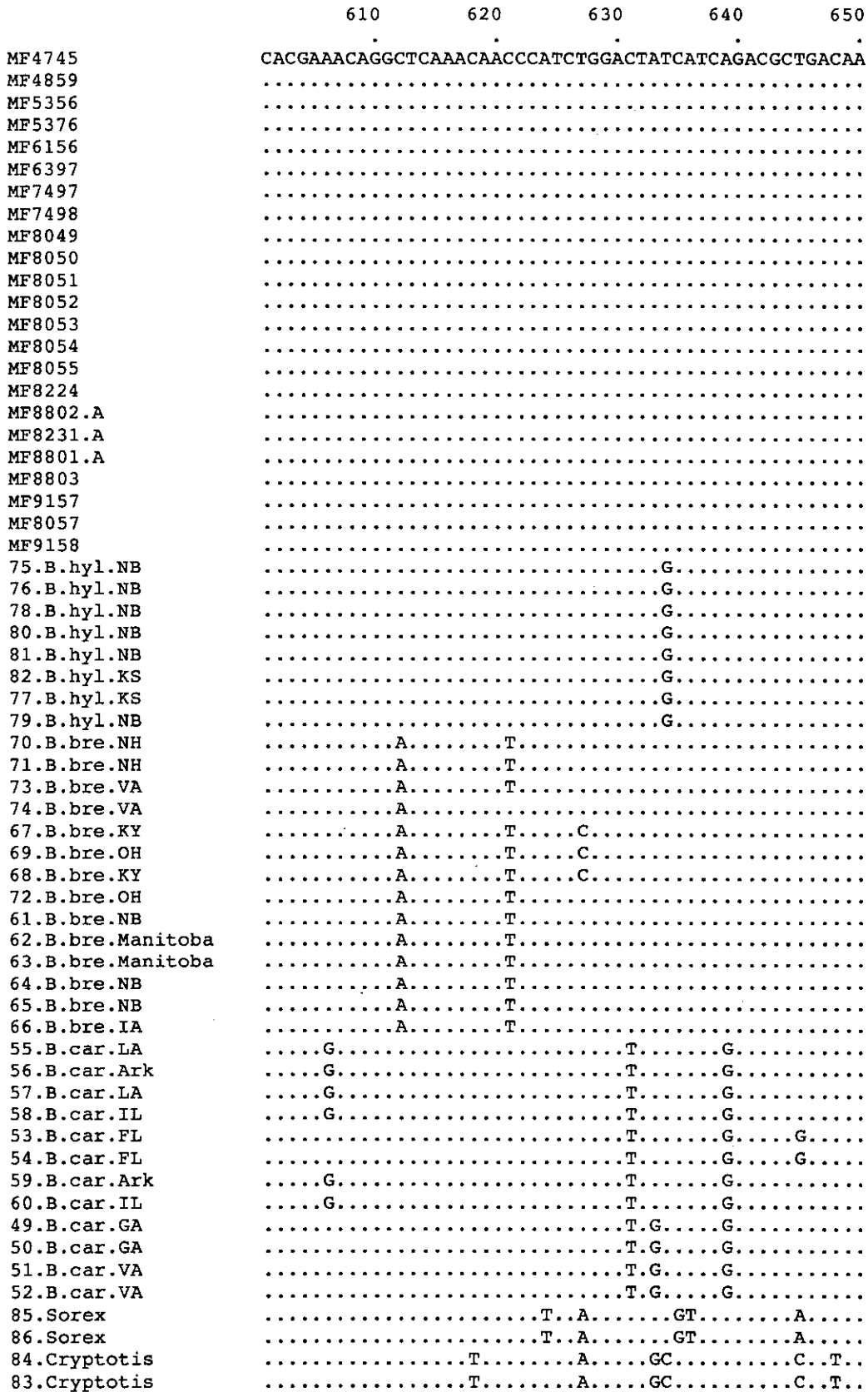
85.Sorex
86.Sorex
84.Cryptotis
83.Cryptotis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78.B.hyl.NB
80.B.hyl.NB
81.B.hyl.NB
82.B.hyl.KS
77.B.hyl.KS
79.B.hyl.NB
70.B.bre.NH
71.B.bre.NH
73.B.bre.VA
74.B.bre.VA
67.B.bre.KY
69.B.bre.OH
68.B.bre.KY
72.B.bre.OH
61.B.bre.NB
62.B.bre.Manitoba
63.B.bre.Manitoba
64.B.bre.NB
65.B.bre.NB
66.B.bre.IA
55.B.car.LA
56.B.car.Ark
57.B.car.LA
58.B.car.IL
53.B.car.FL
54.B.car.FL
59.B.car.Ark
60.B.car.IL
49.B.car.GA
50.B.car.GA
51.B.car.VA
52.B.car.VA
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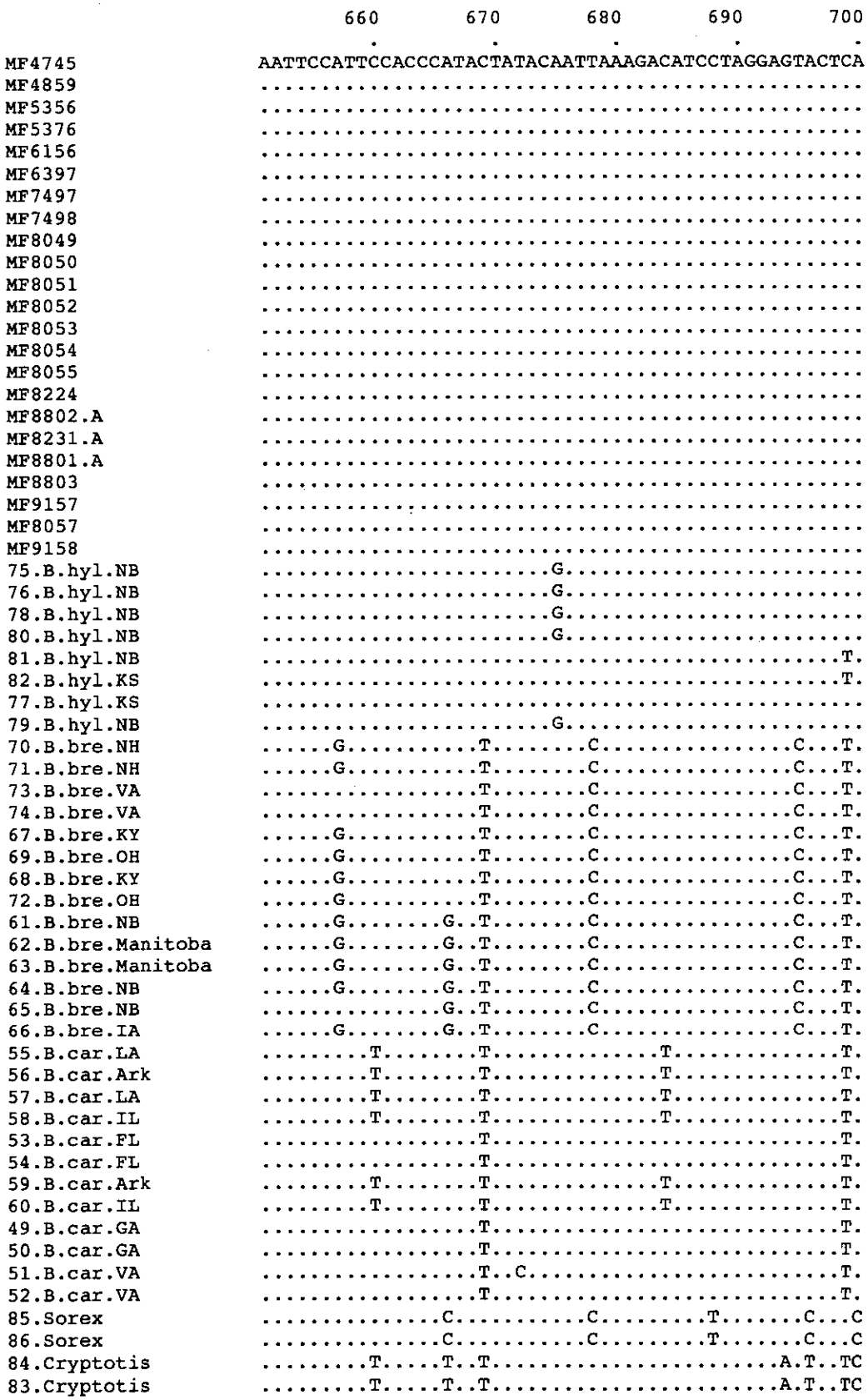
86.Sorex
84.Cryptotis
83.Cryptotis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MF5376
MF6156
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MF8049
MF8050
MF8051
MF8052
MF8053
MF8054
MF8055
MF8224
MF8802.A
MF8231.A
MF8801.A
MF8803
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MF8057
MF9158
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76.B.hyl.NB
78.B.hyl.NB
80.B.hyl.NB
81.B.hyl.NB
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77.B.hyl.KS
79.B.hyl.NB
70.B.bre.NH
71.B.bre.NH
73.B.bre.VA
74.B.bre.VA
67.B.bre.KY
69.B.bre.OH
68.B.bre.KY
72.B.bre.OH
61.B.bre.NB
62.B.bre.Manitoba
63.B.bre.Manitoba
64.B.bre.NB
65.B.bre.NB
66.B.bre.IA
55.B.car.LA
56.B.car.Ark
57.B.car.LA
58.B.car.IL
53.B.car.FL
54.B.car.FL
59.B.car.Ark
60.B.car.IL
49.B.car.GA
50.B.car.GA
51.B.car.VA
52.B.car.VA
85.Sorex
86.Sorex
84.Cryptotis
83.Cryptotis

```
                          710        720        730        740        750
                           .          .          .          .          .
MF4745                    TCTTGATCCTAGTACTAACATGCCTAGTACTATTTTCTCCAGACTTACTA
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MF5356                    ..........................................................
MF5376                    ..........................................................
MF6156                    ..........................................................
MF6397                    ..........................................................
MF7497                    ..........................................................
MF7498                    ..........................................................
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MF8050                    ..........................................................
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MF8055                    ..........................................................
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MF8802.A                  ..........................................................
MF8231.A                  ..........................................................
MF8801.A                  ..........................................................
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MF9157                    ..........................................................
MF8057                    ..........................................................
MF9158                    ..........................................................
75.B.hyl.NB               ..........................................................
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78.B.hyl.NB               ..................................C.......................
80.B.hyl.NB               ..................................C.......................
81.B.hyl.NB               ..........................................................
82.B.hyl.KS               ..........................................C.....C.....
77.B.hyl.KS               ..........................................................
79.B.hyl.NB               ..........................................................
70.B.bre.NH               ..C.....................G.....C.....C.....C.....
71.B.bre.NH               ..C.....................G.....C.....C.....C.....
73.B.bre.VA               ..C.....................G.....C.....C.....C.....
74.B.bre.VA               ..C.....................G.....C.....C.....C.....
67.B.bre.KY               ..C.....................G.....C.....C.....C.....
69.B.bre.OH               ..C.....................G.....C.....C.....C.....
68.B.bre.KY               ..C.....................G.....C.....C.....C.....
72.B.bre.OH               ..C.....................G.....C.....C.....C.....
61.B.bre.NB               ..C.....................G.....C.....C.....C.....
62.B.bre.Manitoba         ..C.....................G.....C.....C.....C.....
63.B.bre.Manitoba         ..C.....................G.....C.....C.....C.....
64.B.bre.NB               ..C.....................G.....C.....C.....C.....
65.B.bre.NB               ..C.....................G.....C.....C.....C.....
66.B.bre.IA               ..C.....................G.....C.....C.....C.....
55.B.car.LA               ..C..........................................C..T..
56.B.car.Ark              ..C..........................................C..T..
57.B.car.LA               ..C..........................................C..T..
58.B.car.IL               ..C..........................................C..T..
53.B.car.FL               ..C.............G.........G.............C..T.G
54.B.car.FL               ..C.............G.........G.............C..T.G
59.B.car.Ark              ..C..........................................C..T..
60.B.car.IL               ..C..........................................C..T..
49.B.car.GA               ..C.....................G...........G...C..T..
50.B.car.GA               ..C.....................G...........G...C..T..
51.B.car.VA               ..C.....................G...........G...C..T..
52.B.car.VA               ..C.....................G...........G...C..T..
85.Sorex                  .AC.C..TT....C.......C.T....CT....C..C..T..T......
86.Sorex                  .AC.C..TT....C.......C.T....CT....C..C..T..T......
84.Cryptotis              ..C.A..................T.........C.....C.....C.....
83.Cryptotis              ..C.A..................T.........C.....C.....C.....
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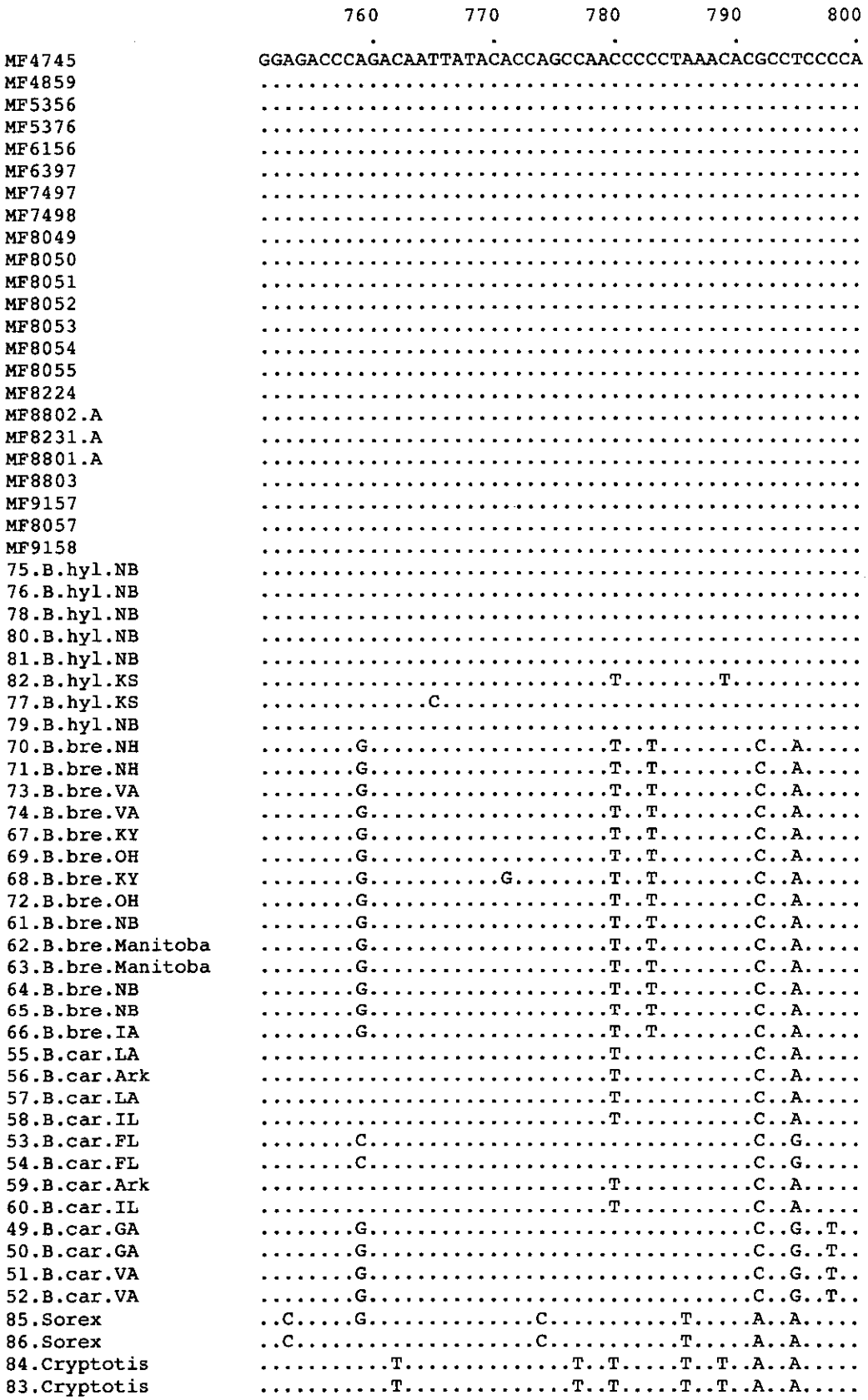
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MF8802.A
MF8231.A
MF8801.A
MF8803
MF9157
MF8057
MF9158
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76.B.hyl.NB
78.B.hyl.NB
80.B.hyl.NB
81.B.hyl.NB
82.B.hyl.KS
77.B.hyl.KS
79.B.hyl.NB
70.B.bre.NH
71.B.bre.NH
73.B.bre.VA
74.B.bre.VA
67.B.bre.KY
69.B.bre.OH
68.B.bre.KY
72.B.bre.OH
61.B.bre.NB
62.B.bre.Manitoba
63.B.bre.Manitoba
64.B.bre.NB
65.B.bre.NB
66.B.bre.IA
55.B.car.LA
56.B.car.Ark
57.B.car.LA
58.B.car.IL
53.B.car.FL
54.B.car.FL
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60.B.car.IL
49.B.car.GA
50.B.car.GA
51.B.car.VA
52.B.car.VA
85.Sorex
86.Sorex
84.Cryptotis
83.Cryptotis

