FINAL PERFORMANCE REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-127-R

F10AP00541

Endangered and Threatened Species Conservation

Population supplementation: a proven means toward endangered species recovery for the Houston toad

Prepared by:

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25 October 2013

INTERIM PERFORMANCE REPORT

STATE: Texas **GRANT NUMBER:** TX E-127-R

GRANT TITLE: Population supplementation: a proven means toward endangered species recovery for the Houston toad

REPORTING PERIOD: 1 Sep 10 to 31 Aug 13

OBJECTIVE(S):
Use field and molecular tools to determine the most efficient means of headstarting the Houston toad as one of the few proven methods to lead to recovery of endangered species.

Segment Objectives:

- **Field Surveys for eggstrands** - visit each chorus pond during daylight and half of each eggstrand will be collected, quantified, and transported to the Houston Zoo for rearing. Once hatching is underway begin genetic analyses confirming the genetic background of each strand.
- **Laboratory analyses**
    - Microsatellites will be amplified and analyzed.
    - Genetic clustering analyses will be used to infer the number of clusters (K), or populations, in the dataset and to assign individuals to a cluster. Prior to all releases we follow strict IUCN approved biosecurity protocol that includes veterinary screening and chytrid testing.

Significant Deviations: None.

Summary Of Progress: See Attachment A.

Location: Bastrop County, Texas.

Cost: Costs were not available at time of this report.

Prepared by: Craig Farquhar **Date:** 25 October 2013

Approved by:  **Date:** 25 October 2013

C. Craig Farquhar

ATTACHMENT A

2013 Final Report

Population supplementation: a proven means toward endangered species recovery for the Houston toad

Section 6 Grant TX E-127

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16 September 2013

INTRODUCTION

The Houston toad was first described from Harris County near Houston, Texas in 1953 (Peterson et al., 2004; Sanders, 1953). In 1970, the Houston toad was the first endemic species from Texas and the first amphibian federally listed as an endangered species (Peterson et al., 2004). A high correlation has been found between the sandy loam soils of the Lost Pines ecoregion and Houston toad (*Bufo houstonensis*) occurrence (Koepp et al., 2004). It has been suggested the Houston toad is a poor burrower (Bragg, 1960), implying that the sandy soils enable them to bury down and aestivate during the cold winter months. Therefore, Houston toads are thought to be restricted to areas of sandy loam soils, and not necessarily pine forests (Brown and Thomas, 1982). To date, nearly all recovery efforts have centered on the Houston toad population in Bastrop County, Texas. Despite those efforts, Houston toad populations have remained in a continual decline consequent of multiple stressors, including habitat fragmentation, urban growth of the city of Bastrop, red imported fire ants, fertilizer and chemical run off, agricultural practices, wildfire, and drought. Although all these factors negatively impact toad populations and disrupt and alter their natural history patterns, the continued drought and the catastrophic wildfire of 2011 are of primary concern for the Houston toad, today.

The Lost Pines region of Texas is a loblolly pine (*Pinus taeida*) and oak dominated forest located at the boundary of the Colorado River and the Carrizo-Wilcox aquifer (Brown and Mesrobian, 2005). The last remaining fragments of this ecosystem are currently found in Fayette, Colorado, Austin and Bastrop Counties (Tabor and Fleenor, 2003). Historically, these loblolly pine forests were naturally maintained by

low intensity wildfires. Natural fires progressed through the forest landscape, and removed the accumulated biomass and leaf litter, recycled soil nutrients, regulated plant succession, and maintained wildlife habitat (Rideout et al., 2003; Cain et al., 1998). Unnatural suppression of the natural fire disturbance regime has occurred for over 100 years (Nordlind and Ostlund, 2003). Fire suppression results in an increase in biomass (i.e., leaf litter and debris), tree stand densities (Kaufmann et al., 2003), and insect killed trees (Schowalter et al., 1981; Fettig et al., 2007; Jenkins et al., 2008), which, in turn, all drastically increase the potential of a catastrophic high intensity, high impact wildfire (Mutch, 1994).

On September 4th, 2011 the Bastrop County Complex fire resulted in the loss of 14,600 ha of Lost Pines habitat. This fire impacted approximately 40% of the remaining habitat patch in the County (Wallace et al. 2011) therefore increasing the difficulty of achieving recovery for this species. We have already published some of the outcomes of both prescribed fire and wildfire in Bastrop County on juvenile amphibian abundance and survivorship, but none of those fires were at the severity nor at the scale of the 2011 wildfire (Brown et al, 2011).

Recent efforts to offset continued declines of the species have included head-starting of individuals with the intent of "bridging" the populations through the current intense drought/fire conditions while increased habitat management and active stewardship efforts are initiated. This management strategy coupled with restoration of suitable habitat may lead to population recovery of the Houston toad. We seek to provide data that is relevant to immediate population remediation for the species in Bastrop County. The following chapters outline the current Houston toad head-starting

program, including genetic tracking of Houston toad head-start releases, and speculate on future plans for this program.

STUDY AREA

Study Area — The 34,400 ha Lost Pines ecoregion of Texas is thought to be a remnant of a pine-dominated forest that occurred in east and east-central Texas approximately 10,000 to 14,000 years ago (Bryant 1977, Al-Rabab'ah and Williams, 2004). It is now separated from the western boundary of the East Texas Piney Woods ecoregion by approximately 80 km. The primary study sites for these projects are the GLR, a 1,900 ha ranch owned by the Boy Scouts of America and Welsh, a neighboring property approximately 184 ha owned by Bastrop County and managed by Texas State University. In 2011 the Bastrop County Complex fire burned approx. 50% of the GLR (See Appendix I) and have given us the rare opportunity to test post wildfire effects on habitat. In addition, we utilized data collected at the 2,400 ha Bastrop State Park (BSP).

RESULTS

Assess and measure the outcomes of head-starting for the Houston toad population since 2007 through the use of pedigree reconstruction.

Introduction – More than 30% of amphibians are listed as threatened, endangered or critically endangered and more than 43% of species are in decline (IUCN et al., 2008). Manipulative practices such as population supplementation have been an attractive strategy for conservation biologists and some species have benefited from supplementation through relocation, repatriation, translocation and head-starting (Griffith et al., 1989). These practices have been very successful with mammals and birds (Seddon et al. 2005; Griffith et al 1989). Head-starting has also been a successful management tool for the conservation of Kemp's Ridley sea turtle (*Lepidochelys kempii*) (Fontaine and Shaver, 2005; Shaver and Wibbles, 2007) and the gopher tortoise (*Gopherus polyphemus*) (Tuberville et al., 2005). However, few translocation, repatriation, or head-starting programs have been reported as successful for amphibians (Dodd and Seigel, 1991).

