

**EARLY JUVENILE ECOLOGY OF THE ENDANGERED HOUSTON TOAD,
BUFO HOUSTONENSIS (ANURA: BUFONIDAE)**

THESIS

**Presented to the Graduate Council of
Texas State University-San Marcos
in Partial Fulfillment
of the Requirements**

for the Degree

Master of SCIENCE

By

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**San Marcos, Texas
May 2004**

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ACKNOWLEDGEMENTS

I would like to begin by thanking my husband, Ryan, for being the foundation of support I always stood on. I would also like to thank my family, especially my mother, for giving me the courage and strength I needed to complete my goals. I would like to express my indebtedness to Todd Swannack and Gina Lobban for their countless hours of well-used advice, support, and strength.

I am very thankful to the members of my thesis committee. My deep appreciation goes to Dr. Baccus for his expertise and absolute generosity. I would also like to express my gratefulness to Dr. Simpson for his wisdom, patience, and selfless concern for his students. Finally, it is to my major advisor, Dr. Forstner, that I owe the most overwhelming debt of gratitude. Because of your persistence, I am finally at a level I never thought I could be. Thank you.

This manuscript was submitted on February 25, 2004.

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CHAPTER 1

THE IMPORTANCE OF JUVENILE ECOLOGY IN THE CONSERVATION AND MANAGEMENT OF THE HOUSTON TOAD, *BUFO HOUSTONENSIS*

INTRODUCTION

The Houston toad, *Bufo houstonensis*, is an endangered species that, despite efforts to obtain life history information, retains many unknowns about its ecology. These ecological unknowns have inhibited management practices to such an extent that only practices benefiting the adult population have been applied (Hatfield et al. in press). To determine the best management practices for any endangered species, data from all stages of the life cycle are necessary. For amphibians, data from the juvenile stage are particularly important, as this stage is subject to higher vulnerability because of rapid desiccation (Werner 1986). Hence, anuran juveniles depend on wetlands and the surrounding upland habitat for their survival (Semlitsch 2000a). To document habitat dependence in *B. houstonensis* juveniles, base line data on survivorship from egg to metamorph are necessary. Once achieved, the subsequent development of techniques for determining juvenile growth, dispersal, and morphological identification can be undertaken. These techniques will allow data collection directly guiding management

practices, including the definition of appropriate buffer zones, toward conservation and eventual recovery of the species.

THE HOUSTON TOAD

The Houston toad was federally listed as an endangered species in 1970 (Peters 1968). The listing was based primarily on its small range, strict habitat requirements, and its scarcity in the face of perceived, and since realized, habitat destruction (Brown 1975). Destruction of habitat has been effected mainly by agricultural use and urban expansion and more peripherally by watershed alteration. The decline of the species also exacerbated by reproductive failure and decreased survival during droughts (U. S. Fish and Wildlife Service 1992). Hybridization with sympatric congeners consequent of landscape changes has also had an impact on the species (Kennedy 1961, Brown 1971, Hillis et al. 1984).

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

The preferred *B. houstonensis* habitat is deep, carrizo sand often in or near pines of the Post Oak Savannah Region of Texas (Kennedy 1962, Brown 1971, Brown and Thomas 1982). Because adult Houston toads are poor burrowers and have difficulty digging into compacted soil (Bragg 1960), they select the soft, pliable sand of central

Texas. However, juveniles may not necessarily be confined to this soil type as they might seek moist shelter under leaf litter (Clarke 1974).

Bufo houstonensis is a small toad 5.0 - 8.5 cm long and is similar in appearance to the American toad, *Bufo americanus*. In fact, *B. houstonensis* is a relict descendant of the narrow skulled *B. americanus* group (Blair, 1972). General coloration varies from light brown to gray or purplish gray, sometimes with green patches. They have pale ventral surfaces, which often have small, dark spots. Males have a dark throat coloration (U. S. Fish and Wildlife Service 1992). In general, an adult Houston toad (*B. houstonensis*) has a characteristic mottled-brown coloration (Figure 1).