```
                          810        820        830        840        850
                           .          .          .          .          .
MF4745                    CATTAAGCCAGAATGATATTTTCTATTTGCCTACGCCATTCTGCGATCCA
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MF5356                    ..................................................
MF5376                    ..................................................
MF6156                    ..................................................
MF6397                    ..................................................
MF7497                    ..................................................
MF7498                    ..................................................
MF8049                    ..................................................
MF8050                    ..................................................
MF8051                    ..................................................
MF8052                    ..................................................
MF8053                    ..................................................
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MF8802.A                  ..................................................
MF8231.A                  ..................................................
MF8801.A                  ..................................................
MF8803                    ..................................................
MF9157                    ..................................................
MF8057                    .................................................T.
MF9158                    ..................................................
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80.B.hyl.NB               ..................................................
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82.B.hyl.KS               ..................................................
77.B.hyl.KS               ..................................................
79.B.hyl.NB               ..................................................
70.B.bre.NH               ......A..........C...T.........T.....C..A.....T.
71.B.bre.NH               ......A..........C...T.........T.....C..A.....T.
73.B.bre.VA               ......A.............T.........T.....C..A.....T.
74.B.bre.VA               ......A.............T.........T.....C..A.....T.
67.B.bre.KY               ......A..........C...T.........T.....C..A.....T.
69.B.bre.OH               ......A..........C...T.........T.....C..A.....T.
68.B.bre.KY               ......A..........C...T.........T.....C..A.....T.
72.B.bre.OH               ......A..........C...T.........T.....C..A.....T.
61.B.bre.NB               ...C..A..........C...T.........T........A.....T.
62.B.bre.Manitoba         ...C..A..........C...T.........T........A.....T.
63.B.bre.Manitoba         ...C..A..........C...T.........T........A.....T.
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65.B.bre.NB               ...C..A..........C...T.........T........A.....T.
66.B.bre.IA               ...C..A..........C...T.........T........A.....T.
55.B.car.LA               ..................................T.....C..A.....T.
56.B.car.Ark              ..................................T.....C..A.....T.
57.B.car.LA               ..................................T.....C..A.....T.
58.B.car.IL               ..................................T.....C..A.....T.
53.B.car.FL               .....................................C..A.....T.
54.B.car.FL               .....................................C..A.....T.
59.B.car.Ark              ..................................T.....C..A.....T.
60.B.car.IL               ..................................T.....C..A.....T.
49.B.car.GA               ......A..............................C..A.....T.
50.B.car.GA               ......A..............................C..A.....T.
51.B.car.VA               ......A..............................C..A.....T.
52.B.car.VA               ......A..............................C..A.....T.
85.Sorex                  ......A..T...........C...........T........C.....A.
86.Sorex                  ......A..T...........C...........T.....C..C.....A.
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83.Cryptotis              T...........G.....C..C.....C..T.....T.....A.....T.
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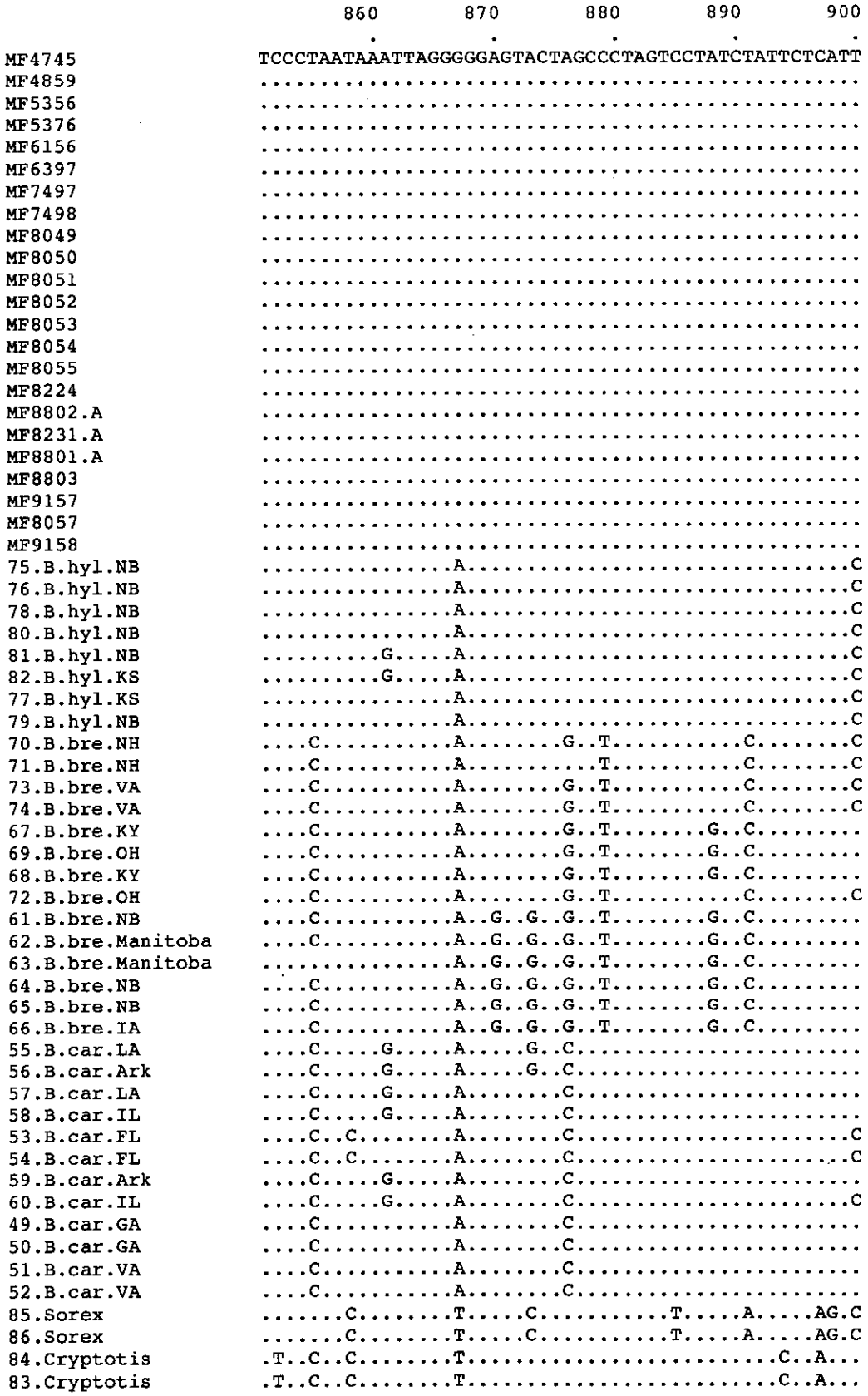
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MF4859
MF5356
MF5376
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MF8050
MF8051
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80.B.hyl.NB
81.B.hyl.NB
82.B.hyl.KS
77.B.hyl.KS
79.B.hyl.NB
70.B.bre.NH
71.B.bre.NH
73.B.bre.VA
74.B.bre.VA
67.B.bre.KY
69.B.bre.OH
68.B.bre.KY
72.B.bre.OH
61.B.bre.NB
62.B.bre.Manitoba
63.B.bre.Manitoba
64.B.bre.NB
65.B.bre.NB
66.B.bre.IA
55.B.car.LA
56.B.car.Ark
57.B.car.LA
58.B.car.IL
53.B.car.FL
54.B.car.FL
59.B.car.Ark
60.B.car.IL
49.B.car.GA
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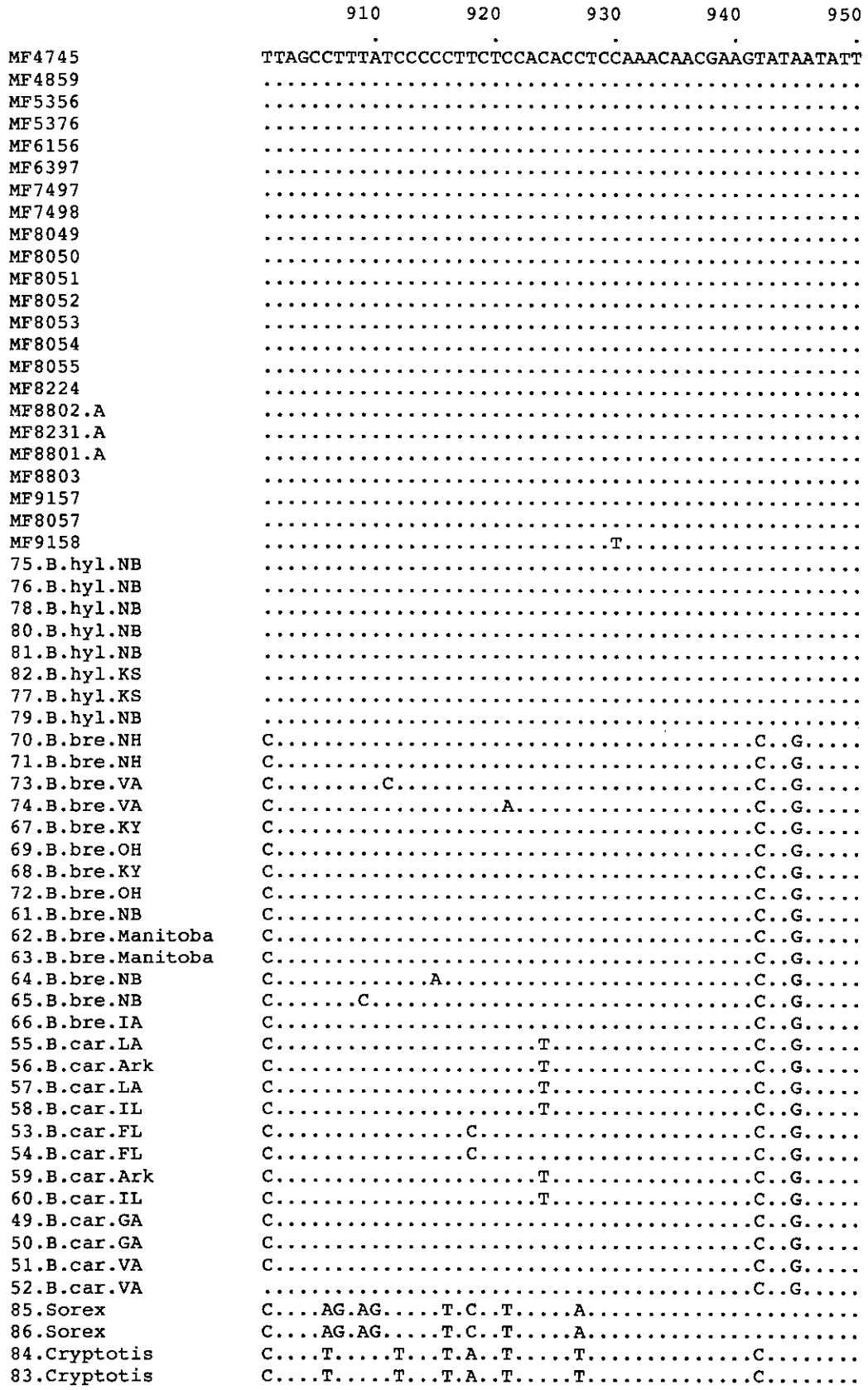
86.Sorex
84.Cryptotis
83.Cryptotis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MF5376
MF6156
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MF8050
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MF8054
MF8055
MF8224
MF8802.A
MF8231.A
MF8801.A
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MF8057
MF9158
75.B.hyl.NB
76.B.hyl.NB
78.B.hyl.NB
80.B.hyl.NB
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82.B.hyl.KS
77.B.hyl.KS
79.B.hyl.NB
70.B.bre.NH
71.B.bre.NH
73.B.bre.VA
74.B.bre.VA
67.B.bre.KY
69.B.bre.OH
68.B.bre.KY
72.B.bre.OH
61.B.bre.NB
62.B.bre.Manitoba
63.B.bre.Manitoba
64.B.bre.NB
65.B.bre.NB
66.B.bre.IA
55.B.car.LA
56.B.car.Ark
57.B.car.LA
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54.B.car.FL
59.B.car.Ark
60.B.car.IL
49.B.car.GA
50.B.car.GA
51.B.car.VA
52.B.car.VA
85.Sorex
86.Sorex
84.Cryptotis
83.Cryptotis

```
                          960        970        980        990       1000
                           .          .          .          .          .
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MF5376                    ..........................................................
MF6156                    ..........................................................
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MF8801.A                  ..........................................................
MF8803                    ..........................................................
MF9157                    ..........................................................
MF8057                    ..........................G.......G.......................
MF9158                    ..........................................................
75.B.hyl.NB               ..........................................C.......
76.B.hyl.NB               ..........................................C.......
78.B.hyl.NB               ..........................................C.......
80.B.hyl.NB               ..........................................C.......
81.B.hyl.NB               ..........................................C.......
82.B.hyl.KS               ..........................................C.......
77.B.hyl.KS               ..........................................C.......
79.B.hyl.NB               ...AAA.....................................C.......
70.B.bre.NH               C..........C..G...C....C..................C.....C.
71.B.bre.NH               C..........C..G...C....C..................C.....C.
73.B.bre.VA               C..........CA.G...C....C..................C.....C.
74.B.bre.VA               C..........CA.G...C....C..................C.....C.
67.B.bre.KY               C..........C..G...C....C..................C.....C.
69.B.bre.OH               C..........C..G...C....C..................C.....C.
68.B.bre.KY               C..........C..G...C....C..................C.....C.
72.B.bre.OH               C..........C..G...C....C..................C.....C.
61.B.bre.NB               C..........C..G...C....C...G..............C.....C.
62.B.bre.Manitoba         C..........C..G...C....C...G..............C.....C.
63.B.bre.Manitoba         C..........C..G...C....C...G..............C.....C.
64.B.bre.NB               C..........C..G...C....C...G..............C.....C.
65.B.bre.NB               C..........C..G...C....C...G..............C.....C.
66.B.bre.IA               C..........C..G...C....C...G..............C.....C.
55.B.car.LA               C..........C..........C......T.....................
56.B.car.Ark              C..........C..........C......T.....................
57.B.car.LA               C..........C..........C......T.....................
58.B.car.IL               C..........C..........C......T.....................
53.B.car.FL               C..........C..........C......T.................C.
54.B.car.FL               C..........C..........C......T.................C.
59.B.car.Ark              C..........C..........C......T.....................
60.B.car.IL               C..........C..........C......T.....................
49.B.car.GA               C..........C..........C......T.......G..C.........
50.B.car.GA               C..........C..........C......T.......G..C.........
51.B.car.VA               C..........C..........C......T.......G..C.........
52.B.car.VA               C..........C..........C......T.......G..C.........
85.Sorex                  C........T..C..........C..................C..A..C.
86.Sorex                  C........T..C..........C..................C..A..C.
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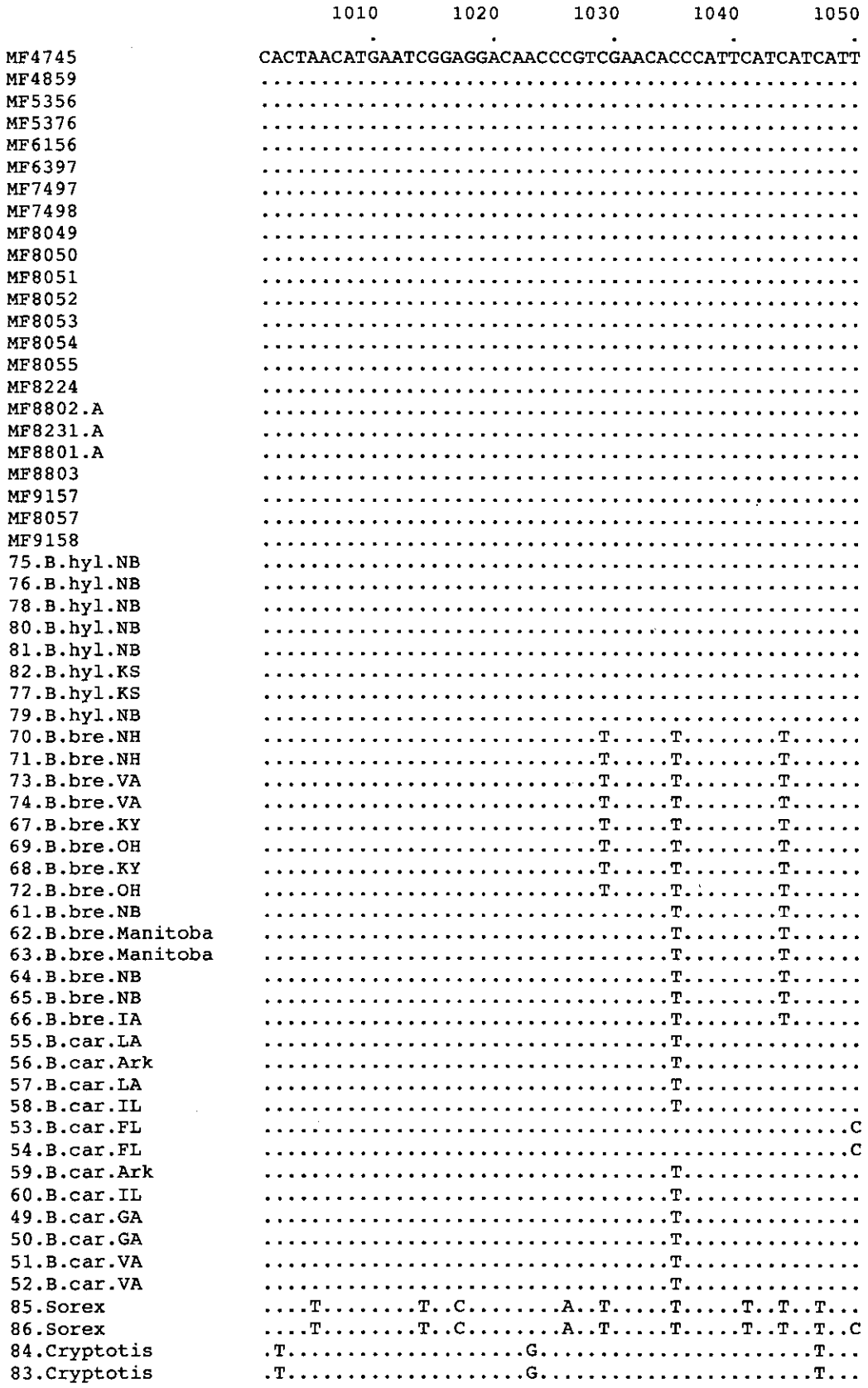
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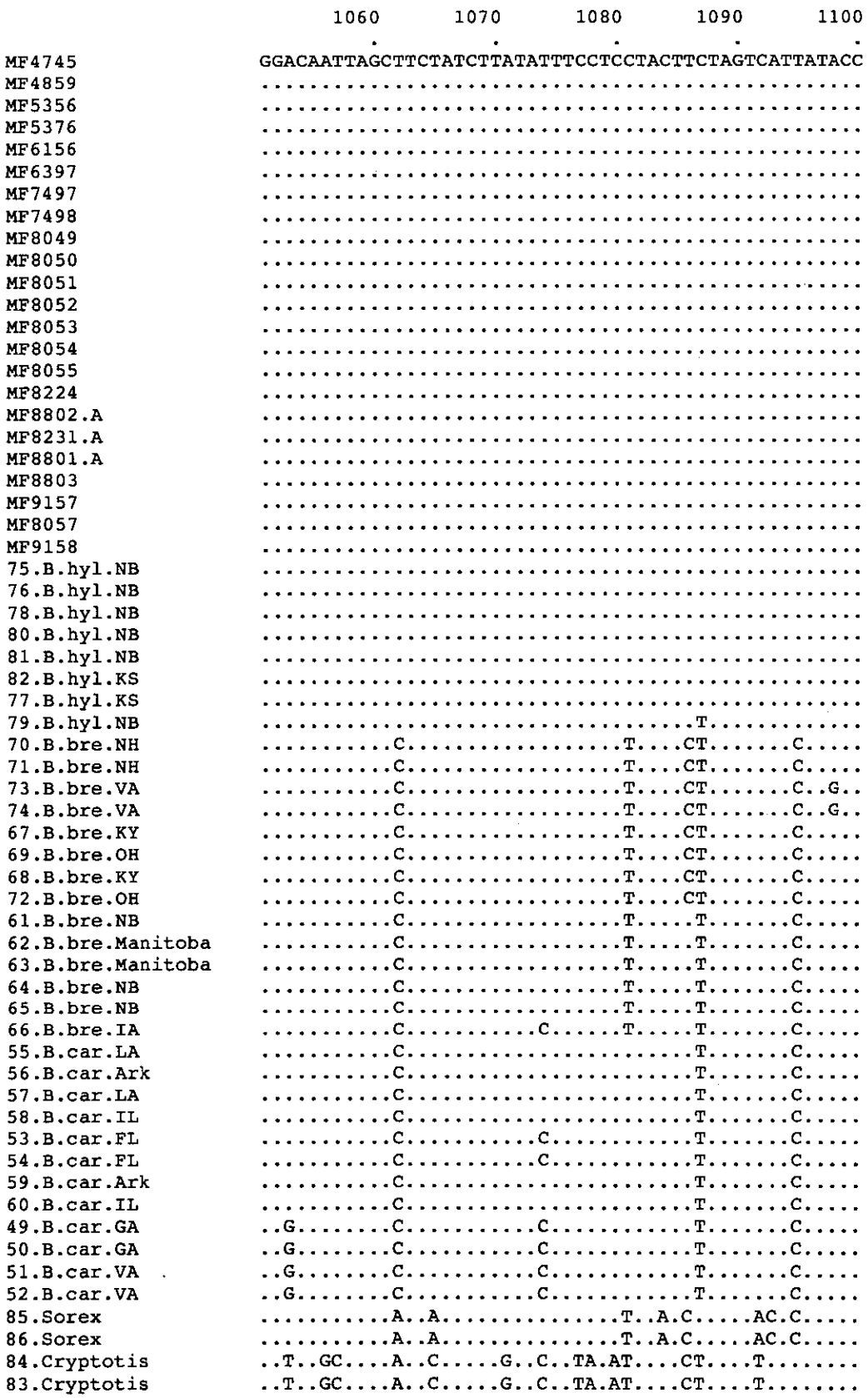
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60.B.car.IL .....C.....T.....C.....
49.B.car.GA ..G.....C.....C.....T.....C.....
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85.Sorex .....A..A.....T..A.C.....AC.C.....
86.Sorex .....A..A.....T..A.C.....AC.C.....
84.Cryptotis ..T..GC....A..C.....G..C..TA.AT....CT....T.....
83.Cryptotis ..T..GC....A..C.....G..C..TA.AT....CT....T.....

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MF6156                    .......................................
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MF8801.A                  .......................................
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82.B.hyl.KS               .......................................G
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79.B.hyl.NB               .......................................G
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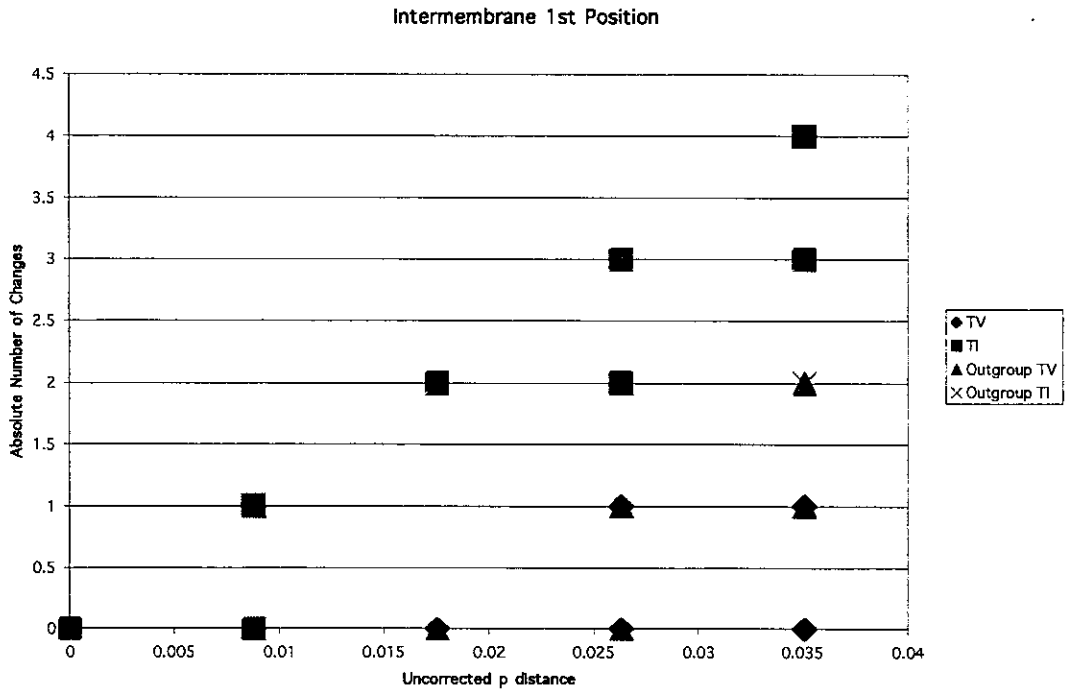
71.B.bre.NH               G......................C................G
73.B.bre.VA               T......................C................G
74.B.bre.VA               T......................C................G
67.B.bre.KY               G........C.............C.G............G
69.B.bre.OH               G......................C................G
68.B.bre.KY               G........C.............C................G
72.B.bre.OH               G......................C................G
61.B.bre.NB               G......................C................G
62.B.bre.Manitoba         G......................C................G
63.B.bre.Manitoba         G......................C................G
64.B.bre.NB               G......................C................G
65.B.bre.NB               G......................C................G
66.B.bre.IA               G......................C................G
55.B.car.LA               .......................................G
56.B.car.Ark              .......................................G
57.B.car.LA               .......................................G
58.B.car.IL               .......................................G
53.B.car.FL               ......................C................G
54.B.car.FL               ......................C................G
59.B.car.Ark              .......................................G
60.B.car.IL               .......................................G
49.B.car.GA               .......................................G
50.B.car.GA               .......................................G
51.B.car.VA               .......................................G
52.B.car.VA               .......................................G
85.Sorex                  T..................T..CC.TC..........G
86.Sorex                  T..................T..CC.TC..........G
84.Cryptotis              .........C...C.T.....T.................SG
83.Cryptotis              .........C...C.T.....T.................G
```

APPENDIX 3

Saturation curves for each codon position within each of three membrane regions of cytochrome *b* for the *Blarina* data set. *Sorex* and *Cryptotis* were the specified outgroups. Ingroup-outgroup saturation is present in third position transitions of all three regions of the protein as well as first codon position transitions of the transmembrane region. Symbols for ingroup and outgroup transitions (TI) and transversions (TV) are noted in the key.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Absolute Number of Changes
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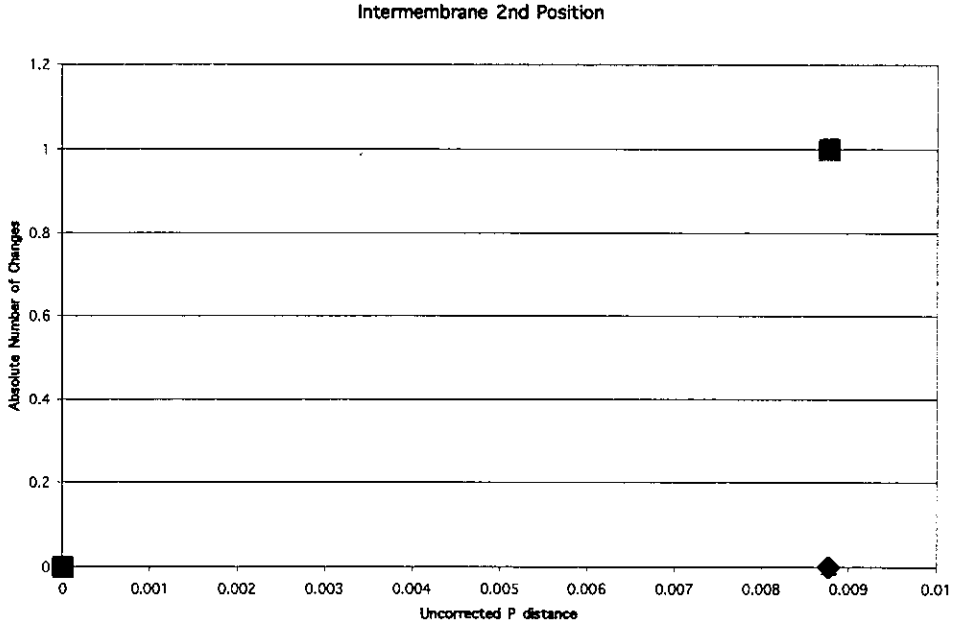
4
3.5
3
2.5
2
1.5
1
0.5
0
0
0.005
0.01
0.015
0.02
0.025
0.03
0.035
0.04
Uncorrected p distance
TV
TI
Outgroup TV
Outgroup TI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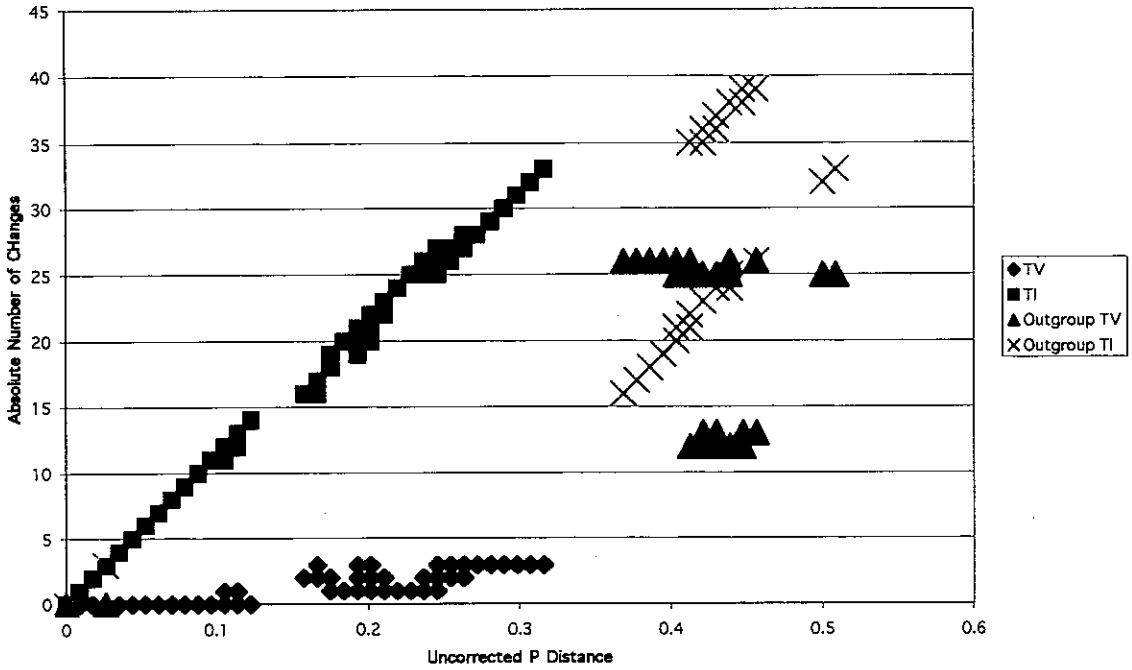
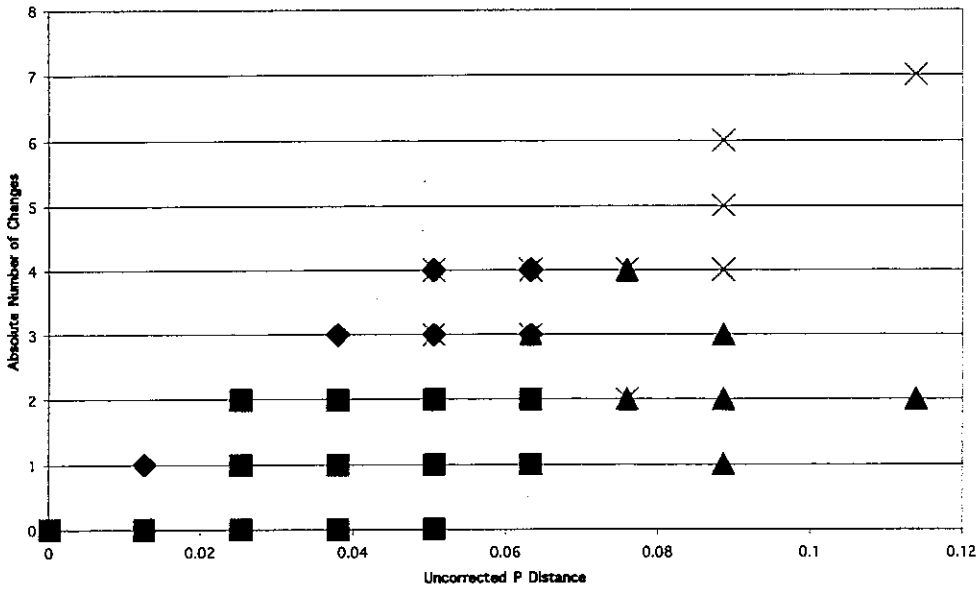
Absolute Number of Changes
1.2
1
0.8
0.6
0.4
0.2
0
0
0.001
0.002
0.003
0.004
0.005
0.006
0.007
0.008
0.009
0.01
Uncorrected P distance