Head-starting is a management practice in which wild individuals of early lifestages (eggs, tadpoles, etc.) are protected in the field or raised to a larger size in captivity (Haskell et al., 1996). Many anurans are explosive breeders in which a single egg strand may consist of thousands of eggs. The mortality rate is highest in the early life stages (eggs, tadpoles and metamorphosed juveniles) (Breden, 1987; Greuter, 2004). By avoiding this mortality through head-starting, it is believed more individuals will be capable of reaching maturity and reproducing (Dodd and Seigel, 1991). For many

species, translocation or head-starting may be the only conservation option for re-establishing or supplementing populations. Conservation practices are limited by time and money, therefore determining the effectiveness and efficiency of these techniques is essential for future or continued programs (Scott and Carpenter, 1987).

Houston toad population restoration efforts using the release of captive propagated juveniles, tadpoles, and eggs was first conducted by the Houston Zoo in the 1980's. The Attwater Prairie Chicken National Wildlife Refuge, located in Colorado County, TX, was selected for this early project because it was located within the historic range of the Houston toad and was thought to have suitable habitat. Egg strands were removed from ponds in Bastrop County, raised at a rearing facility at the Houston Zoo, then released within the Refuge as eggstrands, tadpoles, metamorphs, or adults. This initial translocation program has been historically reported as unsuccessful in failing to yield a sustaining population of Houston toad within the National Wildlife Refuge (Dodd and Seigel, 1991).

In 2007 the Houston Zoo, in cooperation with Texas State University, Texas Parks and Wildlife Department, and U.S. Fish and Wildlife Service facilitated a second head-starting program. In addition to head-starting, this program involved annual chorusing surveys to further monitor populations on public and private lands. Houston toad egg strands were removed from ponds located in Bastrop County, raised to older stages (tadpole, metamorph, or adult) and then returned to their natal ponds. Adult head-start toads were toe-clipped or PIT tagged for future identification. The majority of head-starts were tadpoles or juveniles which cannot be physically marked for future identification making an assessment of head-starting difficult, necessitating molecular

markers and pedigree reconstruction for the identification of captured toads from 2008 – 2013. This enables our assessment of the effectiveness of this head-starting program.

Results from this program showed first year survivorship from egg to metamorph for captive individuals was 77% (Vandewege, 2011). Overwinter juvenile survivorship has been estimated to be 0.1% or 0.3 % (Vandewege, 2011); however, severe drought may have skewed this estimate. Due to a continued drought, the absence of a 2011 breeding period and the catastrophic wildfire, we have yet to further investigate these estimates.

This study assessed the survivorship of head-starts from 2008 - 2013. Houston toad egg strands or tadpoles collected during the breeding seasons of 2008 – 2013 were genotyped through the use of genetic markers for pedigree reconstruction to determine if the reproduction events involved a head-start individual. These individuals were then tested against genotypes from the 2007 – 2012 head-start releases. These genotypes are all retained and will be tested against Houston toad offspring in 2014.

It is possible to estimate the relatedness among pairs of individuals with genetic markers. Relationship categories such as full sibling (sib) or half sib can be estimated from the probabilities derived from a dyad (a pair of individuals) sharing zero, one or two alleles that are identical by descent (Thompson, 1991). A powerful tool derived from relatedness estimations yet to be fully exploited is pedigree reconstruction algorithms. These divide a dataset into sibgroups based on codominant genetic marker data (Blouin, 2003). These partitioning algorithms may be able to determine whether a wild caught toad is directly related to the head-started population providing a unique

genetic mark- recapture method to measure the abundance and distribution of head-started Houston toads on the landscape.

Herein, we describe a method capable of monitoring the supplemented populations through time using molecular markers and sibship algorithms. Many adults were captured after thousands of head-started juveniles were released onto the landscape. Pedigree reconstruction was used to determine how many collected adults were in fact recaptured head-starts thus measuring the impact head-starting has had since 2007.

Methods – Egg strand collection and juvenile tissue acquisition - Areas surveyed included Bastrop State Park (BSP) (Bastrop Co.), Bluebonnet Electric (BBE) (Bastrop Co.), Griffith League Ranch (GLR) (Bastrop Co.), Hilltop lakes (HTL) (Leon Co.), Jim Small Family property (JMS) (Bastrop Co.), Musgrave Family pond (MSV) (Bastrop Co.) and Nava Family pond (NAP) (Austin Co.). If amplextant *B. houstonensis* were observed during surveys, the location was marked and the area surveyed for egg strands the following day. Up to 100% of discovered egg strings were removed from wild habitats and brought back to the Houston Zoo or to the Welsh head-start facility to be reared to different life stages. Head-started juveniles were released at different life stages post hatch to the same site they were collected. DNA samples were taken from each egg strand or tadpole cohort for genetic analysis and were accessioned into the Michael R. J. Forstner frozen tissue catalog at Texas State University.

Adult tissue collections- Tissue was taken from adults in the years after head-starting began to determine how many collected adults were recaptured head-starts. Adults were sampled in three different ways, audio surveys, pit fall traps, and active

searching during FEMA fire clean up operations. During the springs of 2008-2013, between 16 and 26 auditory surveys were conducted between January and May. Listening posts were located to allow chorus monitoring of potential Houston toad breeding sites (~300), most of these occurring in Bastrop Co. (Jackson et al., 2006). Observers listened for 5 minutes for chorusing. Calling males and observed females were collected and a toe was clipped from specimens using sterile scissors and stored in 95% ethanol. Blood samples were also taken from collected toads using a sterile syringe and stored in a blood storage buffer (Longmire et al., 1997). DNA samples were accessioned into the Michael R. J. Forstner frozen tissue catalog at Texas State University.

DNA extraction method and genotyping - DNA was extracted from toe clips or blood using a DNeasy® DNA Tissue kit (QIAGEN Inc.) on an Applied Biosystems 3500xL Laboratory Automation Workstation following the manufacturer's protocol. Extractions will be evaluated by electrophoresis on a 2% agarose gel and visualized under UV light after Gelred staining.