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

Houston toads mate in ephemeral rain pools, flooded fields, and permanent ponds (Jacobson 1989). Breeding ranges from January to June, followed by aestivation in shallow underground refugia until the next spring's rains (Jacobson 1989). Stagnant pools that persist for at least 30 days are required for breeding and for the aquatic larval life stage to develop (Jacobson 1989). Males vocalize from shallow water or from habitat near the breeding pond and can call within a 100 m radius surrounding the breeding pond. However, pairs have arrived to the breeding sites already in amplexus (Jacobson 1989), indicating terrestrial amalgamation.

Adult toads emerge to breed only when conditions are optimal, but can emerge outside the breeding season if habitat disturbance occurs. Adults do not appear to be



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

faithful to particular breeding ponds. This lack of site fidelity allows genetic exchange to occur, alleviating isolated sink populations from the possibility of inbreeding or bottlenecking (Lacy and Seal 1994). However, Houston toads in Bastrop County are part of a metapopulation where two subpopulations are locally spread out and separated by a four-lane highway. This separation has been a cause of concern as such barriers inhibit the genetic exchange in other species (Semlitsch 2000b).

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

Several recommendations were made from examination of the initial Houston toad population and habitat viability assessment (Lacy and Seal 1994). A geographic information system database was proposed to incorporate annual surveys of the adult population, soil maps, road maps, known Houston toad population locations, and habitat type. This database would have determined areas of suitable habitat as well as the amount of isolation and continuity among population localities (Lacy and Seal 1994). Unfortunately, this has yet to be completed.

In the 1994 PHVA analysis, the Conservation Breeding Specialist Group (Lacy and Seal 1994) provided recommendations to improve habitat for the Houston toad, described threats and land use activities, gave management guidelines, and encouraged

public outreach. For habitat improvement, the preferred toad habitat used outside of the breeding season must be characterized. To enhance reproduction, pond construction and restorative techniques must be investigated, but the role of travel corridors between and among breeding ponds must also be explored (Lacy and Seal 1994).

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

Since the publication of the PHVA in 1994, some recommendations have been implemented. However, it is implicit in the first PHVA that all recommendations were based on adult populations. A second PHVA (Hatfield et al. in press) has just been completed and made a point of noting the lack of pertinent data on the other life stages. To date, the aquatic and juvenile life stages have not been addressed and therefore, are imperative for inclusion in management plans. A closer inspection of interactions between *B. houstonensis* life stages and the local environment might increase the potential for recovery.

HOUSTON TOAD ENVIRONMENT

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

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

Small wetlands are, therefore, critical habitat requirements for amphibians (Semlitsch and Bodie 1998) providing both aquatic and terrestrial habitats. Management that accounts for both the aquatic and adjacent terrestrial habitats is required to maintain viable breeding populations (Semlitsch 1998). Juveniles have the greatest abundance in wetland habitats because post-emergent dispersal has not yet begun. Once dispersal

