EFFICACY AND EFFICIENCY OF HEAD-STARTING AND CAPTIVE PROPAGATION OF AN ENDANGERED AMPHIBIAN: IMPLICATIONS FOR CONTINUED POPULATION AND HABITAT MANAGEMENT FOLLOWING CATASTROPHIC WILDFIRE

by

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Chapter II

ABSTRACT

The Lost Pines ecoregion of Texas is a loblolly pine (*Pinus taeda*) and post oak (*Quercus stellata)* dominated woodland forest with remaining fragments in Austin, Bastrop, Colorado and Fayette Counties. Bastrop County continues to support the largest known and best studied population of Houston toads (*Bufo [Anaxyrus] houstonensis*). The Houston toad was first described in Houston, Texas in 1953, and was the first animal from Texas and first amphibian federally listed as an endangered species. To date, nearly all recovery efforts have centered on the "robust" population remnant in Bastrop County, Texas. 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, fertilizers and chemical run off, agricultural practices, drought, and most recently, catastrophic wildfire. The aftermath of the Bastrop County Complex fire of 2011 has left Bastrop County with the need for immediate, active and continual restoration of plant communities on public and private land. This recent fire event now presents us with the rare opportunity to explicitly test habitat suitability and species survivorship pre and post catastrophic wildfire on native amphibian populations. I seek to provide data that are relevant to continued population conservation programs and the ongoing habitat remediation and restoration efforts in Bastrop County. I have 1) investigated the efficacy and effectiveness of head-starting and captive propagated releases of Houston toads; 2) assessed habitat suitability and the effects of catastrophic wildfire on Houston toad populations, and 3) assessed familiarity and community support of recovery efforts for the Houston toad among City of Bastrop residents. My results will guide future management strategies and contribute to conservation recovery efforts for the remaining Houston toads in this altered landscape.

CHAPTER I

INTRODUCTION

The Lost Pines ecoregion of Texas is a loblolly pine (*Pinus taeda*) and post oak (*Quercus stellata)* dominated woodland forest located at the boundary of the Colorado River and the Carrizo-Wilcox aquifer (Brown and Mesrobian, 2005) and currently retains fragments in Austin, Bastrop, Colorado and Fayette Counties (Tabor and Fleenor, 2003). This region represents the westernmost extension of loblolly pine forests in Texas and is thought to be a refugium population of a once continuous Eastern loblolly pine forest (Al-rabab'ah and Williams, 2004; Correll and Johnson, 1970). It is now separated from the western boundary of the East Texas Piney Woods ecoregion by approximately 80 km. Historically, these loblolly pine forests were naturally maintained by low intensity wildfires. Fires moved through these fire adapted forests, removing the accumulated biomass and leaf litter, recycling soil nutrients, regulating plant succession, and maintaining wildlife habitat (Rideout et al., 2003; Cain et al., 1998). Suppression of that disturbance in these fire evolved ecosystems has been occurring for over 100 years (Nordlind and Ostlund, 2003; Pyne et al., 1996). Fire suppression leads to an increase in biomass in the form of accumulated leaf litter and debris, an increase in stand densities (Kaufmann et al., 2003), and an increase of insect killed trees (Schowalter et al., 1981), which in turn all drastically enhance the potential for catastrophic high intensity, high impact wildfires (Mutch, 1994).

Bastrop County, Texas continues to support the largest known, and best studied, population of Houston toads (*Bufo [Anaxyrus] houstonensis*) (U.S. Fish and Wildlife, 1984; Dixon et al., 1990). The Houston toad was first described in Houston, Texas in

1953 (Peterson et al., 2004; Sanders, 1953). In 1970, the Houston toad was the first animal from Texas and the first amphibian federally listed as an endangered species (Peterson et al., 2004). Since the Houston toad was first described, Houston toad populations quickly became scarce. The cause of population decline is unknown, but many speculate the decline was due to the severe drought of the 1950's coupled with the expansion of the city of Houston (U.S. Fish and Wildlife, 1984).

A high correlation has been found between the sandy loam soils of the Lost Pines ecoregion and Houston toad occurrence (Koepp et al., 2004), with small isolated toad populations found between the Colorado and Trinity Rivers. 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. The Houston toad burrows underground or under logs and debris most of the year and only surfaces to forage and breed. Houston toads are therefore thought to be restricted to areas of sandy loam soils, while not necessarily pine forests (Brown and Thomas, 1982). Since 1978, Bastrop State Park and the surrounding areas in Bastrop County have been designated critical habitat for the Houston toad.

To date, nearly all recovery efforts have centered on the "robust" population remnant in Bastrop County. Houston toad populations have remained in a continual decline consequent of multiple stressors, including habitat fragmentation, continued urban growth of the city of Bastrop, red imported fire ants, fertilizers and chemical runoff, agricultural practices, drought, and now catastrophic wildfire. Although all these factors negatively impact toad populations, the Bastrop County Complex fire is of primary concern for the survival of this endangered species. On September 4th, 2011 the

Bastrop County Complex fire began in Bastrop County, which altered 36,000 acres of Lost Pines Habitat. The aftermath of the Bastrop County Complex fire has left Bastrop County with the need for immediate, active and continual restoration of the plant community on public and private land. Restoration actions, along with some of the necessary expenses, will require landowner support and involvement and habitat and wildlife management. In order to guide private and public landowners in effective post fire habitat restoration strategies that will improve habitat for the Houston toad, we must determine optimal habitat recovery options for toad survivorship.

We have surveyed and documented amphibian diversity and abundance before and after prescribed fire in Bastrop County (Brown et al., 2011; Brown et al., 2014; Jones et al., 2006). This recent fire event now presents me with the rare opportunity to explicitly test habitat suitability and species survivorship pre and post catastrophic wildfire on native amphibian populations. Habitat specific suitability for the Houston toad, although speculated, has not yet been tested.

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, habitat remediation, and also to habitat restoration for the species in Bastrop County. I have investigated the efficiency and effectiveness of head-starting Houston toads, determined habitat suitability and effects of catastrophic wildfire on this endangered amphibian, and assessed knowledge and support of Bastrop residents on the recovery of the Houston toad. These projects will provide results to guide future management options in hopes of offsetting additional mortality to Houston toads remaining within the altered landscape.

Study Areas

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 14,000 to 10,000 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 Griffith League Ranch (GLR), a 1,900 ha ranch owned by the Boy Scouts of America and Welsh, a neighboring property of approximately 184 ha owned by Bastrop County and managed by Texas State University. Both properties are recognized by the United States Fish and Wildlife Service (USFWS) as Houston toad habitat and are currently managed primarily through habitat restoration efforts. In 2011 the Bastrop County Complex fire burned approximately 50% of the GLR and has 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). The IACUC permit number for this research is 1011_0501_11 and federal USFWS permit is TE039544-0.

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CHAPTER II

TESTING SURVIVORSHIP OF ADULT HOUSTON TOADS (*BUFO HOUSTONENSIS*) IN SIMULATED WILD HABITAT EXCLOSURES

Introduction

Many studies have shown that fire can have both positive and negative effects on wildlife populations. These fire effects are driven by multiple factors such as fire intensity, fuel load, wind, and relative humidity (Esque et al., 2003). Moseley et al. (2003) suggests low intensity, low impact prescribed fire has little negative effect on wildlife populations. Fire, in many cases, has been shown to increase population densities (Minshall et al., 1989; Greenberg and Waldrop, 2008; Brown et al., 2011). The natural history and behavior of individual species and how quickly they can adapt to the changing environment will also affect how they respond to fire. Compared with other vertebrates, amphibians have much smaller dispersal and movement capabilities (Sinsch, 1990; Bury et al., 2000; Semlitsch and Bodie, 2003; Bowne and Bowers, 2004), which could increase direct mortality. Furthermore, the moist permeable skin of amphibians would increase their vulnerability to smoke and heat and may lead to dehydration (Stebbins and Cohen, 1995; Bury et al., 2000). Habitat type, topography, or the presence of wetlands may create refugia and provide protection to animals during fire (Whelan, 1995). Hossack and Corn (2007) observed a slight increase in amphibian populations at local wetlands post wildfire.

Fewer studies have investigated the effects of catastrophic wildfire on amphibian populations. As fire intensity increases, so may the chances for an increase in direct and indirect mortality of certain species. Direct mortality can be caused by direct exposure to flames, ash, and smoke and occurs immediately as the fire passes across the landscape. Indirect mortality can be caused by changes in habitat, reduction in food, water and shelter, and lower nutrient availability. Brown et al. (2014) reported minimal direct mortality on amphibians post wildfire, and Greenberg and Waldrop (2008) reported a higher abundance of American toads in burned habitats compared to unburned habitats post wildfire.

The frequency of extirpation of a population or the extinction of a species due to a fire event or other habitat disturbance event is low (Thomas et al., 2004). However, the situation in Bastrop, for Houston toads, is abnormal. The species had only one large population center remaining prior to the fire, which itself was coupled with the ongoing extreme drought. This provides a scenario where the catastrophic fire is paired with extreme drought, affecting the only genetically diverse, large, population fragment of a species that remains. As noted in the 1994 population viability assessment (Seal, 1994), extinction or extirpation risk probabilities, for the Houston toad, are truly a serious concern.

While surveys allowed us to examine amphibian diversity and abundance before and after prescribed fire (Brown et al., 2011; Jones et al., 2006) and wildfire (Brown et al., 2014) in Bastrop County, we still need to continue to test habitat suitability pre and post catastrophic wildfire specifically for the Houston toad. Houston toad response to the newly altered landscape following the Bastrop County Complex fire is unknown and at best speculative.

The purpose of this study is to test and compare adult Houston toad survivorship within the various different habitat types that existed prior to the fire, and in forest types remaining after the Bastrop Complex Fire. We will gain insight on habitat suitability for the Houston toad given the current conditions found across Bastrop County. These data will hopefully provide suggestions on immediate and ongoing restoration efforts leading toward habitat and population recovery from the 2011 Bastrop Complex Fire, but also future fire recovery efforts.

Key to enabling this evaluation is the availability of improved microchip technology (BioMark) enabling detection even when the toad is buried up to 10 – 12 cm deep. The availability of different habitat types within these study sites, and the coincident availability of adult Houston toads to release into replicate enclosures in those different habitats able us to test survivorship and possible growth over time. The results from this study will be provided to the relevant management agencies and Bastrop County as quickly as possible in order to help guide habitat and wildlife management for public and private lands in Bastrop County and may also be used as a blueprint for management strategies in other fire prone regions of the Southeast United States. Testing habitat recovery options, then establishing the best management practices on public and private lands is the core benefit of the described studies. The described studies provide results and benefits for future management options in hopes of offsetting additional mortality to Houston toads remaining within the altered landscape.

Quantitatively estimating demographic parameters from mark-recapture (henceforth MR) studies have advanced considerably over the last three decades (Lebreton and Pradel, 1992; Burnham and Anderson, 2002). Currently, most MR studies use multi-model analysis in information-theoretic framework to estimate survival (φ) and the probability of recapture (p) (Burnham and Anderson, 2002; Schmidt et al., 2002).

Statistical inference from model selection under an information-theoretic approach requires rigorous attention to selecting the candidate set of models. Briefly, a candidate model set is developed using a priori hypotheses focusing on the relationship between survival and recapture, and covariates, such as treatment effects, environmental parameters, among others. Models are ranked based a selection criterion, most commonly Akaike's information criterion (AIC_c) (Akaike, 1973), which provides a reliable decision criterion for model selection for both nested and non-nested models (Schmidt and Anholt, 1999; Burnham and Anderson, 2002).

This study used an information-theoretic approach to model selection to choose models that best fit MR datasets collected from exclosure experiments conducted in 2011 – 2012, and 2013 – 2014; where four adult Houston toads were released into large outdoor exclosures representing different habitat types (described above). For each model φ and *p* were estimated.

Materials and Methods

Two adult survivorship and habitat suitability trials were conducted between June 2011 and March 2014. Trial 1 compared adult Houston toad survivorship among three habitat types; pine, oak, and juniper dominated habitat patches in Bastrop County prior to the Bastrop County Complex fire. Trial 1 began June 2011 and was completed in March 2012. A second trial (trial 2) was conducted after the Bastrop County Complex fire of 2011. This second trial compared adult Houston toad survivorship among four habitat types, adding a wildfire burned habitat along with the pine, oak, and juniper habitats. These two trials are intended to illustrate habitat suitability in current habitat patches in Bastrop County.

Trial 1 – Prior to the Bastrop County Complex fire we sought to evaluate habitat and adult head-start toad survival in three habitats using a field exclosure experiment. Three habitats were selected for the preliminary study; loblolly pine dominated, oak dominated, and juniper dominated woodlands or forest patches. Five exclosures were built within each habitat for a total of 15 exclosures. Each exclosure is approximately 10 x 10 m^2, built using galvanized aluminum flashing. The flashing is buried 10 to 12 cm deep within the soil substrate in order to prevent toads from tunneling under and escaping.

Male Houston toad adults, that were captive raised at the Houston Zoo, were released within each of the 15 exclosures. These adults were raised from wild population eggstrands collected in Bastrop State Park and Griffith League Ranch in Bastrop County, Texas. A total of four toads were placed within each exclosure. Upon release, each toad was implanted with a BioMark Passive Integrated Transponder tag or PIT tag. The BioMark PIT tags can be read at a depth of 10 – 12 cm beneath the surface even when the animal is underground and buried under logs and other debris. These PIT tags enabled us to monitor the location and movement of each toad over time with minimal disturbance using the subsurface detection abilities of the BioMark chip reader.

On June 6, 2011 sixty zoo raised male Houston toads were divided evenly and placed within the 15 exclosures. Upon release, each toad was weighed, snout urostyle length (SUL) and head width (HW) measured, and pit tagged. As toads were released, pit tag numbers were recorded so the location of each toad was confirmed. The first week after initial release, toads were checked every other day. As toads were found, they were flagged and numbered and movement was recorded (Figure 1). Toads were then surveyed 2 to 3 times a month usually following rain events, from June 2011 to March 2012.

Survival estimates – Survivorship estimates were conducted in Program MARK (White and Burnham, 1999) using the Cormack-Jolly-Seber mark-recapture model (Cormack, 1964; Jolly, 1965; Seber, 1965). We assumed that capture probability was at 100% at the conclusion of trial 1. Each exclosure was searched extensively upon and post completion of trial 1 until no more toads were discovered. All individuals that were captured in subsequent censuses were known to be alive in any previous census.

Canopy Cover – Canopy cover was collected twice during trial 1 (July 2011 and October 2011) for each habitat. Canopy cover was estimated for each exclosure in the three habitats using a spherical crown densitometer. For each exclosure, estimates were taken from nine points arranged in a grid formation with three rows and three points per row. These nine points were then averaged and percent cover reported for all 15 exclosures. Differences among habitats were assed with a single factor analysis of variance (ANOVA).

Red Imported Fire Ant Counts – Fire ants were trapped, collected, dried, identified, and counted for all 15 exclosures. For each exclosure, nine petri dishes were placed in a grid formation with three rows of three dishes per row. The points used for this procedure where the same nine points used to collect canopy cover. Each dish was baited with one half piece of Vienna sausage link. The bait traps were deployed in each exclosure for 45 minutes then picked up and placed in a Ziploc bag. The bags of ants were put on ice to reduce ant activity. Ants were then dried, then identified and sorted. Differences among habitats were assed with a single factor ANOVA.

Trial 2 – In order to test habitat suitability after the catastrophic wild fire, five additional exclosures were added to the Griffith League Ranch study site in a location that was severely burned during the Bastrop County Complex fire, creating a fourth habitat to be tested. These additional exclosures allowed us to test fire habitat suitability as well as replicate our previous exclosure study. For this second study four Houston toad adults were placed into each of the 20 exclosures for a total of 80 adult toads. Each exclosure contained two males and two female toads, allowing us to compare survivorship among sex. Each toad was implanted with a BioMark PIT tag prior to release. SUL, HW, mass and photographs were collected for each adult toad. Exclosures were monitored 2 to 3 times a month from March, 2013 until March, 2014.

Survival estimates – Survivorship estimates were conducted in Program MARK (White and Burnham, 1999) using the Cormack-Jolly-Seber mark-recapture model (Cormack, 1964; Jolly, 1965; Seber, 1965). We assumed that capture probability was at 100% at the conclusion of trial 2. Each exclosure was searched extensively upon and post completion of trial 2 until no more toads were discovered. All individuals that were captured in subsequent censuses were known to be alive in any previous census.

Canopy Cover – Canopy cover was collected twice during the study (July 2013 and January 2014) for each habitat. Canopy cover was estimated for each exclosure in the four habitats using a spherical crown densitometer. For each exclosure, estimates were taken from nine points arranged in a grid formation with three rows and three points per row. These nine points were then averaged and percent cover reported for all 20 exclosures. Differences among habitats in canopy cover were assed with a single factor ANOVA.

Model selection procedure methods trial 1,trial 2, and males vs females– Using a model selection approach based on information-theoretic methods, Program MARK (White and Burnham, 1999) was used to estimate the probabilities of φ and *p* for adult Houston toads. . Methods followed Cooch and White (2006). Two explanatory factors were used to explore variation in φ and *p*: time and habitat type. Time was considered as constant among sampling periods (•) or variable across periods. Habitat type (*ht*) was treated as a categorical covariate with three levels (juniper, oak, and pine) and used to determine if habitat type affected φ or *p*. Based on these factors, eight candidate models were developed, where each model represented a different biologically-based hypothesis that explored the effects of time and habitat type on estimates of φ and *p*. For example, φ_t p_t represented the Cormack-Jolly-Seber model (CJS) that is fully time dependent for both φ and *p.* Whereas $\varphi_{ht} p_{\bullet}$ represented a model where survivorship varied among habitat types, and *p* remained constant among sampling periods.

The amount of support for each of the eight candidate models was evaluated using a correction factor for AIC (AIC_c) which protects against over-fitting the models, especially with small sample sizes (Hurvich and Tsai, 1989). The model with the lowest AIC_c was considered to best fit the data unless the difference in AIC_c values (ΔAIC_c) among competing models was < 2.0, then the models were considered indistinguishable. Models were ranked from one to eight, with one being the best supported model and eight being the least. If multiple models supported the data, the most parsimonious model was chosen as the best supporting models. Point estimates, standard errors and 95% confidence intervals were recorded for φ and *p* for each model.

In trial 2 we were able to test survivorship and recapture between females and males. Using a model selection approach (described above) we estimated the probabilities of φ and p for males and female adult Houston toads. Two explanatory factors were used to explore variation in φ and *p*: time and sex. Time was considered as constant among sampling periods (•) or variable across periods. Sex (*s*) was treated as a categorical covariate with two levels (males and females) and used to determine if sex affected φ or *p*. Based on these factors, 9 candidate models were developed.

Results

Trial 1

Toad Detection In Exclosures – There is a decrease in total toad detection among all three habitats over time. Total detection started at 68% during census one decreasing to 2% total detection at the conclusion of trial 1 (Figure 2). Detection percentage was calculated using the total number of toads detected each census divided by the number of toads assumed to be alive at each census.

During the study, PIT tags were found among the debris or under the sandy soils. These lone PIT tags were either the only remnant of a mortality event or, likely a PIT tag that had been shed or expelled out through the skin. It is not uncommon for a toad to expel a PIT tag once it has been implanted. Tags can migrate out of the skin from the tag insertion point, leaving a live toad very difficult to detect. The Houston Zoo reports a 10% expulsion rate for PIT tags implanted into their adult Houston toads (Paul Crump, personal comm. 2012). During trial 1, eight PIT tags have been found within the exclosures. One tag found in juniper habitat, two found in pine habitat and five tags found in the oak habitat. These eight tags represents 13% of all pit tags used in trial 1. To prevent bias, we removed individuals corresponding to the lost tags from detection estimates post date of tag discovery because it is unknown if these toads represent live (undetectable) or dead (mortality event) individuals.