Genotyping and allelic diversity- PCR was performed at five microsatellite loci: BBR36 (Simandle et al., 2006), BC52.10, bco15 (Chan, 2007), BM224 (Tikel et al., 2000) and IHHH (Gonzalez et al., 2004) that were previously shown to be highly polymorphic within *B. houstonensis* populations (McHenry, 2010). It was revealed in McHenry (2010) that BM224 contained two unique motifs separated by a conserved region where electromorph size homoplasy was present. An additional reverse primer (BM224DJM) was designed to anneal within the conserved region to amplify the first half of the locus. Single locus statistics were estimated in FSTAT (Goudet, 2001). We

used the estimated population allele frequencies to test the power of relationship inference for three genealogical relationship comparisons: 1) full sibs vs. unrelated 2) half-sibs vs. unrelated and 3) full-sibs vs. half sibs.). Fragment analysis was performed on an ABI 3500xL Genetic Analyzer (Applied Biosystems, Inc.). Fragment results were called using GeneMapper v4.1 and Data Collection v3.1 software from Applied Biosystems.

Sibship reconstruction- Sibship partitions were constructed from a dataset of all sampled individuals within egg strands. All collected adults from Bastrop and Austin counties between 2008 and 2013 were combined with samples from strands 1 and 3 (2007), 4-8 and 10 (2009) to determine the frequency of recaptured head-starts in these counties. Allelic dropout rates and mutation rates were set to 1% and 1.5%, respectively as recommended by Wang (2004). Adults collected between 2008 and 2013 that partitioned with samples taken from captive egg strands were considered potential head-starts given congruent temporal and spatial data. Vandewege (2011) tested 4 algorithms for determining sibship (relatedness) reconstruction in B. *houstonensis* and found COLONY (Wang, 2004; Jones and Wang, 2009) to be the most successful at assigning individuals to families. Therefore, COLONY was used for all pedigree reconstruction for head-start Houston toads.

Results. - Head-starting captures and releases- A total of 38 egg strands were collected for head-starting between 2008 – 2013 from localities within extant populations of the Houston Toad (Figure 1, Table 1). Head-starting releases took place between March and September of each year. Prior to 2013, a mean of 642 individuals were released from each egg strand, the majority of which were tadpoles or newly

metamorphosed juveniles (Table 1). Only a fraction of the egg strands were raised past metamorphosis. On average, 77% of an egg strand survived to metamorphosis in captivity and when releases were accounted for, 24.6% of metamorphs survived to one year in captivity. A mean of 16 samples were collected from each strand for genotyping.

Adult collection- Three hundred and eighty-six toads were sampled across pit fall traps, audio surveys, and FEMA fire clean up operations. Most toads were collected from GLR (between 2008 and 2012) and BSP (2013). Due to drought conditions relatively few toads were heard calling or collected in years 2008, 2009 but a wetter 2009-2010 winter yielded many more in the spring of 2010. Drought conditions persisted and breeding was not documented across the County in 2011. Few toads were collected in 2012 following the Bastrop County Complex Fire that burned over 40% of Houston toad habitat. Finally, several larger rain events occurred the spring of 2013 and breeding was documented at multiple ponds in BSP over a four day period. Almost all individuals collected were males given a significant male bias within the species (Swannack and Forstner, 2007).

Egg strand genotyping- - Seven hundred and sixty eight individuals were genotyped at four or five loci. One missing genotype per individual was allowed, which resulted in 687 completely genotyped samples and 81 samples were missing information at one locus. This is 1.8% missing data. The number of alleles per locus varied from 1 to 6 with a mean of 2.05 alleles per locus per egg strand. Average observed heterozygosities within egg strands ranged between 0.32 and 0.9 with an overall mean of 0.73.

Frequency of recaptured head-starts- Three hundred and eighty-six (44% from GLR and 14% from BSP) adults were collected between the audio and pit-fall trap surveys (Appendix II). Only one individual had a genotype 100% consistent with a

head-started egg strand in Bastrop Co. (Figure 2). The probability an individual having this exact genotype by chance was 1.31e-8 and the probability a random individual has a genotype consistent with egg strand 1 was 1.48e-5 based on global allele frequencies. This male could have been released on July 9th or May 27th of 2007

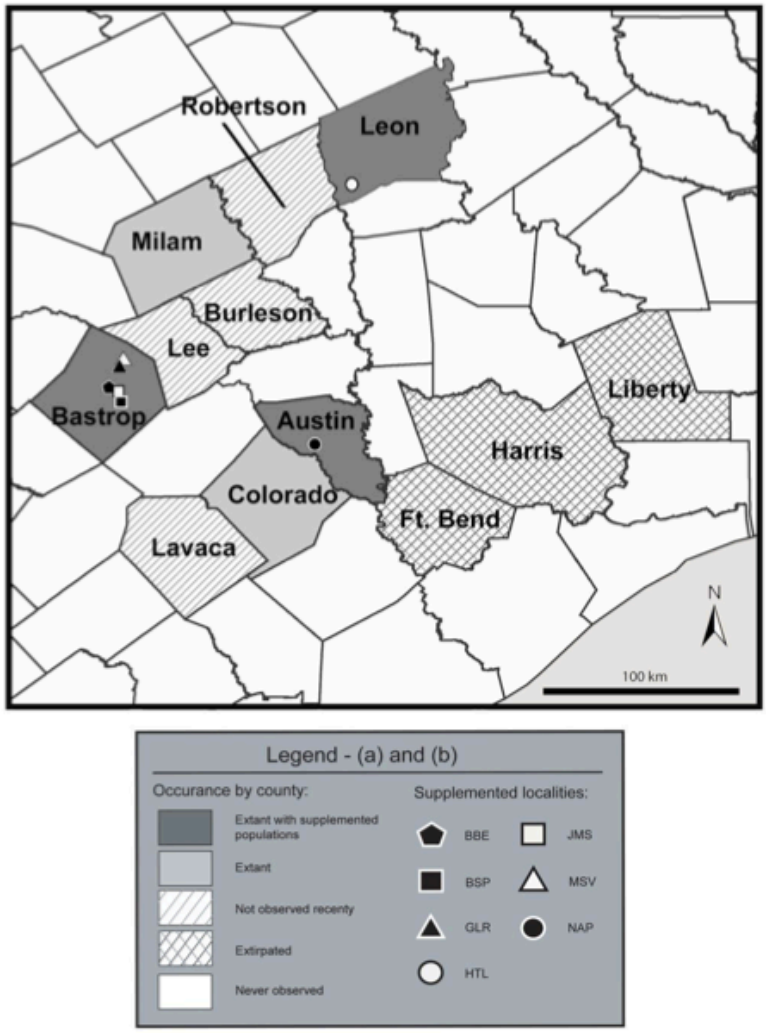

Figure 1. Range map of *B. houstonensis* (a) Occurrence of *B. houstonensis* in Texas by county. (b) Counties surveyed and distribution of supplemented sites

Table 1. Locality and release description data for captive egg strands and zoo adult head-starts. The total number released only includes individuals released up until 2013. The % egg, % tadpole, % juvenile, % adult describe the proportion of each listed release and age class associated with that release.

