Annual Report

Houston toad metapopulation assessment and genetics: Data necessary for effective recovery strategies in a significantly fragmented landscape

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by

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Introduction

The Houston toad (*Bufo houstonensis*) persists on the landscape in widely scattered populations among which isolation and consequent population interactions vary at several spatial scales. The species is ecologically adapted to local extirpation events and subsequent recolonization is believed to be a normal part of the metapopulation cycle. The Houston toad also has a tremendous reproductive potential, thus providing a remarkable innate capacity for population growth and eventual recovery. As a conservation initiative, the current situation for the Houston toad is critical. In the last five years, stakeholder efforts focusing on the toad have become focal to the Bastrop County community. As a result, private landowner Safe Harbor agreements, newly purchased Conservation Lands, and the recently approved County level Habitat Conservation Plan have been incorporated toward Houston toad recovery.

This has coincided with renewed regional-scale research efforts both within Bastrop County (Forstner 2002; 2003) and in adjacent Lee County (Forstner and Dixon 2001). However, most of the current and recently completed research efforts have been focused on habitat use and ecology of the species centered on the scale of ponds and inter-pond movements (Forstner and Swannack 2004). Our own ongoing research seeks to evaluate land use and habitat restoration as means toward recovery of the species in Bastrop County (Forstner 2004). Those efforts are again at a local, single forest fragment scale. If we examine the historic scientific knowledge base for the Houston toad, we find that data primarily exist for two broad areas: distribution and life history. While historical survey data provide distributional evidence (Yantis 1989-1992), nearly all other work on the toad has been at the pond or county scale (Price 2003). Yet, while all of the previous and ongoing research avenues have been guided by the Houston toad Recovery Plan (1984), range-wide biological data remain as important as in 1984 (USFWS 1984) and as unknown today as it was then.

The 1984 Recovery Plan specified range-wide surveys as a primary goal of immediate need in 1984, and Yantis (1989-1992) completed such audio chorus surveys. Since that time, the data from ponds on Bastrop State Park show a dramatic decline in the toads during the 1990s with some evidence of stabilization during the past five years (Price 2003). In this recent period, surveys that sought data at the county-wide scale have

been completed for both Bastrop (Forstner 2002) and Lee Counties (Forstner and Dixon 2001). Beyond those two counties however, virtually no current data exist evaluating chorusing in other historically documented locations. This is a particular concern as many of the locations outside of Bastrop and Lee County are likely to represent very small populations with low numbers of individuals compared to the Houston toads of Bastrop State Park. Those smaller populations are thus at higher risk of extirpation during episodes like the drought of the 1990s and may not be recolonized (Blaustein et al. 1993).

Ultimately, this is a problem for management, as all current thinking about the Houston toad reflects the idea that extinction will be prevented only if conservation efforts focus on metapopulation dynamics (Hatfield et al. 2004). Unfortunately, we have virtually no information at this spatial scale that can help to guide management strategies and conservation efforts. Preventing extinction of the Houston toad over the next several decades is only possible if groups of populations are able to act as fluctuating reservoirs for recolonization as local extirpation of population subsets occur (Hatfield et al. 2004).

Objective: Evaluate the Houston toad range-wide status including metapopulation genetics useful in current management strategies and conservation plans.

Approach:

The survey results allow us to compare the data with the historical survey database for the toad. The collection of the survey data has met or exceeded the guidelines specified currently by the USFWS for audio surveys of the Houston toad. Because many of the locations have not been visited for Houston toad surveys in more than ten years, we have also examined potential habitat adjacent to those specific locations, using the historic site as the starting reference for each region. Subsequently, we ground truthed the locations by visiting the localities, examining potential breeding sites and the characteristics of the habitat. Then, beginning in January 2007, appropriate nightly conditions led to audio surveys of the localities. Chorus surveys for amphibian detection are fairly standardized and we have recently published an analysis of our methods which provides statistical evidence that we are unlikely to fail to detect Houston toads at a location should they occur at that pond (Jackson et al. 2006).