Toad detection was initially high for pine and juniper habitats at 86% and 65% detection for the first census (Figure 3). Detection was at 23% for the first census in the oak habitat, jumping to 50% by census 2, however quickly dropping down to 19% by census 4. Pine was the only habitat where detection remained above 50% until August 16^{th} 2011 (census 11). Detection hit 0% in the juniper and oak habitats by August 25^{th}, 2011 and September 20^{th}, 2011 respectively. On March 25^{th} 2012 we concluded trial 1 and aggressively searched all 15 exclosures for remaining toads. Duff layer was moved along with debris, rocks, and limbs. A single toad was detected in the pine habitat at the end of trial 1.

Although a decreasing detection trend is noticeable, a slight increase in detection within the pine and cedar habitats was noticed on June 30^{th}, 2011 (census 6) (Figure 3). This increase in detection is positively correlated with a 3.81 cm rainfall event that occurred on June 22.

Toad Survivorship trends and MARK recapture φ and p estimates– During the first month of the study, total toad survivorship decreased dramatically. Total survivorship fell below 50% by July 17^{th}, 2011 (census 7) (Figure 4). In the juniper and oak habitats, survivorship decreased by 30% between census 1 and 3. Pine habitat survivorship did not fall below 50% until census 13 (Figure 5). Significant differences between survivorship over time and among habitats were seen in trial 1 (ANOVA: Habitat, $df = 2$, F=47.159, $p = <0.001$; Time, $df=1$, F=291.644, $p=<0.001$).

Although we found deceased toads during these trials, we were unable to determine the fate of the individuals from missing chips. Therefore we did not account for joint live and dead recaptures in our MARK models. Based on ΔAIC_c, model 1 ($\varphi_{ht} p_{ht}$) was the best supported model (Table 1). For this model, φ and *p* varied with habitat type (Table 2), but not across time, with pine having the highest values for both φ (0.92) and *p* (0.79). Juniper had the second highest φ (0.84), but had the lowest *p* (0.6). The other seven models had ΔAIC_c values > 2.0, which indicates that habitat type had a stronger effect on the data compared to the most parsimonious model (Model 3, $\varphi . p .$).

Canopy Cover – Average canopy cover during trial 1 was 78.7 % in the pine habitat, 76.7 % in the oak habitat, and 82.6 % in the juniper habitat. Canopy cover did not differ among the habitats (ANOVA: df = 2, 12, F= 0.223, p=0.804). The juniper habitat contain understory of yaupon holly along with oak, increasing canopy cover. The pine and the oak habitats are clear of understory growth allowing more sunlight to penetrate to the forest floor.

Toads and Red Imported Fire Ants – Several deceased toads were found covered in red imported fire ants (RIFA). It is uncertain whether this is the direct cause of mortality or if the ants acted as opportunists once the toads were dead. We tested for the abundance of RIFA within all 15 exclosures to see if ant abundance was correlated with toad mortality within habitats. Ants were sampled in July 2012 after the trial had been concluded, in order to prevent attracting fire ants to the exclosures when toads were present. We sampled during the summer months when fire ant movement is at its highest. Red imported fire ants did not differ among treatments (Table 3) (ANOVA; df= 2, 12, F= 1.741, p= 0.22).

Trial 2

Toad Detection In Exclosures – Trial 2 began March, 2013 and was concluded April 2014. There is a decrease in total toad detection over time and among all habitats (Figure 6). Detection had a sharp initial decrease at the beginning of the study followed by a continual decrease in detection through the summer and early fall. As temperatures rise during the summer months and then fall during the winter months toads will bury down deep to avoid desiccation. This can decrease chances of detection. Although a decrease in detection is expected, as Spring of 2014 approached, detection continued to decline until detection hit 0% on April 6^{th}, 2014.

Among habitats detection was the highest within the oak and pine habitats (Figure 6). Although detection within the juniper habitat had decreased to 13% by the 6^{th} census, detection increased above 30% following the rain events in May. Detection fell to 0% for all habitats by April 2014.

We found detection to be positively correlated with rain events (2013 – 2014 study) (Figure 7). A small increase in detection was observed on May 26^{th}, 2013, July 27^{th}, 2013 and November 22^{nd}, 2013. Two large rain events also occurred December 15^{th}, 2013 and February 9^{th}, 2014. An increase in detection was not observed following these two rain events.

A total of 19 pit tags were found during this study. This represents 24% of the total number of tags used in this study. Two tags were found in the oak habitat, four in the pine habitat, five in the juniper habitat, and eight in the burned habitat. Individuals that corresponded with these tags were not used in detection estimates post date of tag discovery.

Male vs Female Survivorhip and Detection– There was no difference in survivorship between male or female toads. Out of 80 total Houston toads, 22 males and 22 females were found deceased or a lost PIT was discovered. Therefore sex ratio (M:F) for detected toads was 1:1 for males to females. In the wild, explosive breeding amphibian sex ratios are commonly male biased (Wells, 1977; Davies and Halliday, 1979) caused by several factors such as, unequal sex ratios at birth, differences in male/female mortality rates, differences in male/female migration rates (Swannack and Forstner, 2007), and delayed maturation (Gibbons, 1990). In a controlled environment without breeding pressures and a decrease in natural predator opportunities males and females are able to survive across the landscape equally.

Based on ΔAIC_c, model 1 ($\varphi(.)\,p(.)$ was the best supported model (Table 4). For this model, φ and p were constant across time and between sex (Table 5) with φ (0.88) and p (0.62). The other eight models had ΔAIC_c values > 2.0, which indicates that the most parsimonious model had the strongest effect on the data.

Toad Survivorship trends and MARK recapture φ and p estimates – We report a 5.0% decrease in survivorship per visitation event for pine, 3.5% in oak, 3.6% in juniper and a 3.2% decrease in survivorship in burned habitat (Figure 8). Pine was the only habitat to sustain survivorship above 50% beyond May of 2013 (census 7). Pine survivorship fell below 50% July 17th, 2013. Pine habitat went from 50% survivorship on July 17th to 0% survivorship by August 20th, 2013 (Figure 8). June 1st marks the end of Houston toad breeding season. Breeding events beyond June 1st have been documented, but are rare. Survivorship on June 1st was at 65% in pine, 46% in oak, 27% in juniper, and 16% in burned habitat. During the breeding season (January 1^{st} – June 1^{st}) Houston toad activity should be at its highest. Total survivorship can be seen in Figure 9.

A total of 17 toads (21 %) were found deceased during Trial 2. Five deceased toads were found in the oak habitat, six were found in pine habitat, four were found in juniper habitat, and two were found in the burned habitat. Deceased toads are collected and transported to the tissue collection at Texas State University under federal permit # TE039544-0.

Based on ΔAIC_c, model 1 ($\varphi_{ht} p_{ht}$) was the best supported model (Table 6). For this model, φ and p varied with habitat type (Table 7), but not across time, with oak having the highest values for both φ (0.91) and p (0.78). Pine had the second highest φ (0.89), and second highest p (0.65). The other seven models had ΔAIC_c values > 2.0, which indicates that habitat type had a stronger effect on the data compared to the most parsimonious model (Model 4, $\varphi_{\bullet} p_{\bullet}$).

Canopy Cover – Average canopy cover during this study was 67.6% in the pine habitat, 72.0% in the oak habitat, 78.9% in the juniper habitat, and 4.0% in the burned habitat. Canopy cover was highest in the juniper habitats. The juniper habitat contains understory of yaupon holly which increases canopy cover. The pine and the oak habitats are clear of understory growth allowing more sunlight to penetrate to the forest floor. The burned habitat contains little to no overstory cover due to the severity of the Bastrop County Complex fire as it moved through areas of the Griffith League Ranch (ANOVA; df= 3, 16, F= 18.39, p=<0.001).

Old toad detections – During trial 1, we were monitoring three adult male toads that were part of an initial habitat suitability trial using the 15 original exclosures in three habitats. This initial trial, which began in March of 2010, had complications in detecting Houston toads below the ground. The original PIT tags used were not able to be detected using the biomark pit tag reader, therefore this trial was postponed until stronger chips were received. Toads that could be detected were removed and released in Bastrop County. During trial 1, three adult males from the 2010 attempt were discovered in the exclosures. These individuals had successfully overwintered and were detected the summer of 2011. Two toads were found in the juniper habitat during June of 2011 and the third toad was found in pine habitat in July 2011. Unfortunately these toads had lost the original PIT tags, therefore we cannot compare original SUL and mass. They were identified however as individuals in the original study due to toe clip markings taken as DNA samples before released into the exclosures. These toads were measured, weighed and released back to their original exclosure.

Discussion

The purpose of this study was to compare survivorship among different habitat types located in Bastrop County. Two trials were conducted in order to replicate the experiment during and after the severe drought and fire of 2011. We were able to add a burned treatment in trial 2 to look at fire responses. Habitat sites used in this study are sites that have been designated as optimum Houston toad habitat and therefore have been used as Houston toad head-starting release sites. Since 2007 we have been working with the Houston Zoo and with the USFWS on head-starting the Houston toad. Current head-starting strategies have focused on releasing individuals (adults, juveniles, tadpoles and now eggs) in designated "suitable habitat" within Bastrop County. Natural history of the Houston toad has led us to believe these areas of "suitable habitat" contain dominant stands of loblolly pine accompanied by deep sandy loam soils. Not only have these studies helped us confirm many of these designated areas are indeed suitable habitat, we were able to make predictions on how suitable these areas remained post catastrophic wildfire.

The effects of habitat on survivorship and recapture of adult *B. houstonensis* were significant with differences in survivorship and recapture estimates between habitats but not over time. In both trials pine and oak had the highest survivorship and recapture estimates, therefore further supporting our prior placement of head-start Houston toads in Bastrop County. Based on our data it is difficult to infer if pine or oak is the best habitat. Differences between pine and oak habitats between trial 1 and trial 2 can be due to temporal conditions between each year.

The best supported mark-recapture model for both trials was $\varphi_{ht}p_{ht}$ where survivorship and recapture estimates varied among habitats but not over time. Survivorship and recapture estimates constant over time, suggests temporal conditions were not a driving factor in these estimates. This is also supported by comparing detection and survivorship trend data among the two trials (Figure 10). Trial 1 was conducted in 2011 during an exceptional drought in this region and the majority of the state of Texas. Trial 2 was conducted in 2013 and 2014 where temperatures were cooler and rainfall had increased annually compared to 2011 – 2012. We would expect to see survivorship dependent over time during 2011 as conditions continued to worsen. Although drought conditions may have some affect on Houston toad these data fit a model where time was not a strong factor.

We did not see a difference in survivorship or recapture rates between males and female Houston toads. In the wild amphibian sex ratios are commonly male biased (Wells, 1977; Davies and Halliday, 1979). The selection of this model matches the detection and survivorship trend data between male and females over time, therefore supporting its selection. Differences in male and female mortality rates can be caused by the differences in their behaviors. Males can have increased rates of mortality during the breeding season when they are moving across the landscape multiple nights and actively calling at a pond edge (Swannack and Forstner, 2007). In a controlled environment without breeding pressures and a decrease in natural predator opportunities males and females are able to survive across the landscape equally.

Houston toad survivorship was lowest within the burned treatments ($\varphi = 0.84$). It is not surprising to see this result, however with habitat altered in Bastrop State Park and the Griffith League Ranch by catastrophic fire, it has implication to further management strategies. In 2015 no Houston toads were found in Bastrop State Park or on any burned locations in the Griffith League Ranch. We are currently releasing captive propagated in Bastrop State Park. Testing head-start survivorship has been difficult since 2010 and the 2015 spring season rains have helped create a best case head-start release scenario. We will continue to test head-starting in these burned locations, but if trends continue we may need to focus all our concerns on unburned release locations.

Rain events in 2013 and 2014 confirm that Houston toad adults become more active during these rain events due to detection increases correlating with large rain events. These correlations were during early months of this study and as survivorship and detection decreased, rain events no longer led to an increase in detection the following spring. We would expect to see an increase in detection during spring rains when Houston toad breeding season is occurring. Therefore, we strongly believe that detection is a suitable proxy to survivorship for both studies.

Houston toad mortalities or loss of detections (found PIT tag) were discovered during both trials. In total, 30 out of 140 total toads used in both trials were found deceased and another 27 PIT tags were found in both trials. The numbers of deceased toads for both studies were 20, 6, 10, and 2 and PIT tags were 6, 7, 6, and 8 respectively from pine, oak, juniper and burned habitats. Pine habitat has the highest survivorship during these two trials and counterintuitively, the highest confirmed mortalities. Survivorship is lowest in the burned habitat with the lowest number of confirmed mortalities. We have documentation of toads using shallow burrows in the pine habitat where sand is lose. Toads have been found desiccated while emerging from these burrows (Figure 11). Therefore the mortality events are occurring close to the surface and more readily detected. Burned toads are burrowing deeper to find cooler soils. It is assumed mortalities are high in this habitat but are occurring below the surface and therefore not detected.

We have anecdotal evidence that Houston toads may burrow deeper into these soils during periods of intense drought or suboptimal habitat provided by the three adults from the pilot study. These individuals burrowed beneath our detection threshold of ~20cm. Although detection errors may exist during each census, we are confident however that the survivorship declines are real. While early deaths may have occurred as a result of initial acclimation to the exclosures, we do not believe subsequent mortality to be experimental deaths, but natural mortality for adult Houston toads.

Red imported fire ants were found in most exclosures. Many deceased toads were discovered covered in fire ants or bones had been partially consumed by the ants. Fire ants can drive amphibian mortalities for those individuals emerging from the pond, however are not as commonly linked to adult mortalities. This predation and mortality has been tested in the Houston toad, where predation was linked to newly metamorphosed toadlets (Freed and Neitman, 1988). It is concerning however that fire ants are more prevalent in areas that are suggested to be suitable habitat for the Houston toad. Currently we are managing for red imported fire ants in areas we are conducting head-start releases and will continue to test the effects of these invasive predators.

The results from this study are not encouraging, as adult survivorship is lower in both severe drought and post drought/ post wildfire conditions than predicted from model assessments (Swannack et al., 2009). 2011 was the worst drought in Bastrop County on record (Nielsen-Gammon, 2012). We accept that the realities of the drought, particularly the exceptional severity, may have influenced our results, but the conditions during the second trial were not as severe as in 2011 and thus the strongly negative trends were not expected in 2013 and 2014 during Trial 2. These results improve our understanding of habitat suitability for this species and continue to refine our knowledge of how the current habitats are influencing population persistence. We can only infer from these data that survivorship is low for adult Houston toads and when tested in severe and then again, under more optimal conditions, the results are the same. Wild Houston toads are utilizing habitats that we have tested to be most suitable and we will continue to release captive propagated toads in these areas. However, if we are releasing them in the best habitats in

Bastrop County and we continue to see these trends either 1) Bastrop County no longer has sufficient contiguous suitable habitat 2) another factor is influencing these declines.

Figure 1. Example an adult Houston toad (*Bufo houstonensis*) wild mesocosm exclosures in Bastrop County, Texas. This is an example of a replicate exclosure in the pine habitat. The pink flags represent the last known location of each of the adult male Houston toads. Four adult Houston toads are located within each exclosure.

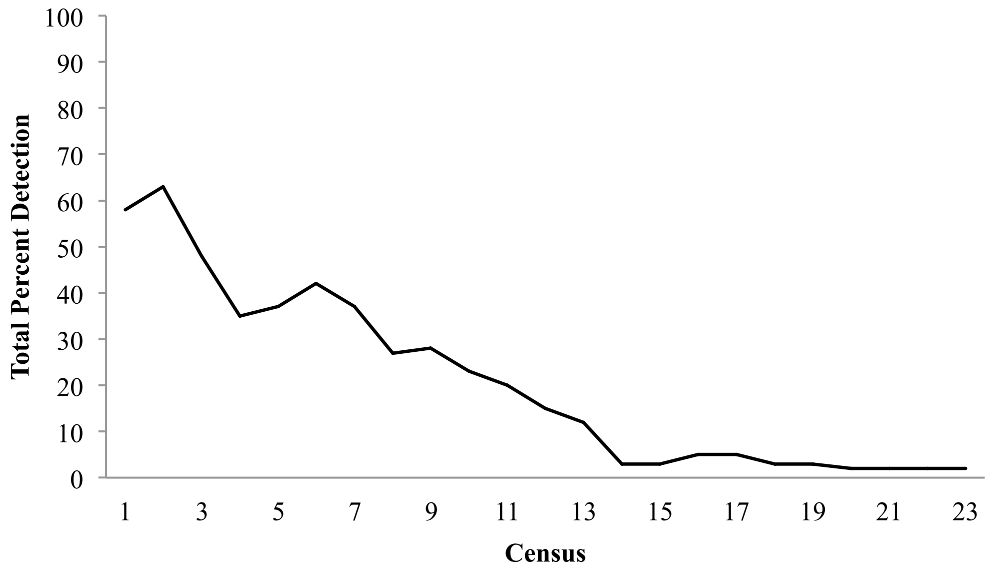

Figure 2. Combined detection over time of adult Houston toads (*Bufo houstonensis*) for all three habitats (juniper, pine and oak). Trial 1 began 11 June 2011 and concluded at census 23 on 25 March 2012.

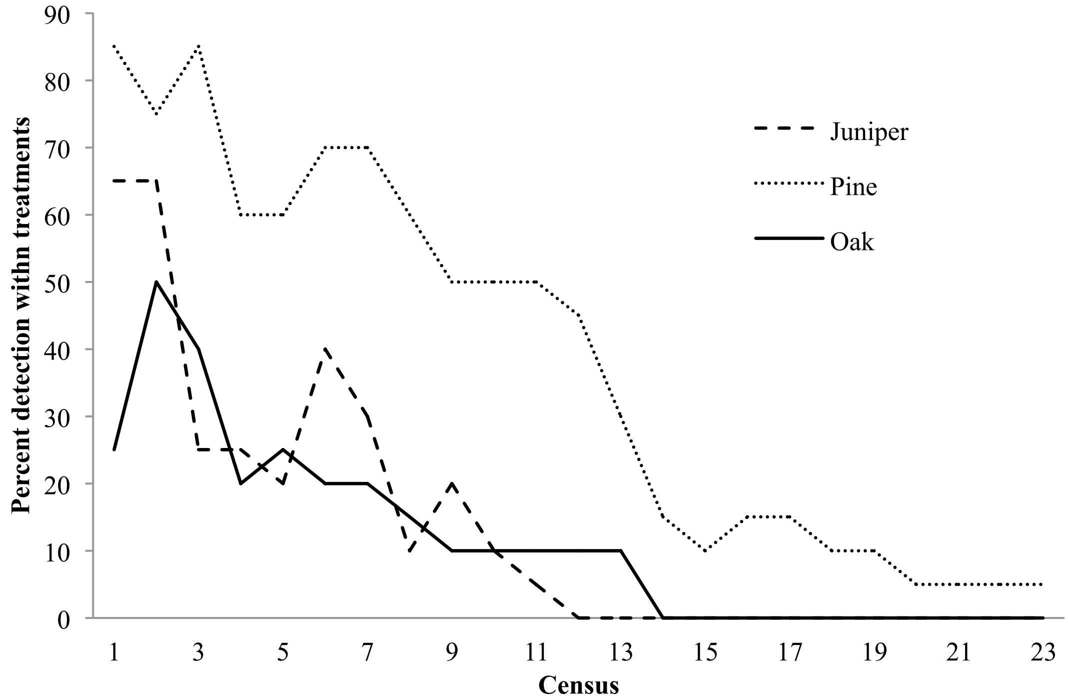

Figure 3. Detection over time of adult Houston toads (*Bufo houstonensis*) within each of the three habitats (juniper, pine and oak). Trial 1 began 11 June 2011 and concluded 25 March 2012.