County	Locality	Date Collected/ Released	# of Egg Strands Collected	Number Released	% egg	% tadpole	% juvenile	% adult
Bastrop	GLR P 2	3/14/07	1	384	-	-	83	17
Bastrop	BBE P 3	3/14/07	1	151	-	-	93	7
Bastrop	BSP P19	3/20/09	1	936	-	-	100	-
Austin	NAP P1	4/18/09	4	2598	-	-	100	-
Bastrop	GLR P13	4/19/09	1	660	-	-	100	-
Bastrop	BSP P11	2/21/10	1	651	-	70	30	-
Bastrop	BSP P8	2/21/10	4	3198	-	89	11	-
Bastrop	BSP P14	2/22/10	2	870	-	100	-	-
Bastrop	GLR P9	2/22/10	1	1275	-	100	-	-
Bastrop	MUS	3/4/10	3	1380	-	100	-	-
Bastrop	JMS P4	3/6/10	1	329	-	-	100	-
Bastrop	GLR P12	3/6/10	1	331	-	-	100	-
Bastrop	GLR P5	3/7/10	1	1000	-	100	-	-
Austin	NAP P1	3/26/10	1	399	-	-	100	-
Bastrop	GLR P13	4/18/10	1	1908	-	-	100	-
Bastrop	BSP P8	3/13/13	2	1500*	-	100	-	-
Bastrop	BSP P3	3/13/13	4	1800*	-	100	-	-
Bastrop	GLR P12	3/9/13	1	62	-	-	-	100
Bastrop	GLR P2	3/9/13	1	63	-	-	-	100
Bastrop	Welsh	4/13/13	3	604	-	-	-	100
Bastrop	JMS	5/13/13	1	800*	-	100	-	-
Bastrop	BSP P8	5/14/13	1	2000*	100	-	-	-
Bastrop	GLR P12	5/14/13	1	2000*	100	-	-	-
Bastrop	GLR P14	5/14/13	1	2000*	100	-	-	-
Bastrop	GLR P15	5/14/13	1	2000*	100	-	-	-
Bastrop	GLR P2	5/22/13	2	4000*	100	-	-	-
Bastrop	GLR P12	5/22/13	1	2000*	100	-	-	-
Bastrop	GLR P14	5/22/13	1	2000*	100	-	-	-
Bastrop	GLR P2	7/20/13	3	230	-	-	100	-
Bastrop	BSP P3	7/20/13	1	30	-	-	100	-

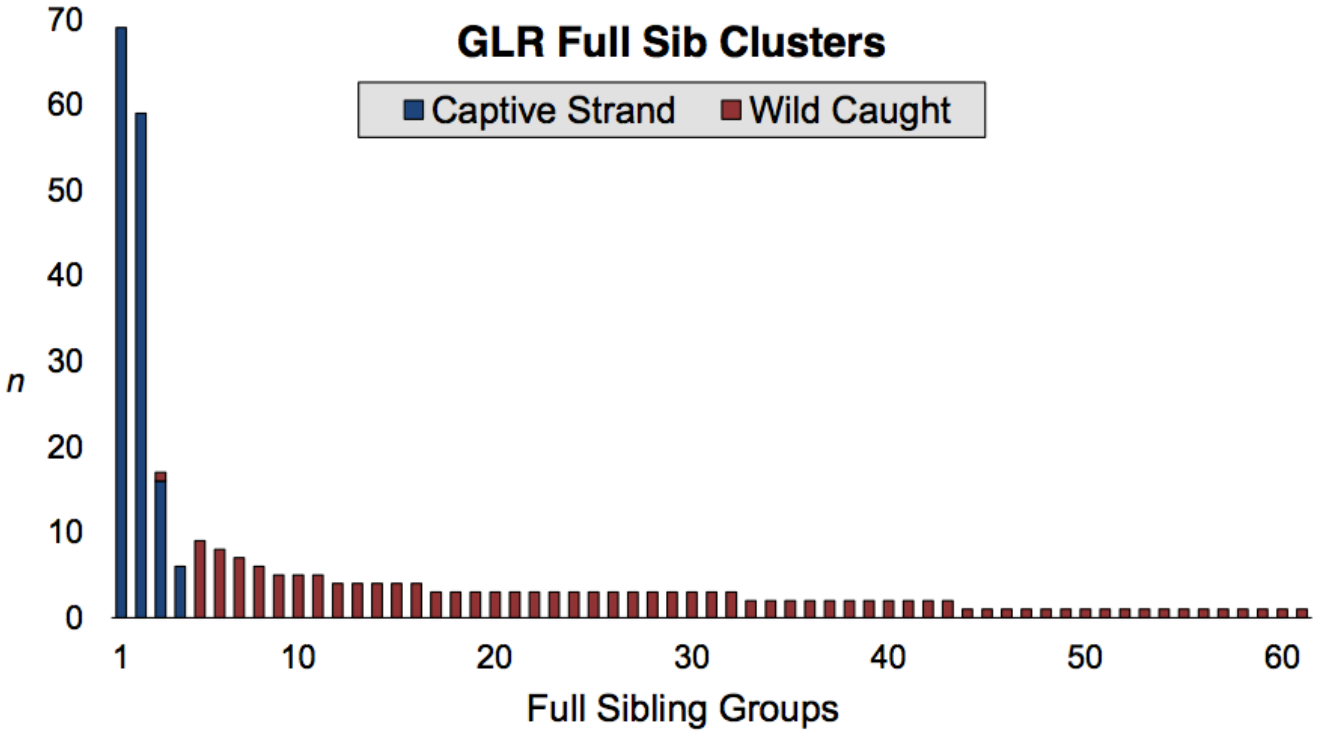

Figure 2. Full sibling reconstruction from GLR. Full sibling partition was constructed from both captive egg strands (1 and 10) and wild caught individuals from GLR collected between 2008 and 2010. Each bar is a different reconstructed sibling group. The y axis represents the number of individuals that partitioned within a single sibling group. Blue represents members from the captive population and red represents individuals collected from the wild. Notice only one individual partitioned with a captive egg strand. This individual portioned with a kin group in egg strand 1 and was collected in 2008.