DNA sampling of Houston toads continues to be non-consumptive with genomic samples obtained from blood/toe during each of the surveys conducted. We have assembled blood samples from several localities using this method during the past five years, routinely recapturing the sampled adults in subsequent chorus nights and years. As all of the samples taken are collected in the field, handling is tied to data recording methods and appropriate sterile technique. All samples have been routinely from living animals by sterile syringe or scissors and placed into sterile cryogenic storage tubes. Should any physical encounters occur for deceased individuals, tissue samples and appropriate voucher specimens are always then salvaged.

The laboratory work has standardized methods (Avise 1994, Smith and Wayne 1996) allowing us to carry on this work with as much confidence as is possible with population genetic studies. All laboratory work is confined to workspaces designed for such work and began in July 2006 on existing samples. All work from the initial DNA collection (see above) through DNA extractions, amplification, and subsequent allele/base pair calling are strictly controlled with appropriate positive and negative controls. Peak height, signal to noise ratios, and size standard controls act to guide the precision of allele calls and accuracy of DNA base pair assignments from the automated sequencing platform. Accuracy of the hardware is specified by the manufacturer as less than 1% error rate, which is itself halved by our complete bidirectional sequencing of all templates. The laboratory analyses examined marker suitability during the first year of funding using already collected samples.

Results

Range wide surveys– We conducted surveys which met or exceeded detection probabilities of 0.90 (Jackson et al. 2006) in the following counties during 2008: Austin, Bastrop, Colorado, Lavaca, Lee, and Milam. We also revisited historical localities and performed less than 10 survey nights in the following counties in 2008: Burleson, Leon, and Robertson. Finally, we extended our surveys into adjacent counties to those known to be occupied by Houston toads, or those with otherwise appropriate habitat: Anderson, Guadalupe, Henderson, and Wilson. These visits were generally site assessment of habitat with canopy identified by aerials, or in consultation with Dr. Jim Yantis during

our review of both habitat and historical sites on his routes. Houston toads were found to be actively chorusing over time and in numbers greater than 10 individual males (total seasonal count) in only one county (Bastrop). A single chorus of more than ten toads was heard one night in Leon, three toads were found and two others heard in Austin, but no significant chorusing was detected in any of the historical locations in any of these counties. Milam County had only two Houston toads detected in 2008. At this time we consider the Houston toad to be likely extirpated in Lavaca County, unlikely to occur in Lee County, and at very low numbers in Austin, Colorado, and Leon counties.

Population genetics– Obviously the difficulty in assessing metapopulation genetics is simply that our work will now be based on significant samples from only one county and as many samples as we have found from the remaining occupied counties, but nonetheless dramatically fewer samples overall outside of Bastrop County. We have made extraordinary progress on all samples on hand to date. As of 29 Aug 2008, 490 Houston toad DNAs have been extracted. Of these, 376 were male, 31 female, 2 likely female, 40 juveniles, 20 tadpoles, and 17 are otherwise unknown gender (not recorded). Tissues resulting in these DNAs were collected across several years. In 2000, 5 were collected, 44 in 2001, 95 in 2002, 41 in 2003, 26 in 2004, 64 in 2005, 74 in 2006, 134 in 2007, 3 in 2008, and 4 tissues have unknown collection dates. See Table 1 for numbers of Houston toads collected by locality.

Ten microsatellite loci have been shown to be homologous to published sequence and are suitably polymorphic in Houston toads: BBR34-2, BBR36, BBR281 (Simandle 2006), BC52.03, BC52.10, BC52.12, bco15 (Chan 2007), BM224 (Tikel et al. 2000), IHHH, and IYY (Gonzalez et al. 2004). Two loci have been tested thoroughly, and while they are homologous to published sequence and polymorphic, this polymorphism turned out to be a result of indels (insertion deletion events not related to the microsatellite locus itself) and thus not changes in number of microsatellite repeats: Bbuf15 (Brede et al. 2001) and BC60.37 (Chan 2007). Six loci amplified in Houston toads but were monomorphic: Bbuf49 (Brede et al. 2001), bco40 (Chan 2007), BM121, BM239 (Tikel et al. 2000), ICCC, and IDDD (Gonzalez et al. 2004). Nine loci amplified but were not microsatellite loci in Houston toads: BC52.04, BC52.11, BC60.20 (Chan 2007), BM128, BM217, BM229, BM279, BM322 (Tikel et al. 2000), and IKK (Gonzalez et al. 2004).