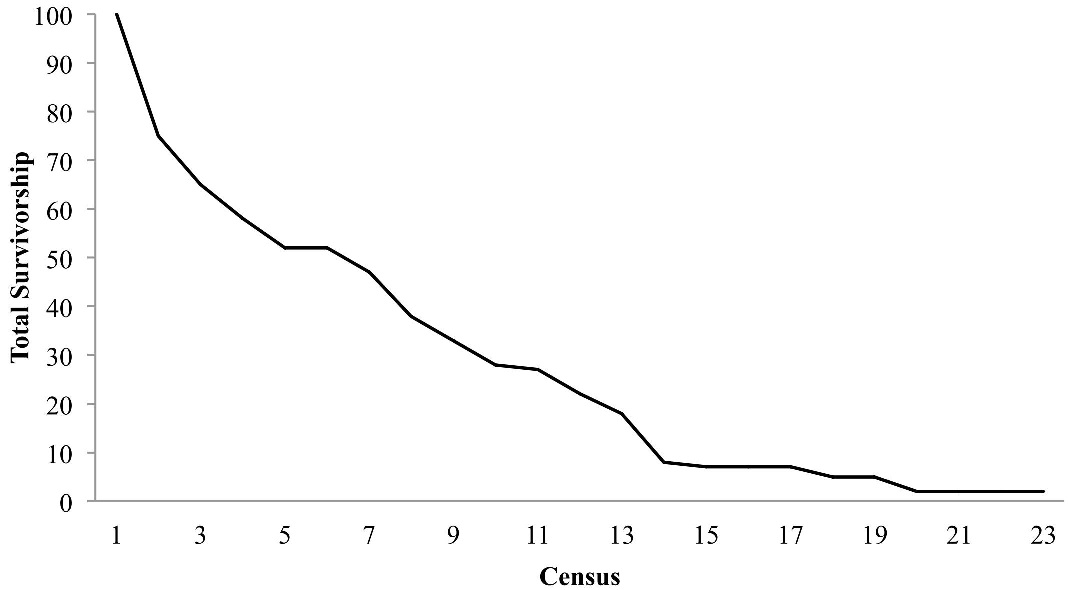

Figure 4. Combined survivorship over time for adult Houston toads (*Bufo houstonensis*) for all habitats (juniper, pine and oak). Trial 1 began 11 June 2011 and concluded 25 March 2012.

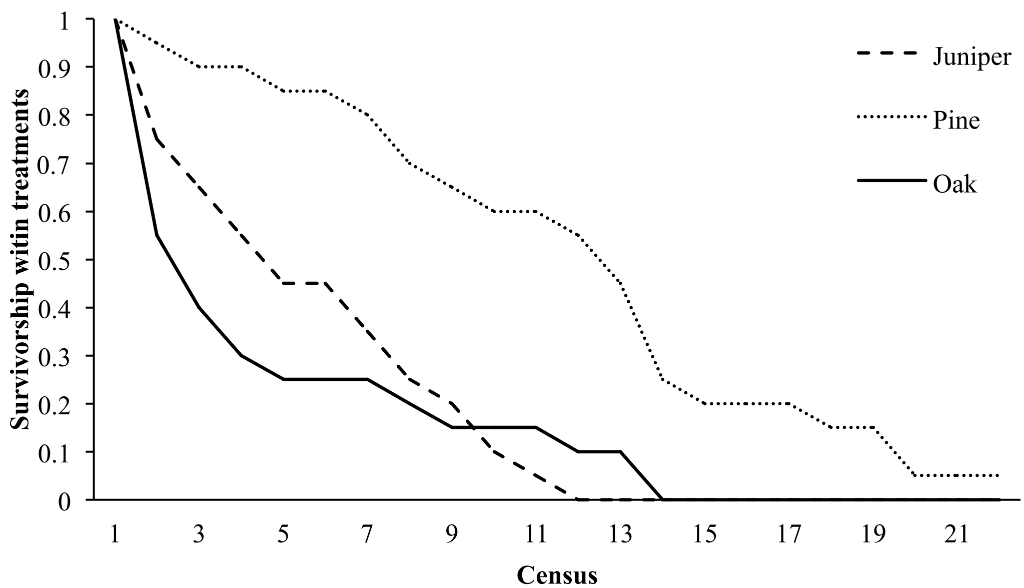

Figure 5. Survivorship over time for adult Houston toads (*Bufo houstonensis*) within all three habitats (juniper, pine and oak). Trial 1 began 11 June 2011 and concluded 25 March 2012.

Table 1. Cormack-Jolly-Seber candidate models and model selection results for trial 1 used for estimating φ and *p* of 60 adult male Houston toads (*Bufo houstonensis)* from a habitat suitability exclosure experiment. Models are listed by most supported to least supported based on AIC_c scores. *t* represents time-specific estimates (one estimate available for each sampling period),• indicates estimates were constant across time, *ht* is a covariate representing the habitat type of the treatment (juniper, pine and oak).

Model	AICc	ΔAICc	AICc Weight	Likelihood	# Pars.	Deviance
1. φ(*ht*)*p*(*ht*)	723.21	0.00	0.97	1.00	6	402.12
2. φ(*ht*)*p*(.)	730.26	7.05	0.29	0.03	4	413.30
3. φ(.)*p*(.)	738.80	15.58	0.00	0.00	2	425.92
4. φ(*t*)*p*(*ht*)	750.74	27.52	0.00	0.00	2	385.59
5. φ(*t*)*p*(.)	761.08	37.86	0.00	0.00	24	400.58
6. φ(*t***ht*)*p*(.)	783.33	60.12	0.00	0.00	50	357.65
7. φ(*t***ht*)*p*(*ht*)	807.50	84.29	0.00	0.00	63	344.84
8. φ(*t***ht*)*p*(*t***ht*)	1038.26	315.04	0.00	0.00	135	290.97

Table 2. Trial 1 estimates for φ and (*p*) in adult Houston toads (*Bufo houstonensis*) based on the model supported by the AIC_c selection criterion using program MARK. The model selected was $\varphi_{ht}\ p_{ht}$ where φ and *p* varied with habitats. Lower and upper confidence intervals (CI) are reported.

Estimator	Estimate	Standard Error	Lower CI	Upper CI
Survivorship (φ)				
Juniper	0.843	0.032	0.769	0.898
Pine	0.921	0.017	0.88	0.949
Oak	0.786	0.043	0.69	0.859
Recapture (*p*)				
Juniper	0.600	0.053	0.494	0.699
Pine	0.796	0.028	0.734	0.845
Oak	0.720	0.061	0.587	0.822

Table 3. Total number of Red Imported Fire Ants (*Solenopsis invicta*) found within each of the habitat replicates in trial 1 adult Houston toad (*Bufo houstonensis*) exclosure study in Bastrop County, Texas. Each replicate was baited at nine points and ants were collected after 45 minutes. We compared these totals among the three habitats and toad mortalities.

Habitat	*Solenopsis invicta*	Total per habitat
Juniper 1	0	
Juniper 2	75	
Juniper 3	0	
Juniper 4	379	
Juniper 5	204	658
Oak 1	0	
Oak 2	0	
Oak 3	75	
Oak 4	0	
Oak 5	0	75
Pine 1	0	
Pine 2	167	
Pine 3	43	
Pine 4	424	
Pine 5	1263	1,897

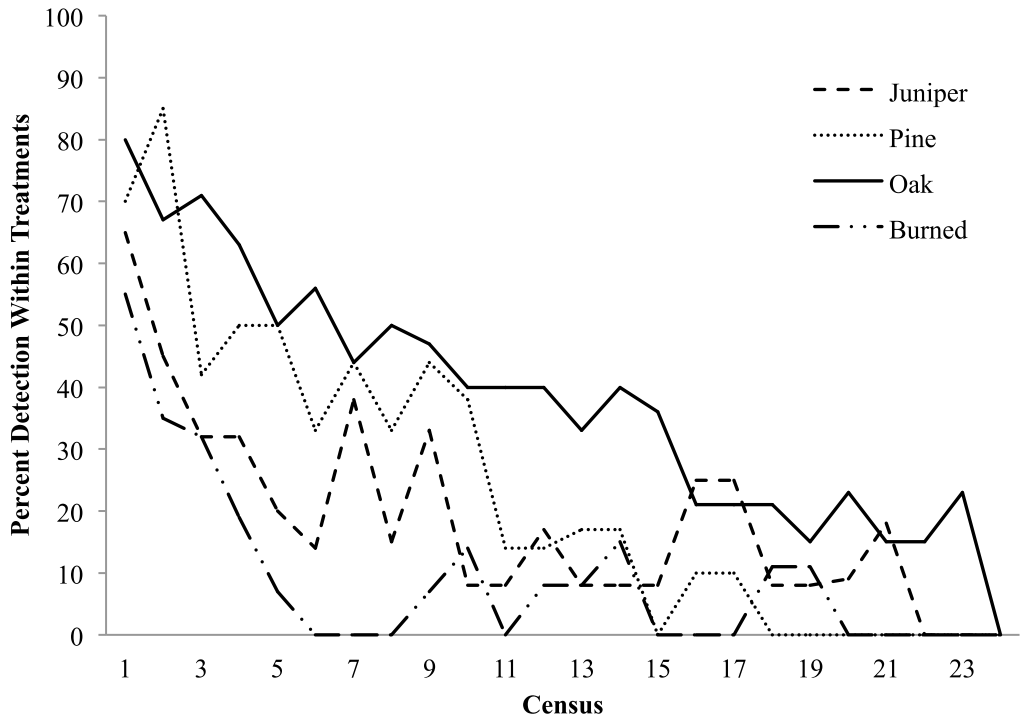

Figure 6. Detection over time of adult Houston toads (*Bufo houstonensis*) among all four habitats during trial 2(juniper, pine, oak and burned). Trial 2 began March 2013 and concluded March 2014.

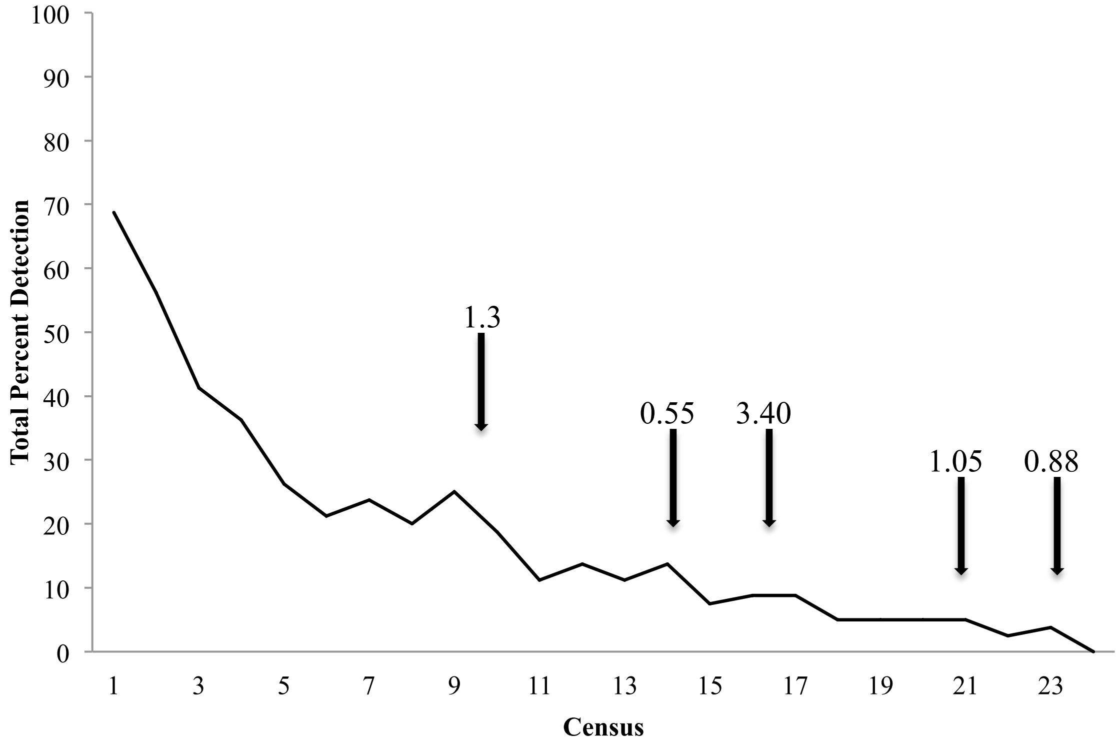

Figure 7. Combined detection over time of adult Houston toads (*Bufo houstonensis*) for all four habitats in trial 2 (juniper, pine, oak and burned). Arrows represent rain amounts from rain events that dropped greater than 0.5 inches of rain in one rain event. Trial 2 began March 2013 and concluded March 2014.

Table 4. Cormack-Jolly-Seber candidate models and model selection results for males and female adult Houston toads (*Bufo houstonensis*) during trial 2 used for estimating φ and *p* from a habitat suitability exclosure experiment. Models are listed by most supported to least supported based on AIC_c scores. *t* represents time-specific estimates (one estimate available for each sampling period),• indicates estimates were constant across time, *s* is a covariate representing sex.

Model	AICc	ΔAICc	AICc Weight	Likelihood	# Par.	Deviance
1. φ(.)*p*(.)	1078.04	0.00	1.00	1.00	2	597.80
2. φ(*t*)*p*(.)	1094.61	16.57	0.00	0.00	25	565.22
3. φ(*s*)*p*(*t*)	1098.41	20.37	0.00	0.00	26	566.76
4. φ(*t*)*p*(*t*)	1128.67	50.63	0.00	0.00	48	544.27
5. φ(*s*)*p*(*s***t*)	1130.59	52.55	0.00	0.00	50	541.10
6. φ(*s***t*)p(.)	1136.18	58.14	0.00	0.00	49	549.23
7. φ(*t*)*p*(*s***t*)	1167.64	89.58	0.00	0.00	72	518.37
8. φ(*s***t*)*p*(*t*)	1177.01	98.96	0.00	0.00	72	527.75
9. φ(*s***t*)*p*(*s***t*)	1223.30	145.25	0.00	0.00	95	503.28

Table 5. Trial 2 estimates with males and females for φ and (*p*) in adult Houston toads (*Bufo houstonensis*) based on the model supported by the AIC_c selection criterion using program MARK using the Cormack-Jolly-Seber mark-recapture model. The model selected was $\varphi_{(.)}p_{(.)}$ where φ and *p* did not vary among time or sex. Lower and upper confidence intervals (CI) are reported.

Estimator	Estimate	Standard Error	Lower CI	Upper CI
Survivorship (φ)				
Males and Females	0.88	0.012	0.852	0.903
Recapture (*p*)				
Males and Females	0.62	0.022	0.579	0.661

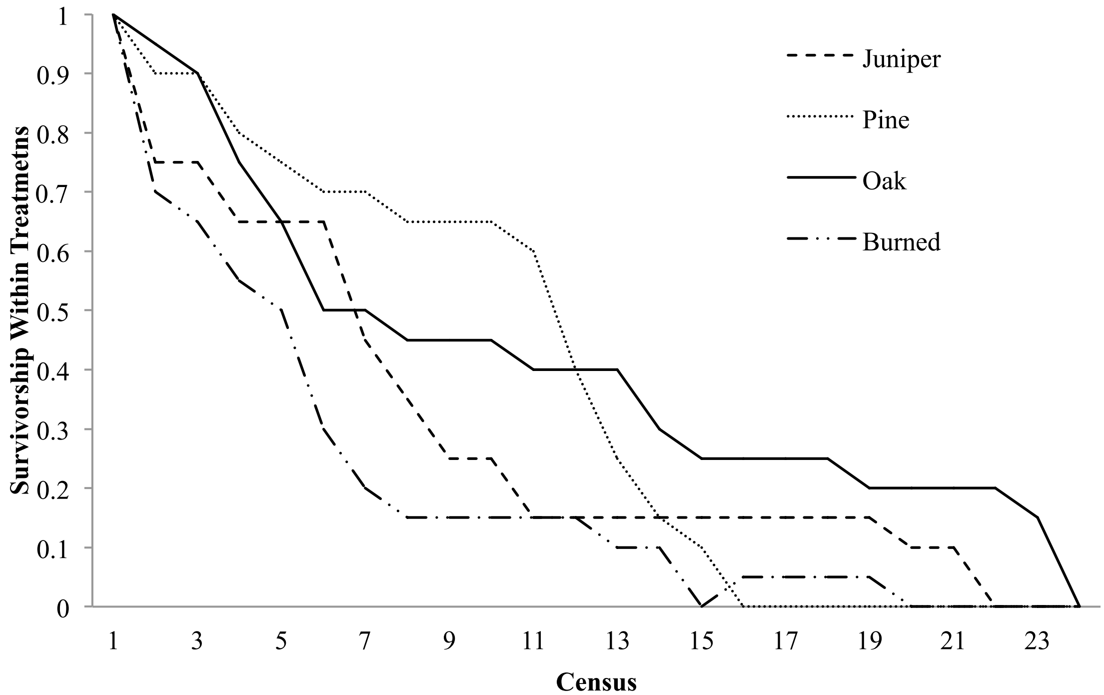

Figure 8: Percent survivorship over time of adult Houston toads (*Bufo houstonensis*) within all four habitats during trial 2 (juniper, pine, oak and burned). Trial 2 began March 2013 and concluded March 2014.

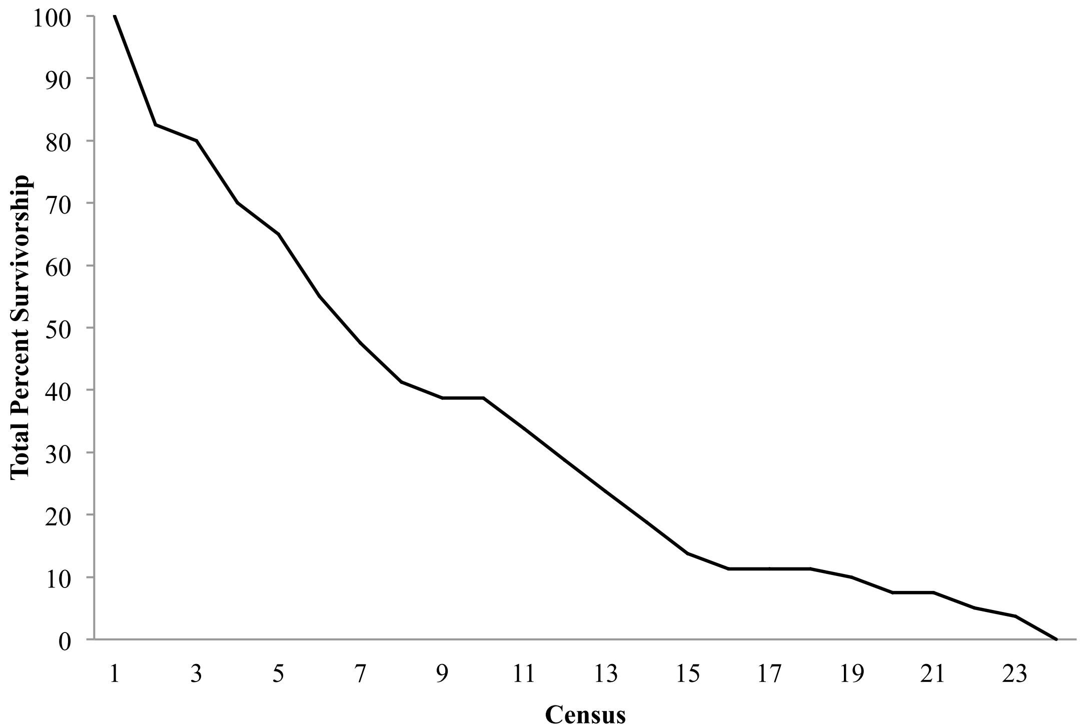

Figure 9. Combined survivorship over time of adult Houston toads (*Bufo houstonensis*) for all four habitats during trial 2 (juniper, pine, oak and burned). Trial 2 began March 2013 and concluded March 2014.