Intermembrane 3rd Position



Matrix 1st Position



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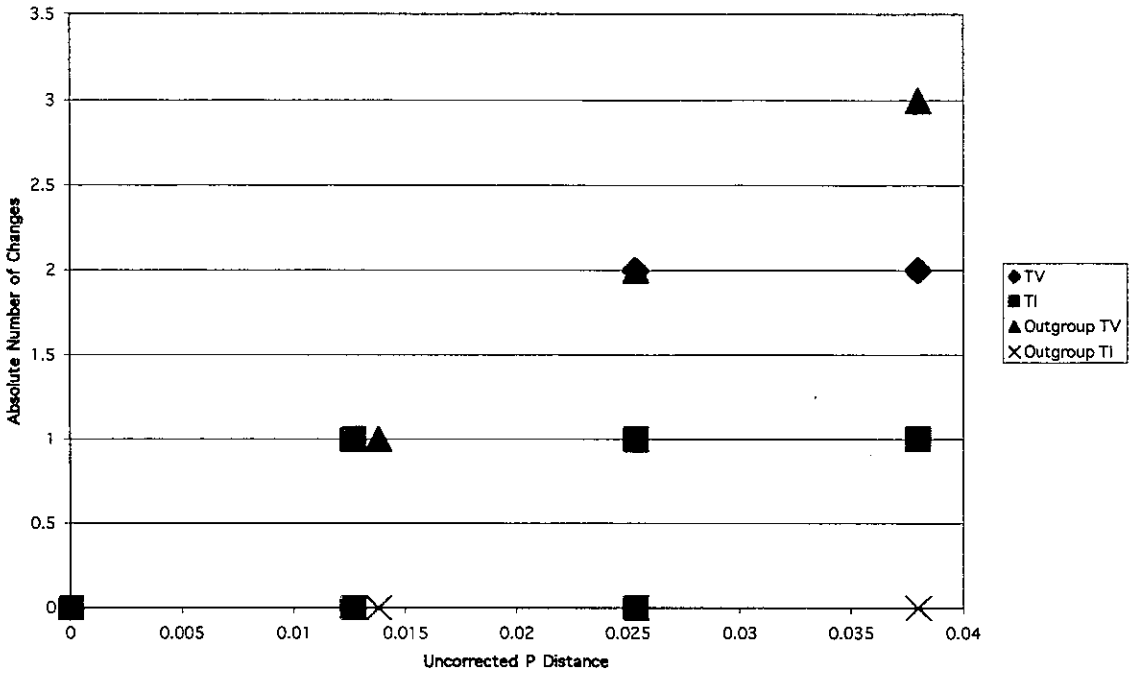

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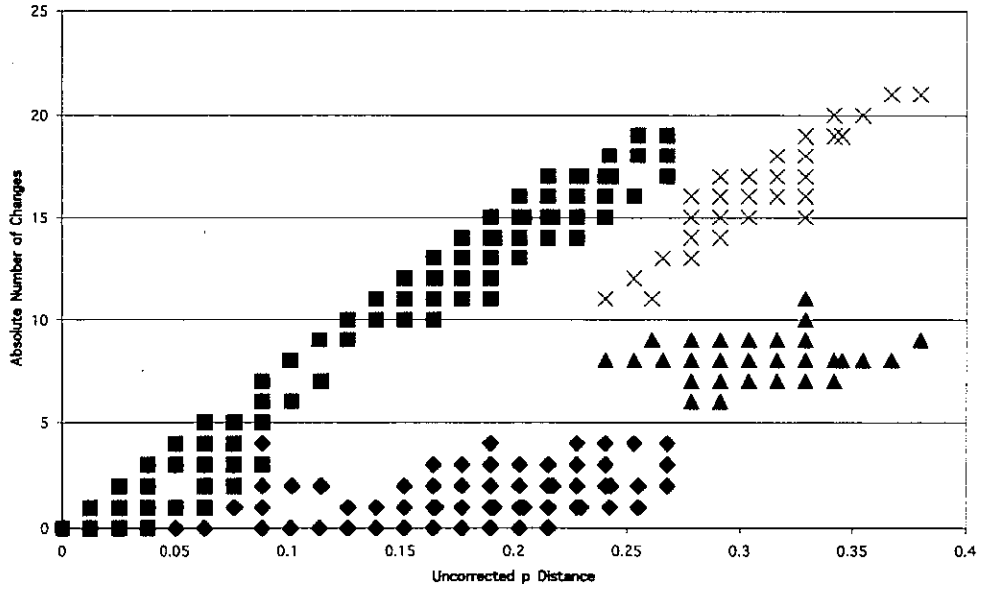
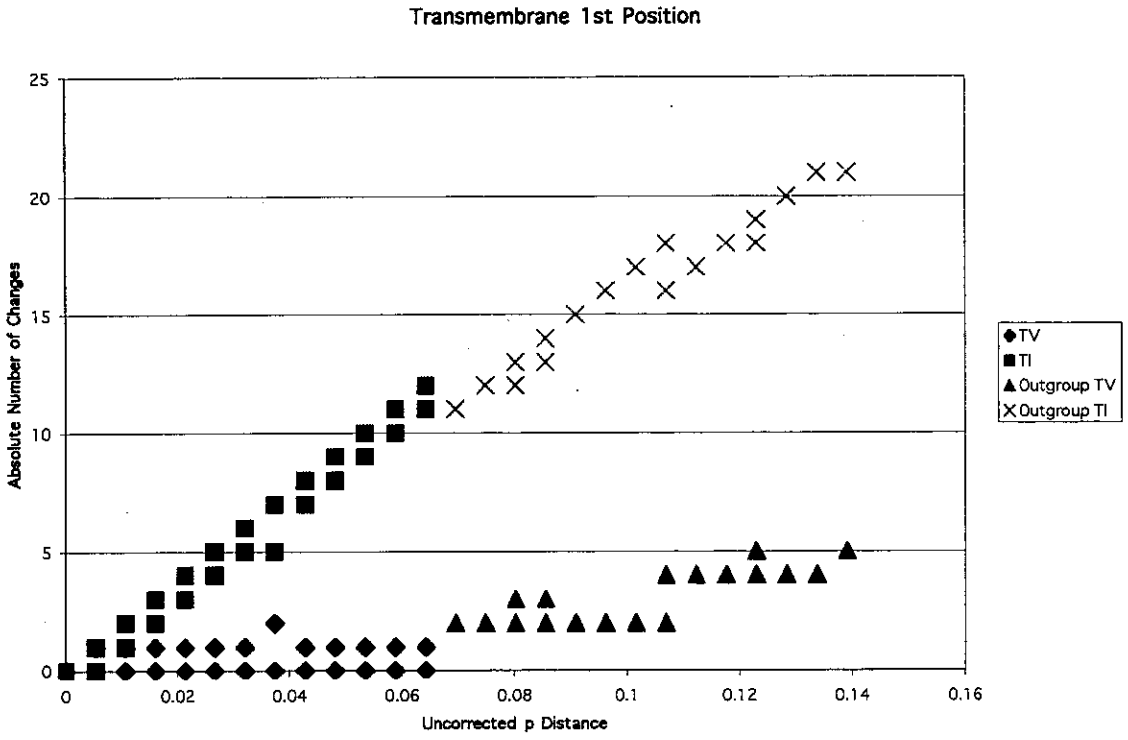
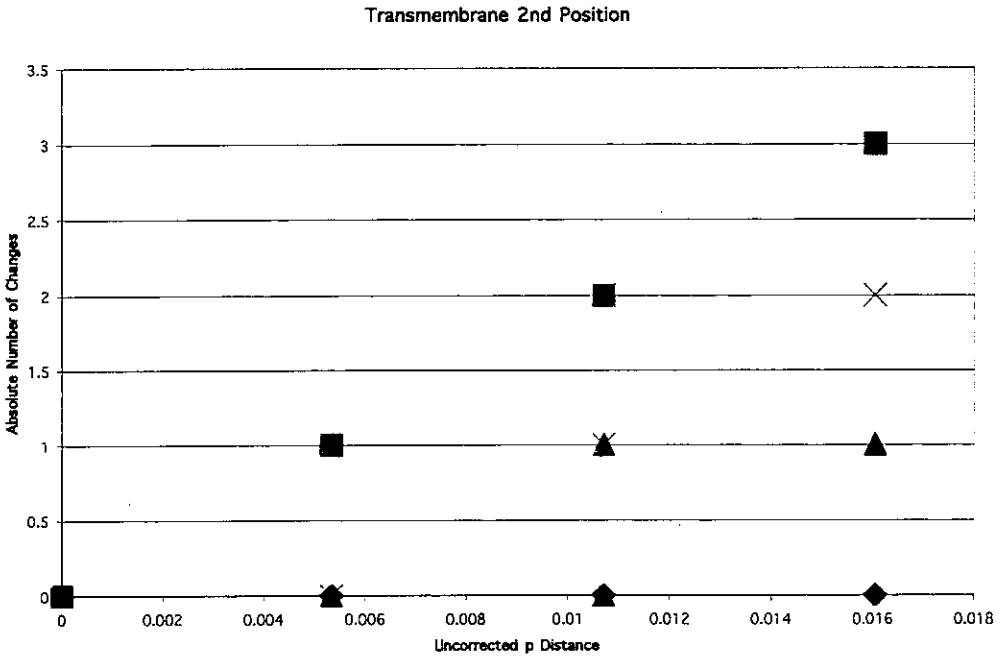



Transmembrane 1st Position
Absolute Number of Changes
Uncorrected p Distance
TV
TI
Outgroup TV
Outgroup TI


Transmembrane 2nd Position
Absolute Number of Changes
Uncorrected p Distance

Chapter 7:
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Houston toad research and surveys

2003 data and final report

for the

CAC-Griffith League Ranch
Bastrop County, Texas

by

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Introduction

The 2003 surveys mark the completion of four consecutive years of audio/physical surveys for the Houston toad on the Griffith League Ranch (GLR) in Bastrop County, Texas. The year also represents the second year of a three year grant exploring aspects of the herptofaunal community composition and multiple life-stage survivorship parameters for Houston toads. Concurrent surveys conducted across multiple counties find the Houston toad to be at very low levels (Forstner 2003). If the current populations are to be assisted in recovery through management it is necessary to generate novel field documentation and execute field research directed at understanding ecological aspects of the species that have remained unexplored. In order to assess areas under their control the Capitol Area Council of the Boy Scouts of America (CAC-BSA) has conducted audio surveys for the Houston toad on the GLR in Bastrop County. The CAC-BSA further sponsored research seeking data which are critical to the design of management plans for Houston toads in Bastrop County and across its remaining range. That involvement includes documentation of occurrence and breeding via night surveys on the GLR, field monitoring using reptile and amphibian collection arrays and mark recapture techniques, and several manipulative experiments designed to examine survival and ecology in the aquatic and juvenile life stages of the species.

The objective of these tasks remains the description of the annual ecology of the Houston toad in the field and under constrained experimental conditions. As we have emphasized previously (Forstner 2002) despite many previous audio surveys in Bastrop county, very few have included direct assessments of reproductive success. Likewise, there were many previous studies which sought to compile data on the Houston toad in the field. However, the current research program on the GLR has begun the process of evaluating the Lost Pines reptile and amphibian community, of which the toad is a part, and has discerned patterns of habitat usage by the toad. These data should be useful for management decisions both on the GLR and elsewhere.

From conception the overall research program is integrated and the data have been collected so as to allow subsequent analyses to provide a measure of the success in the adaptive management programs instituted at the relevant locations. We are in the second year of a long-term commitment to the investigation of how many toads there are, where they are, when they are present above ground, how they utilize their habitat, and determining the quantities in their life table. That life table is best summarized as a simplified model of Houston toads in the wild (or in this case, on the GLR). The parameters within that model are critical to predictive evaluations and discerning trends in the population. As monitoring continues our goal remains the integration of research to provide direction to management, allowing toad population levels on the GLR to increase concurrent with the scope of the CAC-BSA utilization of the property.

Methods

In 2003 the fourth year of audio surveys of the properties during the annual spring breeding season was completed. This involves the completion of up to 20 nights of audio surveys with the goal of detecting and describing the occurrence of Houston toads during the breeding season on the property. Second, the herptofaunal (both reptiles and amphibians are collected) array trapping project has assembled another year of data and increased the scope of the design by adding new trapping arrays in areas of the ranch that were previously surveyed only during the breeding season. This second program requires daily fieldwork every day of the year. Taken together these two approaches allow the current status and distribution of Houston toads on the property to be described. They were supplemented during 2003 by direct observation of adult

Houston toad movement using radio telemetry. In the final portion of the overall project design for 2003 two distinct experiments have been designed to provide answers about Houston toad survivorship and juvenile ecology. The majority of the methods follow those of the previous year (Forstner 2002).

Surveys

Obviously one of the most important aspects about managing endangered species is monitoring the numbers of individuals and reproductive success annually. Nocturnal audio surveys allow development of a trendline for the breeding activity of the Houston toad. Likewise, the data provide the information necessary to define the areas used by the toads during the spring and to monitor changes in the abundance or distribution of the toads over time. Audio surveys for the Houston toad have minimum acceptable guidelines established by the USFWS. The audio surveys completed each year for the CAC-BSA meet and exceed those guidelines.

All audio surveys are conducted on nights when climatic conditions appear favorable to Houston toad breeding events. A series of established listening stations are monitored for a period of (minimally) five minutes and the calls of all amphibians are noted. When Houston toads are heard, attempts are made to physically locate the individuals if this can be accomplished without undue disturbance to the chorus. Individuals are then permanently marked using microchip transponders (AVID) and physical measurements are taken. Upon completion of the data collection all individuals are released back into the breeding pond. Prior to the initiation of the breeding season all ponds are examined and data regarding the water levels and chemistry are collected, this work is followed up after the breeding season. Subsequent to active chorus nights, positive ponds are searched during daylight or early evening to evaluate the success of the breeding chorus by counting the number of egg strings found in each pond. Finally, at the end of the season at the time when juveniles emerge from the ponds, each pond is checked to determine whether or not juvenile Houston toads are seen at the ponds' edges. Performing these activities has assisted in the research phases of the next program. The remaining research sections are presented in order of the lifestage each examines.

Sponsored Research

The overall research design for the GLR has grown to include five distinct but integrated projects. The overall design allows GLR Houston toads to be evaluated and tracked from eggs through adults. The first project (Juvenile Arrays) provides details for Houston toads during the juvenile stages. During the breeding season Houston toad egg strings are located. Three egg strings are selected and monitored (this is in concert with the second project described below). The number of eggs is estimated and then the known strings are followed as the tadpoles hatch. The tadpole group (cohort) will then establish a defined foraging zone. This area along the pond edge is then ringed by concentric ellipses of raised herptofaunal fence. In some cases pit fall traps (1 quart plastic buckets) are used. Generally, these fences will be simply left as barriers to dispersal and will allow the subsequent emergent juveniles to be located, marked, and released. The juveniles receive a cohort mark and total numbers of individuals are monitored throughout the weeks of emergence. The juvenile arrays are left in position until 30 days after the last juvenile is found within them. Toe clips are retained from individuals to allow for potential DNA typing at a later time.

In the second experiment (Experimental ponds) an artificial pond array is used to test specific effects of factors which may influence the survivorship of Houston toad tadpoles. A total of 24 ponds have been created to determine the effects, if any, that slope, insects, and fish play on the eventual emergence of Houston toad juveniles. The design provides both replicates and a control for the three treatments. Each pond is surrounded by a vertical barrier to allow accurate collection and counts of emerging toads. Juveniles which successfully emerge from the artificial pond array are released beside each pond. A perimeter array is in place at 50m from the artificial

ponds and is monitored daily to detect any successful dispersal of the juveniles away from the artificial ponds which lie within a pasture.

The third experiment capitalizes on each of the preceding experiments and the annual survey work. The entire GLR is monitored for Houston toad activity and distribution by a herptofaunal array. The array consists of vertical barriers (aluminum flashing set into the ground and held vertical with stakes), pit fall traps (5 gallon buckets placed into the ground level with the surface), and funnel traps. The funnel traps are placed alongside the vertical "fence" and animals hop into the funnels but are unable to hop out of the funnels. Thus animals are collected by the trapping arrays during all hours. The arrays are checked every day at first light. As rains can flood the subsurface buckets, heavy rains require that some of the buckets be closed until the saturation in the sandy soils has retreated. Similarly, fire ants have been a problem at some locations, these locations are monitored and when necessary treated using commercial fire ant "bait" (Amdro). Mortality within the buckets is kept to a minimum by providing damp cotton towels to insure both humidity and temperature relief to animals trapped in the buckets. Funnel traps are shaded using aluminum flashing to prevent heat stress to animals trapped in the funnel system. The arrays have been placed in a design which varies both the vegetation and the distance from known breeding ponds. By examining the distribution of Houston toad captures it may be possible to draw conclusions about habitat use and movement in and out of the breeding season. The data collected from other species of vertebrates will eventually allow a characterization of the reptile and amphibian community within which the Houston toad exists.

During the 2003 season direct evaluation of the adult toad movements was completed using attached radio telemetry and direct evaluation of juvenile dispersal were to have been examined using transluminant dyes. Microtransmitters (2g) were externally attached by a variety of methods to adult Houston toads at the breeding ponds. The animals were then followed daily to determine individual movements within the habitat. Juveniles leaving the juvenile array experiment were to be dusted with active transluminent dyes and then followed using portable UV transluminators.

Finally, point based vegetation mapping, vegetation density, and duff depths have been completed for the ranch. That work is complementary to large mammal, bird, and burn management planning projects currently prepared for eventual integration within the comprehensive wildlife plan for the GLR.

Results

Audio Surveys overall

The breeding season in 2003 had fairly normal temperatures with two abrupt and deep cold freezes occurring in February and March. In both cases chorusing and breeding had begun and was interrupted during these cold snaps. In a similar result to 2002 an egg string deposited the night prior to the cold snap in February failed to mature subsequent to the freeze (100% egg mortality). The freeze did result in thin marginal icing of the breeding pond but the direct cause of the mortality is not clear. The remaining season brought near average rainfall for the first year in nearly a decade. Complementary to the heavy July rains of 2002, the GLR had abundant pooled water on the surface and several features noted in previous years as potential breeding locations held water for more than 120 days, had male Houston toad chorusing, and in 1 case had Houston toad reproduction occur within them. Two of the new locations had been heard in chorus from Houston toads in previous years but were either not explicitly located (Pond 19 within the flood plain of Alum creek) or went dry within just a few weeks (Pond 6b). The other two new sites are low lying areas within drainage regions which may have been impounded but changes to the general drainage pattern with historical road building and grading on the ranch. Neither of the two new sites (designated as Ponds 20 and 21) had successful breeding during 2003 but both locations had Houston toads in chorus on consecutive nights and during several periods of the breeding season in 2003.

Griffith League Ranch

Table 1 provides relevant data for the 2003 Houston toads audio surveys conducted on the GLR.

Table 1. Houston toad audio survey results for the Griffith League Ranch Bastrop County, Texas for 2003.

Date	7-Jan	21-Jan	28-Jan	2-Feb	12-Feb	13-Feb	14-Feb
Temp (F)	70	66.3	62.9	70.5	68.9	68	74.3
Humidity			89	77	85	90	90
Wind	6	4	1-2	8.9	3	3	2.2-4.8
Moon		0.5	0	0	.40	0	0
Pond 1	pond full	0	0	0	1RS	0	0
Pond 2	pond full	0	1RS, 1BH off property	0	2RS	3BH,4BH nw	1BH,5BH nw
Pond 3	pond nearly full	0	1RS	0	0	0	0
Pond 4	pond full	0	0	1RC,1RS	1RC	0	0
Pond 5a,b	pond 5a full, 5b full	1RS	0	2RS	3RS	1.3BH	1BH,4RS
Pond 6	pond full	0	1RS	0	0	0	0
Pond 6B	Pond ½ full	-	0	0	0	0	1BH
Pond 7	pond full	0	0	0	1RS	0	1BH
Pond 8	pond full	0	1RC	1RS	0	0	0
Pond 9	pond full	1RS	2RS	1RS	0	1BH	0
Pond 10	pond full	5RS	0	0	0	0	0
Pond 11	pond full	0	2RS	0	1RS	0	0
Pond 12	pond very full	2RS	3RS	1BH	2RS	0	3RS
Pond 13	pond full	0	0	0	0	0	0
Pond 14	pond full	0	0	0	0	0	1RS
Pond 15	pond full	0	0	0	0	0	0
Pond 16	pond very full	0	1RS	0	0	0	1RS
Pond 17	pond full	0	0	0	-	0	0
Pond 18	Pond/drainage full	0	0	0	1RS	0	0
Pond 19 (Alum)	(large marsh) Flowing	-	0	0	1RS	0	0
Pond 20	(area very full)	-	-	-	-	1BH(NEWPOND)	0
Pond 21	(area very full)	-	-	-	-	1BH(NEWPOND)	0

Species Key =	0=None	**BX**=*Bufo sp.* hybrid?	**PS**=*Psuedacris streckeri*
AC=*Acris crepitans*		**GC**=*Gastrophyrne carolinensis*	**PT**=*Psuedacris triseriatus*
BH=*Bufo houstonensis*		**GO**=*Gastrophyrne olivaceous*	**RC**=*Rana catesbaena*
BS=*Bufo speciosus*		**HC**=*Hyla cinerea*	**RCL**=*Rana clamitans*
BV=*Bufo valliceps*		**HVC**=*Hyla versicolor/chrysocelous*	**RS**=*Rana sphenacephala*
BW=*Bufo woodhousei*		**PC**=*Psuedacris clarki*	**SH**=*Scaphiopus couchi*

Table 1. Cont. Houston toad audio survey results for the Griffith League Ranch Bastrop County, Texas for 2003.

Date	19-Feb	23-Feb	1-Mar	7-Mar	8-Mar	9-Mar	10-Mar	11-Mar
Temp (F)	59	62	64	70.5	63	54.3	63.8	68
Humidity	88	53	59	39	55	68	57	87
Wind	1.5-3	4	4.6-7	1.1	0	0	0.5	1-3.3
Moon	0	0	0	0.125	.50	0	0.5	0
Pond 1	0	0	0	1RS	0	0,1RS(OFF)	1RS	0
Pond 2	0	No eggs found	No eggs seen	0	0	8BH	7BH	10.1BH
Pond 3	0	0	0	0	0	0	0	0
Pond 4	0	2RS egg masses	0	1RS	0	1RS	0	0
Pond 5a,b	0	7 RS egg masses	0	2RS	0	1RS	5RS	0
Pond 6	0	0	0	0	0	0	2RS	0
Pond 6B	0	0	0	0	0	0	0	0
Pond 7	0	0	0	1RS	0	0	1BH	3RS
Pond 8	0	0	0	0	0	1RS	0	0
Pond 9	0	3RS egg masses	0	1RS	0	0	3RS	0
Pond 10	0	0	0	0	0	2RS	1RS	0
Pond 11	0	0	0	4RS	-	0	5RS	0
Pond 12	2RS	0	0	0	0	3BH	3BH	0
Pond 13	1RS	0	0	0	0	0	0	0
Pond 14	0	0	0	0	0	3RS	0	0
Pond 15	2RS	0	0	0	0	10RS	0	0
Pond 16	0	0	0	0	0	0	0	2RS
Pond 17	0	0	0	0	-	0	0	0
Pond 18	0	0	0	0	-	0	0	0
Pond 19 (Alum)	1RS	0	0	0	-	0	0	0
Pond 20	0	0	0	0	0	1BH	0	1BH
Pond 21	0	0	0	0	0	0	0	1BH

Species Key = 0=None

AC=*Acris crepitans*
BH=*Bufo houstonensis*
BS=*Bufo speciosus*
BV=*Bufo valliceps*
BW=*Bufo woodhousei*
GC=*Gastrophyrne carolinensis*
GO=*Gastrophyrne olivaceous*
HC=*Hyla cinerea*
HVC=*Hyla versicolor/chrysocelous*
PC=*Psuedacris clarki*
BX=*Bufo sp.* hybrid?
PT=*Psuedacris triseriatus*
RC=*Rana catesbaena*
RCL=*Rana clamitans*
RS=*Rana sphenacephala*
SH=*Scaphiopus couchi*
PS=*Psuedacris streckeri*

Table 2. Cont. Houston toad audio survey results for the Griffith League Ranch Bastrop County, Texas for 2002.

Date	14-Mar	16-Mar	28-Apr	18-May
Temp (F)	66.5	74	78	81
Humidity	60	64	-	-
Wind	0.6	5	-	-
Moon	.65	0	0	0
Pond 1	0	0	BH tadpoles	BH juveniles
Pond 2	1HV	2 BH egg strings, HV,RS eggs	0	0
Pond 3	0	0	0	0
Pond 4	3RS	0	0	0
Pond 5a,b	1RS	0	0	0
Pond 6	0	HVC, AC eggs and tads	0	0
Pond 6B	0	0	BH tadpoles	No emergents
Pond 7	0	0	0	0
Pond 8	0	0	0	0
Pond 9	1RS	RC eggs, RS eggs and tads	0	0
Pond 10	0	0	0	0
Pond 11	1BV,2RS	0	0	BH juveniles
Pond 12	0	0	0	0
Pond 13	0	0	0	0
Pond 14	1HV	0	0	0
Pond 15	4RS	0	0	0
Pond 16	1RS	0	0	0
Pond 17	0	0	0	0
Pond 18	0	0	0	0
Pond 19 (Alum)	1RS	0	0	0
Pond 20	0	0	0	0
Pond 21	0	0	0	0

Species Key = 0=None

AC=*Acris crepitans*
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RCL=*Rana clamitans*
RS=*Rana sphenacephala*
SH=*Scaphiopus couchi*

Table 2. Cont. Houston toad audio survey results for the Griffith League Ranch Bastrop County, Texas for 2003.

Date	2-June	19 June
Temp (F)	84	82
Humidity	-	-
Wind	-	-
Moon	-	-
Pond 1	0	0
Pond 2	BH juveniles	No juveniles seen
Pond 3	0	0
Pond 4	0	0
Pond 5a,b	No juveniles	0
Pond 6	0	0
Pond 6B	No juveniles/no tadpoles	0
Pond 7	0	0
Pond 8	0	0
Pond 9	0	0
Pond 10	0	0
Pond 11	2 BH juveniles	No juveniles seen
Pond 12	No juveniles/no tadpoles	0
Pond 13	0	0
Pond 14	0	0
Pond 15	0	0
Pond 16	0	0
Pond 17	0	0
Pond 18	0	0
Pond 19 (Alum)	0	0
Pond 20	0	0
Pond 21	0	0

Species Key =	0=None	**BX**=*Bufo sp.* Hybrid?	**PS**=*Psuedacris streckeri*
AC=*Acris crepitans*		**GC**=*Gastrophyrne carolinensis*	**PT**=*Psuedacris triseriatus*
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BV=*Bufo valliceps*		**HVC**=*Hyla versicolor/chrysocelous*	**RS**=*Rana sphenacephala*
BW=*Bufo woodhousei*		**PC**=*Psuedacris clarki*	**SH**=*Scaphiopus couchi*

The results from GLR for 2003 showed fewer toads than in previous years but with more females and similar levels of reproduction from previous years (Table 1). Two new locations had chorusing beyond locations that have historically shown Houston toad chorusing. Several previous chorusing locations did not chorus in 2003 (Ponds 8, 10, 15, 16). However, four adult Houston toads were collected (not in chorus) at Pond 8 during the breeding season. While I did not observe Houston toads at Pond 6B during my surveys, Houston toads did breed in Pond 6B on a night I did not survey. They were heard by part of the field crew and I observed egg strings in the pond the following morning. Pond 11 had calls in 2002 but did not in 2003, yet Pond 11 did have breeding on a non-survey night as juveniles did emerge from this pond for the first time during my evaluation of the properties ponds. As last year, Pond 16 failed to provide Houston toad breeding or chorusing for the third year in a row after having done so in 2000. Finally, Ponds 17, 4, and 1 have never had Houston toads during any of the four survey years.