Head-starts of 2013 – In 2013 we released 634 adult Houston toad head-starts from Bastrop and Leon Counties in Bastrop County (Griffith League Ranch and Welsh properties respectively). Upon release of adult toads, each toad was individually marked by toe clipping, weighted, measured, and photographed. Toe clippings were collected for DNA analysis. Toe clips are stored in 95% ethanol will be taken for each individual and samples will be accessioned into the Michael R. J. Forstner frozen tissue catalog at Texas State University. We also released 10 egg strands at 4 different ponds on the

GLR property (see chapter 3 for dates and locations of releases). In the spring of 2014 we will continue monitoring Bastrop Co. breeding locations through annual call surveys. Individuals collected in 2014 will be genotyped and tested against the 2013 head-start releases.

Disscussion - Pedigree reconstruction using COLONY resulted as an efficient method of assigning individuals to appropriate sibgroup. Out of 386 wild collected Houston toads only one has been identified as a head-start individual. Head-start releases primarily involved the release of tadpoles and metamorphs. Greuter (2004) reports low survivorship of both tadpoles and juveniles. Therefore it has been suggested that releasing head-starts at these lifestages is inefficient (Vandewege, 2001). Additionally complicating the assessment is the lack of data from Houston toads detected following the extreme drought of 2011, preventing any pragmatic statistical treatment. Although thousands of individuals were released in 2010 as head-starts, the lack of a breeding season in 2011 and the continued drought and wildfire reduced detection of Houston toads everywhere and includes these head-starts.

The next step in this head-starting program is to test the efficiency of releasing captive reared adults. Head-starting and captive breeding has had variable degrees of success in amphibians (Dodd and Seigel, 1991; Griffiths and Pavajeau, 2008). In 2013 a total of 759 adult Houston toads were released on the GLR and Welsh properties in Bastrop Co. With spring rains and a 2014 breeding season, we will be able to continue to test the efficiency of head-starting endangered amphibians using this relatively robust release set of adult individuals.

Review of the 2013 Houston toad breeding survey data and head-start releases in Bastrop State Park and the Griffith League Ranch.

Bastrop state park experienced one breeding event for the 2013 Houston toad season. This event began on March 8th and ended March 9th 2013. On the evening of March 8th, over 40 adult male Houston toads were heard chorusing throughout Bastrop State Park resulting in a minimum of 15 egg strands oviposited between March 8th and March 10th (Table 1). Houston toad surveys were not conducted on March 8th, therefore it is unknown how many males were in chorus for Pond 8 or if chorusing occurred elsewhere in Bastrop State Park. A total of six wild egg strands were collected from Pond 3 and Pond 8 in Bastrop State Park and transported to the head-start facility in Bastrop County (Figure 1).

Table 1. The 2013 Houston toad survey data from Bastrop State Park. Included are the total number of Houston toad males heard calling in Bastrop State Park, the number of egg strands observed, and number of egg strands collected following the breeding event

Pond #	# Males chorusing	Egg strand yield	# Head start egg strands
Pond 1	17	4	0
Pond 3	9	7	4
Pond 5	5	0	N/A
Pond 8	Unknown	4	2
Pond 10	6	0	N/A

Figure 1. Egg strands are held in their plastic collection container until hatched and free swimming. The free-swimming tadpoles are then released into the tadpole head-start tanks. One egg strand is released per tank.

Jim Small Egg Strand 2013 - On April 9, 2013 three Houston toad egg strands were found on the Jim Small property in Bastrop County. This is a Safe Harbor site in Bastrop County, and we were able to unexpectedly, but providentially detect and head-start an eggstrand from this site. One of these egg strands was transported to the head-start facility in Bastrop County. On April 17th the free-swimming tadpoles were released into the holding tank. Only approximately half of the egg strand fully developed into hatchlings. Tadpoles were raised at the head-start facility until the tadpoles reached late stage development (Gosner stage 40). These tadpoles were later released back to the Jim Small property pond.

Zoo Adult Houston Toad Bastrop County Release - On March 16th, 137 adult Houston toads (from Bastrop County) were de-accessioned from the Houston Zoo toad facility and transported to the head-start facility in Bastrop County. Individuals remained in tanks at the head-start facility for two weeks for acclimation enabling our first "soft release" to the Welsh property in Bastrop County. This facility has 42 tanks available, each eight feet in diameter and 2500 gallon capacity. Individuals were kept at

densities of approximately 70 toads per tank (total of two tanks) with males and females in both tanks. The adults were released onto the Griffith League Ranch property (Pond 2 and Pond 12) on March 9th and March 19th. In total, 62 toads were released at Pond 2 and 63 were released at Pond 12. We had a total mortality of nine individuals before releases occurred. Deceased individuals were deposited in the frozen tissue catalog at Texas State University. Upon release, each toad was individually marked by toe clipping.

During the acclimation period, several toads were observed in amplexus and eggs were oviposited in both tanks. Due to the number of egg strands oviposited, we were unable to quantify the total number of egg strands laid during the two week period. Most of these egg strands failed to develop; however, we determined approximately 1/2 of one egg strand in tub # 2 and 1/8 of an egg strand in tub # 1 successfully developed and hatched as free swimming tadpoles. The first metamorphs from these egg strands began emerging on May 18th. Metamorphs were released at ponds 2 and 12 on the Griffith League Ranch respectively. This represents the first non hormone induced captive breeding for the Houston toad.

Zoo Adult Houston Toad Leon County Release - On April 13th, 2013 a total of 634 adult Houston toads (from Leon County) were removed from the Houston Zoo facility and transported to the head-start facility in Bastrop County. These adults were placed in the head-start tanks at densities of 60 – 67 per tank and remained in the tank for up to 11 days for acclimation for soft release on the Welsh property in Bastrop County. Of these 634 toads, 256 adults came from Zoo strand 27 (G25241) with sex ratio of 182/74 (M/F), 280 adults came from Zoo strand 25 (G25239) with sex ratio of

141/139 (M/F) and 95 adults came from Zoo strand 28 (G25242) with sex ratio of 60/35 (M/F). In total, the head-start facility received 383 Leon County males and 248 Leon County females.

Initially males and females were kept separate at the head-start facility with an average of 60 toads per tank. Over the next four days males and females were systematically placed together in order to promote amplexus. Within 4 days, dozens of egg strands were oviposited, however few strands developed. Metamorphs were first observed on May 18th.