Table 6. Candidate models and model selection results for trial 2 used for estimating φ and *p* of 80 adult Houston toads (*Bufo houstonensis*) from an habitat-suitability exclosure experiment. Models are listed by most supported to least supported based on AIC_c scores. *t* represents time-specific estimates (one estimate available for each sampling period),• indicates estimates were constant across time, *ht* is a covariate representing the habitat type of the treatment (juniper, pine, oak, and burned).

Model	AIC_c	ΔAIC_c	AIC_c Weight	Likelihood	# Par.	Deviance
1. φ(*ht*)*p(ht*)	1040.43	0.00	1.00	1.00	8	626.79
2. φ(t)*p*(*ht*)	1055.39	14.96	0.00	0.00	28	598.09
3. φ(*ht*)*p*(.)	1077.15	36.72	0.00	0.00	5	669.71
4. φ(.)*p*(.)	1078.04	37.61	0.00	0.00	2	676.71
5. φ(t)*p*(.)	1094.61	54.18	0.00	0.00	25	644.13
6. φ(t**ht*)*p*(*ht*)	1182.38	141.95	0.00	0.00	99	527.97
7. φ(t**ht*)*p*(.)	1209.55	169.12	0.00	0.00	95	568.44
8. φ(t**ht*)*p*(t**ht*)	1524.50	484.07	0.00	0.00	191	443.37

Table 7. Trial 2 estimates for φ and (*p*) in adult Houston toads (*Bufo houstonensis*) based on the model supported by the AIC_c selection criterion using program MARK using the Cormack-Jolly-Seber mark-recapture model. The model selected was $\varphi_{ht}\ p_{ht}$ where φ and *p* varied with habitats. Lower and upper confidence intervals (CI) are reported.

Estimator	Estimate	Standard Error	Lower CI	Upper CI
Survivorship (φ)				
Juniper	0.870	0.028	0.803	0.916
Pine	0.888	0.024	0.832	0.927
Oak	0.914	0.02	0.866	0.946
Burned	0.841	0.035	0.76	0.899
Recapture (*p*)				
Juniper	0.537	0.049	0.44	0.631
Pine	0.645	0.041	0.56	0.721
Oak	0.777	0.032	0.707	0.834
Burned	0.351	0.056	0.251	0.466

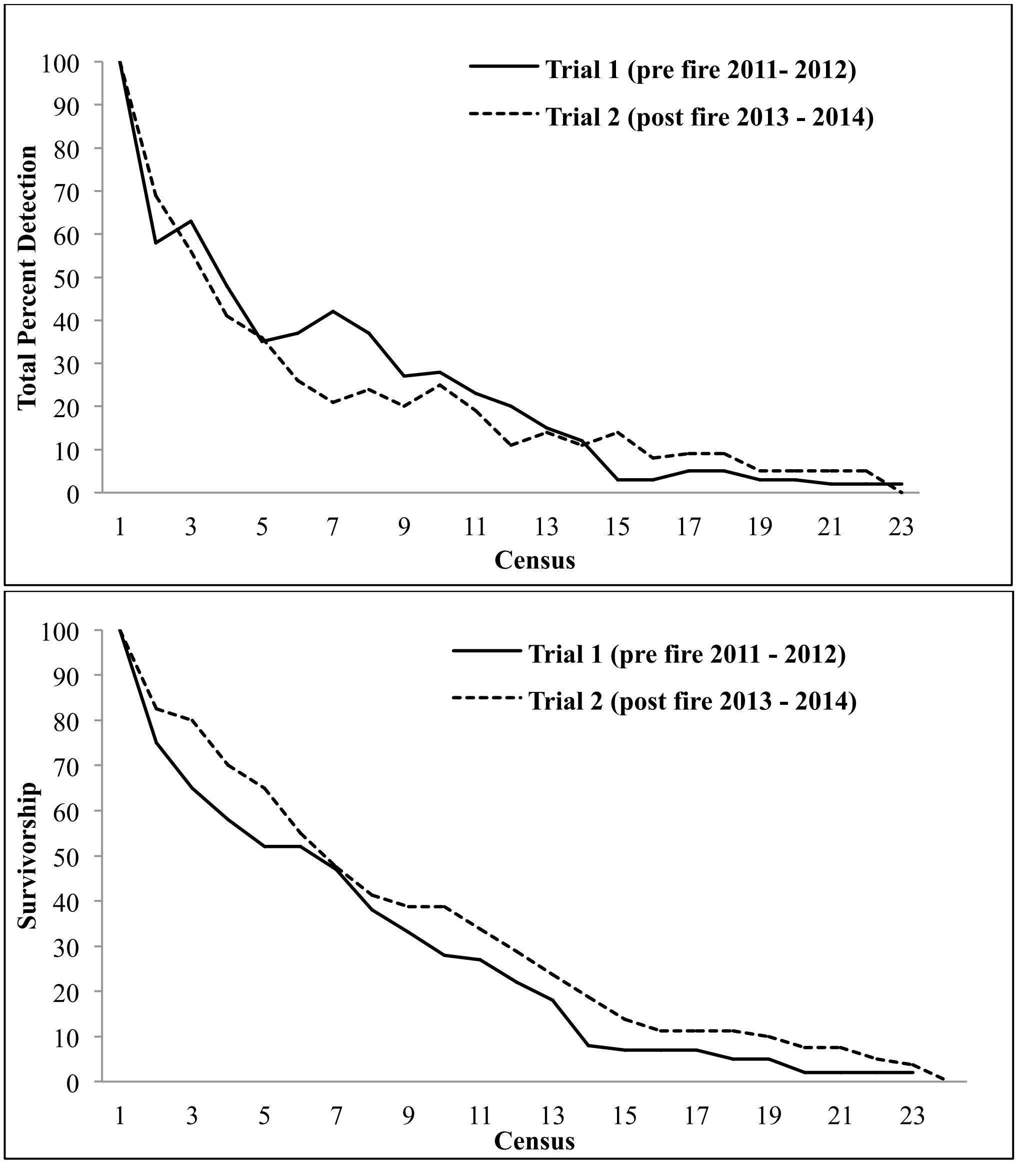

Figure 10. Houston toad (*Bufo houstonensis*) total percent detection and survivorship comparison between trial 1 (2011 – 2012) (top) and trial 2 (2013 – 2014) (bottom). Detection and survivorship trends are comparable across time during the two trials.

Figure 11. Photo of an adult male Houston toad (*Bufo houstonensis*) from trial 1 during the drought of 2011. This individual was found desiccated as it emerges from its shallow burrow. This toad was from pine habitat.

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CHAPTER III

TESTING DENSITY DEPENDENCE IN JUVENILE HOUSTON TOAD (*BUFO HOUSTONENSIS*) IN BASTROP COUNTY, TEXAS

Introduction

Amphibian declines are continuing to accelerate globally. This is in part due to habitat loss, disease, agriculture practices, invasive species, drought, and wildfire. 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 necessity (Dodd and Seigel, 1991).

In order to implement effective conservation management practices, we must be able to determine which life stage is the most effective to use in offsetting these declines. Many studies have looked at various life stages to determine which stage is having the most effect on global amphibian declines. It has been hypothesized that mortality at the egg stage could be the leading factor for the continued declines. Vonesh and De la Cruz (2002) tested egg and juvenile mortality within *Bufo* and concluded that mortality occurring at the juvenile life stage may have a greater impact on amphibian declines rather than embryonic life stages. Berven (2009) reported that juvenile population size of the wood frog (*Rana sylvatica*) was the most important factor that impacted juvenile survivorship alongside the age of female at first reproduction. Harper and Semlitsch (2007) showed juvenile density had strong negative effects on survival, growth and reproduction of the American toad (*Bufo americanus*).

Research on density dependence during juvenile terrestrial life stages is still relatively rare, especially research focusing on endangered amphibians. When species populations are low, density dependence may not be a concern because we assume larval or terrestrial densities will not be large enough to have a negative effect. Determining which life stage drives population regulation can be helpful in developing new or increasing the effectiveness of conservation management strategies.

Houston toad head-starting efforts have focused on the release of thousands of tadpoles, metamorphs (Vandewege et al., 2011) and now eggs onto the landscape onto recently extirpated or current Houston toad locations. Adult toads have been monitored via radio telemetry (Forstner and Swannack, 2004) pit tag, and toe clips (Brown et al., 2011). It is uncertain which Houston toad life stage is most vital to growth rate and species survival. Studies have shown that pre-metamorphic densities have little impact on life factors such as survivorship and growth rates therefore the critical life stage in question may be metamorph and/or juvenile stages. Metamorph or juvenile survivorship and growth rates of the Houston toad have yet been significantly tested.

Preliminary data from the adult toad exclosure experiment was used to determine which habitat (juniper, pine or oak) was the most suitable for Houston toads. Before we can eventually test habitat suitability post catastrophic wildfire, we must determine the optimal juvenile dispersal density for emerging metamorphs. Finding the optimal dispersal density will enable us to eliminate survivorship variables for a post catastrophic fire survivorship study.

Houston toad dispersal of a 50 m radius of the natal pond up to 13 weeks post emergence has been reported in Greuter (2004) and has been used to develop a buffer zone for habitat management. Density of individuals during dispersal and along with conspecifics across a landscape can alter and affect growth rates, resource competition, survival and reproduction (Harper and Semlitsch, 2007). For the Houston toad, conservation management practices have been implemented at various life stages with varying successes.

We are conducting direct assessments of habitat restoration options following the aftermath and recovery efforts from the Bastrop County Complex Fire using juvenile Houston toad exclosure experiments. These exclosures are being applied to assess the density of juveniles required to evaluate future juvenile head-start releases. The density exclosures are within an unburned pine dominated 80% or greater canopy cover habitat. This habitat was chosen based on the adult exclosure study discussed in the previous chapter. These densities are needed to better guide metamorph releases and to enable the eventual repeat of habitat suitability testing for juvenile Houston toads. Once these optimum densities are determined we will also have the ability to test these densities in burned habitats retaining limited canopy with approximately 40% canopy and catastrophically burned habitats with 10% or less canopy cover thus representing 40% of current Houston toad habitat in Bastrop County.

Quantitatively estimating demographic parameters from mark-recapture (henceforth MR) studies have advanced considerably over the last three decades (Lebreton and Pradel, 1992; Burnham and Anderson, 2002). Currently, most MR studies use multi-model analysis in information-theoretic framework to estimate survival (φ) and the probability of recapture (p) (Burnham and Anderson, 2002, Schmidt et al., 2002). Statistical inference from model selection under an information-theoretic approach requires rigorous attention to selecting the candidate set of models. Briefly, a candidate model set is developed using a priori hypotheses focusing on the relationship between survival and recapture, and covariates, such as treatment effects, environmental parameters, among others. Models are ranked based a selection criterion, most commonly Akaike's information criterion (AIC_c) (Akaike, 1973), which provides a reliable decision criterion for model selection for both nested and non-nested models (Schmidt and Anholt, 1999; Burnham and Anderson, 2002).

This study used an information-theoretic approach to model selection to choose models that best fit MR datasets collected from a juvenile exclosure experiment conducted in 2014 – 2015 where juvenile Houston toads were released into outdoor exclosures at six different densities. For each model φ and *p* were estimated.

Materials and Methods

Exclosures for juveniles were 2 x 1 m^2 constructed of 1/8th inch hardware cloth, with covers made from bird netting (preventing immediate bird predation and tree debris in falls) (Figure 1). The exclosures were buried 20 cm deep with walls extending 50 cm above ground. A 10 cm lip was folded along the top and bottom of each pen to prevent toads from tunneling out of the exclosure and prevent toads from scaling the hardware cloth walls and escaping. Each exclosure contained ground cover and woody debris to offer shade and two Tupperware bowl reservoirs filled with sphagnum moss and water to supplement hydration to each exclosure. Moss reservoirs were filled as needed to prevent toad desiccation.

Twenty-seven exclosures were loaded with juvenile Houston toads on August 18th 2014. Densities used for this experiment are 2, 4, 5, 6, 9, and 12 juvenile Houston toads per exclosure. Juvenile Houston toad availability for the study influenced the total number of replicates possible. Replicates for each density were four replicates (density of

2), seven replicates (density of 4), five replicates (density of 5), six replicates (density of 6), three replicates (density of 9) and two replicates (density of 12). A total of 148 juvenile Houston toads were used in this experiment. Upon release each toad was measured (snout urostyle length (SUL) and head width (HW)), weight recorded, and given an individual toe clip number for easy identification. Average mass and SUL for exclosure toads was 1.15 g and 19.2 mm SUL. Houston toad juveniles weighed 0.25 – 3.0 g (mean = 1.16; SD = 0.5019) and SUL length was 9.4 – 26.3 mm (mean = 19.26; SD 2.9840). Houston toad juveniles were assigned to the 27 exclosures and differences were seen among starting mass and SUL of individuals among the six densities (Mass: ANOVA, $df = 5, 21$, $F= 2.434$, $p=0.037$) (SUL: ANOVA, $df = 5, 21$, $F=3.479$, $p= <.005$). The five density exclosures had significantly larger juveniles at the start of this study, however did not affect the overall growth outcome for this study. This study began on August 18th 2014 and concluded March 25th 2015. Initially the exclosures were visited once a week in order to document any initial decrease in detection. Once detection had stabilized, exclosures were visited once every two weeks. During each census, SUL, and mass were taken. A total of 13 censuses occurred during this study.

Drought is a common concern for the survivorship of Houston toads. Before juveniles were released into the exclosure, we simulated a rain event in order to increase soil moisture levels. Each exclosure received an initial 15 gallon treatment of water to simulate a two inch rain event. Exclosures were rehydrated daily using a three gallon pump sprayer at dusk with the intent to slow down instant evaporation associated with Texas summer days. Exclosures were checked each evening on a three day rotation of nine exclosures each day. Data was collected for each exclosure once a week until detection stabilized. Exclosures were then checked bi-weekly.

Analysis of Survivorship – Capture probabilities for each census were calculated in the program MARK (White and Burnham, 1999) using a Cormack-Jolly- Seber (Cormack, 1964; Jolly, 1965; Seber, 1965) model to assess the accuracy of our censuses. For this model all juvenile toads captured in subsequent censuses were known to be alive in all previous census no matter if the individual was not detected. We compared survivorship based on stocking densities then compared to final densities among all six densities. We assumed that capture probability was at 100% at the conclusion of this study.

Model selection procedure in MARK – Using a model selection approach based on information-theoretic methods, Program MARK (White and Burnham, 1999) was used to estimate the probabilities of φ and *p* for juvenile Houston toads. Program MARK methods followed Cooch and White (2006). Two explanatory factors were used to explore variation in φ and *p*: time and density. Time was considered as constant among sampling periods (•) or variable across periods. Habitat type (*d*) was treated as a categorical covariate with six densities (2, 4, 5, 6, 9, and 12) and used to determine if density affected φ or *p*. Based on these factors, six candidate models were developed, where each model represented a different biologically-based hypothesis that explored the effects of time and density on estimates of φ and *p*. For example, $\varphi_t p_t$ represented the Cormack-Jolly-Seber model (CJS) that is fully time dependent for both φ and *p.* Whereas $\varphi_d p_{\bullet}$ represented a model where survivorship varied among density, and *p* remained constant among sampling periods.

The amount of support for each of the six candidate models was evaluated using a correction factor for AIC (AIC_c) which protects against over-fitting the models, especially with small sample sizes (Hurvich and Tsai, 1989). The model with the lowest AIC_c was considered to best fit the data unless the difference in AIC_c values (ΔAIC_c) among competing models was < 2.0, then the models were considered indistinguishable. Models were ranked from one to six, with one being the best supported model and eight being the least. If multiple models supported the data, the most parsimonious model was chosen as the best supporting models. Point estimates, standard errors and 95% confidence intervals were recorded for φ and *p* for each model.

Analysis of Growth **–** To analyze growth we used a linear mixed effects model, repeated measures analysis of variance (ANOVA) to determine differences in SUL among all density treatments over time. Only exclosures that contained at least one detection each census were used in these calculations. At the close of this study, juvenile Houston toads were not detected in any of the 12 density exclosures. Therefore we ran a repeated measures ANOVA using only data from the five densities that were represented at the conclusion of the study (densities 2, 4, 5, 6, and 9). Fixed factors were density and time with exclosure as the random factor.

Soil moisture monitoring – Soil moisture was monitored and recorded for four months (October – January). Ten Decagon Devices EC-5 soil moisture meters were evenly placed among the 27 exclosures. These soil meters measure the volumetric water content (m^3/m^3 VWC) of the soil and have a ~0.2 L measurement volume. Each meter is placed in the center of the exclosure, approximately 10 cm below the surface of the soil. Data from each soil meter was sent to a Decagon Devices Em50 data logger via 15 m extension cables. Soil moisture was set to record at 6 am, 12 noon, and 6 pm each day for four months. Soil moisture was then averaged daily and graphed. Data logger batteries were replaced once every three weeks.

Results

Juvenile Survivorship – One hundred forty seven juvenile Houston toads were released into 27 exclosures on August 18th 2014 and were monitored until March 25th 2015. Upon completion 46 toads (31%) were detected throughout the entire study. Five out of the six density treatments had toads survive throughout the entire study. Exclosures containing 12 juvenile toads failed to provide a single toad detection after October 2014 (census 7). Average density per exclosure was highest in densities four, five, six and nine (Figure 2). Therefore only exclosures with densities of 2, 4, 5, 6 and 9 had individuals that could overwinter. There was a dramatic decrease of total juvenile survivorship during the first two months of the study (Figure 3). This is most likely due to initial stresses involved with transporting toads and then initial acclimation of the new environment. Survivorship hit a plateau just prior to overwintering (November – February). Individuals who survived up to the onset of freezing temperatures were successful in overwintering and surviving until the spring season. Toads in the highest density exclosures experienced the greatest mortality with 0% surviving in exclosures that held 12 Houston toads. When comparing survivorship among the six densities, survivorship was the highest in exclosures containing four juvenile Houston toads (Figure 4).

Based on ΔAIC_c, model 1 ($\varphi_d\, p_.$) was the best supported model (Table 1). For this model, φ varied by density but not across time and recapture was constant (Table 2).

Survivorship was highest in exclosures with five juveniles (φ = 0.93), followed closely by densities of six (φ = 0.92) and four (φ = 0.91) juvenile toads per exclosure. Recapture (*p*) for densities 4, 5 and 6 toads per census was $p = 0.91$, $p = 0.87$, and $p = 0.92$ respectively. The highest recapture estimate for each census were the 12 density exclosures ($p = 0.98$). Model 2 was closely comparable with Model 1, however we chose to select Model 1 due to the high standard deviance seen in Model 2. The other 4 models had ΔAIC_c values > 2.0, which indicates that density had a stronger effect on the data compared to the most parsimonious model (Model 3, φ•*p*•).