We are ahead of schedule and on track for this project. As discussed we have rolled the salary unexpended for 2007-08 forward, using it beginning this spring. Likewise unexpended second year funds are being incorporated as part of our expenditures in our final year in order to meet project goals efficiently.

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Table 1. Number of Houston toad (*Bufo houstonensis*) tissues collected by county, locality, and specific locality.

County	Locality	Specific Locality	Number	Total Per Locality	Total Per County
Austin	TCW pond		2	2	3
	unnamed private pond		1	1	
Bastrop	Bastrop State Park	Melissa's traps	7	64	457
		BSP pond 11	11		
		BSP pond 19	20		
		BSP pond 8	26		
	Bluebonnet Headquarters	BBHQ pond 1	18	55	
		BBHQ pond 2	6		
		BBHQ pond 3	31		
	Bob Long	Bob Long Back Pond	19	19	
	Along 290	Dube Ln & Sandy Creek	5	10	
		Kuhl Site	4		
		Musgrave Pond	1		
	Griffith League Ranch	GLR unknown	9	265	
		GLR traps	54		
		GLR pond 10	3		
		GLR pond 11	4		
		GLR pond 12	39		
		GLR pond 15	2		
		GLR pond 2	111		
		GLR pond 3	2		
		GLR pond 5	7		
		GLR pond 6	5		
		GLR pond 7	11		
		GLR pond 8	4		
		GLR pond 9	13		
		Old Fire Tower Rd & 1441	1		
	Jim Small	JS pond 1	3	37	
		JS pond 2	5		
		JS pond 3	1		
		JS pond 4	20		
		JS pond 5	7		
		JS pond 6 (Jake's mudhole)	1		
	Unknown	Unknown	7	7	
Colorado	CR-52	CR-52, near intersection with Warsehak Schuette Rd	3	3	3
Lee	CR-333	CR-333, 2.7 mi S jct CR-331 & CR-333	1	1	19
	Durham	Durham pond 1	7	17	
		Durham pond 2	10		
	F3 pond 6	F3 pond 6	1	1	
Leon	Hilltop Lakes	Hilltop Lakes, Cherokee Lake	1	1	1
Milam	CR-342	CR-342	4	4	4
Unknown	Unknown	Unknown	3	3	3

Literature Cited

Avise, J.C. 1994. Molecular Markers, Natural History, and Evolution. New York, Chapman and Hall. 511 pp.

Blaustein, A.R., D.B. Wake, and W.P. Sousa. 1994. Amphibian Declines: judging stability, persistence, and susceptibility of populations to local and global extinctions.

Brede, E.G., G. Rowe, J. Trojanowski, and T.J.C. Beebee. 2001. Polymerase chain reaction primers for microsatellite loci in the common toad *Bufo bufo*. Mol. Ecol. Notes 1:308-310.

Chan, L.M. 2007. Twelve novel microsatellite markers for the Great Plains toad, *Bufo cognatus*. Mol. Ecol. Notes 7:278-280.

Gonzalez, Z., D. A. Ray, L. R. McAliley, M. J. Gray, C. Perchellet, L. M. Smith, and L.D. Densmore. 2004. Five polymorphic microsatellite markers for the Great Plains toad, *Bufo cognatus*. Mol. Ecol. Notes 4:9-10.

Forstner, M.R.J. 2002. The 2002 Houston toad surveys in Bastrop County. Final Technical Report. Bastrop County citizen's workgroup and Bastrop County Houston toad project. 20 pg.