The 634 adults were subsequently released at the Welsh property in Bastrop County between April 17th and May 15th 2013 (Table 2). Eggs were laid in the Welsh pond following the first two nights of adult toad releases. Four egg strands were oviposited on the first night and one on the second night. None of the eggs from the four egg strands hatched, and only one egg strand appeared to be fertilized. We observed predation on Houston toad eggs by resident tadpoles within the Welsh pond. These predatory tadpoles were *Scaphiopus* spp..

Table 2. Leon County Adult release dates and number of toads released at the Welsh property in Bastrop Co., TX.

Date of Release	Number of Toads Released
April 17th 2013	176
April 18th 2013	127
April 19th 2013	131
April 27th 2013	87
April 29th 2013	70
May 9th 2013	2
May 11th 2013	4
May 15th	7

Captive Propagated Zoo Egg Strand Releases Of 2013 - On May 14th we released four egg strands from the Houston Zoo to the Griffith League Ranch and

Bastrop State Park (Table 3). Strands 39 – 41 are all crosses from Zoo strand 22 (Female) and strand 23 (Male). Five additional egg strands, from the Houston Zoo, were released on May 22nd.

Table 3. Date and location of egg strand releases from captive breeding event at the Houston Zoo. Release locations are Bastrop State Park (BSP) and Griffith League Ranch (GLR).

Location	Date	Pond	# of strands	Genetic Cross Information
BSP	5/14/13	8	1	Blue Bonnet HQRS Female X BSP Pond 8 Male
GLR	5/14/13	12	1	GLR Pond 12 Female X Jim Small Pond 4 Male
GLR	5/14/13	14	1	GLR Pond 12 Female X Jim Small Pond 4 Male
GLR	5/14/13	15	1	GLR Pond 12 Female X Jim Small Pond 4 Male
GLR	5/22/13	2	2	GLR Pond 2 Female X GLR Pond 12 Male
GLR	5/22/13	2	2	GLR Pond 12 Female X GLR Pond 2 Male
GLR	5/22/13	12	1	Jim Small Pond 4 Female X BSP Pond 8 Male
GLR	5/22/13	14	1	GLR Pond 12 Female X Jim Small Pond 4 Male

Egg strands were either covered with a metal hardware cloth exclosure or left uncovered (Figure 2). Covered egg strands in Ponds 15 and 14 were not observed after the release date. We believe raccoons entered the ponds and removed the egg exclosures from their location. Due to the muddy characteristics of these ponds, we were unable to observe eggs or tadpoles beyond the initial release date. Both covered egg strands released in Pond 12 successfully hatched, and on May 23rd we observed the first metamorphs onto the landscape. The covered egg strands released at Pond 8 in Bastrop State Park and at Pond 2 on the Griffith League Ranch successfully hatched; however, it is unknown if these tadpoles emerged onto the landscape. None of the uncovered egg strands were detected post release date. To date all exclosures are securely fastened to the bottom of the ponds using two 5 inch rebar posts.These metal hardware cloth exclosures seem to be effective in preventing immediate predation on egg strands and can help head-start the egg stage into the tadpole stage.

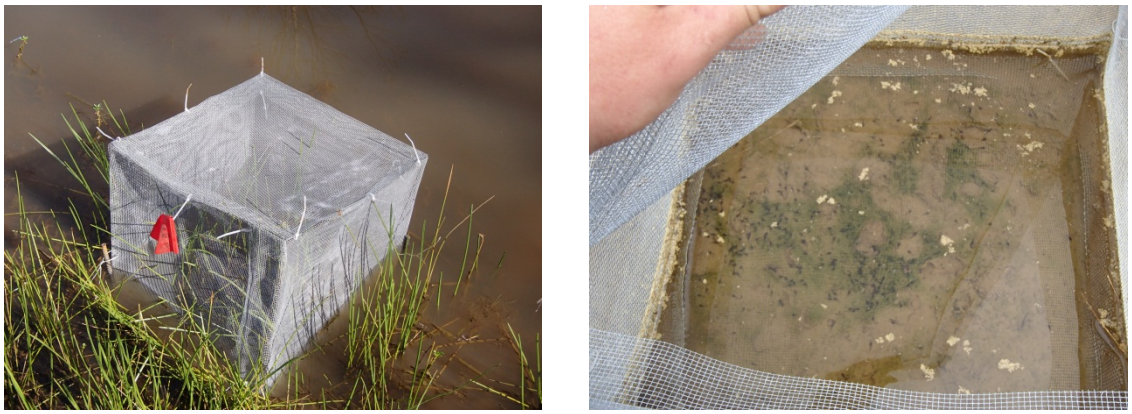

Figure 2. Egg exclosures used to reduce predation on released eggs and tadpoles. These are 1 x 1 m^2 exclosures built using 1/8 in hardware cloth.

Houston Toad Tadpole Head-start Releases Of 2013 - During the 2013 toad season we detected 15 Houston toad egg strands within Bastrop County. From these 15 egg strands we transported eight strands (resulting from four separate ponds) to the head-start facility in Bastrop County. Tadpoles were later transported back to the natal ponds (Table 4). On May 22nd and on June 9th, the Houston Zoo took 13 metamorphs from Pond 3 and 35 metamorphs from Pond 8 and transported them to the Houston toad breeding facility at the zoo. These individuals will be raised to adults and become part of the Houston toad assurance colony.

Table 4. Release dates for the wild collected Houston toad egg strands from across Bastrop County in 2013. These individuals were released as Gosner stage 40 tadpoles.

Pond	Release Date 1	Release Date 2	Release Date 3
BSP Pond 3	April 30th	May 4th	----------
BSP Pond 8	May 5th	May 14th	May 30th
Jim Smalls	May 13th	May 20th	May 28th
GLR Pond 12	Egg strand did not develop	----------	----------

2008 – 2013 Head-start and Captive Propagated Egg Strand Overview – In total, approximately 40,000 Houston toads were released in Bastrop and Austin counties as head-start eggs, tadpoles, juveniles, or adults along with captive propagated egg strands (Table 5). Currently there are plans to continue head-starting wild collected egg strands along with the release of Houston toad captive bred egg strands in cooperation with the Houston Zoo. If this captive propagation production meets our current goals, the zoo would enable us to produce and release 125, 000 – 250, 000 eggs to BSP and GLR in 2014, a truly dramatic change to the current effort (Table 5).

Table 5. Years of all Houston toad head-start release efforts in Austin, Bastrop and Leon Counties. * represents the current forecast for release of head-start egg strands for 2014. The zoo will begin breeding captive frogs in the spring for releases at BSP and GLR.