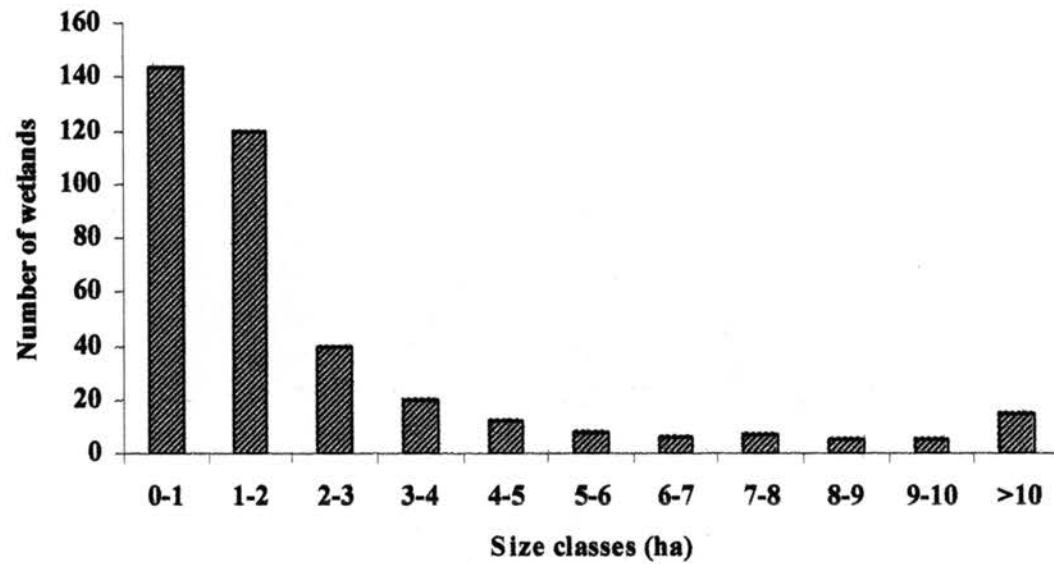


Figure 2. Distribution of natural depression wetland sizes (n=371) from the Savannah River Site on the upper coastal plain of South Carolina (Redrawn from Figure 1 in Semlitsch and Bodie, 1998). The lower limit of detection from geographic information system data was 0.2 ha.

occurs, juvenile amphibians tend to migrate beyond the water's edge into small ephemeral wetlands rather than to larger wetlands (Semlitsch 2000b) due to the lack of aquatic predators (Dayton and Fitzgerald 2001).

Beyond the pond's edge, multiple aspects of the terrestrial life stages occur. For example, beyond growth to maturity, adult yearly hibernation and migration patterns are two aspects of the amphibian life cycle that take place beyond wetland boundaries. Adult amphibians make annual migrations from the terrestrial habitat to breeding ponds (Pechmann and Semlitsch 1986, Pechmann et al. 1989). Therefore, to complete their life cycles, the majority of amphibian species require use of not only wetlands, but also lands adjacent to them.

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

Often insufficient ecological information is available for a species utilizing terrestrial habitats surrounding these small isolated wetlands and for the area immediately adjacent to the water's edge, both of which are required for the survival of these species (Semlitsch 1998). This is especially true in the case of the Houston toad. The use of the adjacent terrestrial habitats, the identification of relevant distances for both communities

and species, and the area required for the life cycle stages are necessary for the accurate determination of buffer zones (Burke and Gibbons 1995). Each of those factors should have a direct consequence on guiding basic principles for managing the remaining Houston toad habitat.

The adult life stage dynamics of the Houston toad are better known as studies have been done on hybridization (Kennedy 1961, Brown 1971, Hillis et al 1984), reproductive ecology (Hillis et al. 1984, Jacobson 1989, Price, unpubl. data) including captive breeding (Quinn et al. 1987), feeding, (Bragg 1960), and conservation (Brown 1975, Brown and Thomas 1982). However, the larval and post-emergent ecology of the Houston toad are poorly characterized. In particular, little research has exclusively focused on juvenile toad dispersal, developmental patterns, and survivorship. Hillis et al. (1984) addressed tadpole development and only briefly mentioned the dispersal of postmetamorphic *B. houstonensis* from the pond site. Thomas and Allen (1997) observed a small number ($n = 25$) of juveniles over a short sampling period (about 1 month) at a native pond. Quinn and Mengden (1984) evaluated captive raised adults from chemically induced egg strands and observed subsequent development. Developmental growth was recorded, but dispersal of juveniles could not be addressed in captivity. In all of these studies, dispersal or growth were the only two aspects of juvenile ecology addressed. Survivorship data and positive identification are also needed to better address population survival estimates and field identification. Yet, neither topic has been previously published.

Previous knowledge of Houston toad juvenile ecology remains incomplete. An investigation is needed to classify and clarify the importance of developmental growth,

juvenile dispersal patterns, morphology, and survivorship in the wild. Such data are particularly useful for their contribution to survivorship calculations and evaluating the PHVA. These data will help to define better management practices compatible with the toad including the relevant size and configuration of buffer zones.