Growth Analysis – Growth rates were significantly reduced in the 12 density exclosures by census 7 (ANOVA: $df = 6, 19$, F= 4.1003; p= 0.0167). During the last census, growth rates were not significantly different among the remaining five densities (ANOVA: $df = 4, 21$, F= 2.3894; p= 0.1044) (Table 3). Toad SUL and mass was measured during each census. Average SUL at the start of the study was 19.23 mm and average SUL at the end of the study was 25.67 mm. Average mass at the start of the study was 1.15 grams and 1.91 grams at the end of the study (Figure 5) resulting in a positive linear regression correlation among total SUL and mass over time. All but density 12 exclosures saw an increase in SUL and mass throughout the study. Individuals in the 12 toad density exclosures lost mass over time and survivorship hit 0% by census 7.

Soil moisture data – Soil moisture was measured from October 2014 to January 2015. Soil moisture was positively correlated with rain events (Figure 6). Juvenile toad detection did not increase following large rain events (Figure 7).

Discussion

The effects of density on survivorship and recapture of juvenile *B. houstonensis* were significant with differences in survivorship and recapture estimates among densities but not over time. Recapture rates were constant among habitats and time. Survivorship estimates were highest in density 5, followed closely by densities of 6 and 4 toads per exclosure. Survivorship was lowest in exclosures containing 12 juvenile toads ($\varphi = 0.73$). Detection and survivorship trend data supports the selection of this model.

Recapture estimates were highest in the 12 and 2 density exclosures, both showing the lowest survivorship estimates. By census 7 toads in the 12 density exclosures were no longer detected. Recapture estimates are high because it is reporting the probability of recapturing an individual each census. If toads are no longer being detected then estimates report a high probability that recapture rate of 0 will occur.

Growth was not significant among densities during this study. Trend data shows that 12 density exclosures were the only density to see a reduction of mass over time. As no detections were made at 12 density exclosures after census 7, this density level could not be included in the repeated measures ANOVA. It can be inferred from trend data that significant differences may have occurred if these data were available to be included. Releasing at lower densities appears to favor the overall health and success of juvenile Houston toads.

Conditions in the exclosures were favorable, with consistent shade and moisture. Artificially supplementing water availability was not required after census 4. Rainfall amounts were enough to maintain moisture within the exclosures. A few conspecifics were found within these exclosures and were removed therefore reducing competition among targeted densities. On two occasions large Gulf Coast toads (*Bufo nebulifer*) were found within two exclosures. Although removed, there was no detection of juvenile

Houston toads in those exclosures. This occurred at census 2 in both occasions and predation is likely the cause of zero detections loss. This emphasizes the need to exhaustively search within newly constructed exclosures, despite the consequent disturbance to the structural habitat within each. Finally, unlike the adult exclosure studies, we have little evidence of red imported fire ants present in these exclosures. On occasion ants were seen in the exclosures, however were not exhibiting aggressive mound behavior. Bird netting was effective in keeping birds from preying on the toads, and kept hog nose snakes (*Heterodon platyrhinos*) from entering the exclosures.

Overwintering was not a period of high mortality as seen in Harper and Semlitsch (2007) with *B. americanus* (American toad). Prior to overwintering total detection stabilized and maintained between 30% and 40% detection until the close of the study. This suggests that at these densities, individuals were not competing for resources and therefore able to maintain body condition before overwinter estivation.

Similar, but not identical stocking densities and replicates were seen in Harper and Semlitsch (2007), testing American toad densities. Due to difficulties in acquiring juvenile Houston toads for this experiment we could not mimic their American toad stocking densities. Fewer replicates could have influenced our results. Results from Harper and Semlitsch (2007) showed survivorship and growth to be highest in lower stocking densities. Therefore, we reduced the number of lowest and highest stocking densities at the design of this experiment, focusing on optimum densities and testing those in this study. It would be optimum to replicate this study and increase in replicates for comparison.

There was no recovery of any dead juvenile toads during this study making it unclear what proximate causes contributed to the loss of detection for 69% of the juvenile toads. The initial decrease in overall detection is likely due to stresses involved in acclimation and time of year of release. These individuals were released in August, one of the hottest months of the year. It was fortunate that large rain events occurred often and greatly decreased the chance of death by desiccation.

From a regulatory and and management perspective, the paradigm for many years takes the position that Houston toads are not active on the surface of the habitat during the months of July to December of each year. This is very clearly false based on both the adult and juvenile exclosure data. Juvenile Houston toads remained actively above ground from August 2014 to March 2015. Rain events did not have an affect on juvenile toad movement as was seen to affect the surface activity by adult Houston toads. We observed juvenile toads above ground throughout the day but as temperatures would rise during the late morning hours, toads were more often observed taking shelter under the provided structure in or near the water pools provided. The daily juvenile Houston toad movement would then increase again in the late afternoon hours. Only when temperatures dropped below 0º C did we observe a decrease in above ground movement within the exclosures. During these freezing temperatures toads were found below the provided structure and many were found tucked up under the water pools. Very few toads were observed buried down into the sandy loam soils even during these freeze events. Therefore little evidence that support juvenile Houston toads spend any considerable amount of time below the soil surface or implement estivation behavior during summer and winter months as do the adults

If density dependence is regulated at the juvenile life stage for the Houston toad then focusing efforts in improving habitat specifically for Houston toad juveniles could potentially increase survivorship at this level (Halpern et al., 2005). Determining the optimal habitat can be a huge help in future conservation strategies for the Houston toad and amphibians in Bastrop County. We need to focus our release efforts in areas that are conducive to housing this rare amphibian. Head-starting has been our key conservation strategy for the Houston toad. We are now one step closer in maximizing the efficiency and efficacy of head-starting Houston toads.

From these results were can continue to optimize our management practices for this endangered species. We know that at high densities these individuals will not thrive, however stocking densities are not confined to a narrow or specific number of individuals. It can be assumed that current populations will not reach high densities naturally, therefore this knowledge can be used to guide future head-starting or captive propagated releases.

Above ground activity can be an important factor in juvenile Houston toad ecology. Juvenile amphibian activity increases once individuals emerge and move out onto the landscape. Dispersal across the landscape allows juveniles to seek out upland habitats or undergo inter-pond dispersal. Therefore this need to disperse would increase activity for juvenile individuals. This study gave us the first opportunity to observe juvenile toads post one year since hatching as tadpoles. During this study there was a steady increase in overall juvenile SUL and mass over time. From this study we conclude that Houston toad juveniles are above ground and active during all months of the year. This can have major implications on future conservation management practices and plans conducted in Bastrop County.

Conservation and management practices implemented for the Houston toad in Bastrop County have strongly been influenced by data gathered from monitoring adult populations. Few studies have discussed Houston toad emergence behavior (Greuter 2004) or juvenile dispersal (Vandewege, 2013). Understanding juvenile ecology and behavior post emergence to one year has not been documented until now. Differences in adult and juvenile Houston toad ecology are significant and can have major management implications.

In the aftermath of the Bastrop County Complex fire, clean-up operations were conducted in cooperation with USFWS in order to decrease or prevent activities that would lead to “take”. Many of these operations were conducted year round in Houston toad habitat. Monitoring for the Houston toad during these operations was heaviest during Houston toad breeding season, continuing a few months into the summer capture emergence and then movement of metamorphs. From this study we conclude that juvenile Houston toads are potentially moving across the landscape all months of the year. If density dependence is regulated at the juvenile life stage in Houston toads, management practices need to shift to incorporate the activity of not only breeding adults, but movement and habitat of juveniles dispersing across the landscape after the initial pond emergence event.

We will be able to use these data collected from this study to look at optimal juvenile densities across various habitats in Bastrop County. As Bastrop County is still undergoing continual habitat management post wildfire, these data will continue to aid in our efforts to manage the habitat and population of this endangered amphibian.

Figure 1. Example of a juvenile Houston toad (*Bufo houstonensis*) wild mesocosm exclosures in Bastrop County, Texas. This represents one of 27 exclosures located in pine habitat in Bastrop County. Toad densities of 2, 4, 5, 6, 9 or 12 toads are located within each exclosure.

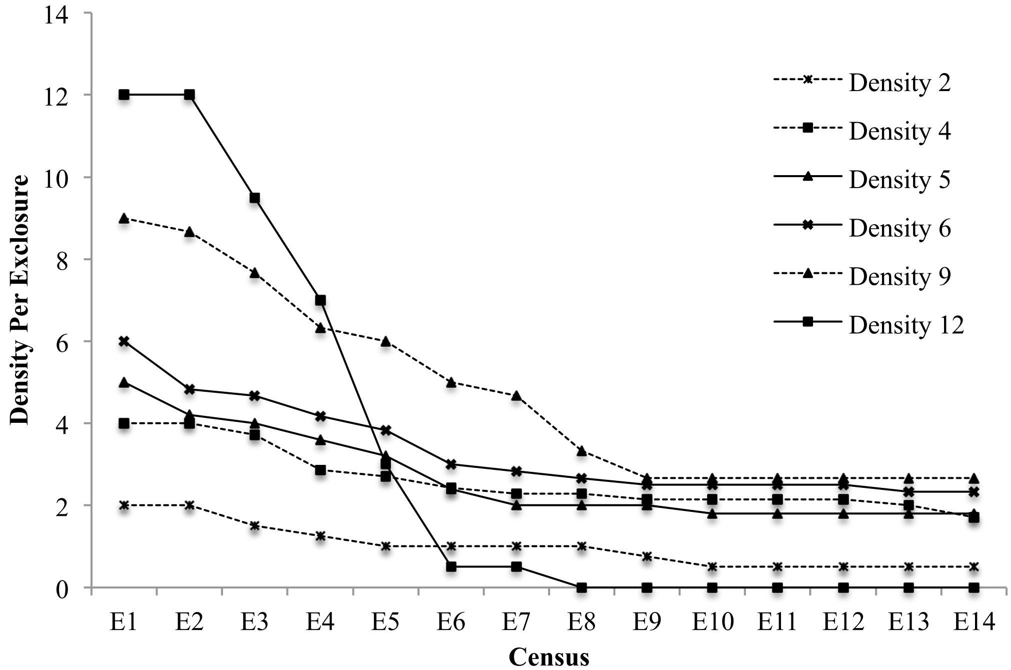

Figure 2. Average density per exclosure of juvenile Houston toads (*Bufo houstonensis*) detected in 26 1 x 2 m^2 outdoor exclosures in Bastrop County, Texas. Each exclosure contains one of six densities of juvenile Houston toads (2, 4, 5, 6, 9 and 12 individuals per exclosure). Initial release was 15 August 2014 (E1) and final census was 25 March 2015 (E14).

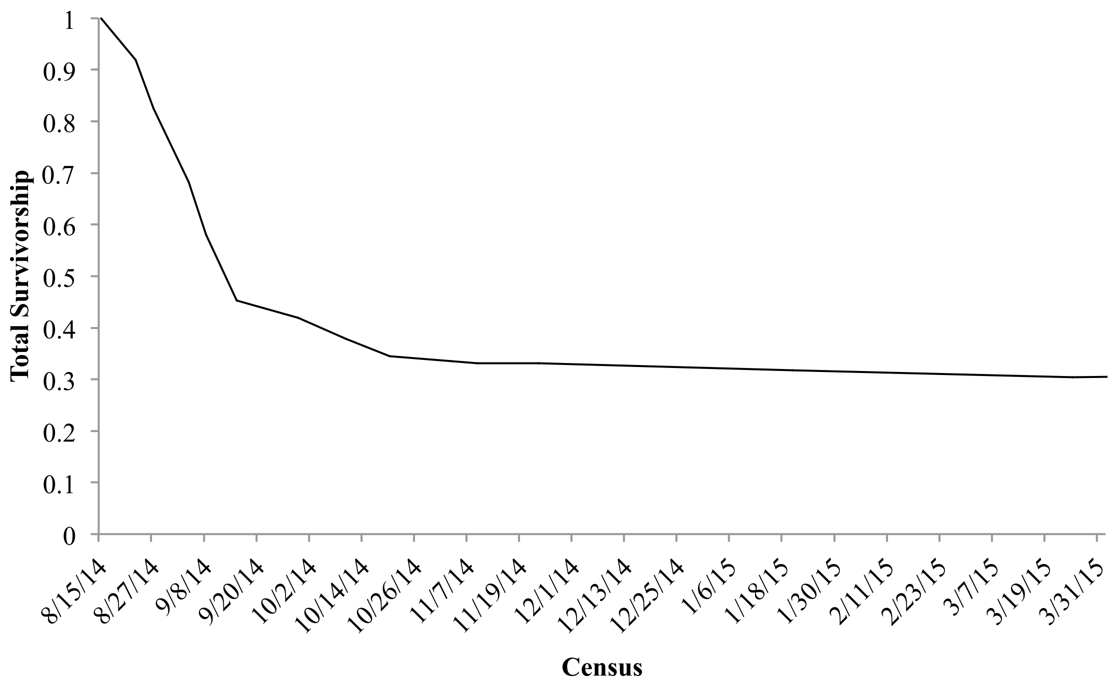

Figure 3. Total survivorship over time of 148 juvenile Houston toads (*Bufo houstonensis*) detected in 26 1 x 2 m^2 outdoor exclosures. Initial release of juvenile Houston toads was 15 August 2014 and final census was 25 March 2015

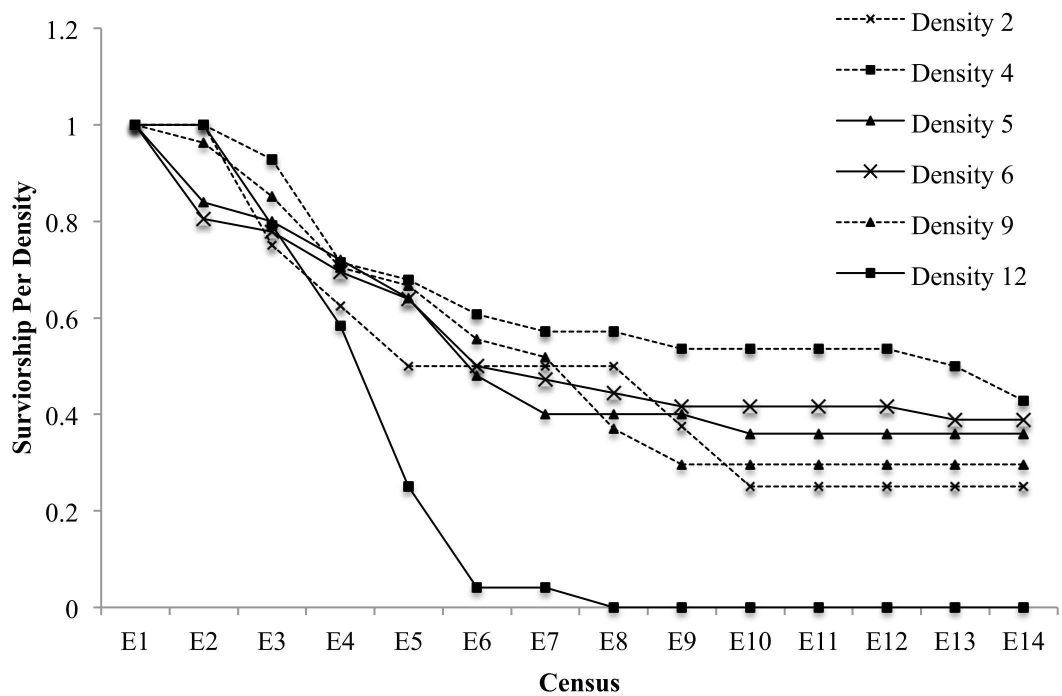

Figure 4. Survivorship per density over time of juvenile Houston toads (*Bufo houstonensis*) detected in 26 1 x 2 m^2 outdoor exclosures. Each exclosure contains one of six densities of juvenile Houston toads (2, 4, 5, 6, 9 and 12 individuals per exclosure). Initial release was 15 August 2014 (E1) and final census was 25 March 2015 (E14).

Table 1. Program MARK Cormack-Jolly-Seber (CJS) model AIC_c comparison for Juvenile Houston toads (*Bufo houstonensis*) in a juvenile density exclosure study. Density is represented by (*d*). AIC_c model chosen reflects survivorship parameter (φ) dependent by density and recapture parameter (*p*) is constant (φ(*d*)*p*(.)).

Model	AICc	ΔAICc	AICc Weight	Likelihood	# Par.	Deviance
1. φ(*d*)*p*(.)	1136.43	0	0.57	1	7	44.06
2. φ(*d*)*p*(*d*)	137.01	0.59	0.43	0.75	12	434.43
3. φ(.)*p*(.)	1152.65	16.23	0	0	2	470.39
4. φ(*t***d*)*p*(.)	1180.55	44.12	0	0	78	332.1
5. φ(*t***d*)*p*(*d*)	1182.38	45.95	0	0	83	321.97
6. φ(*t***d*)*p*(*t***d*)	1287.97	151.54	0	0	154	241.44

Table 2. Survivorship estimate of juvenile Houston toads (*Bufo houstonensis*) from six stocking densities; 2, 4, 5, 6, 9, and 12 toads per 1 x 2 m^2 exclosure. φ(*d*)p(.) was the Cormack-Jolly-Seber (CJS) model used for each treatment. Lower and Upper confidence intervals (CI) are reported.

Estimator	Estimate	Standard Error	Lower CI	Upper CI
Survivorship (φ)				
Density 2	0.889	0.043	0.774	0.949
Density 4	0.911	0.016	0.9	0.963
Density 5	0.939	0.021	0.06	0.945
Density 6	0.918	0.017	0.879	0.946
Density 9	0.902	0.021	0.851	0.936
Density 12	0.725	0.048	0.623	0.809
Recapture (*p*)				
Density 2	0.977	0.023	0.852	0.997
Density 4	0.905	0.02	0.857	0.938
Density 5	0.871	0.028	0.807	0.916
Density 6	0.915	0.019	0.87	0.945
Density 9	0.923	0.021	0.869	0.956
Density 12	0.978	0.022	0.861	0.997

Table 3. Linear mixed effects model, repeated measures analysis of variance (ANOVA) between SUL among five of the six toad densities (2, 4, 5, 6, 9, and 12) over time using juvenile Houston toads (*Bufo houstonensis*). Only exclosures that contained at least one detection for each census were used in these calculations. At the end of the study juvenile Houston toads were not detected in any of the 12 density exclosures. SUL was the measured variable with density and census as factors and exclosure as the random factor.