Houston toads were heard in chorus west of Pond 1, directly across the west fence of the property at a distance of less than 200m. Egg strings from Houston toad females were found at Ponds 2, 5a, 6B, and must have existed in Pond 11 during 2003. The first Houston toad chorusing was heard on 28 January and the last choruses occurred on 15 March, demonstrating a far shorter overall breeding period than in previous years. Likewise other species of amphibians showed much diminished chorusing on the GLR from levels seen in previous survey years. Despite those decreases we recaptured two of a series of male Houston toads originally marked in 2000 (toe clips 300 and 330). These toads were originally marked at Pond 9 after collection in the drainage adjacent to the Pond. One of those animals was recaptured at Pond 2 having traveled a minimum straight-line distance of 7300' (2263m) or 1.4 miles.

Research experiments

Juvenile arrays

This summer was extremely productive with *Bufo houstonensis* juvenile recaptures going almost all the way into August. A summary is as follows:

Pond 2- Elliptical arrays caught 262 initial captures and 453 recaptures for a total of 715 from 2 egg strands on the north bank. 23 Captures were from pond 2 but not from the two *Bufo houstonensis* egg strands (believed to have dispersed from neighboring property) and these could be either *B. houstonensis* or *B. valliceps* as it was late in the summer. Juveniles were found around the north and south permanent Y-arrays that parallel the perimeter fence. Dispersal for juveniles captured in the elliptical arrays was found to be 50m around the pond in either direction a few days after leaving the arrays and up to 70m away dispersing into the woods. Because of heavy rain followed by intense heat and dryness, juveniles were not found in the artificial refugia as had occurred as last year. Dispersing *Bufo* juveniles, whether they were *houstonensis* or *valliceps,* were found throughout the summer in or near the GLR traps, with a concentration around the pond 12 traps, pond 5 traps, and the pond 2 traps. Larger juveniles (July 2003) had started to develop morphological differences and pictures were taken to record the data.

Egg Counts-3 *Bufo houstonensis* egg strands were counted in the early spring of 2003. The egg strand from pond 5 died (potentially from exposure to the cold temps the next night) and the two at pond 2 were the first to be counted using several egg strand estimation techniques. Physical counts of the half of the egg strand taken showed an underestimation of the strand numbers by ~45%. Several weeks later *B. valliceps* were laying eggs and four measurement techniques were used on their eggstrands: full wire

model, wire piece estimation, the spherical technique, and physical counting. The wire piece method was found to be the best for *in situ* efforts. 8 *Bufo valliceps* egg strands were laid in these bins, 3 of which died before the eggs could mature to tadpoles. However, after 8 egg strands, 4 measurement techniques each, and 3 attempts per technique, the egg estimation technique from *in situ* egg strings has greatly increased in precision and accuracy.

Artificial Arrays (see below for additional artificial array information) -58 juveniles were captured out of 24-pond arrays. Only 5 ponds had juveniles with pond 3 having 55 emergent juveniles. Measurements were taken; tissue was not taken as tissue had been collected from the same cohort at pond 2. There were no recaptures around the artificial arrays most likely due to the lack of shade, lack of moisture, and the presence of fire ants.

Pond 11-Egg strands were missed as juveniles were found already in dispersion on 5/10/03. There were 82 initial captures and 11 recaptures. A low recapture count was likely a consequence of no specific arrays for capture and recaptures were by walking along the ground and spotting them. As the juveniles were not discovered until May 10th, they were larger in size and could have emerged earlier. A prediction model already published (Greuter and Forstner in press) may help in establishing the approximate time of emergence.

Artificial ponds

The twenty-four man-made experimental ponds on the Griffith League Ranch in Bastrop County, TX were monitored from March 23 – July 19, 2003. In late winter, each pond was pumped (using rented equipment) to facilitate removal of remaining predators and tadpoles. The water from the experimental ponds was re-used to maintain flora. The ponds were subsequently stocked with 100 non-*Bufo* tadpoles; these were primarily *Rana* tadpoles although some *Hyla* tadpoles were included. Predators were stocked according to Figure 1. Predator treatments were randomly assigned to ponds within the two slope treatments. All ponds that required fish were stocked with one bass and one perch, or one bass and four juvenile perch approximately 3 cm in length. The insect treatment ponds each received 24 Odonate larvae. All fish, insects, and tadpoles were obtained from other ponds on the Griffith League Ranch.

This year screens were constructed and used to cover the ponds throughout the season. They were in use soon after the ponds were stocked. On March 23, each pond was stocked with 183 *Bufo houstonensis* tadpoles from two egg strands that were taken from Pond 2. The tadpoles from each egg strand were divided equally among the 24 ponds; they were counted out in groups of 30 using small buckets and a plastic eyedropper. Ponds were treated as necessary with Amdro throughout the summer to kill fire ants within the flashing.

The first Houston toadlet emerged on May 12 from Experimental Pond (EP) 3, and the last toadlet emerged on July 4 from EP-3. In total, 58 toadlets emerged. The ponds where emergence occurred were EP 3, 5, 12,13, and 18. These ponds represent all treatments with the exception of that with only insect predators.

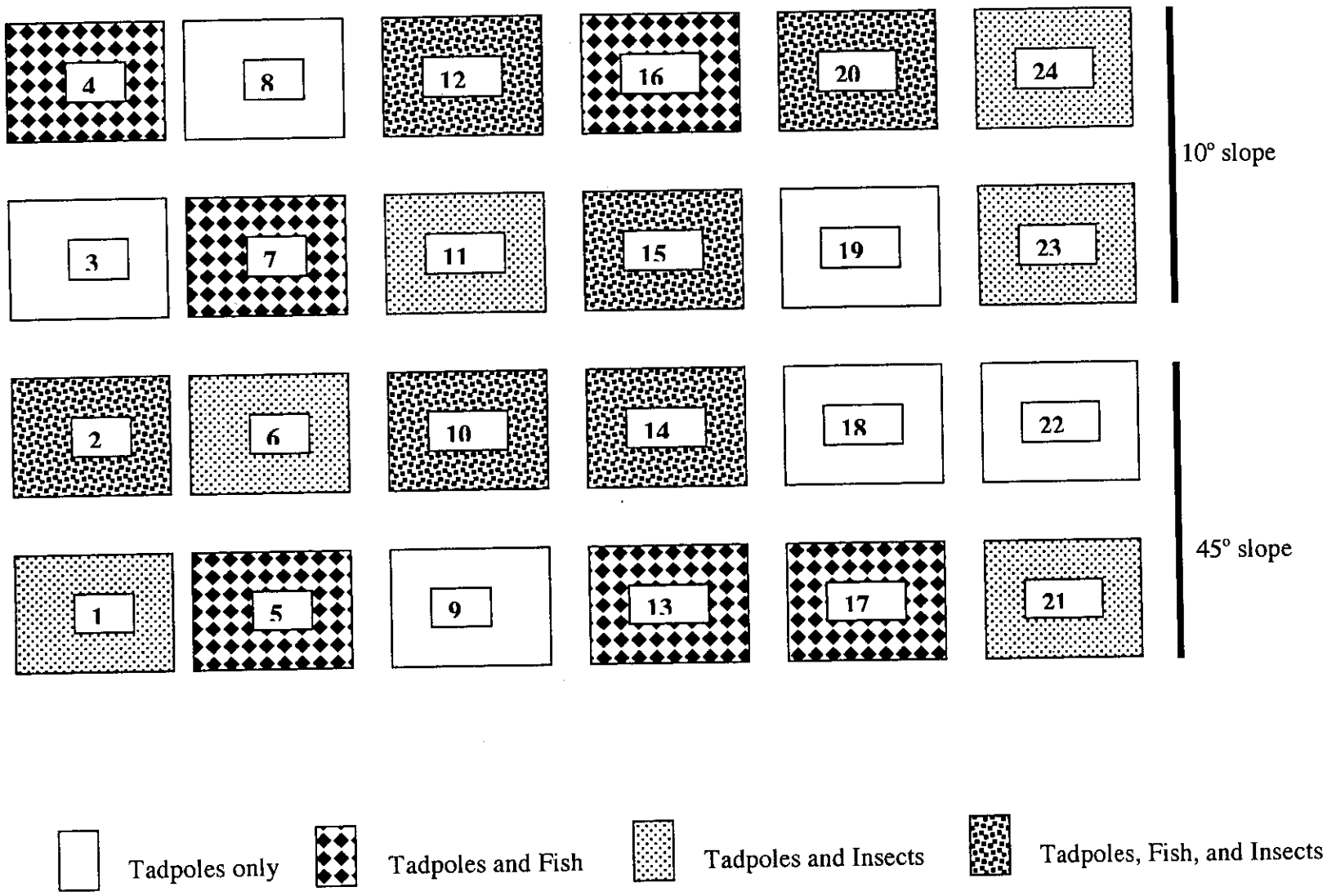

Figure 1. The 24 experimental ponds located on Griffith League Ranch with respective numbers and treatments (as indicated in key) for 2003.

The low number of toadlets emerging may be attributed to several factors. The toadlets emerged much later than their cohort that was left in the natural pond, probably due to lower water temperature attributed to shading by the new screens. In 2002, the pond temperatures varied from 81.6- 96.6 degrees F; in 2003, the temperature varied from 79-80 degrees F on July 9. The lower temperature prolonged the larval stage thus increasing the opportunity for predation by fish and insects. The *Rana* stocked in the ponds could have been predators; since *Rana* were stocked early in the season, the tadpoles were in their second year of development and therefore quite large. Although checking ponds every 24 hours should ensure that toadlets remain near the water after emerging, increased vegetation around the ponds may hide toadlets.

Additionally, this vegetation harbors potential predators such as wolf spiders, fishing spiders, and lizards.

Statistical analysis shows that there is no difference between 10 and 45-degree angle ponds ($F = 0.92$, $p = 0.35$), no difference among predator treatments ($F = 1.02$, $p = 0.41$), and no interaction between factors ($F = 0.93$, $p = 0.45$). However, the amount of zeros in the data set could skew results. Additional analyses may extract more meaningful results from the data.

Herptofaunal array

In 2003, 24 toads were captured. 17 were captured in the traps; the remaining 7 were captured at breeding ponds. Figure 2 shows the distribution of the toads per treatment type. The treatments are as follows: **Pond 2:** 4 traps surrounding Pond 2 (1, 2, 3, A); **Pasture:** all 5 pasture lines (however toads were only captured on the periphery of the pasture as in the previous years; **Treatment 1:** 3 traps closest to Pond 5 (B, C, 4); **Treatment 2:** traps near ponds 6 & 7 (5, 6, 7); **Treatment 3:** traps near pond 12 (15, 16, 17). Fewer toads were captured in the traps in 2003 (17) than in 2002 (23). More toads were captured in traps in 2003 (17) than in 2001 (6), the caveat being we had fewer traps in 2001 (Figure 3). Four toads were recaptured in 2003 that were initially captured in 2002. One male individual moved from Pond 5 to Pond 2 (PIT: 113664121 A).

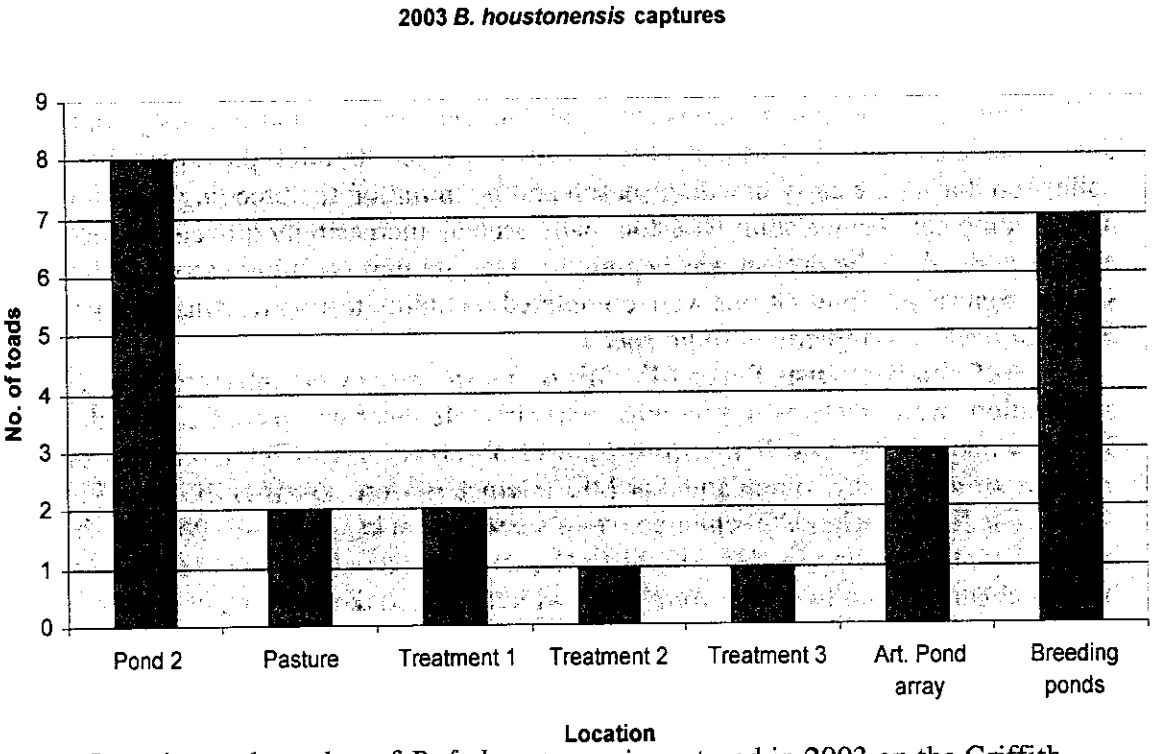

Figure 2. Location and number of *Bufo houstonensis* captured in 2003 on the Griffith League Ranch in Bastrop County, Texas.

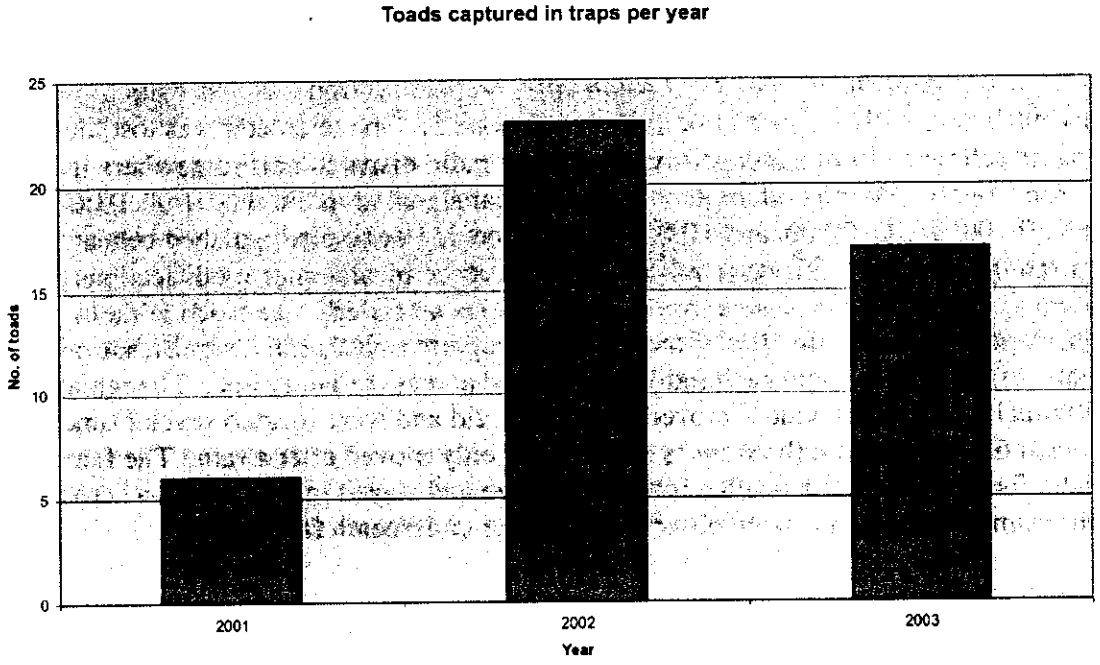

Figure 3. Toads captured per year in herptofaunal arrays on the Griffith League Ranch in Bastrop County, Texas.

Adult telemetry

Telemetry work with adult Houston toads was initiated in March of 2003 on the GLR. Several practical difficulties emerged that were corrected as the study continued during the late spring. Unfortunately the delay in project initiation prevented data collection during the early breeding season and by initiation the breeding season was nearly complete. Hence adult Houston toads became increasingly difficult to locate exactly at the time the project was beginning. Despite these administrative difficulties several significant observations were completed including testing the final technology on *Bufo valliceps*, a sympatric bufonid species.

Bufo houstonensis-Seven (4M:3F) *B. houstonensis* were telemetered in 2003. 34 observations were made over 3 months. The first telemeter was placed on a male on 3/8/03 at pond 5. The last *B. houstonensis* was observed on July 3rd in the belly of a *N. erythrogaster*. During March and April the telemeters were consistently "shed" by the toads. The first attachment technique was a spandex "jacket". These proved to be quite ineffective at remaining in place on the toads, most lasted only 3 – 4 days. In April beaded chains around the waist were used, which enabled the tracking of 2 male *B. houstonensis* over more than a month. However the chains rusted after any significant rainfall and stainless steel chain should prevent this problem in the fall of 2003 and 2004 breeding season.

From the results that were compiled in 2003, both male and *female B. houstonensis* stayed near (within 20m) of a breeding pond, during the breeding season. All males sought refuge in mammal burrows, under leaf litter, or in the crevices under fallen trees. Two of the females were found in self-dug burrows about 1 in deep. After the breeding season, a single male moved over 50m from its breeding pond (Pond 5). The others stayed within 20 m of their respective ponds.

Bufo valliceps-4 (2M:2F) *B. valliceps* were telemetered this year with 3 individuals originally trapped at pond 2, one at pond 5. One telemeter was lost, due to signal interference from a competitive frequency radio dispatch unit somewhere in Bastrop County. Despite attempts to obtain a clear signal at midnight, 01:00, 03:00, 06:00, 07:00, 08:00, 09:00, and 10:00 – 19:00 it could not be distinguished beneath the dispatch interference. Efforts to minimize interference by antennae modifications or even elimination with the consequent loss of signal radius all failed. The toads without interference stayed within 10m of their point of capture point, until a significant rain event. After such an event each individual moved across the landscape. The remaining individuals (1M:1F) at pond 2 moved across the grid and were located several times between 65 – 80 m from the water's edge. They only moved after a rain. The female at pond 5 found a hole underneath a rotten cedar tree and stayed in that location across the entire summer. All toads were observed to shelter underneath fallen trees.

Vegetation mapping

Vegetational transects of the property during all four seasons have been completed during 2003. The final compilation of those data are expected to be available within 3 months, concurrent with the completion of several M.Sc. degrees dependent upon those data. Additional transects have been placed, but additional work is currently on hold pending funding to examine fire ecology of the Houston toad. Duff depth and

distribution were compiled with the assistance of the Texas Forest Service (TFS) to allow both compatibility and continuity with their database for the county. The TFS is currently compiling that information independently of the study on the GLR

Discussion

Surveys

Houston toads are active on the majority of the Griffith League Ranch in forested habitat. The only impoundments not actively used for chorusing by the Houston toad during the breeding season remain Pond 1 and 4. While Pond 17 never has chorusing it is located adjacent to two chorusing/breeding ponds (Ponds 11 and 19). It remains distinct that Pond 17 is otherwise suitable habitat but remains disconnected from canopy areas by more than 50m. Pond 19 is likewise in open non-canopied ephemeral wetland habitat but lies just out of the canopy of Alum creek. As noted in the previous report (Forstner 2002) these exceptions continue and remain important in defining the distinctions within suitable habitat for actual breeding use of a water body.

Each year of assembled data continues to refine our understanding of the dynamics of Houston toad breeding and reproductive success. This year Houston toads again demonstrated that breeding pond use and subsequent reproduction are not strongly correlated. In only a few locations during any one year does a breeding chorus lead to successful reproduction. This may be a simple consequence of female rarity or be representative of environmental parameters or dynamics as yet not understood. Houston toads reproduced in Ponds 2, 5, 6B, and 11 during 2003. This is greater by one pond than the previous season, however, as in 2002 most of those egg strings failed to lead to successful juvenile Houston toads. Only in Pond 2 and Pond 11 were any emergent Houston toads found.

Other changes include both loss of chorusing at sites and additional areas found from which Houston toads were in chorus. The lack of breeding activity at Pond 16 continues while Ponds 6B, 20, and 21 were added to the number of locations on the GLR which have had multiple nights of chorusing in a given year. In the 2000 survey, Pond 16 was the first pond to provide Houston toad calls and one of only two locations to have had egg strings within it. In 2001 calls were heard at the inlet areas of the pond. Yet in both of the last two years no toads have called from this site. Among 2003 new locations, Pond 6B went further than simple chorusing by having an egg string deposited within it. Unfortunately as has been the case with Pond 6 during past years, 6B did not provide successful emergence of juvenile toads.

It was quite unexpected (by me) that additional chorus sites were likely to be found on the ranch. To my chagrin and despite extensive efforts on my part during 2000-2002 additional chorus sites continue to be found. However, 2002-2003 were the first "normal" rainfall years in nearly a decade. This reinforces the requirement of, at minimum, three years of consecutive surveys in order to document the occurrence of Houston toads on a given parcel. With only a single year or even two years of data several areas which support toad breeding would have been overlooked on the GLR.

At this point it is clear that our definitions for and distinctions among water bodies in the range of Houston toads is worthy of explicit presentation. We formally define a potential breeding pond as any water body of sufficient size and depth to persist more than 120 days in the spring and which occurs within the potential range of Houston toads. Chorus ponds are then locations that meet that criterion and have had male

Houston toads call from them. We usually refine this to be multiple nights of chorusing as we normally encounter individual male toads calling from roadside ditches or puddles for short periods (hours) or for a single night. Breeding ponds are locations having all of the above and providing evidence of successful reproduction in the form of either Houston toad egg strings or tadpoles. Finally and arguably most importantly, emergence ponds are locations from which successful emergence of juvenile Houston toads has occurred. For the three new locations named in 2003 on the GLR, one (6B) is now a breeding pond and two (Ponds 20 and 21) are chorus ponds. However, we recognize that only in rare abundant rainfall conditions will Ponds 20 and 21 be likely to ever support breeding. Indeed, these two locations are more likely to lead to unsuccessful reproduction given their relative ephemeral nature in most years and they are not consistent features of the landscape.

The survey data during the first three years had shown a consistent increase in both the number of individual male Houston toads heard and in the number of locations from which they are heard calling. During 2003 one new breeding location was mapped (6B) and one new emergence pond defined (Pond 11), but the overall number of toads heard decreased from 2002 levels. This is also correlated with diminished chorus activity by other amphibian species from 2002 levels.

Research

Juvenile trapping arrays

The successful trapping, monitoring, and measuring of juvenile Houston toads continues to reveal aspects of juvenile Houston toad ecology likely to be directly applicable to management both on the GLR and in the range of the toad generally. Techniques developed to determine the number of eggs in an egg string without disturbing the egg strand were not previously available. The work on the GLR has led to the development, testing, and evaluation of several methods seeking to obtain the raw number of Houston toad eggs in naturally deposited egg strings. The data from growth of juvenile toads in the wild have been submitted for publication and is now in press (Greuter and Forstner in press). The data reinforce the critical nature of the immediate pondside habitat to juvenile toads and their post emergent use of the adjacent uplands. Emergence ponds are rare among chorus ponds on the GLR, hence if that trend is true as a general case in the range of the Houston toad, protecting emergence ponds will positively influence Houston toad populations. Survivorship from the egg string stage through to emergence appears to be lower than 1%, compilation of the data thus far collected should enable explicit calculation of the survival through these life stages. We anticipate publication of that information in the spring of 2004. We would expect to locate the first recaptures of juveniles marked during the 2002 season in the upcoming 2004 breeding months and may then be able to calculate the survival through to the adult stage.

Artificial ponds

One of the primary limitations of the first year of experimental pond work was controlling the insect colonization of the ponds. We have ameliorated the majority of colonization by fabricating and placing screen covers over each of the ponds. This

decreased the amount of light reaching the pond surface and delayed the maturation times of tadpoles placed into the ponds by several weeks beyond that of the other half of the egg strands which remained in the native ponds. The raw number of Houston toad eggs that were available during 2003 was much smaller than the number used in 2002 due to the average smaller size of the egg strands found in the wild ponds. We will attempt to combine the 2003 data with 2002 and reanalyze the data taking into account the zero sum data available from many of the 2003 ponds. At this time while the experimental design remains robust the implementation of the design is hampered by the limitations on the number of Houston toad tadpoles that can be used in any one year. It may be as informative to use tadpoles of a much more common sympatric toad (Gulf coast toad) in 2004 to generate sufficient data to provide an adequate statistical test of the factors.