With little preexisting data and that information being neither extensive nor complete, this study was designed to assemble biological and ecological characteristics of the juvenile Houston toad. This research sought data on developmental growth, dispersal patterns, morphological distinctions and the determination of survivorship from egg to metamorph. The results will hopefully provide an evaluation for the Houston toad juvenile habitat and developmental requirements, a justification for buffer zone placement, and provide calculations of survivorship and the methods required for making those assessments.

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

CHAPTER 2

FIELD TECHNIQUES THAT AID IN THE DETERMINATION OF HOUSTON TOAD, *BUFO HOUSTONENSIS*, JUVENILE SURVIVORSHIP

INTRODUCTION

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

Survivorship calculations range from simple percentages (number of individuals survived/total number initial individuals) (Shirose and Brooks 1995) to highly complex

models as those implemented in MARK (White and Burnham 1999), but the techniques used to determine the raw data for these calculations (i.e., the actual number of individuals) have rarely been explored. Published data on egg and tadpole counting techniques are limited to estimates from: (1) counting the number of gravid females, (2) total masses, or (3) individual eggs.

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

Even fewer techniques seek to avoid handling eggs during counting. Because of clear, shallow water, large eggs, and small masses, Anderson et al. (1971) counted eastern tiger salamander (*Ambystoma t. tigrinum*) eggs without removing masses from the environment and with a minimum of disturbance. Biologists in Canada have used a technique that avoided manipulation of amphibian eggs by usually estimating the number of eggs in multiples of ten (Ministry of Environment, Lands, and Parks 1998).

Manipulation of any part of the embryonic stage of an amphibian could contribute to mortality (Anderson et al. 1971) and is, therefore, generally avoided, especially when dealing with an endangered species, such as the Houston toad. However, little research has explored methods for egg counts without manipulation or displacement. Therefore, I: (1) designed 4 estimation techniques, and (2) tested these techniques on *Bufo houstonensis* and *B. valliceps* egg strands so that having determined the best estimation technique, I could then calculate survivorship from egg to metamorph in *B. houstonensis*.

METHODS

STUDY AREA

The Griffith League Ranch (1,963-hectares; 4,848-acres) is located in Bastrop County and is owned by the Boy Scouts of America. This ranch is an ideal site for observing Houston toads due to its numerous ponds, favorable habitat conditions, and known Houston toad populations. Seventeen ponds on the Griffith Ranch have had Houston toad chorusing and 7 of these are known breeding sites. Pond 2 was chosen for this experiment due to the abundance of Houston toads, characteristic habitat of carrizo sand and mixed hardwood/pine forest, and easy access.

The IUCUC ID No. for this research is HGVMAD_02. *B. houstonensis* eggs were naturally laid in Pond 2, so traps were needed to capture juveniles upon emergence to determine metamorphic survivorship. A drift fence with pitfall traps were curved around the egg strand so when metamorphosis occurred, juveniles would be captured in the traps. The trapping array was placed 2 m, 5 m, and 8 m from the egg strings. Juveniles were followed up to 13 weeks post-emergence.

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

INVOLVED SPECIES

Only 2 *B. houstonensis* egg strands were found in the breeding pond (pond 2). Gulf coast toad egg strands were then included in the evaluation due to the small number of *B. houstonensis* strands and the need to perform multiple estimates for analyses. Because *B. valliceps* has egg strands similar to *B. houstonensis*, is not endangered, and readily available on site, the Gulf coast toad was considered an appropriate substitute. Eight *B. valliceps* egg strands were estimated.

Three of the *B. valliceps* strands died after initial development and before hatching (before stage 13, Werner, 1986) due to a lack of fertilization or some other natural cause. One *B. houstonensis* egg strand died in the pond before the eggs could hatch (before stage 13, Werner, 1986) in early March due to extreme cold weather. All bufonid strands were estimated, but those failing to develop were not included in the data due to the inability to complete survivorship calculations. Therefore, only 2 *B. houstonensis* strands and 5 *B. valliceps* strands were considered as the data used in the experiment.