Year	Number of Individuals Released	Wild Detections
2007	535	---
2008	75	29
2009	4000	30
2010	14730	193
2011	60	5
2012	0	68
2013	21089	61
2014*	250000	---

PROJECT SUMMARY

Over the past several years, there has been an increase in the global loss of biodiversity (Griffith et al., 1989). Therefore population supplementation practices such as; captive-breeding, head-starting, and translocation programs have increased in popularity (Dodd and Seigel, 1991). For the Houston toad, head-starting may be the only conservation option for re-establishing or supplementing populations of this endangered species. Although head-starting efforts for the Houston toad began in 2007, multiple stressors led to a decrease in over all detection across Bastrop County (see Ch. 3 Table 5). Close to 15,000 head-starts were released in 2010, however the successes of this robust release have yet to be fully assessed. The spring of 2011 failed to yield a single reproductive event due to stressors caused by extreme drought. Furthermore the Bastrop County Complex Fire created additive effects that led to yet another failed breeding season of 2012.

We will not be able to evaluate the survivorship of the 2013 head-starts until the spring of 2014. Between 2007 and 2012 approximately 19,500 head-starts have been released on BSP and GLR. Wild survivorship from egg to adult has been estimated between 0.01 – 0.03 % (Vandewege, 2011). With these releases and suriviorship estimates, we should expect these head-start events to yield 20 adult Houston toads. Therefore out of 386 wild individuals collected during this study, it is encouraging that one individual has been found to be a head-start. Results from Vandewege 2011 suggest head-start survivorship is equivalent to wild survivorship.

Future head-starting efforts are currently working to increase the efficiency of this population supplementation tool. We know head-starting for the Houston toad can be successful for this endangered species. In 2013, along with wild collected egg strands released as head-starts, we were able to captive propagate egg strands at the Houston Zoo and release these in Bastrop County. We plan to increase these captive propagated egg strand releases and hope to release over 200,000 head-starts in 2014. Increasing the number released will yield to higher numbers of individuals surviving to adulthood and therefore increase our detection of head-starts come the following spring breeding seasons.

To futher increase head-starting efficiency, we will use data from the habitat suitability study to guide future releases and to continue to manage Houston toad habitat through a continued drought and post catastrophic wildfire.

AKNOWLEDGEMENTS

Thanks to Texas Parks and Wildlife and US Fish and Wildlife Service for providing funding that allowed these projects to take place. Thanks to Paul Crump the Houston Zoo for donating samples for this research. Appreciation goes to the following: for allowing access to property used in these studies: Jim Small, Bob Long, Bastrop State Park, LCRA, the Boy Scouts of America and Blue Bonnet Electric Cooperation. Activities associated with this research were permitted under the IACUC permits 0713_0428_07, 0810_0208_11, and 1011_0501_11 and United State Fish and Wildlife Service permit TE039544-0.

APPENDIX 1

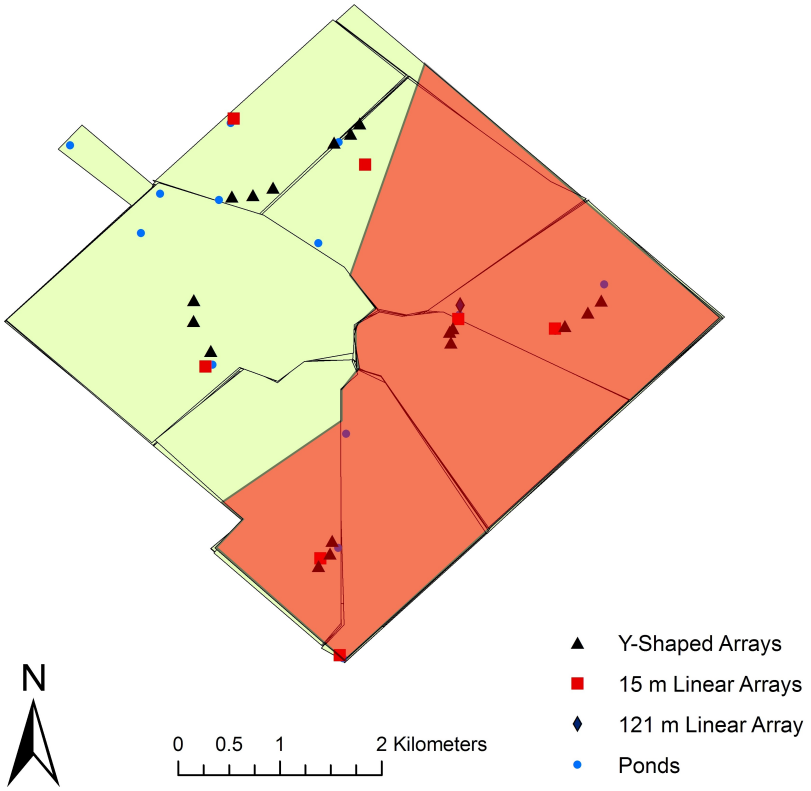

Figure 1. Map showing burned areas of GLR from the Sept 4th 2011 wildfire. Area shaded in red represents burned landscape

APPENDIX 2

Year sampled, number of individuals collected at that location, coordinates (WGS84), country, state, county and locality description for all Houston toads sampled in Bastrop County between 2008- 2013.

Year Sampled	Number of Indiviuals Collected	Latitude	Longitude	County	Locality Description
2008	2	30.214500	-97.232849	Bastrop	GLR P6-A1
2008	20	30.216261	-97.241722	Bastrop	GLR Pond 2
2008	4	29.877890	-96.352943	Austin	TCW Pond
2008	2	29.872459	-96.363861	Austin	Hinkel Road
2008	1	30.196119	-97.243736	Bastrop	GLR P12-A1
2009	9	29.883341	-96.361549	Austin	Nava Pond
2009	2	29.879910	-96.359619	Austin	McMurray Pond
2009	4	30.188950	-97.232536	Bastrop	GLR Pond 13
2009	6	30.194889	-97.243584	Bastrop	GLR Pond 12
2009	1	30.212351	-97.229988	Bastrop	GLR Pond 7
2009	1	30.199181	-97.221970	Bastrop	GLR Pond 9
2009	1	30.178730	-97.232468	Bastrop	GLR Pond 14
2009	1	30.209320	-97.242912	Bastrop	GLR Pond 5
2009	1	30.216261	-97.241722	Bastrop	GLR Pond 2
2009	1	30.198931	-97.221878	Bastrop	GLR L-9
2009	1	30.196119	-97.243736	Bastrop	GLR 15 B-S
2009	1	30.194750	-97.244202	Bastrop	GLR L12
2009	1	30.212540	-97.230087	Bastrop	GLR L7
2010	19	30.245670	-97.221649	Bastrop	Musgrave Pond
2010	4	30.237520	-97.211517	Bastrop	Dube Lane
2010	8	30.209320	-97.242912	Bastrop	GLR Pond 5
2010	13	30.199181	-97.221970	Bastrop	GLR Pond 9
2010	1	30.205601	-97.234230	Bastrop	GLR Pond 8
2010	14	30.194750	-97.244202	Bastrop	GLR L12
2010	37	30.194889	-97.243584	Bastrop	GLR Pond 12
2010	5	30.201990	-97.208992	Bastrop	GLR Pond 11
2010	1	30.196119	-97.243736	Bastrop	GLR T15
2010	1	30.198931	-97.221878	Bastrop	GLR L-9