The collection of data is not without benefit however as a publication has been accepted reporting the data collected from wild ponds and attempting to correlate Houston toad breeding use to a variety of abiotic factors. Forstner and Ahlbrandt (in press) reports those data for other researchers and management authorities to incorporate into their own projects.

Herptofaunal array

The results from the herptofaunal array remain some of the most informative and descriptive field data we collect on Houston toads. We continue to capture Houston toads both in and out of the breeding season. We also collect juveniles with the implicit value when found as future recaptures. We have, indeed, successfully recaptured previously marked Houston toads from 3 years ago. None of the current recapture data would suggest that the population of Houston toads on the property is larger than predicted nor are we failing to collect a significant portion of the toads on the property. That claim is supported by the number of recaptures and consistency of trapping locations from year to year. In 2003 additional trapping arrays were placed between Pond 12 and the proposed location of Lake 1. These new arrays have collected Houston toads during 2003 and thus the primary baseline information for subsequent management actions have been assembled.

Last year we began the process of conceptually exploring the differential sex ratio of males to females found in Houston toads. That has led to the submission of a manuscript (Swannack and Forstner in review) that seeks to explain the approximately 10:1 male biased sex ratio found in Houston toads. If our model is correct then this ratio is not an error induced by sampling at the breeding ponds, but rather a consequence of differential maturity of female toads in the wild. This has important implications for Houston toad management, but also for understanding amphibian sex ratios in general. That publication is a direct result of the correlation of breeding pond survey data and the information collected from the trapping arrays.

The distribution of captures on the GLR continues to demonstrate mainly canopy forest captures (Figure 3). Indeed none of the evidence collected thus far would allow the characterization of Houston toad habitat as being other than primary canopied forest over deep sandy soils. In 2003 we increased the recorded distance for an individual Houston toad to have moved to 1.4 miles or 2263m. This movement took 3 years and is the farthest distance recorded for an individual toad dispersal. A second individual was recaptured in 2003 within 100m of the original capture site three years previous. In 2003 no hybrid phenotypes nor audio calls were found among toads on the GLR.

Vegetational mapping

The vegetational and soil surface descriptions have been completed for a full year of seasons and from multiple locations. It will be critical to properly place this vegetation context alongside the dispersal data and the collecting locations for Houston toads on the GLR. We have requested and received federal permit permission to conduct a controlled burn on the GLR subsequent to an approved burn plan. We are now in the process of working with the TFS to best design a plan and provide the prescription to the Austin USFWS office for approval.

Conclusions

Over the course of the past 4 years of survey work and research the current Houston toad population on the GLR has become much less mysterious. We have collected data demonstrating the most prevalent Houston toad numbers on the property exist near Pond 2 during the breeding season. Houston toads are present in all forested regions of the ranch with suitable soils, but have not been collected in the areas near Pond 1 nor Pond 4. While the physical number of adult Houston toads heard or collected decreased in 2003 the number of females remained fairly consistent. Similarly the number of chorus, breeding, and emergence ponds remained consistent with last year. The locations of each of those categories have tended to rotate among locations on the ranch since 2000 and this was true of the 2003 season.

During 2003 two recaptures were 3 years between capture date and recapture. One of those individuals has moved more than a mile (1.4 mile) in the intervening years. This is longest known dispersal for a Houston toad thus far documented. We also collected baseline information on Houston toads and the herptofaunal community in general for the area on the GLR to be inundated by the proposed Lake 1 construction.

Significant data has been collected on ecological aspects of the Houston toad that were poorly known or undocumented. Three current manuscripts provide these novel aspects in the form of scientific publications. Forstner and Ahlbrandt (in press) describes the abiotic factors examined and their relative effect on Houston toad breeding use of a given pond. Greuter and Forstner (in press) describes the growth of juvenile Houston toads in the wild. Finally, Swannack and Forstner (in review) provides a model of sex ratio evolution in Houston toad that seeks to explain the reason for male bias in the wild. These publications demonstrate our dedication to dissemination of our results in a timely fashion. While not broadly synthetic each paper contributes to the overall knowledge of the Houston toad and can now be directly cited in current recovery planning and management discussions. Our current plans include publication of the dispersal data for Houston toad adults and juveniles, new methods of estimating egg number for *in situ* egg strands, and a detailed treatment of the specifics of microhabitat use by the Houston toad throughout the year.

Houston toad research and surveys

2002 data and final report

for the

CAC-Lost Pines & Griffith League Ranch
Bastrop County, Texas

by

Michael R.J. Forstner, Ph.D.

August 16, 2002

Introduction

The Houston toad is an endangered native Texan. Currently at very low levels in every county in which it occurs, the toad can benefit from field documentation and field research directed at understanding its ecology. In order to assess areas under their control the Capitol Area Council of the Boy Scouts of America (CAC-BSA) has conducted audio surveys for the Houston toad on their properties in Bastrop County. The CAC-BSA has further sponsored research seeking data which are critical to the design of and management plans for Houston toads in Bastrop County and across its remaining range. That involvement includes documentation of occurrence and breeding via night surveys at the Lost Pines (LP) and Griffith League Ranch (GLR) BSA camps and field monitoring using reptile and amphibian collection arrays on the GLR.

The objective of these tasks is to describe the life of the Houston toad in the field and under different conditions. Many previous night surveys have been conducted, however, few have included the aspects of the program currently in place on the GLR and LP. Likewise, there have been many previous studies which sought to compile data on the Houston toad in the field. However, the current research program on the GLR is designed to evaluate the reptile and amphibian community, of which the toad is a part, and to attempt to discern habitat usage by the toad for management uses both on the GLR and elsewhere.

Each of these programs is integrated and the data have been collected so as to allow subsequent analyses to provide a measure of the success in the adaptive management programs instituted at the relevant locations. Conceptually, we have begun a long-term commitment to the investigation of how many toads there are, where they are, when they are present above ground, how they utilize their habitat, and what are the quantities in their life table. That life table is best summarized as a simplified model of Houston toads in the wild (or in this case, on the GLR). The parameters within that model are critical to predictive evaluations and discerning trends in the population. As monitoring continues it is our goal to provide direction that allows toad population levels on the GLR to increase alongside the scope of the CAC-BSA utilization of the property.

Methods

Two basic programs are underway on the CAC-BSA properties during 2002. The first of those represents a continuation of the previous years of endangered species consulting via audio surveys of the properties during the annual spring breeding season. This portion of the data gathering involves the completion of up to 20 nights of audio surveys with the goal of detecting and describing the occurrence of Houston toads on the property. The second program reflects a continuation of the herptofaunal (both reptiles and amphibians are collected) array project begun in 2000. This second program requires daily fieldwork every day of the year. Taken together these two approaches allow the current status and distribution of Houston toads on the property to be described. Furthermore, specific experiments have been designed in the research portion of the programs to provide answers about Houston toad habitat use, survivorship, and juvenile ecology.

Surveys

Obviously one of the most important aspects about managing endangered species is knowing where they are and how many of them are left. Conducted on an annual basis, audio surveys allow development of a trendline for the breeding activity of the Houston toad. Likewise, the data provide the information necessary to define the areas used by the toads during the spring

and to monitor changes in the abundance or distribution of the toads over time. Audio surveys for the Houston toad have minimum acceptable guidelines established by the USFWS. The audio surveys completed each year for the CAC-BSA meet and exceed those guidelines.

All audio surveys are conducted on nights when climatic conditions appear favorable to Houston toad breeding events. A series of established listening stations are monitored for a period of (minimally) five minutes and the calls of all amphibians are noted. When Houston toads are heard, attempts are made to physically locate the individuals if this can be accomplished without undue disturbance to the chorus. Individuals are then permanently marked using microchip transponders (AVID) and physical measurements are taken. Upon completion of the data collection all individuals are released back into the breeding pond. Prior to the initiation of the breeding season all ponds are examined and data regarding the water levels and chemistry are collected. Subsequent to active chorus nights, positive ponds are searched during daylight or early evening to evaluate the success of the breeding chorus by counting the number of egg strings found in each pond. Finally, at the end of the season at the time when juveniles emerge from the ponds, each pond is checked to determine whether or not juvenile Houston toads are seen at the ponds' edges. Performing these activities has assisted in the research phases of the next program.

Sponsored Research

The overall research design for the GLR has four distinct but integrated projects. The overall design allows GLR Houston toads to be evaluated and tracked from eggs through adults. The first project provides details for Houston toads during the juvenile stages. During the breeding season Houston toad egg strings are located. Three egg strings are selected and monitored (this is in concert with the second project described below). The number of eggs is estimated and then followed as the tadpoles hatch. The tadpole group (cohort) will then establish a defined foraging zone. This area along the pond edge is then ringed by concentric ellipses of raised herptofaunal fence. In some cases pit fall traps (1 quart plastic buckets) are used. Generally, these fences will be simply left as barriers to dispersal and will allow the subsequent emergent juveniles to be located, marked, and released. The juveniles receive a cohort mark and total numbers of individuals are monitored throughout the weeks of emergence. The juvenile arrays are left in position until 30 days after the last juvenile is found within them. Toe clips are retained from individuals to allow for potential DNA typing at a later time.

In the second experiment an artificial pond array is used to test specific effects of factors which may influence the survivorship of Houston toad tadpoles. A total of 24 ponds have been designed and placed to allow the effects, if any, that slope, insects, and fish play on the eventual emergence of Houston toad juveniles. The design provides both replicates and a control for the three treatments. Each pond is surrounded by a vertical barrier to allow accurate collection and counts of emerging toads. Juveniles which successfully emerge from the artificial pond array are released beside each pond. A perimeter array is in place at 50m from the artificial ponds and is monitored for dispersal of the juveniles away from the growth ponds.

The third experiment capitalizes on each of the preceding experiments and the annual survey work. The entire GLR is monitored for Houston toad activity and distribution by a herptofaunal array. The array consists of vertical barriers (aluminum flashing set into the ground and held vertical with stakes), pit fall traps (5 gallon buckets placed into the ground level with the surface), and funnel traps. The funnel traps are placed alongside the vertical "fence" and animals hop into the funnels but are unable to hop out of the funnels. Thus animals are collected by the trapping arrays during all hours. The arrays are checked every day at first light. As rains can flood the subsurface buckets, heavy rains require that some of the buckets be closed until the saturation in the sandy soils has retreated. Similarly, fire ants have been a problem at some locations, these locations are monitored and when necessary treated using commercial fire ant "bait" (Amdro). Mortality within the buckets is kept to a minimum by providing damp cotton towels to insure both humidity and temperature relief to animals trapped in the buckets. Funnel

traps are shaded using aluminum flashing to prevent heat stress to animals trapped in the funnel system. The arrays have been placed in a design which varies both the vegetation and the distance from known breeding ponds. By examining the distribution of Houston toad captures it may be possible to draw conclusions about habitat use and movement in and out of the breeding season. The data collected from other species of vertebrates will eventually allow a characterization of the reptile and amphibian community within which the Houston toad exists.

Finally, all of these experiments and the surveys generally will be put into a context reflective of the habitat present on the GLR. Current vegetation mapping, vegetation density, and duff depths are being completed for the ranch. At the completion of this project we will be able to place an appropriate habitat reference context onto the results for toad distribution and breeding.

Results

Surveys overall

The 2002 season was a very unpredictable spring. Temperatures were abnormally cold and two very hard freezes occurred relatively late into the season. Similarly the normal spring cold fronts failed to bring significant rain events with any regularity. As a consequence the breeding chorus events for Houston toads began on schedule but then abruptly stopped. When the temperatures had warmed again by mid March the toad returned and called for several weeks but seemingly compressed the overall breeding season into just a two week span. While many nights of Houston toad chorusing were heard, only a few of those nights provided any female Houston toads at the ponds. This is in keeping with previous years. We have located an additional breeding location on the GLR (also in keeping with previous years). Pond 18 lies along the powerline easement due south of Pond 10. This drainage basin retains several deep holes which filled with water this spring and attracted a Houston toad to chorus. Houston toads were not heard on the Lost Pines property. They were heard in close proximity (within 500m) of the eastern perimeter fence and across the lake at two sites.

Lost Pines survey

Table 1 provides the compilation of results for the 2002 surveys of Lost Pines.

Table 1. Houston toad audio survey results for the Lost Pines BSA property, Bastrop County for 2002.

Date	18-Jan	17-Feb	19-Feb	8-Mar	18-Mar	5-Apr	9-Apr
Temp (F)	71	75	52	75	68	69	65
Humidity	?	67	?	?	70	46	65
Wind	15	1	1	0	1	0	4
Moon	0	0	0.333	0	0	0	0
Post 1	1RS	0	0	1BH (SE)	2BH (SSE)	0	0
Post 2	1AC	0	0	5RS	3RS	1RS	2RS, 2HVC
Post 3	0	0	0	0	0	0	0
Post 4	0	0	0	0	0	0	3BH (S)

Species Key

= 0=None
AC=*Acris crepitans*
BH=*Bufo houstonensis*
BS=*Bufo speciosus*
BV=*Bufo valliceps*
BW=*Bufo woodhousei*
BX=*Bufo sp.* hybrid?
GC=*Gastrophyrne carolinensis*
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*designations within parentheticals represent a compass direction to the calls heard

Griffith League Ranch

Table 2 provides relevant data for the 2002 Houston toads audio surveys conducted on the GLR.

Table 2. Houston toad audio survey results for the Griffith League Ranch Bastrop County, Texas for 2002.

Date	7-Jan	22-Jan	6-Mar	7-Mar	8-Mar	9-Mar	17-Mar
Temp (F)	75	75	70	64	72	50	62
Humidity				75		17	61
Wind	9	4	7	3	5	3	0
Moon		0.5	0	0	0	0	0
Pond 1	pond 3/4 full	0	3BH (W)	0			0
Pond 2	pond full	0	3.3BH	6BH, 1RS		0	0
Pond 3	pond empty	0	0	0	0	0	0
Pond 4	pond 1/2 full	0	1RS	0	0		0
Pond 5a,b	pond 5a empty, 5b full	1RS	1BH	2.1BH, 1RC, 1RS	5BH, 1RS	0	0
Pond 6	pond empty	0	0		0		0
Pond 7	pond full	0	0		0		0
Pond 8	pond full	0	7AC	0	0		0
Pond 9	pond full	3RS	1RS	0	0	0	0
Pond 10	pond full	0	0	0	0	0	0
Pond 11	pond full	6RS	0	0	0		0
Pond 12	pond very full	0	0	0	2BH, 3RS	0	0
Pond 13	pond 1/2 full	0	0	0	0		0
Pond 14	pond 1/2 full	0	0	3RS	0		0
Pond 15	pond full	0	0	0	0		0
Pond 16	pond very full	0	0	0	0		0
Pond 17	pond full	0	0	0	0		0
Pond 18	not visited (unknown)				1BH (NEWPOND)		0
Pond 19 (Alum)	flowing	0	0	0	0		0

Species Key =	0=None	**BX**=*Bufo sp.* hybrid?	**PS**=*Psuedacris streckeri*
AC=*Acris crepitans*		**GC**=*Gastrophyrne carolinensis*	**PT**=*Psuedacris triseriatus*
BH=*Bufo houstonensis*		**GO**=*Gastrophyrne olivaceous*	**RC**=*Rana catesbaena*
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BW=*Bufo woodhousei*		**PC**=*Psuedacris clarki*	**SH**=*Scaphiopus couchi*

Table 2. Cont. Houston toad audio survey results for the Griffith League Ranch Bastrop County, Texas for 2002.

Date	18-Mar	19-Mar	22-Mar	23-Mar	24-Mar	27-Mar
Temp (F)	70	60		63	72	68
Humidity	68	69			68	36
Wind	0	15		5	5	0
Moon	0	0		0.5	0.5	1
Pond 1	1HVC		2RS	0	1BV, 4RS	0
Pond 2	2AC, 18.5BH, 15HVC, 5RS	5.1BH, 8HVC, 1RS	5AC, 3RS	4RS	10BH, 1BV, 1BX, 10HVC	1AC, 1RS
Pond 3			1RS	0	1RS (1BH @COPE)	0
Pond 4			5RS	0	3RS	0
Pond 5a,b	1BH	1BH	6AC, 3RS	4HVC	1BH	1HVC,3RS
Pond 6	0	STORM	0	2HVC	0	0
Pond 7	3BH	ABORT	2RS	0	0	0
Pond 8	0		2AC, 1RS	0	2HVC	
Pond 9	1RS		5RS	1RS	0	1RS
Pond 10	1AC		8HC, 1RS	0	0	0
Pond 11	0		5AC, 7RS		0	4RS
Pond 12	3RS		2RS	1RS	0	2RS
Pond 13	1BH, 1RS		0	1RS	1BH	2AC, 2RS
Pond 14	1HVC		3RS	1RS	0	0
Pond 15	0		10RS	0	0	0
Pond 16	0		0	0	5HVC, 1RS	0
Pond 17	0				0	0
Pond 18	0				0	0
Pond 19 (Alum)	4BH				1BH	0

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Table 2. Cont. Houston toad audio survey results for the Griffith League Ranch Bastrop County, Texas for 2002.

Date	28-Mar	29-Mar	8-Apr	10-Apr
Temp (F)	74		73	78
Humidity	74		89	
Wind	2		3	3
Moon	1		0	0
Pond 1	0		1HVC	5AC, 10RS
Pond 2	10.2BH, 5AC, 20.3HVC, 4RS	18BH, 5AC, 2BV, 30HVC, 3RS		10AC, 5HVC, 10RS
Pond 3	4HVC	4HVC	2HC, 5HVC	0
Pond 4	0	3RS	2RS	1.1RC
Pond 5a,b	2HVC, 2RS	1BH	9BH, 1BV, 2HC, 10HVC, 10RS	8AC, 2HVC, 10RS
Pond 6	0	1BH	2BH, 3BV, 5HVC, 10RS	10AC, 2HVC, 3RS
Pond 7	0	5BH	0	4AC, 4RS
Pond 8	3HVC	1BH	5AC, 2BH, 4BV, 3HVC, 4RS	0
Pond 9	0	5BH, 2BV, 2HC, 13HVC, 1RC	3BH, 2BV, 5HVC, 3RS	5RS
Pond 10	1BH	1BW, 3BH, 1BV	1BH	5AC, 2RS
Pond 11	0	3BH	2BH, 1BV, 5RS	10AC, 5RS
Pond 12	2RS	4BH, 1BV	4BH, 2RS	2AC
Pond 13	0	1BH, 3RS, 5AC	0	1HVC, 1RS
Pond 14	0	1BH	0	2RS
Pond 15	0	0	0	0
Pond 16	0	0	0	1RS
Pond 17	0		0	
Pond 18	0		2BH	
Pond 19 (Alum)	0		0	

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Table 2. Cont. Houston toad audio survey results for the Griffith League Ranch Bastrop County, Texas for 2002.

Date	16-Apr	17-Apr	23 May	12 June
Temp (F)	77	80	70	83
Humidity	87	67		27
Wind	3	1		A: 12
Moon	0.2	0.125		
Pond 1	3BV, 6HC, 5HVC	2BV, 2HC	No toadlets	RS tadpoles
Pond 2	20AC, 3.1BH, 5RS, 6BV, 10CH, 10HVC		BH toadlets	RS, HVC tadpol
Pond 3	4BV		No toadlets	no tadpoles dr
Pond 4	5HC, 5HVC, 6RS	10AC, 1BV, 4HVC, 2RS	No toadlets	RS,HVC tadpol
Pond 5a,b	4BV, 5HC, 3HVC, 2RS		BH toadlets	RS tadpoles, 5a
Pond 6	2BV, 1HC, 5HVC		No toadlets	dry
Pond 7	5SH		BH toadlets	SH tadpoles
Pond 8	3BV, 1HC, 10HVC		No toadlets	nearly dry
Pond 9	5AC, 4BV, 3HVC		Not examined (ordnance)	RS tadpoles
Pond 10	3BV		Not examined (ordnance)	no tadpoles
Pond 11	5HVC	2HC, 10HVC, 2RS	Not examined (ordnance)	RS tadpoles
Pond 12	10AC, 1BH, 5BV, 4RS		No toadlets	RS, RC tadpole
Pond 13	0		No toadlets	dry
Pond 14	1RS		No toadlets	RS,HVC tadpol
Pond 15	0		No toadlets	no tadpoles
Pond 16	2BV, 2HVC, 4RS		No toadlets	no tadpoles
Pond 17	3RS	0	Not examined (ordnance)	RS tadpoles
Pond 18	1BH		Not examined (ordnance)	dry
Pond 19 (Alum)	5BV	3BV	Not examined (ordnance)	no tadpoles

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The results from GLR are predictable in most cases given the previous years of surveys. Ponds which have historically shown Houston toad chorusing, did so in 2002. As happened in 2001 not only are additional ponds now known to attract toads, but a new site was found which had male Houston toads calling from it (Pond 18). This year Pond 13 and 14 had Houston toads appear at the pond for the first time. While I did not observe Houston toads at Pond 6 during my surveys, Houston toads did breed in Pond 6 on a night I did not survey. They were heard by part of the field crew and I observed egg strings in the pond the following morning. Pond 15 had calls in 2001 but did not in 2000, nor in 2002. Notably Pond 16 failed to provide Houston toad breeding for the second year in a row after having done so in 2000. Finally, Ponds 17, 4, and 1 have never had Houston toads during any of the three survey years. Houston toads have been heard in 2001 and again in 2002 west of Pond 1, directly across the west fence of the property at a distance of less than 200m. A total of 14 Houston toad females were observed on the entirety of the GLR during 2002. Eleven of those females were found at Pond 2. The remaining females were seen singly at Pond 5b, Pond 6, Pond 7, and Pond 10. Egg strings from those females were found at all ponds except Pond 10 on the day after the breeding chorus was observed. While the greatest number of females appeared on nights with the largest choruses (5 females at a chorus of 18 males), females were also seen when only 3 or less males were present and calling. A total of 145 males calling were heard across the entire season on the GLR. The first of these calls occurred on March 6th and the last calls were heard on the 16th of April. Not all of those calls were from unique individuals, thus approximately 70 total toads were found on the GLR found during the 2002 season.

Research experiments

Juvenile arrays

The 2002 season was challenging for this experiment. Egg strings were tightly clustered in both time and space. As part of the design sought to examine only the reproductive output from a single female, compromises were made to allow data collection. Three egg strings were surrounded by bank side fencing, but two of the collecting arrays overlapped on the outer ellipses. Approximately 400 toadlets emerged and were marked during the 2002 season. Cohorts spent a week or so near the waters edge before disappearing into the forest leaf litter. During dry periods between rains, juveniles tended to cluster in shaded areas like root overhangs near the waters edge. They remained there for many days, in some cases, well beyond the normal 7-10 day period of near water residence. Weights and growth measurements were taken and will be compared with previously available data from captive growth experiments. Preliminary analyses indicate the growth in the post-emergent stage is comparable to that found in captivity. The majority of juveniles emerged from the ponds within a day or so of the entire cohort. For example, on May 26, we located 65 toadlets, but on the next two days a total of 5 unmarked individuals were found. Likewise a similar pulse occurred in the second juvenile trapping array on May 31st, with 23 toadlets found and low numbers of individuals found on the next two successive days.

Artificial ponds

The artificial ponds were successfully stocked and monitored for the 2002 season. Difficulties emerged in preventing the invasion of the array by amphibian "volunteers". These included *Hyla sp.*, but also a significant number of *Rana sphenocephala*. This

creates difficulties in the precise evaluation of the results as the numbers of individual tadpoles in each pond varied with influx of volunteer breedings. Among the variables tested, slope did not appear to influence Houston toad survivorship. The presence of fish did not influence the results of tadpole survivorship, which remained low overall in the presence of invertebrates. In a similar situation to that of the volunteer amphibians, invertebrate colonization of the ponds occurred rapidly and may have influenced the results from 2002. These influences will be prevented in 2003 by preventative covers placed over the array. Houston toad survivorship did not significantly differ among the treatments.

Herptofaunal array

The herptofaunal arrays have collected literally thousands of individual specimens. The majority of those are lizards (n=1795), specifically *Cnemidophorus* and *Sceloporus,* along side 53 snakes, and 19 turtles. However, most significantly there were 138 collections of *Bufo houstonensis* (including three suspected hybrid individuals) during 2001-2002 (Table 3). Most notably among those are collections accomplished outside of the breeding season. Approximately 60 (or about half) of the Houston toads collected were juveniles and were collected during the summer. Adults were collected as early as December 2nd, or approximately two months before animals were seen at the breeding ponds and again as late as August, well out of the breeding season. During this year we collected our first recaptures from animals marked during the previous year. At this time it is not yet valuable to calculate from those recaptures statistics what the overall population size might be, but with additional years of data that measurement will be forthcoming. Overall amphibian collections exceeded 550 individuals (data not included here).

It is notable that while the focus of our work remains Houston toads, we have now documented the occurrence of two species not previously reported from Bastrop County as a result of this project. The tiger salamander (*Ambystoma tigrinum*) and the Canebrake rattlesnake (*Crotalus horridus*) are now both valid county records originating from the GLR. In fact, we have now collected more than 40 tiger salamanders and have had several instances where individuals were recaptured. Likewise we have now documented nearly all of the expected reptile and amphibian fauna that is known from the county and region. Some expected taxa have not been found to date, these would include the snakes in the genus *Lampropeltis,* for example.

The distribution of Houston toad captures is likewise an important overall feature of the array design. With two exceptions all Houston toads have been collected in forested sites. Two Houston toads have been collected in buckets approximately 50m from the forest edge within a pasture. These are the only two specimens to have been found in the open grassland/pasture areas of the ranch. There has been no indication that Houston toads are found as a component of those pastures, nor does it appear likely that they cross wide open spaces. The pasture captures of Houston toads occurred along an edge of the large forested tracts or within a narrow "neck" of pasture which is border on both edges by forested tracts. More importantly, perhaps, no juvenile toads were found in any of the pastures.

Vegetation mapping

Several transects of the property have been completed during the spring of 2002. Additional transects are planned for the summer. Likewise vegetation types have been recorded and are being developed into a rough vegetation map for use during the winter of 2002 and spring of 2003. Duff depth and distribution are being compiled with the assistance of the Texas Forest Service to allow both compatibility and continuity with their database for the county.