EGG STRAND ESTIMATION TECHNIQUES

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

Full Wire Technique

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

Wire Section Technique

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

Spherical Technique

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

$$\begin{array}{ll} 1 & e = \# \text{ eggs/cm}^3 \\ 2 & V_{\text{sphere}} = 4/3 \pi r^3 \quad \text{where } r \text{ is the radius in cm} \\ 3 & r = d/2 \quad \text{where } d \text{ is the diameter of the estimated egg sphere in cm} \end{array}$$

so that

$$4 \quad E = V * e \quad \text{where } E \text{ is the total number of eggs}$$

Individual Counting Technique

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

Actual Egg Number

Once all 4 estimation techniques were performed, they were compared to the actual number of eggs to determine the precision of each technique. The actual number of eggs was obtained by counting the number of immediately hatched tadpoles from each egg strand. To minimize displacement of the endangered species, half of each *B. houstonensis* strand was placed into a 5-gallon bucket. Once the eggs hatched in the bucket, tadpoles were individually counted. The eggs remaining in the breeding pond were allowed to develop normally as this explicit counting will not be possible in most situations. The number of eggs in the natural pond were assumed to be the same as counted in the bucket. Each *B. valliceps* strand was displaced into a 5-gallon bucket until the tadpoles hatched. Once hatched, the tadpoles were individually counted and placed back into the holding enclosure until metamorphosis.

SURVIVORSHIP CALCULATIONS

A simple survivorship formula was used to calculate survivorship (Krebs, 1999). The number of metamorphs to emerge is divided by the estimated or actual number of eggs and multiplied by 100 to obtain a percentage (metamorphosis survivorship). The equation is:

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

where $\hat{S}_0$ = finite survival rate

N_t = number of individuals alive at end of time period

N_0 = number individuals alive at start of time period

Survivorship from emergence to 13-week-old juvenile was calculated similarly. However, to determine the number of individuals that survived to 13 weeks (1 quarter of a year), a population census was estimated using mark-recapture data (details in Chapter 3). The Schnabel-Schumacher method (Krebs 1999) was used to determine the population census at 13 weeks.

RESULTS

EGG STRAND ESTIMATION TECHNIQUES

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

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

Some techniques were difficult to perform. Multiple hours were spent on each egg strand. If these techniques are to be used in a realistic situation, they must be