ANOVA	Degrees of Freedom	F-value	*P*-value
Density	4	2.3894	0.1044
Census	12	2.2671	0.0112
Density:Census	48	1.2249	0.1779

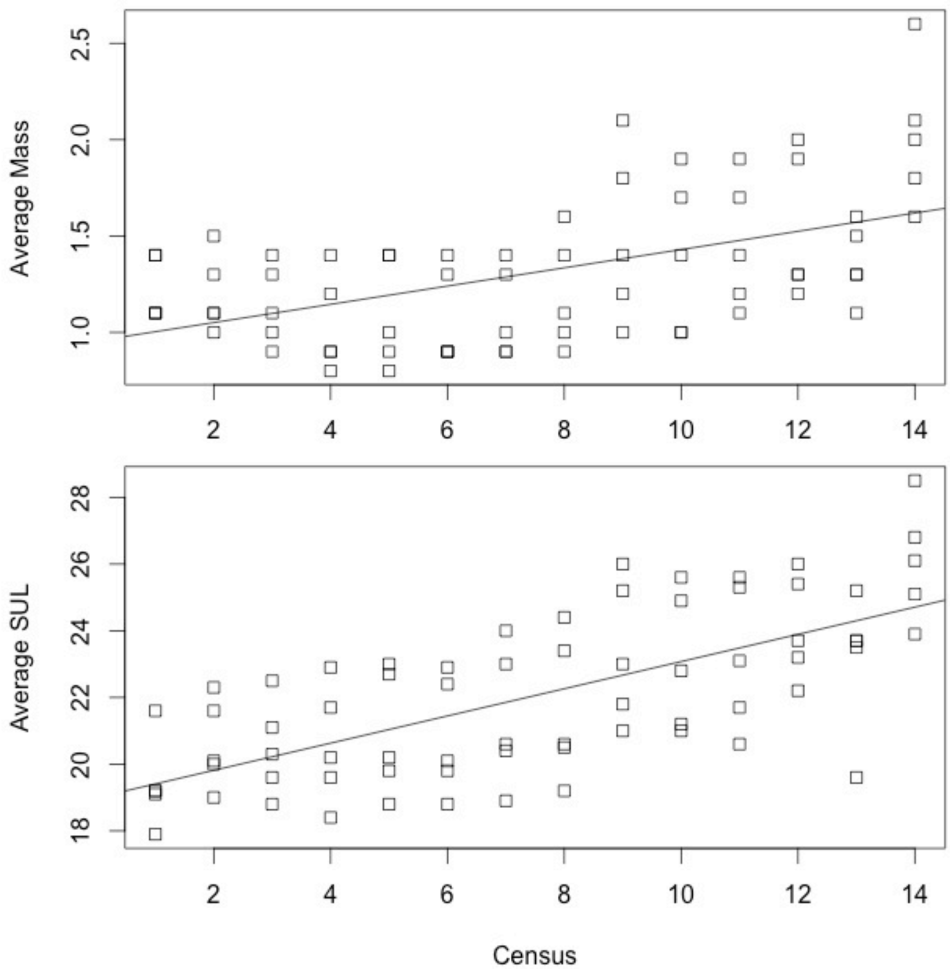

Figure 5. Linear regressions of 1) Total juvenile Houston toad (*Bufo houstonensis*) SUL over time (top) and 2) Total juvenile Houston toad mass over time (bottom) for all individuals who were detected during the final census.

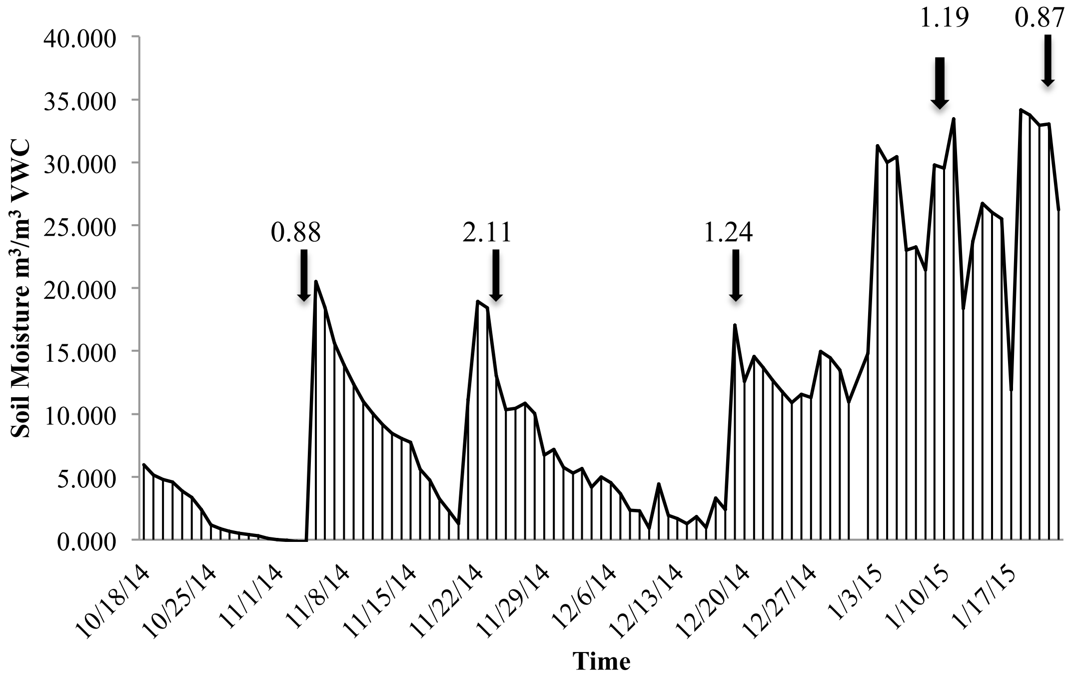

Figure. 6. Changes in soil moisture at the juvenile Houston toad (*Bufo houstonensis*) pine habitat density exclosures in Bastrop County, Texas during October 2014 and January 2015. Soil moisture is measured by m^3/m^3 VWC and was recorded every 30 min, 24 hours a day. Rain events (more than 0.5 inches) are indicated by downward arrows.

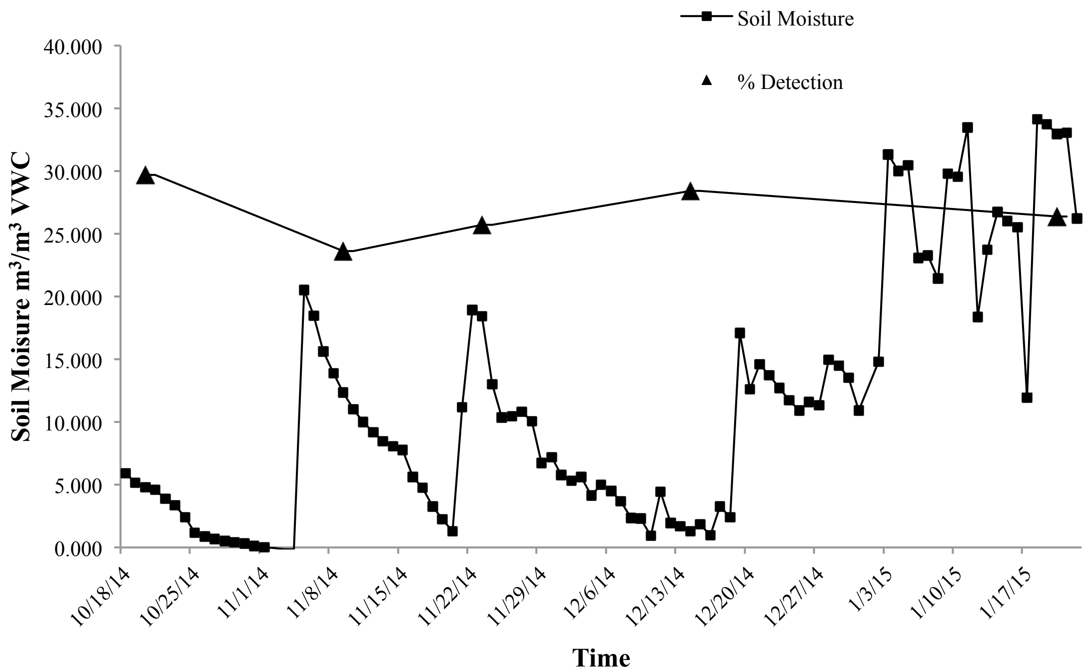

Figure 7. Changes in soil moisture with juvenile Houston toad (*Bufo houstonensis*) detection over time from October 18th 2014 until January 17th 2015. Juvenile Houston toad detection did not change as soil moisture changed over time.

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CHAPTER IV

EFFICACY AND EFFECTIVENESS OF HEAD-STARTING AND CAPTIVE PROPAGATION OF THE ENDANGERED HOUSTON TOAD (*BUFO HOUSTONENSIS*)

Introduction

Translocation, relocation, repatriation and head-starting can be effective conservation management tools and have been used to establish and restore populations of once extirpated species (Griffith et al., 1989). These practices have been very successful with mammals and birds (Seddon et al., 2005; Griffith et al., 1989). They have also been a successful management tool for the conservation of Kemp's Ridley sea turtle (*Lepidochelys kempii*) (Fontaine and Shaver, 2005) and the gopher tortoise (*Gopherus polyphemus*) (Tuberville et al., 2005; Lohoefener and Lohmeier, 1986). However, few translocation, repatriation or head-starting programs have been successful for amphibians (Dodd and Seigel, 1991). Head-starting is a technique of rearing a species in captivity and then releasing them once they have reached a size that might protect them from higher rates of predation usually associated with a smaller size (Haskell et al., 1996; Fontaine and Shaver, 2005). 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 were 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 egg strands, 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 in order 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, adult) and then returned to their natal ponds. Adult head-start toads were toe-clipped or PIT tagged for future identification. Molecular markers and pedigree reconstruction were then used for future identification of captured toads from 2009 – 2011 in order to assess the effectiveness of this head-starting program (Vandewege, 2011).

The majority of head-started releases to date were either tadpoles or metamorphs. Given that survivorship is low for tadpoles and metamorphs, we have now also been given the opportunity to test the release of adult Houston toads. The cost of raising a toad to adulthood is high, however if head start adults are more likely to successfully breed upon release then this could be a cost effective way to supplement the population. This study further assessed the overwinter survivorship of the 2010 juvenile head-start toads and tested the efficacy and efficiency of releasing captive raised adults in 2012. Houston toads egg strands or tadpoles collected during the breeding seasons of 2011 – 2014 were genotyped through the use of genetic markers for pedigree reconstruction to determine if the reproduction event involved a head-start individual. Genotypes from 2009 – 2010 were be retained and also tested against future Houston toad offspring.

Materials and Methods

DNA collection methods of wild toads and head-starts – We continued to conduct amphibian call surveys each year during the breeding season to quantify Houston toad populations on the Griffith League Ranch and Bastrop State Park. If amplectant pairs were observed, the location was flagged and the area surveyed for egg strands the following day. If eggs were discovered, each egg strand was removed, transported, acclimated and housed at the head-start facility in Bastrop County (Figure 1). Upon metamorphosis, these individuals were released back to the natal pond as head starts. A portion of the egg strands was brought back to the Houston Zoo to be reared to different life stages. DNA samples were collected from each egg strand or tadpole cohort for genetic analysis and accessioned into the Michael R. J. Forstner Frozen Tissue Catalog at Texas State University – San Marcos. Sampled egg strands were raised to juveniles and then released at their natal pond or location.

DNA extraction method and genotyping – DNA was extracted from toe samples using a DNeasy® DNA Tissue kit (QIAGEN Inc.) on an Applied Biosystems 3500xL Laboratory Automation Workstation following the manufactuer's protocol. Extraction success was evaluated by electrophoresis on a 2% agarose gel and visualized under UV light after Gelred staining.

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). Fragment analysis was performed on an ABI 3500xL Genetic Analyzer (Applied Biosystems, Inc.).

Sibship reconstruction - COLONY was used for all future pedigree reconstruction for head start *B. houstonensis*. 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.

Results

Head-start captures and releases – We released 729 adult Houston toad head-starts reared from the Houston Zoo in 2012 and approximately 4,100 Houston toad tadpoles in 2013 (Table 1). Upon release each adult toad was individually marked by individual toe clip and a toe sample was taken for DNA analysis. Toe clips were stored in 95% ethanol and accessioned into the Michael R. J. Forstner frozen tissue catalogue at Texas State University. Adult toads were released on the Griffith League Ranch and tadpoles were released in Bastrop State Park at natal pond locations. Each adult toad was PIT tagged for subsequent identification using AVID transponder chips.

Wild collected adult and juvenile Houston toads – From 2011 to 2013, 137 wild adult, juvenile, or tadpole Houston toads were collected in Bastrop County. Only six toads were collected in 2011, all from the Griffith League Ranch. Drought conditions persisted, however in the wake of the Bastrop County Complex fire, FEMA clean-up operations and small chorusing events yielded 69 wild Houston toads in 2012 (13 from

Bastrop State Park; 13 from the Griffith League Ranch, and 43 from FEMA recovery efforts off private property, across the burned zone of the Bastrop County Complex Fire). In 2013 we collected 63 toads from numerous locations across Bastrop County. Since 2013 less than 12 Houston toads have been found during 2014 and 2015 breeding seasons.

Almost all individuals collected were males given the known significant male bias within the species (Swannack and Forstner, 2007) and male bias during call surveys. Calling males were easier to locate along the pond edge. This male bias could have an effect on assessing the effectiveness of head-start or captive propagated releases.

Frequency of recaptured head-starts – One hundred thirty seven (33% from FEMA, 41% BSP, 14% GLR, 8% Jim Smalls, and 4% Musgrave) adults/juveniles/tadpoles were collected between the audio and pit-fall trap surveys and fire clean-up operations between 2011 and 2013. Although 137 individuals were genotyped, some of these represent the same cohort based on wild egg sampling or emergence event where multiple individuals were collected. Therefore this reduces our unique wild collected genotypes down to a maximum of 104 to be tested (Figure 2). Many juveniles collected during the FEMA operations were collected on different days but could be part of the same emerging cohort found at same locations. These wild individuals were genotyped at a minimum of 4 loci and then sibship compared against the head start cohorts from 2011.

Discussion

Pedigree reconstruction using COLONY has resulted as an efficient method of assigning individuals to appropriate sibgroup (Vandewege, 2011). In 2010, Vandewege

(2011) was able to test prior head start releases and determined one individual was in fact a head start toad. Head-starting efforts continued to increase resulting in 2010 being the largest head start release effort at that time. Drought conditions of 2011 and the Bastrop County Complex Fire resulted in no known Houston toad reproduction events in 2011 and 2012. Individuals released in 2010 would then be three years of age by the Spring of 2013, decreasing the chances to detect individuals from the 2010 releases.

However, out of 137 wild toads collected between 2011 and 2013, not a single wild individual was found to be a head-start Houston toad (Figure 2). Greuter (2004) reports low survivorship of both tadpoles and juveniles. Therefore it has been suggested that releasing head starts at these life stages was inefficient (Vandewege, 2011). Additionally complicating the assessment was the lack of data from Houston toads detected following the extreme drought and wildfire 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 across Bastrop County.

The next step in this head-starting program is to test the efficiency of releasing the captive propagated egg strands of 2014 and 2015. Head-starting and captive breeding have had variable degrees of success in amphibians (Dodd and Seigel, 1991; Griffiths and Pavajeau, 2008). With record spring rains of 2015, we will be able to continue to test the efficiency of head-starting endangered amphibians using this relatively robust release of Houston toad eggs.

Up until 2014, the 2010 head start releases have been the largest head start effort for the Houston toad. Unfortunately we conducted these releases prior to the severe drought of 2011, followed by low numbers of wild toads collected in 2012 and 2013. We, however, have the potential to re-test the efficiency of these efforts in 2016. Wild Houston egg strands have not been found in numbers as past years. Continual weather patterns coupled with multiple population stressors are leading to a continued steep population decrease. Therefore, the Houston Zoo has now shifted efforts on captive propagation releases.

In 2014 only one breeding event was documented in Bastrop County. This event occurred on March 27th and resulted in an egg strand found near the outflow of pond 8 on Bastrop State Park. This egg strand was moved to pond 8 and covered with a protective exclosure to reduce egg and tadpole predation.

During the spring of 2015 Houston toads were heard chorusing at four locations in Bastrop County. Five Houston toad males were found chorusing on the Griffith League Ranch at Pond 12. This was the largest chorus detected in 2015. Unfortunately this event did not lead to egg strands. To date, no Houston toad head-starting has occurred in 2015.

Captive Propagated Zoo Egg Strand Releases 2013 – 2015 – April13th 2013 Houston Zoo and Texas State University conducted the first Houston toad release since the Bastrop County Complex fire in 2011. We successfully released approximately 2,000 Houston toad tadpoles into Bastrop State Park Pond 3. On May 14th we released four egg strands from the Houston Zoo to the Griffith League Ranch and Bastrop State Park. Five additional egg strands, from the Houston Zoo, were released on May 22nd. This was the first year egg strands were captive propagated and then released. In 2014, 45 egg strands were released in Bastrop and Austin Counties and in 2015 we released 99 egg strands in Bastrop County (Table 2).

Head-starting efforts have continued despite setbacks caused by drought and wildfire. Head-starting can only occur if wild breeding occurs in Bastrop County. These breeding events have been few and far between since 2011, therefore we have shifted our conservation efforts to releasing captive propagated Houston toads. Until 2013 Houston toads were not successfully breeding in captivity. In 2013 we began releasing captive propagated tadpoles and egg strands in Bastrop County. This strategy is more cost effective, and by protecting the released eggs, allows this management strategy to become an efficient and effective way to head-start the Houston toad. Since 2013 we have placed over 490,000 Houston toad adults, juveniles, tadpoles, or egg strands within Bastrop and Austin Counties.

We have continued to increase our head-starting efforts. Yet, we are no longer getting sufficient chorusing and in turn breeding for the Houston toad. This suggests the Bastrop County Complex Fire, coupled with an already decreasing population, may have been the extinction event for the wild Houston toad population. Furthermore, other amphibian species in Bastrop County have shown no negative effect on population following wildfire (Brown et al., 2014). Therefore captive propagation could be a successful management tool for increasing Houston toad populations.

Figure 1. Wild Houston toad (*Bufo houstonensis*) egg strands removed from natal ponds and acclimated at the Houston toad head-start facility in Bastrop County, Texas. These individuals are raised until Gosner (1960) tadpole stage 40 or metamorphs and then released back to their natal ponds.

Table 1. Locality and release description data for Houston toad (*Bufo houstonensis*) head-start releases in 2013 in Bastrop County, Texas. The % egg, % tadpole, % juvenile, % adult describe the proportion of each listed release and age class associated with that release. * Number referenced is an approximation of individuals released.