Table 3. 2001-2002 Houston toad collections made by the herptofaunal array on the Griffith League Ranch in Bastrop County, Texas.

Date	Location	Genus	Species	Sex	Weight	SVL	HW	#	Capture	PIT
3/12/01	10-1	*Bufo*	*houstonensis*	M	24	59	22	10	1	
3/30/01	4	*Bufo*	*houstonensis*	M	24.5	58.55	21	1	1	
3/31/01	Pond 9	*Bufo*	*houstonensis*	M				10	2	122812234 A
3/31/01	Pond 9	*Bufo*	*houstonensis*	M	22	59.15	21.85	NA	1	122467456 A
3/31/01	Pond 10	*Bufo*	*houstonensis*	M	24	62	23.3	NA	1	122526737 A
3/31/01	Pond 11	*Bufo*	*houstonensis*	M	16	54	19.4	NA	1	122562752 A
3/31/01	Pond 11	*Bufo*	*houstonensis*	M	15	54	20.5	NA	1	122568177 A
3/31/01	Pond 11	*Bufo*	*houstonensis*	M	13	51	19	NA	1	122526311 A
3/31/01	Pond 11	*Bufo*	*houstonensis*	M	16.5	55	20.75	NA	1	122932124 A
4/16/01	10-2	*Bufo*	*houstonensis*	F	31	67	28.3	NA	1	122548357 A
4/16/01	1	*Bufo*	*houstonensis*	F	18	51	21	NA	1	122824117 A
5/11/01	b/w10-2&10-3	*Bufo*	*houstonensis*	F	M	22.5	50	NA	1	122529321 A
5/26/01	1-N	*Bufo*	*houstonensis*	J	< 1.0	10.95	4.55	1	1	
5/27/01	1-1	*Bufo*	*houstonensis*	J	<1.0	10.75	4	2	1	
6/9/01	1-1	*Bufo*	*houstonensis*	J	<1.0	10.95	4.95	3	1	
6/10/01	Pond 2	*Bufo*	*houstonensis*	J	<1.0	8.6	3.75	4	1	
6/11/01	P2 - N	*Bufo*	*houstonensis*	J	<1.0	10.3	4.3	6	1	
6/11/01	P2 - E	*Bufo*	*houstonensis*	J	<1.0	11.6	4.8	7	1	
6/11/01	P2 -W	*Bufo*	*houstonensis*	J	<1.0	7.5	3.4	5	1	
6/13/01	P2 - E	*Bufo*	*houstonensis*	J	<1.0	8.45	3.7	8	1	
6/13/01	P2 - E	*Bufo*	*houstonensis*	J	<1.0	9	3.8	9	1	
6/13/01	COMM	*Bufo*	*houstonensis*	J	<1.0	14.7	5.7	15	1	
6/13/01	COMM	*Bufo*	*houstonensis*	J	<1.0	13.05	5.1	11	1	
6/13/01	P2 - E	*Bufo*	*houstonensis*	J	<1.0	11.4	4.75	7	2	
6/15/01	1-E	*Bufo*	*houstonensis*	J	<1.0			15	2	
6/16/01	P2-S-3	*Bufo*	*houstonensis*	J	<1.0	7.85		NA	NA	
6/16/01	1-E	*Bufo*	*houstonensis*	J	<1.0	8.3	3.65	13	1	
6/16/01	P2-W-N9M	*Bufo*	*houstonensis*	J	<1	12.95	4.75	12	1	
6/16/01	COMM	*Bufo*	*houstonensis*	J	<1	17.6	6.1	14	1	
6/17/01	1	*Bufo*	*houstonensis*	J	<1	14.55	5.8	15	3	
6/17/01	1	*Bufo*	*houstonensis*	J	<1	10	4.15	16	1	
6/17/01	1	*Bufo*	*houstonensis*	J	<1.0	11.25	4.55	17	1	
6/17/01	P2 N	*Bufo*	*houstonensis*	J	<1	7.75	3.5	18	1	

Table 3. (Cont.)

Date	Location	Genus	Species	Sex	Weight	SVL	HW	#	Capture	PIT
6/17/01	P2 - N	*Bufo*	*houstonensis*	J	<1	7.5	3.25	19	1	
6/17/01	P2 - N	*Bufo*	*houstonensis*	J	<1	8.45	3.2	20	NA	
6/17/01	P2-N-2	*Bufo*	*houstonensis*	J	<1	8.1	3.55	4	2	
7/3/01	P2 - N	*Bufo*	*houstonensis*	J	<1	8.15	3.55	40	1	
7/3/01	near 7 m	*Bufo*	*houstonensis*	J	<1	10.3	4.1	31	1	
7/7/01	4-E	*Bufo*	*houstonensis*	J	3	29.15	11.5	60	1	
7/7/01	4-1	*Bufo*	*houstonensis*	J	2	27	10.25	61	1	
7/7/01	P2 - SE	*Bufo*	*houstonensis*	J	< 1	12.95	4.85	62	1	
7/8/01	P2 - N	*Bufo*	*houstonensis*	J	?	10.5	4.45	63	1	
7/12/01	4-W	*Bufo*	*houstonensis*	J	2	24.9	9.15	64	1	
7/14/01	1-S	*Bufo*	*houstonensis*	J	2.5	34	11.4	25	1	
7/14/01	1-N	*Bufo*	*houstonensis*	J	<1	13.3	6.4	26	1	
7/15/01	1-E	*Bufo*	*houstonensis*	J	3.5	35.7	11.5	27	1	
8/18/01	1.5	*Bufo*	*houstonensis*	J	1.0	22.6	8.9	85	1	
8/19/01	1.1	*Bufo*	*houstonensis*	J	<1.0	18.5	7.5	21	R	
8/30/01	1-S	*Bufo*	*houstonensis*	J	2	29.4	9.9	50	R	
12/2/01	4-1	*Bufo*	*houstonensis*	M	23.5	59	12	32	1	036*825*298
12/16/01	7-1	*Bufo*	*houstonensis*	J	14	50.25	11.7	33	1	
2/18/02	Pond 2	*Bufo*	*houstonensis*	M	20.00	50.9	17.3	34	1	
2/18/02	Pond 2	*Bufo*	*houstonensis*	M	21.00	58.3	17.8	35	1	
2/18/02	Pond 2	*Bufo*	*houstonensis*	M	19.00	57.0	18.4	NA	1	113822134A
2/19/02	1-E	*Bufo*	*houstonensis*	M	17.5	51.2	12	36	1	
3/1/02	14-5	*Bufo*	*houstonensis*	F	47.5	75.15	23.1	NA	1	036*820*835
3/7/02	1-1	*Bufo*	*houstonensis*	M	20	58.7	18.5	37	1	NA
3/7/02	Pond 2	*Bufo*	*houstonensis*	M	22	62			R	14749455A
3/7/02	Pond 2	*Bufo*	*houstonensis*	M	20	56.6			1	115513131A
3/7/02	Pond 2	*Bufo*	*houstonensis*	M	18	57			1	116339123A
3/7/02	Pond 2	*Bufo*	*houstonensis*	M		59			1	116311493A
3/7/02	Pond 2	*Bufo*	*houstonensis*	F	32	63			1	
3/8/02	10-2	*Bufo*	*houstonensis*	M	19	58	27.6	NA	1	037*094*354
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	19	57.7	17.6	NA	R	116339123 A
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	20	56.6	17.1	NA	1	036*838*075
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	21	64.2	18.4	NA	1	036 793 068
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	17	54.5	18.3	NA	1	036 814 372

Table 3. (Cont.)

Date	Location	Genus	Species	Sex	Weight	SVL	HW	#	Capture	PIT
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	17	56.9	17.7	NA	1	036 812 564
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	19	59.2	17.8	NA	R	116311493 A
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	25	64.2	18.8	NA	1	036 818 032
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	21	60.7	17.6	NA	1	036 576 325
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	19	58.3	17.7	NA	1	036 576 029
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	25	62.2	19.5	NA	R	114749455 A
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	20	57.7	19.4	NA	1	036 817 280
3/8/02	POND 2 E	*Bufo*	*houstonensis*	F	26	62	20.8	NA	1	036 628 071
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	19	57.4	18.8	NA	1	113822134 A
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	19	56.9	17	NA	1	036 788 885
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	18	54.8	18.4	NA	1	037 091 815
3/8/02	POND 2 E	*Bufo*	*houstonensis*	M	20	60.4	18	NA	1	036 598 882
3/9/02	10-2	*Bufo*	*houstonensis*	M	14	52.4	16.6	38	1	NA
3/9/02	10-2	*Bufo*	*houstonensis*	M	17	58.8	18	39	1	NA
3/9/02	3-1	*Bufo*	*houstonensis*	M	22	59.5	17.65	41	1	NA
3/9/02	3-S	*Bufo*	*houstonensis*	M	19	60.7	18.3	NA	R	036 793 068
3/9/02	3-S	*Bufo*	*houstonensis*	M	20	57.8	18	NA	R	036 598 882
3/13/02	Pond 7	*Bufo*	*houstonensis*	M	31	60.6	19	NA	1	036 582 283
3/13/02	Pond 7	*Bufo*	*houstonensis*	M	22	50.3	18	NA	1	037 097 040
3/13/02	Pond 7	*Bufo*	*houstonensis*	M	23	62	18.1	NA	1	036 791 864
3/13/02	Pond 7	*Bufo*	*houstonensis*	M	34	59.9	17.5	NA	1	036 616 057
3/14/02	Pond 2	*Bufo*	*houstonensis*	M	20	59.5	18	NA	R	113822134 A
3/14/02	Pond 2	*Bufo*	*houstonensis*	M				NA	R	036 838 375
3/14/02	Pond 2	*Bufo*	*houstonensis*	F	31	61.5	19	NA	1	036 816 811
3/14/02	Pond 2	*Bufo*	*houstonensis*	M	21	58.6	17	NA	1	036 624 524
3/14/02	Pond 2	*Bufo*	*houstonensis*	M	25.5	62.4	17.3	NA	1	036 584 804
3/14/02	Pond 2	*Bufo*	*houstonensis*	M	17.5	55	15.5	42	1	
3/14/02	Pond 5	*Bufo*	*houstonensis*	M	19	57.1	16.9	43	1	
3/14/02	Pond 5	*Bufo*	*houstonensis*	M	25	62.5	17.8	44	1	
3/14/02	Pond 5	*Bufo*	*houstonensis*	M	30	62.6	18.2		1	
3/18/02	6-E	*Bufo*	*houstonensis*	J	6.8	38.9	13.1	45	1	
3/18/02	COMM	*Bufo*	*houstonensis*	M	22	59.1	18.2	46	1	
3/20/02	1-1	*Bufo*	*houstonensis*	M	21	54.9	16.5	NA	1	036 838 375
3/20/02	2-E	*Bufo*	*houstonensis*	M	24	58.8	16.5	NA	1	036 599 376

Table 3. (Cont.)

Date	Location	Genus	Species	Sex	Weight	SVL	HW	#	Capture	PIT
3/20/02	2-W	*Bufo*	*houstonensis*	M	17	53.3	17.5	NA	1	036 794 039
3/20/02	6-S	*Bufo*	*houstonensis*	M	16	53.6	16.2	NA	1	036 613 047
3/20/02	6-1	*Bufo*	*houstonensis*	M	22	55.6	17	NA	1	036 829 539
3/21/02	2-S	*Bufo*	*houstonensis*	M	17	57.4	16.35	NA	R	036 599 376
3/23/02	comm	*Bufo*	*houstonensis*	J?	0.94	25.8	9.2	47	1	
3/25/02	Pond 2	*Bufo*	*houstonensis*	M	18.9	58.7	17.5	4005	1	
3/25/02	Pond 2	*Bufo*	*houstonensis*	M	23	60.8	17.6	NA	1	114936332A
3/25/02	Pond 2	*Bufo*	*houstonensis*	M	18	59.8	19.7	NA	1	114624521A
3/30/02	C-F1	*Bufo*	*houstonensis*	M	18	53	18	NA	1	114621225A
4/7/02	Pond 6	*Bufo*	*houstonensis*	M	20.0	55.4	12.1		1	115319480A
4/7/02	Pond 6	*Bufo*	*houstonensis*	M	21.5	62.6	12.5		1	115115363A
4/7/02	Pond 6	*Bufo*	*houstonensis*	M	27.5	63.0	12.7		1	114634183A
4/8/02	10-2	*Bufo*	*houstonensis*	M	27	57.9	21.3		1	037 092 848
4/8/02	10-2	*Bufo*	*houstonensis*	M	22	54	19.5		1	037 092 520
4/8/02	B-F4	*Bufo*	*houstonensis*	M	19	58.6	16.9	61	1	
4/8/02	Pond 2	*Bufo*	*houstonensis*	M					R	113822134 A
4/8/02	Pond 2	*Bufo*	*houstonensis*	M					R	036 584 804
4/8/02	Pond 2	*Bufo*	*houstonensis*	M					R	116339460 A
4/8/02	Pond 2	*Bufo*	*houstonensis*	M	22	58.8	17.5		1	036 605 830
4/8/02	Pond 2	*Bufo*	*houstonensis*	M	17	53.5	17		1	037 608 070
4/8/02	Pond 2	*Bufo*	*houstonensis*	M	19	57.4	17.2		1	036 632 256
4/8/02	Pond 2	*Bufo*	*houstonensis*	M	17	55.5	17.1		1	036 614 296
4/8/02	Pond 2	*Bufo*	*houstonensis*	M	25	66	21.9		1	122462573 A
4/8/02	Pond 2	*Bufo*	*houstonensis*	M	20	60	17.6		1	037 090 520
4/8/02	Pond 2	*Bufo*	*houstonensis*	M	19	57	17.7		1	036 588 595
4/8/02	Pond 2	*Bufo*	*houstonensis*	M	13	51.5	16.4	67	1	
4/8/02	Pond 2	*Bufo*	*houstonensis*	M	19	59.2	16.6	68	1	
4/8/02	Pond 2	*Bufo*	*houstonensis*	M	21	58.3	17.7	69	1	
4/8/02	Pond 10	*Bufo*	*houstonensis*	F	21.5				1	
4/8/02	Pond 2	*Bufo*	*houstonensis*	M	26.5	63.7	21.2		1	114623351A
4/9/02	1-N	*Bufo*	*houstonensis*	M	30	65.8	19.1	93	1	
4/11/02	10-2	*Bufo*	*houstonensis*	F	26	62.4	18.2	95	1	
4/11/02	5-S	*Bufo*	*houstonensis*	M	22	56.5	16.7	96	1	
4/16/02	6-F6	*Bufo*	*houstonensis*	J	7.8	41.1	13.5	45	R	

Table 3. (Cont.)

Date	Location	Genus	Species	Sex	Weight	SVL	HW	#	Capture	PIT
4/17/02	B-1	*Bufo*	*houstonensis*	M	16	56.65	18	99	1	
5/28/02	6.1	*Bufo*	*houstonensis*	M	9.0	60.9	13.8		1	114615790A
9/5/01	1-S	*Bufo*	*toadlet*	J	5.5	39	13.3	4	3	
9/6/01	13-3	*Bufo*	*toadlet*	J	1	29.4	10	28	1	
9/13/01	1-N	*Bufo*	*toadlet*	J	3	35.2	12.7	22	1	
9/24/01	4-1	*Bufo*	*toadlet*	J	<1	10.5	5.5	20, 30, 2		
10/9/01	1-W	*Bufo*	*toadlet*	J	0.3	15	6	30	1	
10/9/01	4-E	*Bufo*	*toadlet*	J	0.3	14.6	5	31	1	
10/12/01	4-P	*Bufo*	*toadlet*	J	0.3	10.9	5.5	24	1	
10/12/01	1-W	*Bufo*	*toadlet*	J	0.6	17.6	5.8	25	1	
10/12/01	1-S	*Bufo*	*toadlet*	J	1.6	26.5	8.8	27	1	
10/19/01	1-N	*Bufo*	*toadlet*	J	1.5	21.9	6.9	28	1	
10/22/01	1-1	*Bufo*	*toadlet*	J	0.5	12	6	29	1	
11/9/01	4-W	*Bufo*	*toadlet*	J	0.8	21.9	8	**30**	1	
7/9/02	B-1	*Bufo*	*toadlet*	J	1.7	25.3	9.8	800	1	
7/9/02	1-W	*Bufo*	*toadlet*	J	> .1	8.2	4	300	1	
8/2/02	B-N	*Bufo*	*toadlet*	J	3.6	34.9	11.2	802	1	
8/2/02	B-N	*Bufo*	*toadlet*	J	0.2	14.2	5.3	803	1	
8/5/02	B-N	*Bufo*	*toadlet*	J	0.2	12.6	5.5	804	1	
8/8/02	A-1	*Bufo*	*toadlet*	J	0.2	15.7	5.5	805	1	
9/13/01	1-1	*Bufo*	*valliceps*	J	16	59.4	18.5	23	1	
11/17/01	1-N	*Bufo*	*valliceps*		14	54.4	13.3	**30**	1	
4/4/01	Pond 2	*Bufo*	*woodhousei / houstonensis*	M	20	57.65	21.85	NA	1	122462573 A

A: 35

Discussion

Surveys

The cumulative results from the three years of Houston toad audio surveys on the Griffith League Ranch provides clear evidence that Houston toads utilize the vast majority of the property. In fact the only area of the property which is not currently utilized for breeding by the Houston toad are the ponds near the front gate (Pond 1 and Pond 4) and the only pond which lies isolated by more than 50m of pasture from any wooded tract (Pond 17).

I believe these exceptions are important. Ponds 1 and 4 lie over soils which are considered to be unsuitable for the Houston toad. Note that both ponds are within ~500m of ponds that are both over appropriate soils and which have Houston toads choruses. In the case of Pond 1, there is an active Houston toad chorus off the GLR near the west fence roughly due west of Pond 1 itself. Similarly, Pond 4 is very near active Houston toads calling from Pond 5a,b and a feeder drainage at the COPE course. Thus, by consistently not supporting Houston toad chorusing these two ponds may indicate that, at least in the presence of ponds over suitable soils, Houston toads will not use ponds which lie over unsuitable geological formations for breeding. Pond 17 is a case where the soils beneath and surrounding it are suitable and preferred Houston toad soils. This pond lies less than 500m from Pond 11 which has active chorusing from Houston toads occurring every year surveyed and a similar distance from Alum Creek which has likewise shown choruses for the past two years. Pond 17 is a shallow seasonal pond which does not have fish and indeed supports a large colony of fairy shrimp (Anostraca) each spring. The shrimp are a classic indicator of shallow ephemeral pools devoid of vertebrate predators. This pond is seemingly more suitable for Houston toad breeding than either the fish rich environment of Pond 11 or the flowing system of Alum creek. Why then does it not support Houston toad reproduction? The only feature which distinguishes this pond from literally every other pond on the Griffith league ranch is its isolation from woodland/forested tracts by more than 50m. Pond 17 lies at the edge of the north fence line and within a large pasture track which wraps westward from Alum creek and north along that fenceline. The pond does have large trees immediately at its banks, but they are a single layer deep and it is possible that the pasture between this pond and the main forested areas of the GLR are preventing it being used as a breeding site for the toad.

Other changes include both loss of chorusing at sites and additional areas found from which Houston toads were in chorus. The lack of breeding activity at Pond 16 and several new sites in which chorus activity was heard are all new data for 2002. In the 2000 survey, Pond 16 was the first pond to provide Houston toad calls. In 2001 calls were heard at the inlet areas of the pond. Yet this year no toads have called from this site. One potential difference among these years would be that during the 2000 season the pond was very low with broad shallow banks. Not only was chorusing present in 2000, but breeding was observed and eggs deposited. For the last two years this site has had a full basin and consequent steep narrow banks. These factors may be related to the lack of Houston toad reproduction at the site. Ponds 13 and 14 have always appeared to be suitable sites for the toad. However, only this year have these sites actually provided calling or sitting males. One of the new sites identified this year was Pond 18. This is a series of deep (1.5m) but small diameter (3m) basins within the drainage due south of Pond 10. A single male Houston toad was heard calling from this location and subsequently found. This location lies quite nearly beneath the powerline easement running ~east-west on the property. Importantly this pond attracted Houston toads, and

yet lies within a cleared corridor in the forest. The ground cover in this corridor at this location is primarily herbaceous growth and heavily shaded vegetation providing mostly open sparsely vegetated ground. A second new site from which Houston toads called this year is the drainage directly east of the COPE course. This area is a broad shallow drainage which retains several pools after heavy rains. In 2001 a single individual was seen in this area but no calling was noted. In 2002 several male Houston toads were heard calling from this drainage. The survey data during the past three years has shown a consistent increase in both the number of individual male Houston toads heard and in the number of locations from which they are heard calling. It was quite unexpected (by me) that additional chorus sites were likely to be found on the ranch. To my chagrin and despite extensive efforts on my part during 2000 and 2001 additional chorus sites continue to be found. I believe there is strong evidence from this dataset in support of the requirement of, at minimum, three years of consecutive surveys in order to document the occurrence of Houston toads on a given parcel. With only a single year or even two years of data several areas which support toad breeding would have been overlooked on the GLR.

Research

Juvenile trapping arrays

The results from the 2002 season provide the only available field data for the growth of juvenile Houston toads just after emergence from the natal ponds. The anecdotal evidence collected this year indicates that the juveniles remain at the ponds' edge for a brief time and then depart. Efforts will be made in subsequent years to better qualify to where precisely they go when they depart. A significant design benefit of the marking of these individuals will be their potential recapture either as larger juveniles or as adults in the herptofaunal array or at the breeding ponds. Some measure of survivorship may be possible to calculate once or if, we begin to recapture these individuals. Most significant are the downward revisions of currently applied estimates of egg to emergent survival. Currently modeled estimates place survival of the juveniles at 1%. Survival from eggs to emergence would be a far larger percentage than survival to adulthood. Yet our estimates place survival from egg to emergence at less than 0.5%. These estimates need to be refined with additional precision in the measurement and counting of eggs present in a string. However, the estimate of 0.5% is more likely to be revised downward than upward by that increased precision.

Artificial ponds

The first year of the artificial pond array was successfully completed but several factors complicate the interpretation of the results from this year. It was unexpected to have volunteer amphibian breeding in the ponds. We erroneously believed that the barrier fencing would be sufficient to prevent animals from reaching the ponds from the outside. Obviously the many *Hyla* and *Rana* which came to the ponds differed strongly with our preconceptions. Thus the average tadpole load varied among treatments and was uncontrolled. Similar problems evolved from invertebrate colonization. We had planned to have the ponds filled and ready literally timed to the breeding activity of the Houston toad. Unfortunately the weather during the spring of 2002 delayed the toad breeding cycle and allowed time for invertebrates to colonize the artificial pond array. Hence while the experiment sought to control the numbers of potential invertebrate predators within each treatment this was only partially accomplished. Both of these

complications to the analyses will be overcome in the spring of 2003 by providing protective cover screens or the equivalent to the pond array.

Given those disclaimers, the results from 2002 do not agree with our initial hypothesis. While not statistically significantly different, the highest survivorship occurred in ponds which contained tadpoles and invertebrates, with a fairly steep slope (45 degrees). This conflicts with our belief that fish predation upon invertebrates and non-toad tadpoles species coupled with little predation by fish on the tadpoles of the Houston toad would mean the greatest survivorship. Hence we predicted that tadpoles plus fish plus invertebrates would provide the greatest overall survival of Houston toad tadpoles. This was not the case in 2002. We believe that the aforementioned complications, alongside smaller than expected stocked juvenile fish led to the results of the current year. We believe the smaller fish (Largemouth bass) were simply too small to effectively predate upon the late instar dragonfly (Odonata) larvae which were stocked alongside them. This would then have defeated the purpose of the treatment in placing both invertebrates and fish into the tadpole ponds in that treatment. We will correct these complications and the experiment will repeated during 2003.

Herptofaunal array

The results from the herptofaunal array are very positive. We are successfully capturing Houston toads both in and out of the breeding season. We are collecting juveniles and all individuals are being marked for future recaptures. We have, indeed, successfully recaptured previously marked Houston toads in the array. Over the coming years as the number of total individuals marked increases so will the recapture probabilities rise. It is possible to speculate that the total number of toads on the property cannot be vastly greater than what we are currently capturing in total between surveys and the herptofaunal array. The reason this is true is simply that recaptures are being found. Thus if the number of toads on the property were orders of magnitude greater than what we have seen then recaptures would be very unlikely. Therefore, recaptures are both good news and bad news. The good news is that we are efficiently collecting Houston toads on the GLR. The bad news is that the total number of toads is much lower than most estimates of Houston toad density would speculate for a parcel the size of the GLR. We collect significantly more males in the traps than we do females. This may begin to address the long held question regarding the gender bias observed in Houston toads.

It has long been questioned whether or not the paucity of females Houston toads seen in comparison with males was simply a result of sampling bias. That is, males come to the ponds and make noise and are therefore conspicuous. Females never call and thus are not as easily found as males. Alternatively, it has been proposed that female Houston toads come to the ponds for only a single night, or do not come to the ponds unless they are in physical condition to allow breeding. If either of those scenarios were true then it might be possible that the numbers of male and female Houston toads are roughly equal in the wild. We simply do not encounter the females as often. From the trapping system we would predict that a smaller bias would be found as the trapping system is not at the breeding pond itself. The traps provide a significantly male dominated bias in Houston toads. Far more males (n=86) are caught than females (n=9). This may be due to males moving more during the spring to seek breeding choruses, but females should likewise be moving through the habitat to forage and find shelter. Hence, it seems reasonable to speculate that the data are revealing the actual percentage of males and females in the

population and female Houston toads are far rarer than males. This differential survival of the sexes will be important to accurately model the populations.