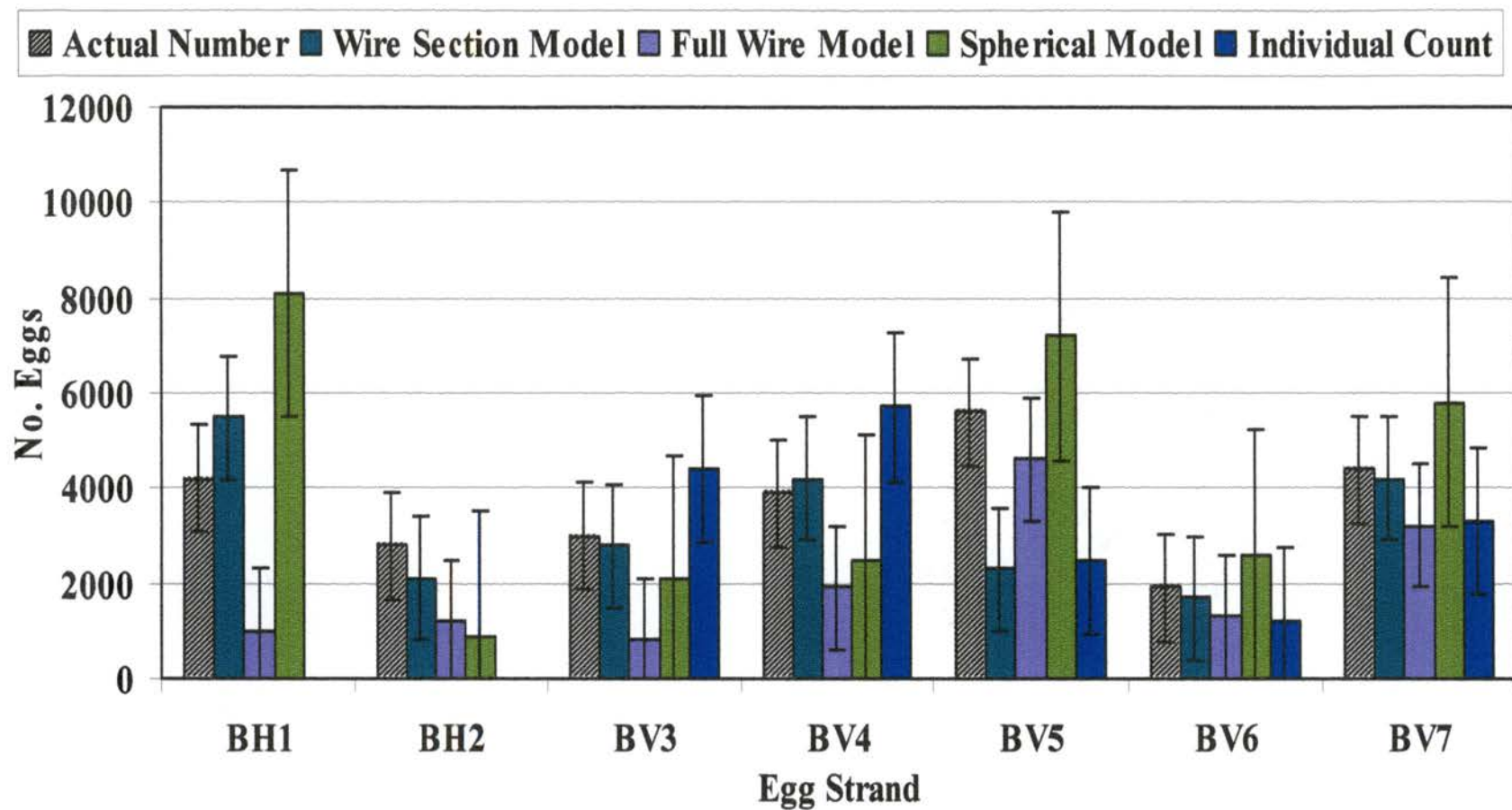


Figure 1. Average Houston and Gulf coast toad egg strand number comparison among four estimation techniques to the actual number of eggs counted. BH=*Bufo houstonensis*. BV=*Bufo valliceps*.

practical in nature. The degree of difficulty and the amount of time each technique took on average is explained in Table 1.

SURVIVORSHIP CALCULATIONS

Survivorship for the egg strands was difficult to determine due to the lack of individual enclosures. The close proximity of the two *B. houstonensis* egg strands in the open pond (wild) prevented individual traps. Table 2 illustrates the estimated and actual percent survival from all 7 cohorts. Survival of *B. houstonensis* metamorphs was overestimated using the estimation techniques (5.29%) when compared to the actual number of juvenile survival (4.73%). When averaged together, the estimation techniques provided a 1.36% and 0.76% survival of *B. valliceps* metamorphs as compared to the actual number, which determined to be 1.20% and 0.64%. The estimated percentage was not significantly different from the actual percentage ($P = 0.89$).

Survivorship from metamorph to 13-week-old juvenile is even lower than that of metamorphic survivorship. The Schnabel-Schumacher method revealed an estimated population size of a 15% decline in population from 332 individuals to 281 over the course of 13 weeks.