County	Locality	Date Released	Number Released	% Egg	% Tadpole	% Juvenile	% Adult
Bastrop	BSP P8	3/13/13	1500*	-	100	-	-
Bastrop	BSP P3	3/13/13	1800*	-	100	-	-
Bastrop	GLR P12	3/9/13	62	-	-	-	100
Bastrop	GLR P2	3/9/13	63	-	-	-	100
Bastrop	Welsh	4/13/13	604	-	-	-	100
Bastrop	JMS	5/13/13	800*	-	100	-	-

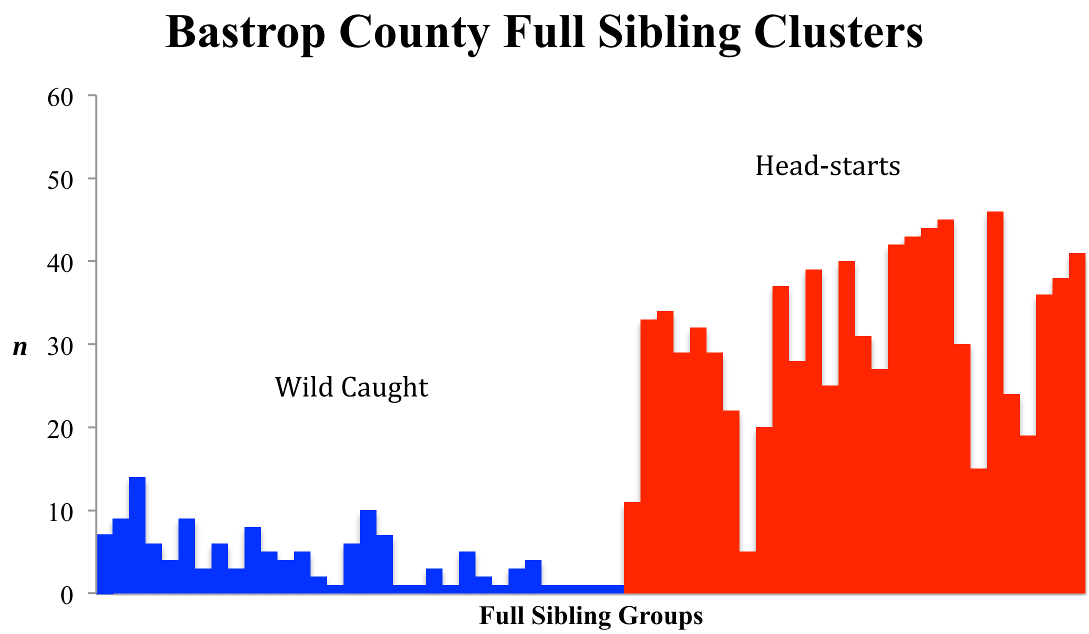

Figure 2. COLONY sibling-ship reconstruction output. The blue bars represent the wild Houston toads (*Bufo houstonensis*) collected from 2011 to 2013. The red bars represent the cohort groups of head-start tadpoles or metamorph Houston toads released in Bastrop County, Texas from 2007 and 2010. After conducting pairwise relatedness in COLONY, no wild collected Houston toad paired with a head-start cohort

Table 2. Locality and release description data for captive propagated Houston toad (*Bufo houstonensis*) egg strands, tadpole and zoo adult Houston toad releases in Bastrop and Austin Counties of Texas. The % egg, % tadpole, % juvenile, % adult describe the proportion of each listed release and age class associated with that release. * Number referenced is an approximation of individuals released.

County	Locality	Date Released	Number Released	% egg	% tadpole	% juvenile	% adult
Bastrop	GLR P12	3/9/13	62	-	-	-	100
Bastrop	GLR P2	3/9/13	63	-	-	-	100
Bastrop	Welsh	4/13/13	604	-	-	-	100
Bastrop	BSP P8	5/14/13	2000*	100	-	-	-
Bastrop	GLR P12	5/14/13	2000*	100	-	-	-
Bastrop	GLR P14	5/14/13	2000*	100	-	-	-
Bastrop	GLR P15	5/14/13	2000*	100	-	-	-
Bastrop	GLR P2	5/22/13	4000*	100	-	-	-
Bastrop	GLR P12	5/22/13	2000*	100	-	-	-
Bastrop	GLR P14	5/22/13	2000*	100	-	-	-
Bastrop	GLR P2	7/20/13	230	-	-	100	-
Austin	NAVA	2/2014	22,000*	100	-	-	-
Austin	NAVA	2/27/14	5,500*	100	-	-	-
Bastrop	BSP P3	2/27/14	12,000*	100	-	-	-
Bastrop	BSP P8	2/27/14	2,000*	100	-	-	-
Bastrop	GLR P12	2/27/14	6,000*	100	-	-	-
Austin	NAVA	3/6/14	3,000*	100	-	-	-
Bastrop	GLR P2	3/6/14	15,000*	100	-	-	-
Austin	NAVA	3/27/14	3,000*	100	-	-	-
Bastrop	BSP P3	3/27/14	3,000*	100	-	-	-
Bastrop	GLR P2	4/3/14	7,000*	100	-	-	-
Bastrop	GLR P12	4/10/14	6,000*	100	-	-	-
Austin	NAVA	4/17/14	6,000*	100	-	-	-
Bastrop	GLR P2	4/17/14	5,000*	100	-	-	-
Bastrop	BSP P3	3/7/15	33,500*	100	-	-	-
Bastrop	GLR P12	3/7/14	4,500*	100	-	-	-
Bastrop	BB	3/7/15	7,000*	100	-	-	-
Bastrop	GLR P2	3/12/15	18,000*	100	-	-	-
Bastrop	BSP P3	3/12/15	14,000*	100	-	-	-
Bastrop	BSP P10	3/12/15	17,500*	100	-	-	-
Bastrop	BSP P27	3/22/1	35,000*	100	-	-	-
Bastrop	GLR P2	3/22/15	7,500*	100	-	-	-
Bastrop	GLR P5	3/22/15	9,500*	100	-	-	-
Bastrop	BSP P3	3/22/15	8,500*	100	-	-	-
Bastrop	BSP P2	3/28/15	11,000*	100	-	-	-

Table 2 continued

Bastrop	BSP P18	3/28//15	26,000*	100	-	-	-
Bastrop	GLR P2	3/28/15	7,000*	100	-	-	-
Bastrop	GLR P12	3/28/15	7,500*	100	-	-	-
Bastrop	BSP P30	4/9/15	33,500*	100	-	-	-
Bastrop	GLR P2	4/9/15	16,000*	100	-	-	-
Bastrop	GLR P12	4/9/15	10,000*	100	-	-	-
Bastrop	BB	4/9/15	5,000*	100	-	-	-
Bastrop	BSP P3	4/18/15	18,500*	100	-	-	-
Bastrop	BB	4/18/15	4,500*	100	-	-	-
Bastrop	BSP P30	4/18/15	10,500*	100	-	-	-
Bastrop	GLR P2	4/18/15	10,500*	100	-	-	-
Bastrop	GLR P12	4/18/15	26,000*	100	-	-	-
Bastrop	BSP P30	4/26/15	20,500*	100	-	-	-
Bastrop	GLR P12	4/26/15	5,000*	100	-	-	-
Bastrop	GLR P2	4/26/15	4,500*	100	-	-	-
Bastrop	BSP P3	4/26/15	12,500*	100	-	-	-
Total			**495,959**				

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CHAPTER V

PREDATOR EXCLUSION DEVICE AS AN EFFECTIVE WAY TO HEAD-START AN ENDANGERED AMPHIBIAN

Introduction

Many conservation or habitat studies involve the trapping or the housing of captured animals in order to collect data. Difficulties arise with these techniques when animals are involved in activities leading to a higher risk of predation (Dodd and Scott, 1994) or desiccation than would occur naturally (Jenkins et al., 2003). Literature discusses using predator exclusion devices (PEDs) in studies using pitfall traps which are common in the collection of amphibians, reptiles, invertebrates and small mammals (Ferguson et al., 2008; Ferguson and Forstner, 2006; Jenkins et al., 2003). However PEDs can be developed and used in not only data collection but in conservation management practices such as head-starting.

Head-starting is the technique of rearing a species in captivity and then releasing them once they have reached a size that might protect them from higher rates of predation (Haskell et al., 1996; Fontaine and Shaver, 2005). 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).

Since 2007, Texas State University in partnership with United State Fish and Wildlife Department, Texas Parks and Wildlife Department and the Houston Zoo have been conducting amphibian population management through head-starting for the endangered Houston toad (*Bufo houstonensis*) in Bastrop County, Texas. Head-starting efforts have included the release of adults, juveniles, metamorphs and tadpoles. Head-starting efforts can be costly and require adequate facility space depending on the life stage at which individuals are released from captivity. The longer these individuals are raised in captivity, the fewer numbers that will be released. Adults, although reproductively able to add to the population upon release, can't be released in high numbers due to cost and space to raise them to adulthood. Eggs, however, are cost effective to produce, can be transported immediately to the wild, and can be released by the thousands. Survivorship to adulthood, however, decreases as individuals are released at earlier life stages. Survivorship from egg to adult in the Houston toad is 0.1% (Swannack et al., 2009). It is key to increase survivorship for these released individuals without the difficulties of time and cost. We developed a predator exclusion device that would protect amphibian egg strands in the natural environment, that would allow us to release the maximum amount of individuals to the environment, and that would greatly increase survivorship to metamorphosis.

In 2013 we discovered one Houston toad breeding event occurring on March 13th resulting in nine egg strands at pond 3 in Bastrop State Park. This was the only breeding event to have been documented in Bastrop State Park in 2013. In efforts to keep these eggs and tadpoles viable, an egg predator exclusion device (PED) was constructed and placed over six of the egg strands. Within two days all three of the uncovered egg strands were gone, while all six of the covered egg strands continued to develop and subsequently hatched into free swimming tadpoles. Tadpoles continued to utilize the shelter of the cage exclosures in the weeks leading up to emergence.

Avian predators are a primary concern for eggs and tadpoles. In 2012 a wild egg strand was discovered in Bastrop State Park. The eggs hatched, however evidence in the form of heron tracks where the tadpoles once were, suggested the fate of these individuals (Figure 1). In 2014, we tested the cage predator exclusion device using Houston toad egg strands captive propagated from the Houston Zoo. It's clear that through the use of PEDs, successes in tadpole growth and metamorphosis could be obtained.

Study Areas - This study was conducted on three properties in Austin and Bastrop Counties. The Griffith League Ranch is a 1,900 ha ranch in Bastrop County, owned by the Boy Scouts of America and Bastrop State Park located in the City of Bastrop, TX. The third property is the NAVA property located in Austin County near the city of New Ulm. The IACUC permit number for this research is 1011_0501_11.

Materials and Methods

A total of 45 Houston toad egg strands were used for this study. Forty-two of the egg strands were captive propagated eggs produced at the Houston Zoo and transported within 24 hours of the breeding event. Three egg strands were the result of wild breeding in Bastrop State Park and the Griffith League Ranch in 2014. Over the course of four months, we were able to conduct eight egg release events. During each release 20% of the egg strands were left uncovered and the remaining egg strands were fit with a predator exclusion exclosure to prevent predation from aquatic, avian, and mammalian predators. Each exclosure is 1 x 1 m^3 constructed from $1/16^{th}$ inch hardware cloth. The exclosures are closed on five sides, leaving the bottom side open in order to place over the egg strands and sit flush against the pond floor (Figure 2). Once in place the exclosures were secured to the pond floor using 1 m rebar steaks that were wired to the exclosure and hammered into the substrate. This prevented raccoons and hogs from being able to flip the exclosures over and destroy the eggs. One week after the eggs hatched and individuals were free swimming tadpoles, flaps were cut along the bottom of each of the four sides of the exclosure. This allowed larger tadpoles to move freely into and out of the exclosure (Figure 3). Exclosures remained in the ponds until all emergence was complete.

Results

A total of 35 out of the 45 egg strands were covered with a mesh exclosure leaving 10 egg strands uncovered. Thirty-one of the 35 covered egg strands were fertilized, developed, and successfully hatched as free swimming tadpoles. Four of the covered egg strands never developed, and therefore were believed to have not been successfully fertilized. The remaining covered egg strand was fertilized, however, eggs were eaten by other tadpoles (*Scaphiopus. hurterii*) during elongation. Only two of the 10 egg strands that were left uncovered hatched into free swimming tadpoles. Eight of the egg strands left uncovered experienced predation within one to two days of being placed into the pond. None of the 35 covered egg strands (fertilized or not) experienced predation while only two of the 10 uncovered egg strands survived (Figure 4).

During the spring of 2014 we discovered Houston toad emergence from all three study sites. Survivorship estimates of Houston toads are 0.01% from eggs to adults returning to the breeding pond (Swannack et al., 2009); therefore, total survivorship from our 104,500 free swimming tadpoles is estimated as 104 adult individuals.

Discussion

This study clearly shows how effective egg exclosures can be for amphibian conservation. We were able to get these individuals to metamorphs, using the natural environment instead of raising them in captivity. This is a very efficient form of head-starting and enables us to place the maximum amount of individuals upon the landscape while potentially increasing survivorship during this critical life stage where predation is highest.

In 2015 we were able to double our egg strand release numbers and place 99 egg strands in Bastrop County resulting in over 300,000 eggs. These releases occurred on three properties in Bastrop County; Griffith League Ranch (three ponds), Bastrop State Park (six ponds) and Blue Bonnet Electric (one pond). Egg strands were covered with the hardware cloth exclosures and monitored after release. To date we have documented successful emergence at three of the eight locations. A full list of egg releases from 2014 and 2015 can be found as Appendix A.

Figure 1. Houston toad (*Bufo houstonensis*) tadpoles were discovered on 7 March 2012 at pond 8 in Bastrop State Park in Bastrop County, Texas. A return visit on 8 March 2012 revealed heron tracks where the tadpoles were located. No tadpoles could be found. The red flag highlights the location of the wild egg strand

Figure 2. An example of a predator exclusion device (PED) used to cover Houston toad (*Bufo houstonensis*) head-start egg strands captive propagated from the Houston Zoo. Egg PEDs were used in 2014 and 2015 for captive propagated egg strand releases.

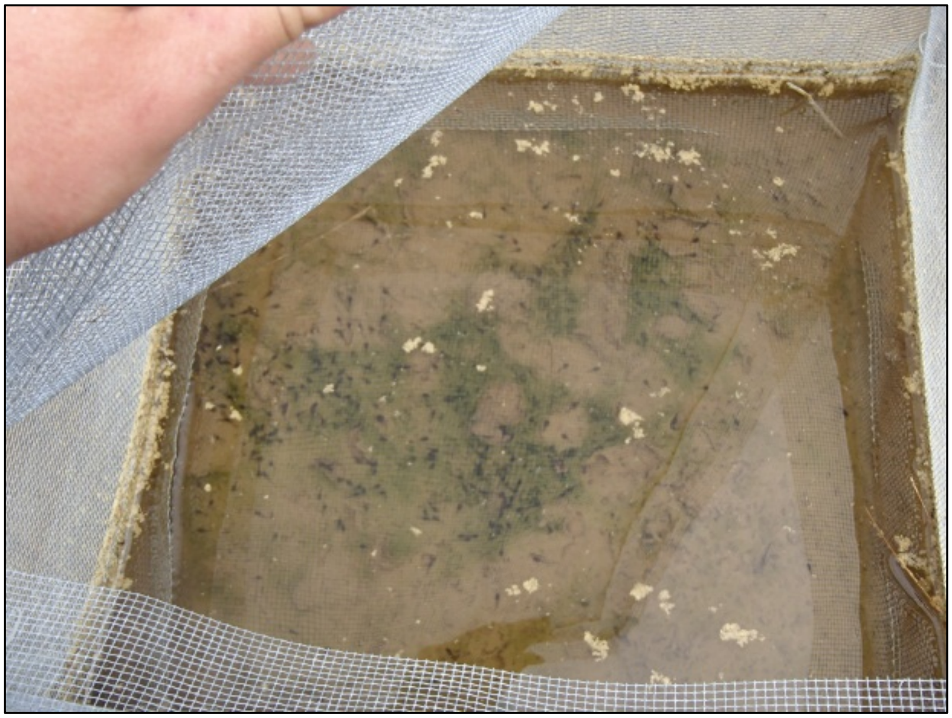

Figure 3. Hatched and free-swimming Houston toad (*Bufo houstonensis*) tadpoles actively utilizing the egg exclosure (PED). This egg strand was located at pond 12 on the Griffith League Ranch in Bastrop County, Texas.

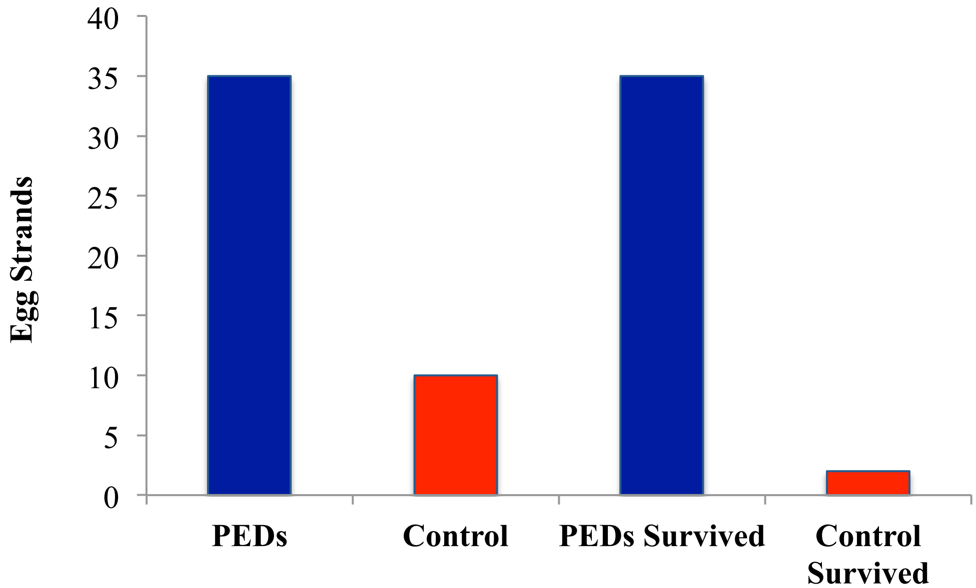

Figure 4. Houston toad (*Bufo houstonensis*) egg strand survivorship while testing the efficiency of the predator exclusion devices (PEDs). In 2014, 45 egg strands were released in Bastrop and Austin Counties in Texas. Thirty-five strands were covered with PEDs. All strands that were covered (fertilized or infertile) either hatched or infertile eggs remained until decomposition.

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CHAPTER VI

ASSESSMENT OF PUBLIC KNOWLEDGE AND SUPPORT FOR RECOVERY OF THE ENDANGERED HOUSTON TOAD (*BUFO HOUSTONENSIS*) IN BASTROP, TEXAS [1]

Introduction

Community support is important for recovery success of threatened and endangered species, particularly when the species occurs largely on private property (Hatch et al., 2002). Endangered species can benefit from community support through landowner-instituted habitat conservation initiatives (e.g., Safe Harbor agreements; Toombs, 2005), monetary, property and volunteer contributions to conservation or research (Alberts and Grant, 2003; Chase et al., 2000), and favorable attitudes that help influence decision-making processes (e.g., proactive land-use planning; Broberg, 2003). Alternately, endangered species can be harmed by intentional or unintentional direct mortality and habitat destruction or degradation (Doremus and Pagel, 2001).

Education can cultivate positive attitudes and actions toward endangered species recovery (Bjorkland and Pringle, 2001; Caro et al., 1994). Education pos- itively influenced conservation and recovery initiatives for a wide range of species, from Kirtland's warbler (*Dendroica kirtlandii*) (Solomon, 1998) to the Florida manatee (*Trichechus manatus latirostris*) (Aipanjiguly et al., 2003). Negative attitudes toward species listed under the Endangered Species Act are usually harmful to species recovery (e.g., Lueck and Michael, 2003).

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[1] Jones, M.C., Donald J. Brown, Ivana Mali, Audrey McKinney and Michael R. J. Forstner. 2012. Assessment of Public Knowledge and Support for Recovery of the Endangered Houston Toad (*Bufo houstonensis*) in Bastrop, Texas. Human Dimensions of Wildlife: An International Journal, 17:3, 220-224

The federally endangered Houston toad (*Bufo* [*Anaxyrus*] *houstonensis*) (Gottschalk, 1970) is endemic to east-central Texas. Texas is over 94% privately owned (Texas Center for Policy Studies, 2000), and the persistence of this species across its range depends heavily on spatially and quantitatively sufficient suitable habitat located on private property. Since the 1970s Bastrop County has housed the majority of Houston toads, and currently it is the only county retaining fairly robust populations (Brown, 1971; McHenry, 2010). The current extinction vulnerability for this species is high. Most of the Bastrop County toad populations are found within 15 km of the city of Bastrop, including Bastrop State Park, which is located adjacent to the city.