Large numbers of amphibians and reptiles are being collected throughout the year by the herptofaunal array. The system is designed to examine both the overall herptofaunal community of which toads are a part, and to examine how toads utilize the habitat. Houston toads do not utilize pastures. Nearly half of the total trapping arrays lie in pastures bordered by forest and adjacent to known Houston toad ponds. In fact the design for the pasture fences was specific in placing the fences between known breeding ponds at different distances from the forest edge. The only toads found have been at the nearest bucket to the edge of the forest at distances less than 50m. It seems clear that Houston toads do not normally transverse large cleared or open areas during their spring activities above ground.

Another way to approach this issue would be comparing the overall amphibian activity with the results from Houston toads. Ultimately we would like to correlate results from the behavior of more abundant species (e.g. *Rana* or *Hyla*) to behavior found in the seldom encountered Houston toad. Amphibians are collected in the pastures. However the species which are most typically associated with ponds and pond edge habitat (ie. *Ambystoma* and *Acris*) are seldom found in the central pasture areas. Some of the array transverses drainages and those drainages typically have some or even significant tree canopy cover. These traps often have both of the pond edge fauna and would support the idea that drainage patterns provide a mechanism to provide dispersal of amphibians across open pasture land. However, those drainages require canopy cover to provide that corridor. This would not indicate that dispersal corridors are a particular viable option for Houston toads but that our evidence indicates the use of such areas by amphibians which share capture patterns with the Houston toad.

Finally it is notable that several hybrid or putative hybrid animals were found on the GLR during the past year. One of these was clearly intermediate in phenotype between Houston toads and Woodhouse's toad. The others appear to be aberrant phenotype Gulf Coast toads (*Bufo valliceps*). Both sympatric species were seen at ponds with Houston toads during active Houston toad chorusing and also later in the season. There did not appear to be any correlation between the position of the pond relative to the forest and the prevalence of Woodhouse or Gulf Coast toads. It has been proposed that these two species prefer open spaces and ponds in grasslands over those in dense forest. This is not true on the GLR. The highest prevalence of these two species on the ranch is at the same pond with the highest prevalence of Houston toads.

Vegetational mapping

The vegetational and soil surface descriptions are underway. It will be critical to properly place context on the dispersal data and the collection locations for Houston toads. As future management options will certainly require controlled burns, it will be invaluable to have the data from this and subsequent years to compare with the post burn data. The duff layers in the GLR are incredibly deep. Some areas have pine needle beds in excess of 10cm thickness. While potentially providing cover for toads, it is likely that this level of floor fuel is a long term detriment to the toad.

Conclusions

The third year of survey data and the first year of a three year research program have been completed. The results provide information which is directly applicable to the management of the Houston toad. Houston toads breed from February to April but are active above ground from December until August with sporadic occurrences in October and November. Adults however, remain concentrated in the months of January to April. Warm periods in December allow activity for adults above ground. The long held paradigm of Houston toads being directly impacted by human activities only during the breeding period needs to be subtly revised to include juvenile activity during the summer months directly adjacent to known breeding ponds. Juvenile toads are most easily disrupted by activity at or near the pond edges during the critical larval and juvenile post emergent stages, thus potential significant impacts are possible in these zones.

Each year of the surveys has provided additional data clarifying the distribution and occurrence of the Houston toad on the property. Every year has seen additional ponds or locations demonstrating chorus activity by the toad. Likewise some locations have only had activity during a single year of the three survey years. It is very apparent to this researcher that the currently required three year survey outline is well supported by our results on the GLR. However, our data would not support six visits as sufficient to accurately discern Houston toad breeding activity. Detection at several sites has been only achieved on a single night and decreasing the number of survey visits would have likewise decreased the chance of locating those chorus locations. Nearly all of the GLR supports Houston toad breeding. Notable exceptions include those ponds lying in unsuitable soils and a pond which is isolated from the forested areas by more than 50m of open pasture.

Each of the research experiments have been installed and large amounts of data collected during the past year. Each of these provides differing insights into the ecology of the Houston toad. Juveniles follow a common pattern of post-emergent behavior among amphibians, remaining near the natal pond for several days before dispersing. The marking of juvenile Houston toads should allow far more accurate measurements of juvenile survival than have been previously available. Indeed the only way to obtain that data is by the process we have undertaken. Similarly, determining the factors which influence tadpole survival are important. The experimental ponds allow tests in a field situation with reduced variables much more like laboratory experiments. Admittedly controlling those variables in the field is more difficult as we learned this year. However, we have adjusted our planning to account for these difficulties and will correct those problems in the spring of 2003. The results from the integration of these two projects with that of the herptofaunal array will be seen as the marked individuals are recaptured in the coming years.

The final level of scale integration will be the incorporation of the vegetation maps currently in development with the patterns of occurrence found for the Houston toad. Likewise it will be necessary to compare the duff layer characteristics to determine if an influence of this fundamental component of the toad's environment plays a role in the distribution or abundance of the species on the GLR.

From the standpoint of overall design for obtaining information deemed critical by the BSA-CAC, the USFWS, and Houston toad biologists, the plan is working. We are obtaining data on scales which have not been available for the Houston toad. There are several aspects which remain to be addressed. Foremost among these would be telemetry

of the adult toads. Radio tracking the individuals would provide dispersal data for this life stage which we currently do not have. The technology exists but at this time the funding is not available to us to purchase necessary equipment to perform that part of this work. Juvenile dispersal cannot be achieved with telemetry but other methods (eg. Fluorescent powder tracking and grid based forest litter searches) may be attempted. We are currently working on methods to obtain more accurate counts of the number of eggs in a given eggstring in the wild. This will be a necessary component if we are to accurately measure the survival to the emergence stage of Houston toads in the wild. Similarly, the presentation of these results in scientific journals has begun. The records for new taxa in Bastrop County have already been submitted and individual reports for other aspects of the project are currently in draft form. It is our intention to publish subsets of the overall project results to insure the most rapid availability of our results in a format that permits citation of this body of work.

2001 Houston toad survey results and summary report for the Griffith League Ranch, Bastrop County, TX

Michael R. J. Forstner

June 7 2001

The second year of a three year survey for Houston toads on the Griffith League Ranch in Bastrop County, TX was begun on January 4, 2001 and concluded on 4/15/01. During the first year of the study Houston toads were found to occur and to breed on the property (see 2000 Griffith League Toad Survey data). The second year of survey on the Griffith League Ranch enhanced our knowledge of both Houston toad occurrence and ponds used for successful breeding. It was a particularly good year for Houston toad surveys due to increased rain and generally mild temperatures in the late winter and early spring. During the 2001 season eight new breeding sites for the Houston toad were added to the list of active ponds (Table 1). I believe two factors played an important role in the increased breeding activity, the break in previous drought conditions and the minimization of livestock impacts on several ponds.

Table 1. Summary of the occurrence of Houston toad breeding chorus activity on the Griffith League Ranch in Bastrop County, TX during the 2001 breeding season.

Pond ID	Houston toads Present	*Bufo* tadpoles present	Juvenile toads observed	Mark/Recapture study site
Pond 1	No	No	No	No
Pond 2*	Yes	Yes	Yes	Yes
Pond 3	Yes	Not detected	Not detected	No
Pond 4	No	No	No	No
Pond 5a, b	Yes	Yes	Not detected	Yes
Pond 6*	Yes	Yes	Not detected	Yes
Pond 7*	Yes	Yes	Yes	Yes
Pond 8*	Yes	Not detected	Not detected	Yes
Pond 8-B?	Yes	Not detected	Not detected	Yes
Pond 9	Yes	Yes	Yes	Yes
Pond 10	Yes	Yes	Not detected	Yes
Pond 11*	Yes	Not detected	Not detected	No
Pond 12*	No	No	No	No
Pond 13	No	No	No	No
Pond 14	Yes	Not detected	Not detected	No
Pond 15	Yes	Not detected	Not detected	No
Pond 16*	Yes	Not detected	Not detected	No
Pond 17	No	No	No	No
Spicer Creek	No	No	No	No
Price Creek	No	No	No	No
Alum Creek	Yes	Not detected	Not detected	No
* indicates a pond which was positive for Houston toads in 2000				

There were several additional sites not on the ranch property, but adjacent to it, which had Houston toad breeding choruses. There was also an additional chorus heard to the NW of Pond 8, which has not been located (Table 1, Pond 8b?). There is no particularly likely spot on topographic nor wetland maps. I believe it to lie adjacent to the drainage N of Pond 8. Likewise there was a broad shallow marsh on the east side of Alum creek that had calling males. Despite repeated efforts on several nights, I was unable to locate individuals within the marsh area and no tadpoles were subsequently observed in the marsh, nor in Alum creek.

In summary there were eight additional sites which had breeding or calling males present during the 2001 season. This brings the total number of sites which had calling males and or breeding pairs to 15 sites across the first two years of the survey (Figure 4 amended). One of those 15 sites (Pond 12) did not have Houston toads calling from the pond nor the adjacent drainage during the 2001 season, but did so during the 2000 season. Roughly 70% of all water bodies deeper than six inches had Houston toads visit them during the previous two years. Toads do not appear to utilize the drainage systems on the Ranch for breeding. Using the USFWS accepted dispersal distances of 0.95 mile (Price TPWD unpublished report), I have generated a map outlining the theoretical

utilization of the property by the Houston toad using the data available up to the current year (Figure 1).

Notably. several ponds which had been previously impacted by cattle utilization during previous years and which did not have Houston toads in them during the 2000 season were used by Houston toads in the 2001 season. The Boy Scouts of America choose to remove the cattle, upon consultation with experts, in an attempt to determine if minimizing pond usage by livestock could enhance the habitat for the Houston toad. The results of their decision have been dramatic. The changes in pond edge habitat were quite apparently beneficial to the toad using the pond and have resulted in three ponds (Pond 3, 5a,b, and 9) that failed to demonstrate breeding choruses in 2000, having breeding in them during the spring of 2001. The pond edges have revitalized quickly and now have both vegetation and invertebrate fauna that are logically conducive to amphibian reproduction. Furthermore, Pond 11 demonstrated a significant Houston toad chorus and many more amphibians generally with decreased compaction and vegetation loss at the pond edges upon the minimization of cattle use of that pond. Finally, the ponds that did successfully breed this year have a robust cohort of juvenile toads at the pond edges which might otherwise have been affected by heavy livestock use. I have included the raw field notes for the 2001 season in Appendix 1.

I look forward to the results of our ongoing collaborative efforts toward the eventual recovery of this species. If you require additional information or clarification on any of these issues please do not hesitate to contact me at your earliest convenience. It is my expectation to be in the field during the months of July and August 2000, returning to San Marcos in September 2000.

Sincerely,

Michael R.J. Forstner

23 Aug 2000

Agency: Boy Scouts of America
Capitol Area Council
7540 Ed Bluestein Blvd.
Austin, TX 78723

From: Michael R.J. Forstner
2751 Old Seguin Rd
San Marcos, TX 78666

Final Report

Griffith League Ranch

Houson Toad Survey 2000

Bastrop Co. TX

I. INTRODUCTION

The primary goal of the 2000 survey was to examine the Griffith League Ranch in Bastrop County, Texas for the presence of Houston toads (*Bufo houstonensis*). A secondary goal was to specifically examine target areas of the property. Those target areas have been tentatively depicted as regions which may be altered during the eventual development of the site as an adventure camp for the Boy Scouts of America. Where possible a more general survey of other amphibian activity and general abundance was also conducted.

The property contains pastureland, pine forest, and mixed pine/oak uplands. The majority of the property overlies the Carrizo formation which is very often a good indicator of Houston toad habitat. The majority of the water bodies on the property appear to represent engineered livestock tanks. However, the relative age of several impoundments is quite old. The property has been ranched since 1832 and the size and relative age of many of the pines growing out of the impoundment dams would support a long history of water at many of the ponds on the property. There is an active perennial stream, Alum creek, which enters and exits the property. Furthermore, spring flow was evident in one of the drainage systems. Thus, there are both standing and flowing water sources on the property. Although many of these are the result of human modifications to the habitat, those modifications appear to have been engineered many years in the past.

II. METHODS

Twenty two visits were made between February 7, 2000 and May 22, 2000. The first of those visits was the site reconnaissance and was made during daylight hours. On March 15, 2000 the night survey was conducted by Phil Koepp, Marsha Koepp, and Martin Payne. I was injured and was unable to physically complete surveys again until the 22nd. The latter half of the survey (April 7 – May 22; 10 visits) was performed during afternoons and early evening hours. These survey trips were specifically designed to examine the specific areas of the property which are suggested for modification on the Spring 2000 site plan. The general study consisted of physical examination of known pond sites and drainages on the property. Each pond and drainage was specifically identified by a number and whenever possible with GPS coordinates.

Each site was examined by listening for Houston toad calls after dark on nights when the ambient temperature exceeded 57 F. Nights immediately after rainfall were considered especially suitable. Each night's surveys also included playing pre-recorded Houston toad audio tapes to elicit response calls and physically walking the perimeter of each pond to explore the presence of any amphibians present but not actively calling nor responding to the tapes. Each evening other vertebrate activity at each pond was noted (fish, feral hogs, etc..) and finally the number and variety of tadpoles present in each pond was investigated as the breeding season progressed. When possible audio recordings of Houston toad chorus events were made on site.

To increase the efficiency of the total survey once a pond was verified to have active Houston toads it was excluded in subsequent survey visits. Thus, subsequent efforts were more focused on ponds which had not yet demonstrated occurrence of Houston toads.

The initial proposal specified 12 survey visits. The number of visits was expanded in April by the addition of 8 further visits to allow examination of any Houston toad breeding activity (and that of any other amphibians) specifically within the drainage areas of the property. Drainage systems were surveyed during daylight and early evening hours and all water bodies were examined for tadpole activity.

After the completion of the survey I was able to obtain information from Andy Price of the Texas Parks and Wildlife Department regarding his survey of the property in

1993. However, this previous report was not available to me at the inception of the 2000 survey. Consequently the identification of potential breeding sites was performed by examination of USGS wetland maps and by truck survey of the property.

III. RESULTS

SITE AND SAMPLING LOCATION DEFINITIONS

The 5,000 acre site is primarily deep sandy soils with pine forest containing numerous cleared pastoral areas and associated engineered stock ponds. Within the forested areas several additional artificial and natural ponds and/or wetland areas exist. Based on maps derived from USGS wetland survey data 15 ponds were initially identified (ponds #1-15 in Table 1).

Two additional ponds were identified during the current survey which were not depicted on the wetlands maps. One of the otherwise unmapped ponds (#16) was located on the first site visit on Feb 7, 2000 and the second pond (#17) was subsequently located on March 20, 2000 by Phil Koepp (Table 1).

During the final phase of the 2000 survey two additional sites (#18 and #19) within drainages were identified as potential habitat. These two marshy areas were discovered during the final days of the survey and should be included in any subsequent survey efforts. Both sites rest at the end of their respective drainage system and lie at or adjacent to the fenceline of the property. However, neither of those two aquatic habitats held any tadpoles nor emerged toadlets on the days examined. Both areas lie within drainage systems which might prevent their use by Houston toads due to relatively steep adjacent banks and potential of heavy runoff.

Perennial water appears to be available in both the Alum Creek drainage and within Drainage B (Table 2). Drainage B contains both running water and pooled areas of standing water within the spring bed. Both are tentatively assumed (potentially erroneously) to be spring fed.

SURVEY TIMELINE AND DEVELOPMENT

During the first site visit by Dr. J.R. Dixon and myself on Feb 7, 2000 a total of 16 ponds were physically visited (ponds #1-16 in Table 1, Figure 1). Ponds #17–19 were not located at this time. It was the consensus of both Dr. Dixon and myself that many of the water bodies were suitable Houston toad habitat. Furthermore, we observed *Bufo*

tadpoles in one of the ponds (#8) on this day. It is very likely that those tadpoles were *Bufo houstonensis.* Subsequent to this visit a proposal for a presence/absence survey was submitted and was accepted by the Boy Scouts of America.

Table 1. Griffith League Ranch Houston toad survey 2000 pond identification. Ponds were numbered, provided with descriptive names, and if possible GPS coordinates were determined.

Number	Name	Houston toads	Coordinates
1	Scripta Pond	-	N 30 12' 51" W 97 15' 20"
2	Log Pond	Y	N 30 12' 26" W 97 15' 02"
3	Culvert	-	N 30 12' 43" W 97 14' 55"
4	Cowtank 1	-	N 30 12' 23" W 97 14' 57"
5a	Marsh 1	-	N 30 12' 34" W 97 14' 35"
5b	Cowtank 2	-	N 30 12' 33" W 97 14' 32"
6	Seep	Y	N 30 12' 54" W 97 13' 58"
7	Atta Pond	Y	No GPS coverage
8	House Pond	Y	N 30 12' 20" W 97 14' 04"
9	Cowtank 3	-	N 30 12' 07" W 97 13' 23"
10	Steer Pond	-	N 30 11' 53" W 97 12' 48"
11	Elm Pond	Y	N 30 12' 07" W 97 12' 33"
12	Finger Pond	Y	No GPS coverage
13	E Roadside Pond	-	N 30 10' 37" W 97 13' 59"
14	W Roadside Pond	-	No GPS coverage
15	Crosslog Pond	-	N 30 10' 36" W 97 13' 58"
16	Reargate Pond	Y	N 30 10' 09" W 97 13' 57"
17	Fenceline Pond	-	N 30 12'17" W 97 12' 27"
18	South Gate drainage Fenceline Pond	-	No GPS coverage
19	S Fork Price Creek Fenceline Pool	-	No GPS coverage

Figure 1. Topographic map of the Griffith League Ranch. Pond and drainage areas are labeled in their approximate positions. Ponds are numbered 1-19. Drainages are designated by letters, A-I.

Standing water areas

Seven of the 19 identified water bodies had Houston toads positively confirmed during the 2000 season. Ponds #2, #6,# 7, #8, #11, #12, and #16 each demonstrated positive occurrence of *Bufo houstonensis* (Table 1). A positive for Houston toads at a given location means that Houston toads were located at that site by active calls heard, adults seen or tadpoles seen or a combination of the three.

Drainages

As the potential exists for standing water within the drainages of the property, these were included in physical surveys to examine their relative suitability as toad breeding habitat. The surveys included all nine major drainages on the property (Table 2, Figure 1).

Standing water was located in six of the drainages systems. Two of these (#F and #G) are included as pools in the impoundment tabulation (#18 and #19 in Table 1). Flowing water was found in Drainage B (Table 2) and in Alum Creek. Drainage B had Houston toads calling from within its banks during the 2000 season, but no evidence of successful breeding was found in this drainage during subsequent visits. The remaining drainages did not contain running water nor significant pooled waters.

Several of the drainages were surveyed within a day of significant local rains. The major drainage systems, excepting those three sites mentioned just above (#B, #F, #G), appear to flush quickly and completely after runoff accumulations. The drainages are steep sided and do not appear to provide suitable habitat for Houston toads beyond the 3 exceptions mentioned above.

One of the 9 surveyed drainages had Houston toads calling from within water standing in the drainage (#B, Table 2). Houston toads thus were observed at 37% of the ponds and within 11% of the drainages on the property.

Table 2. Griffith League Ranch Houston toad survey 2000 drainage identification. Drainages were designated by letter, provided with descriptive names, and surveyed for Houston toads.

Number	Name	Standing water	Flowing water	Houston toads
A	N Arm Finger Pond Drainage	Y	-	-
B	S Arm Finger Pond Drainage	Y	Y	Y
C	Atta Pond Drainage	Y	-	-
D	SE Drainage	-	-	-
E	Spicer Creek Drainage	-	-	-
F	S Gate Drainage	Y	-	-
G	S Fork Price Creek	Y	-	-
H	N Fork Price Creek	-	-	-
I	Alum Creek	Y	Y	-

Amphibian activity

The activity of all amphibian taxa was monitored on each survey night (Table 3, Appendix 1). Seven different amphibian species were found to occur on the property during the survey period. The southern leopard frog (*Rana sphenocephala*) and the southern Cricket frog (*Acris crepitans*) were the most often encountered species. The bullfrog (*Rana catesbiana*) was a common species in nearly all ponds. Large aggregations of grey treefrogs (*Hyla versicolor*) were observed during the spring breeding period for that species. Narrowmouth toads (*Gastrophryne sp.*) also were found to occur in a limited area of the property as were spadefoot toads (*Scaphiopus sp.*).

The first occurrence of *Bufo houstonensis* was registered during the first survey on February 7, 2000 and the last date emerged toadlets were noted was May 22, 2000. Breeding aggregations for Houston toads were noted on February 16th and 18th, again on March 2nd, 15th, and a final chorus on the 22nd of March. The largest of these aggregations occurred on March 2nd and 22nd with up to 16 males noted on the 2nd and 5 amplexing pairs seen with additional males (n~5) calling on the 22nd.

Table 3 summarizes the activity data for all amphibians at each pond by date. During the final half of the survey no attempt was made to detail amphibian occurences at the ponds as these visits were devoted to upland and drainage pattern surveys (Table 3 April 7 – May 22). The unsummarized raw field notes for the survey are included as Appendix 1.

PREVIOUS SURVEY RESULTS

On June 15, 2000 a meeting was conducted with Dr. Andy Price of the Texas Parks and Wildlife Department in Austin. Dr. Price previously surveyed Griffith League Ranch for Houston toads (Appendix 2). While much more limited in both visits (2 vs. 22 visits) and scope (8 vs. 19 ponds), his survey in 1995 provides additional data regarding the distribution of Houston toads on the site. Furthermore, his 1993 survey differs from the 2000 survey in that it was conducted during a year of much higher rainfall than the 2000 season. Not only was the 1995 survey performed in a wetter year, the preceding years were not as drought affected as the years 1996- 2000 have been. His results are presented here as the 1995 survey results are available only as field notes from Dr. Price's visits. Thus, this inclusion will allow all available information regarding Houston toad populations on the Griffith League Ranch up to the 2000 survey to be contained within the current report.

Dr. Price found Houston toads at three additional sites from those found during the 2000 survey. The correlation of his notes to the current (year 2000) pond identification system was achieved by both our meeting and discussion with Dr. Price on the 15th of June, 2000 and examination of his field notes (Appendix 2). He found a lone male at pond #14, observed adults and/or tadpoles in pond #13, and observed *Bufo* tadpoles in a temporary pool on the north side of the roadway leading to pond #6. This depression on the north side of the route to pond #6 was noted by both myself and Dr. Dixon on the February 7 visit to the site. It has not held water at any time during the spring 2000 season.

The 1995 survey also differed in the relative abundance of Houston toads on the site. In the 1995 survey the largest chorus was heard in pond #12 where a very large chorus was heard on March 2nd, 1995 (Appendix 2). In the 2000 survey the water levels were much lower and very few calls were ever heard from this pond. The 1995 survey found large numbers of individuals in and around pond #6, but the 2000 survey located

only 3 adults in this area. While in 1993 pond #16 did have Houston toads there were relatively few calls heard. However, Andy Price has anecdotal reports from Jim Small that the residents living adjacent to this pond have reported loud chorus nights of Houston toads in the past. The 2000 survey located Houston toads in several ponds which were either not visited by Andy Price in 1993 or which were not found to be calling there in 1993. This would include pond #2, pond #7, and pond #11. Both surveys reported Houston toads present in pond #6, pond #8, pond #12, and pond #16.

Thus, the overall data supports the negative effects of the current drought history of the last few years on the populations of Houston toads as revealed by fewer and smaller overall breeding aggregations on the property. The wetter period of the mid 1990s demonstrated much larger chorus groups than any chorus in the 2000 survey. The 1995 survey also located Houston toads at ponds which were not found to be positive for Houston toads in the 2000 survey (Appendix 2).

IV. CONCLUSIONS

The site has many natural or naturalized impoundments within a pine dominated forest landscape in deep sandy soils of the Carrizo formation in Bastrop Co., TX. This habitat is typical for Houston toads and does, in fact, support Houston toad populations. The comparison of a previous survey in 1995 with the 2000 survey allow us to draw several inferences from the data available. There are differences in the two surveys which are likely to be as a direct result of differences in the rainfall conditions over the intervening years. The prevailing drought conditions in the past few years has very likely affected the overall abundance and distribution of the toad on the site. However, breeding aggregations were observed and while at low density on a given night those populations remain.

The Griffith league ranch also demonstrates that at least over a time period of decades or longer, Houston toads will utilize man made or modified water impoundments for breeding. Using the information from the 1993 survey we know that there are several low lying areas which will fill and hold water given sufficient rainfall. Several of these areas might be further improved by engineered or modified dams which would increase the amount of surface water available to the toads in a given year. Particularly valuable would be an examination of the depression on the North side of the pond #6 roadway

which was noted by Dr. Dixon and myself on the first site visit and as a positive for toad breeding by Dr. Andy Price in the 1995 survey. It might be possible to modify that depression facilitate higher water retention and thus provide another suitable breeding site adjacent to the two ponds currently in that region. It is particularly notable as two of the historically engineered sites held active breeding Houston toads during the 2000 season. While it is possible that Houston toads used the sites prior to human modification, it is also likely that this is evidence that breeding habitat can be created and will subsequently be used by Houston toads.

There are numerous other such depressions distributed throughout the site which may allow minimal modifications to provide better impoundment and retention of water for the spring months. This could provide additional breeding habitat for Houston toads and lends itself to scientific investigation of such use by the toad. This type of information would be valuable to general as well as local conservation efforts in amphibians and the Houston toad specifically.

Should you require any further information or clarification of these results or the 2000 survey, please contact me at your earliest convenience (mf11@swt.edu).

Table 3. Summarized amphibian activity on the Griffith League ranch for the spring of 2000. Abbreviations for amphibian taxa are as follows: southern leopard frog (*Rana sphenocephala*) = RS, Cricket frog (*Acris crepitans*)= A, bullfrog (*Rana catesbiana*) =RC, grey treefrogs (*Hyla versicolor*) = H, Narrowmouth toads (*Gastrophryne sp.*) = G, spadefoot toads (*Scaphiopus sp.*) =S?, and Houston toads *Bufo houstonensis* =BH. Sites which had no amphibians are marked by a zero (0) and sites not visited are marked by NV.