Forstner, M.R.J. 2003. Final Technical Report. Final report of the 2003 Bastrop County coordinated Houston toad surveys. Bastrop County Citizen's Workgroup. 12pgs.

Forstner, M.R.J. 2004. Safe harbor habitat restoration and metapopulation connectivity goals for the Houston toad on the LCRA/Lost Pines Scout Reservation. Final Technical Report. Submitted to the BSA/CAC and USFWS. Dec 1, 2004. 13pgs.

Forstner, M.R.J. and J.R. Dixon. 2000. Final Technical Report. An overview and genetic assessment of the occurrence of Houston toads on the Three Oaks lignite mine site. Jackson and Sjoberg, LLC. Austin, Texas. 19 pp.

Forstner, M.R.J. and J.R. Dixon. 2001. Final Technical Report. Report of Houston toads (*Bufo houstonensis*) surveys in Lee County, TX. David Jackson, Jackson and Sjoberg, LLC. Austin, TX. 7 pp.

Forstner, M.R.J. and T.M. Swannack (eds.). 2004. The Houston toad in Context. Final Technical Report. Submitted to the Capitol Area Council, Boy Scouts of America and Texas Parks and Wildlife Department in completion of United State Fish and Wildlife Service Section 6 Grant "Habitat use of the Houston toad." 746 pp.

Forstner, M.R.J., J.T. Jackson, and T.M. Swannack. 2005. Final Technical Report. Final Report of the Long Property, Bastrop County, TX Houston Toad Survey 2005. Submitted to Bob Long and Environmental Defense. 13 pp.

Hatfield, J.S., A.H. Price, D.D. Diamond, and C.D. True. 2004. Houston toad (*Bufo houstonensis*) in Bastrop County, Texas: Need for protecting multiple subpopulations. Pages 292-298 in H.R. Akçakaya, M.A. Burgman, O. Kindvall, C.C. Wood, P. Sjögren-Gulve, J.S. Hatfield, and M.A. McCarthy (eds.), Species Conservation and Management: Case Studies. Oxford University Press.

Jackson, J.T., F.W. Weckerly, T.M. Swannack, M.R.J. Forstner. 2006. Imperfect detection and number of auditory surveys for Houston toads. J. Wildlife Management. 70:1461-1463.

Price, A.H. 2003. The Houston Toad in Bastrop State Park 1990 - 2002: A Narrative. Occasional Papers Wildlife Division, Texas Parks & Wildlife Department. 1:1-21.

Price, A.H. and J.H. Yantis. 1993. Houston Toad (*Bufo houstonensis*) status survey, final report, Job No. 8. Texas Parks and Wildlife Department. Austin, Texas.

Simandle, E. T., M. M. Peacock, L. Zirelli, and C. R. Tracy. 2006. Sixteen microsatellite loci for the *Bufo boreas* group. Mol. Ecol. Notes 6:116-119.

Smith, T.B. and R.K. Wayne. 1996. Molecular Genetic Approaches in Conservation. Oxford University Press.

Tikel, D., D. Paetkau, M. N. Cortinas, R. Leblois, C. Moritz, and A. Estoup. 2000. Polymerase chain reaction primers for polymorphic microsatellite loci in the invasive toad species *Bufo marinus*. Mol. Ecol. 9:1927-1929.

U.S. Fish and Wildlife Service. 1984. Houston toad recovery plan. U.S. Fish and Wildlife Service, Albuquerque, New Mexico. 73 pp.

Yantis, J.H. 1989. Houston toad distribution and habitat status. Performance report, Job No. 76. Texas Parks and Wildlife Department. Austin, Texas.

Yantis, J.H. 1990. Houston toad distribution and habitat status. Performance report, Job No. 76. Texas Parks and Wildlife Department. Austin, Texas.

Yantis, J.H. 1991. Houston toad distribution and habitat status. Performance report, Job No. 76. Texas Parks and Wildlife Department. Austin, Texas.

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