2010	1	30.169291	-97.232361	Bastrop	GLR L16
2010	1	30.209860	-97.240028	Bastrop	GLR TC
2010	4	30.212351	-97.229988	Bastrop	GLR Pond 7
2010	2	30.216261	-97.241722	Bastrop	GLR Pond 2
2010	3	30.216600	-97.241722	Bastrtop	GLR L2
2010	5	30.206499	-97.249901	Bastrop	GLR Pond 4
2010	1	30.199511	-97.210449	Bastrop	GLR P10-A2
2010	3	30.188950	-97.232536	Bastrop	GLR Pond 13
2010	1	30.197800	-97.213272	Bastrop	GLR Pond 10
2010	1	30.198090	-97.213341	Bastrop	GLR L10
2010	3	30.000000	-97.000000	Bastrop	Clay Pond B
2010	1	30.200081	-97.222656	Bastrop	GLR T10-1
2010	1	30.200159	-97.221413	Bastrop	GLR Trap 10-5
2010	2	30.177950	-97.233803	Bastrop	GLR Pond 15
2010	17	29.883341	-96.361549	Austin	Nava Pond
2010	8	31.066980	-97.171227	Leon	Hill Top Lakes
2010	1	29.889959	-96.361229	Austin	Waldrop Pond
2010	3	29.879910	-96.359619	Austin	McMurray Pond
2010	1	30.142307	-97.195801	Basttop	Bob Long Marsh
2010	7	30.137722	-97.243355	Bastrop	Jim Smalls Pond 2
2010	5	30.126329	-97.233704	Bastrop	Jim Smalls Pond 5
2010	12	30.126381	-97.239342	Bastrop	Jim Smalls Pond 4
2010	3	30.400280	-97.242500	Bastrop	Jim Smalls Pond 3
2010	2	30.212540	-97.230087	Bastrop	GLR L7
2010	1	30.210520	-97.238258	Bastrop	GLR TB
2010	1	30.177151	-97.234230	Bastrop	GLR P14-A3
2011	2	30.107320	-97.249240	Bastrop	BSP Pond 3
2011	1	30.200081	-97.222656	Bastrop	GLR T10-1
2011	1	30.086150	-97.237900	Bastrop	BSP Pond 19
2011	1	30.194889	-97.243584	Bastrop	GLR Pond 12
2012	9	30.095700	-97.239500	Bastrop	BSP Pond 8
2012	1	30.107320	-97.249240	Bastrop	BSP Piond 11
2012	1	30.107320	-97.249240	Bastrop	BSP Pond 3
2012	2	30.099500	-97.245600	Bastrop	BSP Pond 6
2012	1	30.201990	-97.208992	Bastrop	GLR Pond 11
2012	9	30.194889	-97.243584	Bastrop	GLR Pond 12
2012	2	30.209320	-97.242912	Bastrop	GLR Pond 5
2012	1	30.214270	-97.232530	Bastrop	GLR Pond 6
2012	5	30.157900	-97.210000	Bastrop	Quarter Horse Lp
2012	1	30.149190	-97.198950	Bastrop	Mustang Dr.
2012	2	30.151780	-97.203720	Bastrop	Alum Creek
2012	3	30.081990	-97.231360	Bastrop	Alum Creek Dr
2012	1	30.103450	-97.225280	Bastrop	BSP Pk Rd 1C

2012	2	30.077980	-97.233780	Bastrop	Texas Kiln
2012	1	30.008800	-97.238700	Bastrop	BSP Pond 19
2012	1	30.096600	-97.265300	Bastrop	Pinedale
2012	1	30.088680	-97.284240	Bastrop	Peace Haven
2012	4	30.143650	-97.248360	Bastrop	Linda Ln
2012	1	30.142640	-97.255160	Bastrop	Lisa Ln
2012	5	30.136600	-97.253200	Bastrop	Hill Crest
2012	1	30.133910	-97.248940	Bastrop	Pine Hill Dr
2012	5	30.143520	-97.257620	Bastrop	Pine Tree Lp
2012	1	30.096980	-97.148160	Bastrop	Sage Rd
2012	1	30.092500	-97.287090	Bastrop	Royal Pines
2012	1	30.052920	-97.151640	Bastrop	Pine Hill Lp
2012	4	30.140840	-97.251210	Bastrop	HWY 21
2012	1	30.249000	-97.236400	Bastrop	Welsh Property
2012	1	30.085700	-97.241000	Bastrop	BSP Stuart Tract
2013	1	30.10717	-97.24996	Bastrop	BSP Harmon Rd
2013	1	30.105	-97.27077	Bastrop	BSP Pk Rd 1C
2013	14	30.10732	-97.24924	Bastrop	BSP Pond 3
2013	6	30.10119	-97.24757	Bastrop	BSP Pond 5
2013	11	30.13943	-97.25118	Bastrop	Jim Smalls
2013	8	30.19521	-97.24412	Bastrop	BSP Pond 1
2013	2	30.10623	-97.26212	Bastrop	BSP Pond 10
2013	3	30.19521	-97.24412	Bastrop	GLR Pond 12
2013	6	30.0957	-97.2395	Bastrop	BSP Pond 8
2013	1	30.11266	-97.25209	Bastrop	BSP Pond 2
2013	1	30.10732	-97.24924	Bastrop	BSP Pond 11
2013	1	30.08696	-97.26042	Bastrop	Pine Shadows
2013	6	30.24574	-97.22159	Bastrop	Musgrave

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