Political conflicts involving the City of Bastrop residents, state and federal wildlife agencies, and the endangered Houston toad date back to 1970 when the toad was first listed as federally endangered (Peterson et al., 2004). These conflicts spawned negative attitudes toward the Houston toad from residents and landowners in and around the City of Bastrop (Brown and Mesrobian, 2005). Beginning in 2000, however, the U.S. Fish and Wildlife Service took a more proactive, cooperation-based approach to Houston toad recovery in Bastrop County. This approach culminated in a landowner and developer-inclusive Habitat Conservation Plan for Houston toad habitat (KES Consulting, Loomis Austin, and Forstner, 2007). Residents living in designated critical habitat for the species are involved at the regulation level of the Habitat Conservation Plan and therefore should be familiar with the federal status of the Houston toad. The human population in the city of Bastrop, however, is increasing rapidly and newer residents may have limited or no knowledge of the Houston toad.

The U.S. Fish and Wildlife Service (USFWS) conservation efforts have been primarily focused on rural landowners in Bastrop County, through the implementation of habitat conservation plans and safe harbor agreements. Conservation initiatives, however, involving the urban and suburban sectors, are becoming increasingly important. These sectors are located adjacent to or within designated critical habitat, are growing rapidly, and are contributing to habitat loss and fragmentation. It is important to gauge the knowledge and opinions about the Houston toad in these sectors as a precursor to future collaborative efforts. In this article, we quantified the level of knowledge about the Houston toad for residents of the City of Bastrop and determined current and future education outlets.

Materials and Methods

We used a door-to-door survey that was designed to be completed within one minute. To minimize sampling bias surveyors did not answer any questions regarding specific information about the Houston toad until participants completed the survey. Survey teams consisted of one male and one female per team to minimize sex-based bias. When requesting participation, surveyors introduced themselves as Texas State University graduate students requesting participation on a research study. Only subjects 18 or older were asked to participate in the survey. To sample a range of population demographics we selected sectors that varied in establishment period, housing costs, and surrounding landscape. The surveyed sectors included the historic district, founded in 1832, Hunters Crossing, developed in 2008, and Tahitian Village, located within the critical habitat boundaries defined by the Houston toad recovery plan (USFWS, 1984; Figure 1).

The survey contained four demographic questions (i.e., sex, age, occupation, residency). Respondents were asked if they have ever heard of the toad. If the respondent had not heard of the Houston toad, the survey was finished and handed in, however if the respondent had heard of the toad we asked them to further answer three questions involving where they had heard of the toad and to what extent was their knowledge of this species. The final three questions asked for the respondents' opinions related to the environmental impact they believe the species has on themselves personally and on the environment (Figure 2).

Results

We visited 193 homes across the three sectors, which resulted in 132 completed surveys (46, 40, and 46 in the Historic District, Hunters Crossing, and Tahitian Village, respectively). The age distribution was: >60 [24%], 51–60 [19%], 41–50 [18%], 31–40 [22%], 18–30 [16%]. Sex was distributed as 45% male and 55% female.

We found that 63% (83 individuals) of the survey respondents had heard of the Houston toad. Among the three sectors, 70%, 63%, and 55% of surveyed respondents in Tahitian Village, Historic District, and Hunters Crossing, respectively, had heard of the Houston toad, however the differences between sectors were not significant (p = .392). Of the 83 respondents who had heard of the Houston toad, 94% knew the Houston toad was an endangered species, and 63% knew the Lost Pines region contained the largest Houston toad population. Furthermore, 50% of the respondents thought the Houston toad benefits them personally, 84% thought the Houston toad population benefits the ecosystem, and 81% cared if the Houston toads went extinct. When survey responses were compared based on sex, no significant differences were observed. For age class comparisons, responses to all questions but one were not significant. When asked, do you think the Houston toad benefits “you” in any way, a significant difference was found (p = .008) (Table 1). All 12 respondents from the 18–30 age class replied “no” for this question.

Discussion

Recovery success in urbanized regions often depends on management programs that benefit target species and simultaneously are socially acceptable (Wilcove et al., 1998). Education increases public awareness and can assist in placing value on an object, and values provide the basis for attitudes (Tarrant et al., 1997). In our study, over half of the survey respondents had some knowledge of the Houston toad. Knowledge was not correlated with sector location; however, residents living outside critical habitat are still gaining knowledge on this endangered species. Attitudes toward the Houston toad were generally favorable among all three sectors. Our results further indicated the majority of survey respondents believe there is an ecosystem-level value in the toads’ existence, but relatively few made the connection between having value to the ecosystem and value to people. To further educate the public (especially younger residents) and promote community support for Houston toad recovery we recommend increasing education at the K−12 level through informative presentations, and increasing education to the general public. Further education efforts should not only provide information about the Houston toad, but also include general information on the role of amphibians in ecosystems and how ecosystem health benefits human populations.

On September 4, 2011 a catastrophic wildfire began in the Lost Pines ecoregion of Bastrop County. Bastrop State Park and Tahitian Village were within the boundary of this 13,800 ha wildfire. Overstory tree mortality was nearly 100%, and understory vegetation was completely removed throughout much of this area. The dead and dying trees are currently being removed, leaving behind currently unsuitable Houston toad habitat patches. The aftermath of the catastrophic wildfire has left Bastrop County with the need for immediate and active restoration of the plant community in order to restore the integrity of the Lost Pines ecoregion. Restoration actions, along with some of the necessary expenses, will require landowner support and involvement. It will be necessary to educate these landowners on best management practices for the Houston toad. The USFWS has compiled this information (2011), and we recommend that it be widely distributed among landowners in Houston toad habitat.

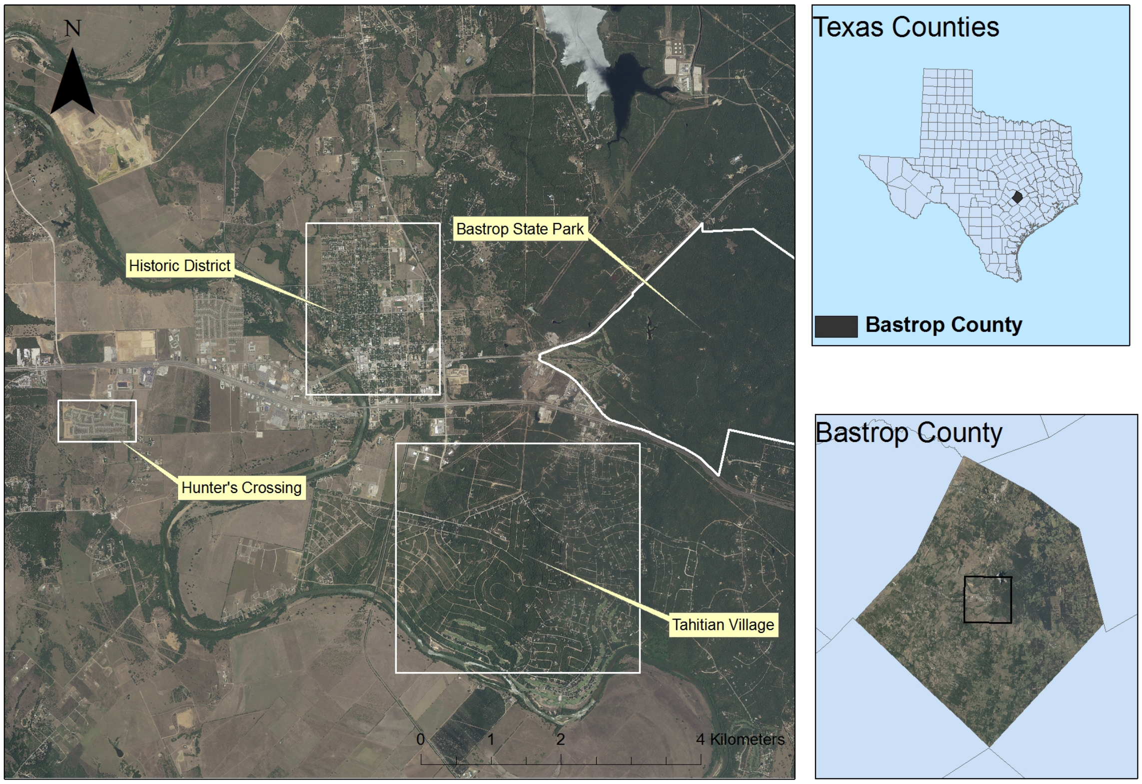

Figure 1. Location of Bastrop State Park and subdivisions within the City of Bastrop, Bastrop County, Texas, that were surveyed in 2011 to assess knowledge and support of the endangered Houston toad (*Bufo houstonensis*).

HOUSTON TOAD SURVEY	
Age class (circle):	18-20 21-30 31-40 41-50 51-60 > 60
Sex (circle):	M / F
Occupation:	Business Construction Food Service Homemaker Military Rancher Retail Retired Unemployed Other: ____________
Are you a resident of Bastrop County?	Yes / No If No, list county of residence ________
Have you ever heard of the Houston toad (circle):	Yes / No (if no, survey is complete)
IF YES:	
Where did you hear about it? (circle):	Bastrop State Park Lost Pines HCP School Other: ____________
Did you know the Houston toad is an endangered species?	Yes / No
Did you know the Lost Pines region has the largest Houston toad population in the world?	Yes / No
Do you think the Houston toad benefits you in any way?	Yes / No Why? ____________ ____________
Do you think the Houston toad benefits the ecosystem in any way?	Yes / No Why? ____________ ____________
Do you care if the Houston toad goes extinct?	Yes / No Why? ____________

Figure 2. Figure of the door-to-door survey used to evaluate City of Bastrop residents knowledge of the Houston toad (*Bufo houstonensis*).

Table 1. Results from Fisher's exact tests (*p*-values) used to determine if knowledge and opinions about the endangered Houston toad (*Bufo houstonensis*) differed between sexes and among age classes for residents of Bastrop Texas based on a door-to-door survey conducted in 2011.

Survey Question	Sex	Age classes
Have heard of the Houston toad	0.465	0.061
Knew the Houston toad was endangered	0.388	0.344
Thought the Houston toad benefited them personally	0.402	0.008
Thought the Houston toad benefited the ecosystem	1.000	0.418
Would care if the Houston toad went extinct	0.792	0.086

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CHAPTER VII

CONCLUSION

Over the past several years, there has been an increase in the global loss of biodiversity. Therefore population supplementation practices such as captive-breeding, head-starting, and translocation programs have increased in popularity. 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 overall detection across Bastrop County. 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.

The head start releases of 2013 and 2014 have also remained untested due to the lack of breeding in 2014 and 2015. Between 2007 and 2015 approximately 520,000 head starts have been released on BSP and GLR. These head starts include eggs, tadpoles, metamorphs, and adults. Wild survivorship from egg to adult has been estimated between 0.01 – 0.03 %. With these releases and survivorship estimates, we should expect these head start events to yield 500 adult Houston toads. These numbers are encouraging, however, it is unknown what the 2016 season will bring. If we experience a 2016 breeding season, this will be the perfect year to test the 2014 and 2015 head start egg releases. Males and females will be reaching maturity and should return to the ponds to breed.

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. To further 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. We will use the combined knowledge learned from all the habitat studies to further conduct head-starting in a way to provide the most potential for successes.

Houston toad detections continue to decrease despite increased survey efforts across the historic range of this species. We successfully found Houston toads in Robertson County in 2014 in larger numbers than have been detected in Bastrop since 2005. Houston toads have not been detected in Robertson County since 2010, and only in small choruses. Therefore, it is imperative to continue to monitor for this species in order to estimate current occupancy and trends in abundance. Finding these small isolated populations can be encouraging and may give us other avenues for head-starting in the coming years.

APPENDIX SECTION

APPENDIX A

Appendix A. Houston toad (*Bufo houstonensis*) egg releases in Bastrop County, Texas in 2015. To date we have released approximately 400,000 eggs in Bastrop County in 2015. Eggs/tadpoles have been released in three ponds on the Griffith League Ranch, six ponds in Bastrop State Park, and one pond at Blue Bonnet Electric. An "A" next to the strand number indicates a strand laid using a new hormone protocol.

Strand	Date Released	Egg Estimate	Release Location	C/U	Current Status
104	3/7/15	5,000	BSP Pond 3	Uncovered	Free Swimming
105	3/7/15	5,000	BSP Pond 3	Uncovered	Free Swimming
106	3/7/15	3,000	BSP Pond 3	Covered	Free Swimming
107	3/7/15	3,000	BSP Pond 3	Covered	Free Swimming
108	3/7/15	4,000	BSP Pond 3	Covered	Free Swimming
109	3/7/15	4,000	BSP Pond 3	Covered	Free Swimming
111	3/7/15	4,500	BSP Pond 3	Covered	Free Swimming
112	3/7/15	5,000	BSP Pond 3	Covered	Free Swimming
113	3/7/15	4,000	GLR Pond 12	Covered	Unfertile
114	3/7/15	1,000	Blue Bonnet	Covered	Free Swimming
115	3/7/15	3,000	Blue Bonnet	Covered	Free Swimming
116	3/7/15	3,000	Blue Bonnet	Covered	Free Swimming
117	3/7/15	5,000	GLR Pond 2	Covered	Free Swimming
118	3/7/15	4,000	GLR Pond 2	Uncovered	Free Swimming
119	3/7/15	500	GLR Pond 12	Covered	Unfertile
120	3/12/15	5,000	GLR Pond 2	Covered	Free Swimming
121	3/12/15	4,500	BSP Pond 3	Covered	Free Swimming
122	3/12/15	5,000	BSP Pond 3	Covered	Free Swimming
123	3/12/15	3,000	BSP Pond 3	Covered	Free Swimming
124	3/12/15	800	GLR Pond 2	Covered	Free Swimming
125	3/12/15	5,000	GLR Pond 2	Covered	Free Swimming
126	3/12/15	5,000	BSP Pond 10	Covered	hatched
127	3/12/15	4,500	BSP Pond 10	Uncovered	hatched
128	3/12/15	4,000	BSP Pond 10	Covered	hatched
129	3/12/15	4,000	BSP Pond 10	Covered	hatched
130	3/12/15	4,000	BSP Pond 3	Covered	Free Swimming
131	3/12/15	4,000	GLR Pond 2	Covered	Free Swimming
133	3/12/15	3,500	BSP Pond 3	Covered	Free Swimming
134	3/12/15	4,000	BSP Pond 3	Covered	Free Swimming
135	3/12/15	4,000	GLR Pond 2	Covered	Free Swimming
136	3/22/15	4,000	BSP Pond 27	Covered	Free Swimming
137	3/22/15	4,000	BSP Pond 27	Uncovered	Few seen/FS

138	3/22/15	4,500	BSP Pond 27	Covered	Free Swimming
139	3/22/15	3,500	GLR Pond 2	Covered	Free Swimming
140	3/22/15	4,000	BSP Pond 27	Covered	Free Swimming
141	3/22/15	4,000	BSP Pond 3	Covered	Free Swimming
142	3/22/15	5,000	GLR Pond 5	Covered	Free Swimming
143	3/22/15	3,500	BSP Pond 27	Covered	Free Swimming
144 A	3/22/15	4,000	BSP Pond 27	Covered	Free Swimming
145 A	3/22/15	4,000	BSP Pond 27	Uncovered	1/2 hatched
146 A	3/22/15	2,500	BSP Pond 27	Covered	Free Swimming
147 A	3/22/15	4,000	GLR Pond 2	Covered	Free Swimming
148 A	3/22/15	4,500	BSP Pond 3	Covered	Free Swimming
149 A	3/22/15	4,500	BSP Pond 5	Covered	Free Swimming
150 A	3/22/15	3,500	BSP Pond 27	Covered	Free Swimming
151 A	3/28/15	4,000	BSP Pond 2	Covered	Elongation
152 A	3/28/15	4,000	BSP Pond 18	Covered	Elongation
153 A	3/28/15	4,000	GLR Pond 2	Covered	Elongation
154 A	3/28/15	4,000	BSP Pond 18	Covered	Elongation
155 A	3/28/15	3,000	BSP Pond 18	Uncovered	Elongation
156 A	3/28/15	4,000	GLR Pond 12	Covered	Cloudy Eggs
157 A	3/28/15	4,000	BSP Pond 18	Covered	Elongation
158	3/28/15	3,500	BSP Pond 18	Uncovered	Elongation
159	3/28/15	3,500	BSP Pond 18	Covered	Elongation
160	3/28/15	4,000	BSP Pond 18	Covered	Elongation
161	3/28/15	3,000	GLR Pond 2	Covered	Elongation
162	3/28/15	3,500	BSP Pond 2	Covered	Elongation
163	3/28/15	3,500	GLR Pond 12	Covered	Cloudy Eggs
164	3/28/15	3,500	BSP Pond 2	Uncovered	Elongation
165	4/9/15	4,000	BSP Pond 30	Covered	N/A
166	4/9/15	5,000	BSP Pond 30	Covered	N/A
167	4/9/15	5,000	GLR Pond 2	Covered	N/A
168	4/9/15	5,000	GLR Pond 12	Covered	N/A
169	4/9/15	5,000	GLR Pond 2	Covered	N/A
170	4/9/15	3,000	GLR Pond 2	Covered	N/A
171	4/9/15	3,500	GLR Pond 2	Covered	N/A
172	4/9/15	4,000	BSP Pond 30	Covered	N/A
173	4/9/15	4,500	BSP Pond 30	Covered	N/A
174	4/9/15	4,000	BSP Pond 30	Covered	N/A
175	4/9/15	5,000	GLR Pond 12	Covered	N/A
176	4/9/15	5,000	Blue Bonnet	Covered	N/A
177	4/9/15	4,000	BSP Pond 30	Covered	N/A
178	4/9/15	4,000	BSP Pond 30	Covered	N/A
179	4/18/15	N/A	BSP Pond 30	Covered	N/A
180	4/18/15	6,566	BSP Pond 30	Covered	N/A

181	4/18/15	4,726	BSP Pond 3	Covered	Free Swimming
182	4/18/15	6,146	BSP Pond 3	Covered	Free Swimming
183	4/18/15	7,625	BSP Pond 3	Covered	Free Swimming
184	4/18/15	N/A	GLR Pond 2	Covered	N/A
185	4/18/15	N/A	GLR Pond 2	Covered	N/A
186	4/18/15	6,900	GLR Pond 12	Covered	N/A
187	4/18/15	10,084	GLR Pond 12	Covered	N/A
188	4/18/15	8,873	GLR Pond 12	Covered	Free Swimming
189	4/18/15	4,590	Blue Bonnet	Covered	N/A
190	4/18/15	1,247	GLR Pond 2	Covered	Free Swimming
191	4/18/15	4,879	GLR Pond 2	Covered	Free Swimming
192	4/18/15	3,893	GLR Pond 2	Covered	Free Swimming
193	4/18/15	N/A	BSP Pond 30	Covered	N/A
194	4/26/15	4,000	BSP Pond 30	Uncovered	Hatched
195	4/26/15	4,500	BSP Pond 30	Uncovered	Hatched
196	4/26/15	5,000	BSP Pond 30	Uncovered	Hatched
197	4/26/15	5,000	BSP Pond 30	Uncovered	Hatched
198	4/26/15	5,000	GLR Pond 12	Covered	Hatched
199	4/26/15	4,500	GLR Pond 2	Covered	Hatched
200	4/26/15	4,500	BSP Pond 3	Covered	Hatched
201	4/26/15	4,000	BSP Pond 3	Covered	Unfertile
202	4/26/15	4,000	BSP Pond 3	Covered	Hatched
203	4/26/15	2,000	BSP Pond 30	Uncovered	Hatched
TOTAL		394,329			