DATE	Feb 7	Feb 11	Feb 12	Feb 16	Feb 18	Feb 23	Feb 28	Mar 2	Mar 6	Mar 9	Mar 15	Mar 22	Apr 7	Apr 17	Apr 28	May 2	May 3	May 4	May 9	May 10	May 19	May 22
Temp	80F	82F	71F	80F	74F	76F	73 F	78F	75F	80F	60 F	79F	80 F	82 F	83 F	81 F	84 F	82 F	84 F	85 F	83 F	85 F
Pond 1		0	A	0	RS A	RS	RS A	0	RS	RS	0	RS										
Pond 2		A	NV	0	RS A	RS	RS A	BH	NV	H RC RS A	0	BH			BH							
Pond 3		RS	0	0	0	0	RC RS A	0	0	0	RC H RS	RS H										
Pond 4		0	NV	RS RC A	RS RC A	RS RC	0	RS	RS	RS	0	H										
Pond 5		A RS	A RS	RC A	RC A	H RC RS A	RS A	RS	RS	RS	RC	RS	H									
Pond 6		BH A	BH	BH RS	0	H RS	A	BH	NV	0	0	0								BH S G		
Pond 7		A	A	NV	RS	RS	0	0	0	RS A	BH	NV							S BH RS	BH S H RS	BH S H RS	BH S H RS
Pond 8	BH	BG A RS	BH RS	NV	NV	NV	A RS	0	NV	NV	NV	0	A									

MICRURUS FULVIUS TENER (Texas Coral Snake). **DIET.** The diet of *M. fulvius* generally consists of small lizards and snakes (Greene 1984. Univ. of Kansas Mus. of Nat. Hist. Spec. Publ. 10:147–162). On 20 October 2001 a female *M. f. tener* (SVL 734 mm; weight 64 g) was captured on the Griffith League Ranch in Bastrop County, Texas, USA. The snake was taken to the lab for measurements. During transport, the snake regurgitated a male *Masticophis flagellum* (SVL 476.3 mm; weight 32 g) and died. The *M. flagellum* was 64.9% of the *M. f. tener*'s length and 50% of its weight, which is slightly higher than the mean body mass relationship for coral snakes and their prey determined by Greene (*op. cit.*). There are few records of coral snakes eating larger colubrids (Neill 1968. Florida Wildlife 21:22–25; Greene, *op. cit.*), and we were unable to locate references to *M. flagellum* being eaten by *M. fulvius*. Both were deposited in the Texas Wildlife Cooperative Museum at Texas A & M University (*Micrurus*: TCWC84720; *Masticophis*: TCWC84719).

We thank Rob Bryson and Jeff Mink for their assistance capturing the snake, and Martha Dollar for expediting the delivery of Greene's paper.

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BUFO HOUSTONENSIS (Houston Toad). **GROWTH.** To determine behavior and development, emergent juvenile *Bufo*

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houstonensis were followed during post-emergence at the Griffith League Ranch, Bastrop County, Texas, USA during the early summers of 2001 and 2002. The data for juveniles reported herein were collected from 12 March to 17 June 2001 and from 18 April to 25 June 2002. Observations were made in the morning (ca. 0700–1000 h), weather conditions were generally hot and/or humid with occasional rain.

Three *Bufo houstonensis* egg strands were each surrounded by three concentric aluminum fences placed 2 m, 5 m, and 8 m from the water line to capture juveniles upon emergence. Pitfall traps (2.5 L plastic paint buckets) were placed every 2 m along the fences. However, most juveniles were collected throughout the vegetation encircled by the traps rather than in the pitfall traps. Standard measurements were taken at the time of capture. Weight (WT) was measured to the nearest 0.001 g with an Acculab portable scale (model PP2060D). Snout–vent length (SVL), and head width (HW) were measured to the nearest 0.01 mm with 20 cm vernier calipers. Juvenile *B. houstonensis* were marked as a cohort, with each cohort receiving the same toe clip pattern. Juveniles were released 10 minutes after capture on the other side of the fence in which they were trapped in an attempt to determine movement and dispersal patterns.

Over the 68-day period, 336 juvenile Houston toads were captured, measured, and marked with 509 recaptures during the same period. Because of the cohort marking, individual identity could not be determined and, therefore it is unknown how many times an individual was recaptured.

Our data are compared with the results of work performed at the Houston Zoo (Quinn 1981. Final Report of the Captive Propagation/Release Program of the Houston Toad, *Bufo houstonensis*. Unpubl. Rept. to the U.S. Dept. of Interior, Fish and Wildlife Service, Office of Endangered Species. Submitted by Dept. of Herpetology, Houston Zoological Gardens. 51 pp.). Those data have not been published, however the authors have made those data available for these comparisons. Development of the Houston toad is poorly understood especially in the juvenile stage. Quinn and Mengden's (1983. Unpubl. ms.) summary of the measurement of Houston toad growth between 1981 and 1982 is one of the few data sets that can be compared in examining Houston toad development. However, those studies were performed in captive animals and thus may be only loosely comparable to results from the wild.

The span of 68 days was broken up into three periods to determine a more exact growth rate. SVL was determined to be an important indicator for growth. For the first period (18 April–10 May), SVL ranged from 7 to 14 mm (mean 8.6 mm; SD ± 1.28 mm). For the second period representing a second cohort emerging from 11 May to 2 June, SVL ranged from 8 to 13 mm (mean 9.8 mm; SD ± 1.59 mm). For the final cohort, during the period from 3 to 25 June, SVL ranged from 8 to 19 mm (mean 13.4 mm; SD ± 4.43 mm).

Captive juveniles (Quinn and Mengden, *op. cit.*) were not significantly larger than the wild-caught *B. houstonensis* reported herein. At age 1.8 months the captive juveniles measured 9–13 mm SVL (mean 11 mm; SD 1 mm); at 2.4 months 21–24 mm (mean 23 mm; SD 2 mm). Thus the growth rates determined in captivity (Quinn and Mengden, *op. cit.*) are very nearly those found for *B. houstonensis* in the wild.

Body weight was also determined to be an important indicator of growth and development. Quinn (1981) did not measure weight until 11.4 months of age, and therefore weight measurements cannot be compared between the two studies. However, in the wild, for the first period, mean weight was 0.084 g (SD ± 0.048 g) and average growth rate was 0.004 g/day; for the second period average growth rate was 0.006 g/day with a mean weight of 0.113 g (SD ± 0.046 g). During the last period mean individual weight was 0.382 g (SD ± 0.321 g) with an average growth rate of 0.019 g/day. Considerable variation was found among individuals over the time period for both size and weight within a cohort.

Quinn (1981) determined that growth was most rapid during the first few weeks of life and then gradually declined as the toads reached adult size during the first year for males and second year for females. During the past two years, we have collected and observed juveniles during the first months of the year (Jan–Mar). This is important given the belief that breeding maturation can occur during the first year of life. Such parameters are critical for efficient population modeling of the species.

We thank H. Quinn, G. Mengen, staff of the Houston Zoo, and J. R. Dixon for assistance in obtaining background information from captivity. We thank M. Gaston, T. Swannack, and T.L. Ahlbrandt for assistance in the field. This work was supported, in part, by a collaborative grant to MRJF from the United States Fish and Wildlife Service, Texas Parks and Wildlife Department, Southwest Texas State University, and the Capital Area Council-Boy Scouts of America. Additional funding was provided by ALCOA, Inc.

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BUFO OCELLATUS (Ocellated Toad). **DIET.** *Bufo ocellatus* occurs in Minas Gerais, Mato Grosso, Goiás, and Pará in Brazil (Frost 1985. Amphibian Species of the World. Allen Press, Inc. and Assoc. Systematics Collections. Lawrence, Kansas. 732 pp.). Aspects of the trophic ecology of this species have been little investigated in natural populations from central Brazil. We analyzed the stomach contents of 13 specimens (43.3 ± 4.3 mm SVL) collected during the Corumbá I Hydroelectric Power Plant reservoir formation (17°59'S, 48°31'W), Caldas Novas municipality, Goiás State, between Sept 1996 and April 1997. Although isolation of populations, and therefore variation in food resource availability may occur during reservoir formation (Paiva 1999. Conservação da Fauna Brasileira. Ed. Interciência. Rio de Janeiro. 260 pp.), these analyses indicated the most frequently taken prey items were Coleoptera (N = 16; 3.6%), Hemiptera (N = 2; 0.45%), Hymenoptera (N = 298; 67.1%, Formicidae), Isoptera (N = 124; 27.9%), and miscellaneous (N = 3; 0.7% plant material; N = 1; 0.2% stones).

The volume of the items were: Coleoptera 418.4 mm^3, Hemiptera 17.8 mm^3, Hymenoptera (ants) 7045 mm^3, and Isoptera 4893.3 mm^3. Coleoptera were present in nine stomachs, Hemiptera in one, Hymenoptera (ants) in thirteen, Isoptera in five, plant matter in three, and stones in one. The diet of the 13 *B. ocellatus* was dominated numerically and volumetrically by Hymenoptera (Formicidae).

CROTALUS HORRIDUS (Timber Rattlesnake). USA: TEXAS: BASTROP Co: Griffith League Ranch, a 5000-acre site owned by the Capitol Area Council of the Boy Scouts of America (30°12'52"N, 97°13'58"W). 18 May 2002. Lee Ahlbrandt, Kensley Jones, Michael Forstner. University of Texas Arlington (UTA Slide No. 26921). Verified by Kathryn Vaughn. Specimen collected from a trap in a herpetofaunal array; first record from the county (Dixon 2000. Amphibians and Reptiles of Texas. Second Ed. Texas A&M Univ. Press, College Station. 421 pp.) This record bridges a gap in the distribution of the species between Lee County to the northeast and Caldwell County to the southwest.

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BUFO HOUSTONENSIS (Houston Toad). USA: TEXAS: LEE Co: 2.7 mi S jct. Lee County Road 331 and CR 333 on CR 333 (30°18'46.1"N, 97°09'08.9"W). 2 April 2001. James R. Dixon and Michael R. J. Forstner. Verified by R. Kathryn Vaughn. TCWC 84556. DOR. Represents first recorded occurrence of this endangered species in Lee County (Dixon 2000, Amphibians and Reptiles of Texas. Second Ed. Texas A&M Univ. Press, College Station. 421 pp.).

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AMBYSTOMA TIGRINUM (Eastern Tiger Salamander). USA: TEXAS: BASTROP Co: Griffith League Ranch, a 5000-acre site owned by the Capitol Area Council of the Boy Scouts of America (30°12'58.6"N, 97°14'30.6"W). 25 May 2001. Todd Swannack and Michael R. J. Forstner. Verified by R. Kathryn Vaughn. TCWC 84707. Specimen collected from a trap in a herpetofaunal array; first record from the county (Dixon 2000, Amphibians and Reptiles of Texas. Second Ed. Texas A&M Univ. Press, College Station. 421 pp.).

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Swannack, Todd, William E. Grant, James R. Dixon, and Michael R. J. Forstner. 2004. **Abstract.** Spatial Distribution and Population Dynamics of the Houston Toad (*Bufo houstonensis*). Texas A&M University Annual Report. 2003.

The Houston toad is an endemic Texas amphibian that is currently restricted to 9 counties in the Eastern-Central portion of the state with the largest population found in the Lost Pines region of Bastrop County. Although the toad has been legally endangered for over 30 years, the majority of previous studies have focused mainly on the breeding biology of the toad. *Bufo houstonensis* is an explosive breeder that breeds intermittently between February and May, after which adults are rarely seen above ground. Data are lacking regarding the non-breeding season activity of the adults, including habitat use and movements of adults once they leave the breeding pond. Also, few data have been collected on post-metamorphic juveniles and little information is available on the population dynamics of the species. These natural history components must be illuminated in order for the conservation efforts for this endangered amphibian to move forward.

The goals of this study are 1) to determine how both adult and juvenile *B. houstonensis* utilize their habitat during both the breeding and non-breeding season and 2) examine the population dynamics of *B. houstonensis*.

Habitat utilization will be examined through 1) an extensive drift fence-pitfall system composed of 24 traps in 5 different habitat types placed throughout the Griffith League Ranch (1951 ha ranch in the Lost Pines region of Bastrop County), 2) radio telemetry, and 3) the creation of artificial refugia used to capture post-metamorphic juveniles.

Population dynamics will be examined by using the data collected from the drift fences and breeding ponds using standard mark-recapture techniques. Also, based on these data an age structured and sex structured population dynamics simulation model will be built as a stochastic compartment model based on difference equations with a daily time step. The model will be used to project population trends into the future. This study was funded through grants awarded to MRJF by United States Fish and Wildlife, Texas Parks and Wildlife, ALCOA, U.S. Geological Survey, and the Boy Scouts of America.

Ferguson, Adam W., and Michael R. J. Forstner. 2004. **Abstract.** Active Predation of Pitfall Traps at a Study Site for the Endangered Houston Toad (*Bufo houstonensis*) in Bastrop County, Texas. 51st Meeting of the Southwestern Association of Naturalists, April 2004.

The use of pitfall traps in sampling reptile, amphibian, and small mammal populations is a common field technique utilized throughout the United States. Studies have addressed mortality within the buckets, pointing to such problems as desiccation, drowning, and freezing. Few studies, if any, have looked at the removal of animals from the buckets by vertebrate predators. We tested the amount and kinds of vertebrate predators potentially removing animals from 18 pitfall arrays set up to monitor the

endangered Houston toad (*Bufo houstonensis*) at the Griffith League Ranch in Bastrop County, Texas. Thirty-eight out of 95 buckets were monitored for predatory behavior. Each bucket was surrounded by a 2 m diameter circle of cleared earth filled with sand media for recording the tracks of potential predators. The track stations were monitored 4 times per month. Track type and patterns were recorded and identified to species when possible. Regular visitors to the transect buckets included (in order from most common to least) raccoon (*Procyon lotor*) 26.40%, American crow (*Corvus brachyrhynchos*) 4.50%, gray fox (*Urocyon cinereoargentius*) 2.88%, several unidentified snake species 1.08%, Virginia opossum (*Didelphis virginiana*) 0.90%, and coyote (*Canis latrans*) 0.18%. Having identified the situation, work during the active toad season has begun to evaluate the most efficient predator exclusion methods. Our results highlight the impact predation might have on herptofaunal and mammalian survey data using drift fence/pitfall arrays and the consequent caution required to assess active predation and prevent it.

Swannack, Todd, and Michael R. J. Forstner. 2003. **Abstract.** A Possible Cause of the Disparity in the Sex Ratio of Adult Houston Toads. 51st Meeting of the Southwestern Association of Naturalists, April 2004, 107th meeting of the Texas Academy of Sciences, March 2004, 63rd Meeting of the Texas Herpetological Society, October 2003.

Sex ratios are difficult to determine for explosively breeding anuran populations. For taxa spending the majority of their lives underground the problem is further exacerbated. However, it is important to obtain accurate estimates, especially for endangered species, because a naturally biased sex ratio decreases effective population size. A population of Houston toads was studied at the Griffith League Ranch in Bastrop County, Texas from March 2001 – August 2003 by both drift fence / pitfall traps and extensive breeding pond surveys. Houston toads appear to remain within or immediately adjacent to canopied forest. A male biased sex ratio was evident for both trapping methods. From the results of a simulation model, adult Houston toad populations are likely male biased as a consequence of the mortality associated with delayed female maturation. It may be possible to predict the mortality of juvenile female Houston toads in the wild using refined models of adult sex ratios.

Greuter, Kensley, L., and Michael R. J. Forstner. 2003. **Abstract.** Postmetamorphic Dispersal Patterns in the Juvenile Houston Toad (*Bufo houstonensis*). 63rd meeting of the Texas Herpetological Society, October 2003.

The Houston toad, *Bufo houstonensis*, has been considered endangered since 1970 due to its strict habitat requirements, scarcity in habitat, and concurrent habitat destruction. Unfortunately, while local environments and adult breeding behaviors are becoming well known, the postmetamorphic biology of the Houston toad is poorly characterized. Postmetamorphic Houston toad juveniles were studied at the Griffith League Ranch, Bastrop County, Texas, USA during 12 March 2001 - 17 June 2001, 18

April 2002 - 25 June 2002, and again beginning 23 April 2003 - 15 August 2003. Dispersal patterns were monitored through several trapping techniques including drift fences, artificial refugia, and quadrat plot sampling. Results indicate that upland habitat surrounding the pond of emergence is extremely important, especially areas of moisture. The pond edge is critical juvenile Houston toad habitat for at least 11 weeks following emergence.

Marquardt, Aaron L., and Michael R. J. Forstner. 2003. **Abstract.** Early Identification of the Endangered Houston Toad (*Bufo houstonensis*) using Mitochondrial DNA markers. 63rd meeting of the Texas Herpetological Society, October 2003.

Anuran larvae have little in common with their much larger, better known adults. Although tadpoles typically inhabit specific aquatic habitats for much longer periods than do their adults, they are difficult to find and are nearly always more difficult to identify morphologically. Most tadpoles are drab in coloration and pattern and even distantly related species have very similar appearances. These animals present morphological trits that can be used to distinguish among species. However, only the most dedicated investigator can easily differentiate among most forms. Studies in the field have revealed the high degree of difficulty in the identification of *Bufo sp.* tadpoles. Accurate identification is critical to the study of the endangered Houston toad (*Bufo houstonensis*). With numbers in decline, time wasted studying the behavior, development, and habitat of an animal that might or might not be *B. houstonensis* may prove to be its downfall. Novel, primers, Cyt ba and Bufo r1 were developed to amplify the highly variable d-loop region of the mitochondrial genome in all Bufo. This amplified region was then sequenced for all unknown toads using fluorescent dye terminator reactions and compared to known sequences, rapidly and correctly identifying *Bufo houstonensis* samples. Subsequent primers allow PCR identification by differential fragment length of *B. houstonensis* from among sympatric congeners.

Morris, Susannah R., and Michael R. J. Forstner. **Abstract.** Effects of Pond Slope and Aquatic Predators on Survivorship of Larval Houston Toads (*Bufo houstonensis*). 63rd meeting of the Texas Herpetological Society, October 2003.

The endangered Houston toad (*Bufo houstonensis*) breeds in small freshwater ponds in Bastrop County in the Lost Pines of Central Texas. Ensuring survival of larval Houston toads is important in the conservation of this rare and declining species. Possible predators on Houston toad larvae include fish and aquatic insects; however, fish may also be a keystone predator that curbs aquatic insect populations. Twenty-four experimental ponds constructed in 2002 were stocked with four different predator treatments: insects, fish, insects and fish, and no predator. All ponds had a 90 degree slope on three sides; the remaining side was sloped 10 degrees in twelve ponds and 45 degrees in twelve ponds. Each pond was stocked with 183 Houston toad tadpoles, and toadlet emergence was

observed and quantified for the 2003 season until no tadpoles remained. In total, 60 toadlets emerged from all ponds, with the majority (54) emerging from one pond which contained no predators. A two.factor ANOVA showed no statistical difference among predator treatments or between slope treatments, and no interaction between the two factors ($p > 0.10$). However, the only treatment which completely prevented emergence was the insects-only treatment. More research is needed to determine the ideal conditions for Houston toad breeding ponds.

Morris, Susannah, Richard W. Manning, and Michael R. J. Forstner. 2003. **Abstract.** Systematics of Locally Endemic Short-Tailed Shrews *Blarina* (Insectivora: Sorcidae) in Bastrop and Aransas Counties, Texas. 83rd meeting of the American Society of Mammalogists, June 2003.

Two species of short-tailed shrews are found in Texas; *Blarina carolinensis* inhabits the eastern third of the state, and *Blarina hylophaga* is found only on the Texas-Oklahoma border in Montague County. Two isolated populations of *Blarina* have been noted outside of these ranges, one in Bastrop County in the Lost Pines of central Texas, and the other on the Gulf Coast in Aransas County. Only six specimens from Bastrop County and nine from Aransas County have been examined previously, and attempts to identify these specimens based on morphological characters have been inconclusive. The purpose of this study was to determine the taxonomic status of these two isolated populations of short-tailed shrews in Texas using morphological and molecular methods. Cytochrome *b* sequences were obtained for *Blarina* from Bastrop and Aransas counties, aligned with sequences from Brant and Ortí's 2001 study that were available on GenBank. Phylogenetic analyses were performed using maximum likelihood and maximum parsimony. Preliminary results based on fifteen specimens from Bastrop County indicate that this population is allied with *Blarina hylophaga.*

Greuter , Kensley, L., and Michael R. J. Forstner. 2003. **Abstract.** Emergent Behavior in the Juvenile Houston Toad, *Bufo houstonensis.* 106th meeting of the Texas Academy of Sciences, March 2003.

The Houston toad, *Bufo houstonensis*, has been considered endangered since1970 due to its strict habitat requirements, scarcity in habitat, and rampant habitat destruction. Being poor burrowers limits their ecological range to the sandy soils of central Texas. The fire ant invasion of central Texas has also aided in the demise of the adult and especially the juvenile stages of life. Predation from large vertebrates is still being researched at this time. Habitat destruction caused by human development has scattered the population to just a few counties with Bastrop, Burleson, and Lee counties having the majority of the extant metapopulations.

The Houston Toad is a year-round resident where found, although its presence can most easily be detected during the breeding season, when males may be heard calling. Houston Toads may call from December through June. Most breeding activity takes place in February and March, and is stimulated by warm evenings and high humidity. Toads emerge from hibernation to breed only if moisture and temperature conditions are favorable. Unfortunately, while local environments and adult breeding behaviors are becoming well known (Hillis et al. 1984), the post-emergent ecology of the Houston toad is poorly characterized.

Juvenile *Bufo houstonensis* have been followed during post-emergence at the Griffith League Ranch, Bastrop County, Texas, USA during the years of 2001 and 2002. Movement, length, and body weight were measured over a course of 68 days in the summer of 2002. Movement was determined to have been directed towards shady regions around the pond of emergence. Juveniles stayed near the pond for an estimated 20 days post emergence and then sought shelter in the shady, damp undergrowth of the surrounding oak-pine forest. Length and body weight were determined and compared to an unpublished study (Quinn and Mengden 1983).

There are just a few published studies on the biology of the Houston toad and within this limited information, very little research has exclusively focused on juvenile toad movement and growth patterns. With the current research on growth being published, hopefully this information will inform a wider audience of the imperativeness in saving this diminishing amphibian.

Morris, Susannah, Richard W. Manning, and Michael R. J. Forstner. 2003. **Abstract.** Systematics of Locally Endemic Short-Tailed Shrews *Blarina* (Insectivora: Sorcidae) in Bastrop and Aransas Counties, Texas. 106th meeting of the Texas Academy of Sciences, March 2003.

Three species of short-tailed shrews (Insectivora: Soricidae) inhabit the United States: *Blarina brevicauda*, *Blarina hylophaga*, and *Blarina carolinensis*. In Texas, *B. carolinensis* is affiliated with the Piney Woods of east Texas and *B. hylophaga* has only been found in Montague County, near the southern boundary of its range. Two isolated populations of *Blarina* exist in Texas, one in the Lost Pines of Bastrop County in central Texas and the other on the central Gulf coast in Aransas County. Attempts to identify these shrews based on morphology have been unsuccessful. The purpose of this study was to identify the isolated populations of shrews based on mitochondrial DNA sequences of cytochrome *b* and 16S rRNA. Preliminary results based only on cytochrome *b* indicate that the Bastrop population is affiliated with *Blarina hylophaga*.

Swannack, Todd M., and Michael R. J. Forstner. 2003. **Abstract.** Distribution of Houston Toads (*Bufo houstonensis*) in Heterogeneous Habitats. 106th meeting of the Texas Academy of Sciences, March 2003.

The Houston Toad (*Bufo houstonensis*) was federally listed as an endangered species in 1971, and is currently found in only 9 Texas counties with the largest populations found in Bastrop County. Very little is known about the biology of this endemic Texas amphibian outside of the breeding season. A study began at the Griffith Ranch (1951 ha) in Bastrop County in March 2001. Drift fences with pitfall traps were placed in either open clearings or mixed hardwood forest (6 in clearings, 2 in forest). In February 2002, seven additional traps were added (6 in hardwoods, 1 in natural clearing). Traps were checked daily, and nightly toad surveys were conducted during the breeding season. From March 2001 – November 2002, 84 (74M:10F) *B. houstonensis* were captured (27 in traps, 56 at breeding ponds). Toads were not captured in open pasture, but were captured moving along the periphery of cleared areas. The sex ratio was significantly male–biased for toads collected both in traps and at breeding ponds. Females were significantly larger than males in all measurements (snout-vent length, head width, and weight).

Lanier, William, Michael R.J. Forstner, Dana M. Garcia, and Joseph R. Koke. 2001. **Abstract.** Identification of Houston toads using mtDNA sequences. 104th meeting of the Texas Academy of Sciences, March 2001.

The Houston toad (*Bufo houstonensis*) is one of sex species found within the Americanus Group of toads. It is a "flagship" endangered species for the United States Fish and Wildlife Service and a Texas endemic. Unfortunately, the very short breeding aggregation season for the Houston toad can complicate determining its occurrence. Many amphibians can be identified to the species level using morphological characters of their tadpoles. However, in the case of young bufonid tadpoles, morphology alone will not allow unambiguous species determination. In such instances, DNA sequence data may provide informative characters that allow the determination of the various taxa. Mitochondrial DNA sequence data from the hypervariable D-Loop region was generated to provide molecular characters distinguishing *B. houstonensis* from other sympatric bufonids. A comparative dataset for sympatric species was developed from samples of *B. cognatus*, *B. speciosus*, *B. valliceps*, and *B. woodhouseii*. In all phylogenetic analyses, each of the species were clearly definable. Therefore, sufficient sequence divergence does exist within this mitochondrial fragment to allow identification at the species level. This study provides a mtDNA sequence marker that is applicable to unambiguously identify *B. houstonensis*. These molecular data should prove useful in evaluating presence/absence and potential breeding habitat of this elusive endangered species.