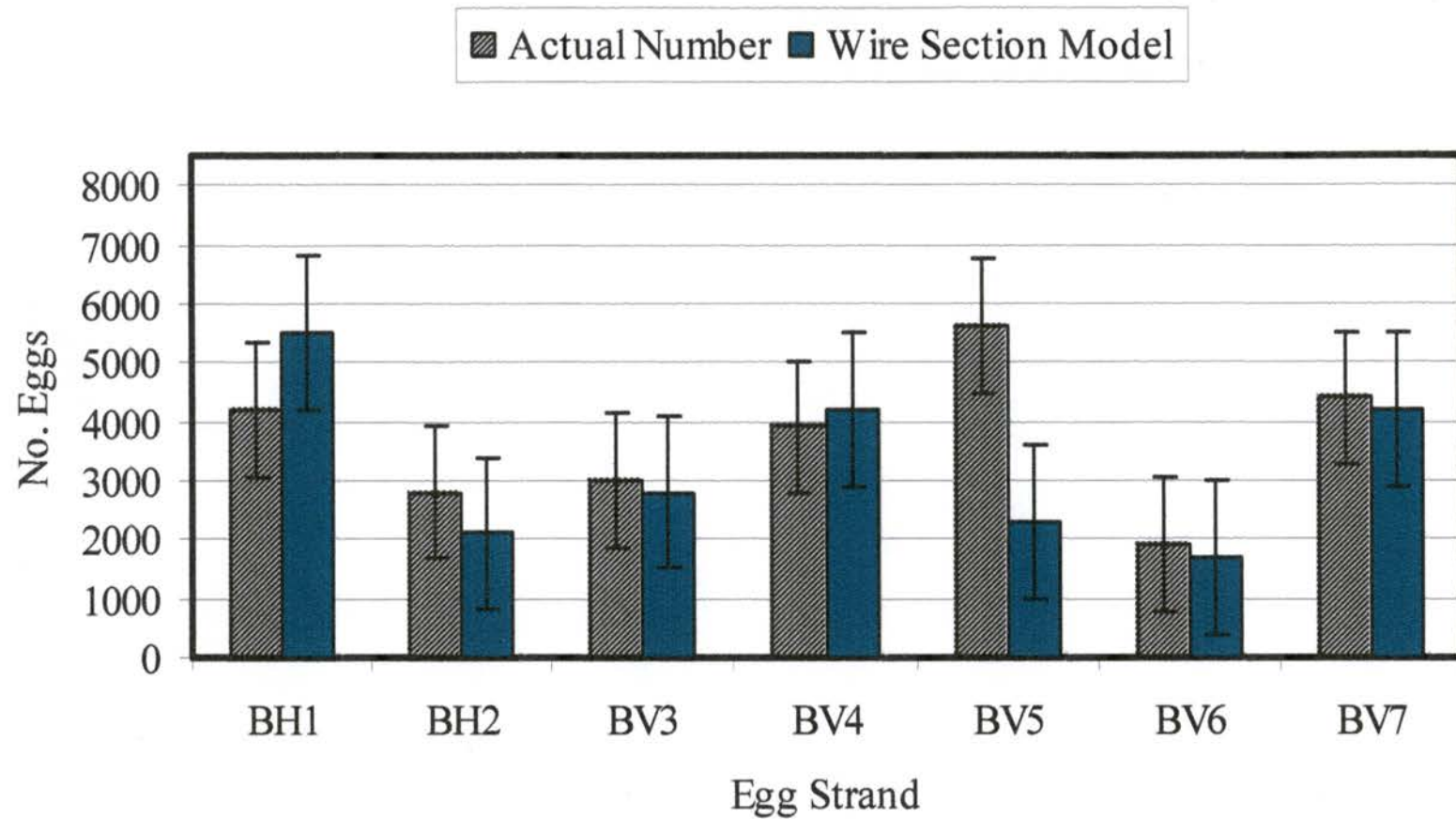


Figure 2b. Average Houston and Gulf Coast toad egg number (No. egg/strand) estimated by the wire section model and compared to the actual egg number of each strand. BH1 and BH2 are *B. houstonensis* egg strands. BV3 through BV7 are *B. valliceps* egg strands. $P = 0.55$, No significant difference between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

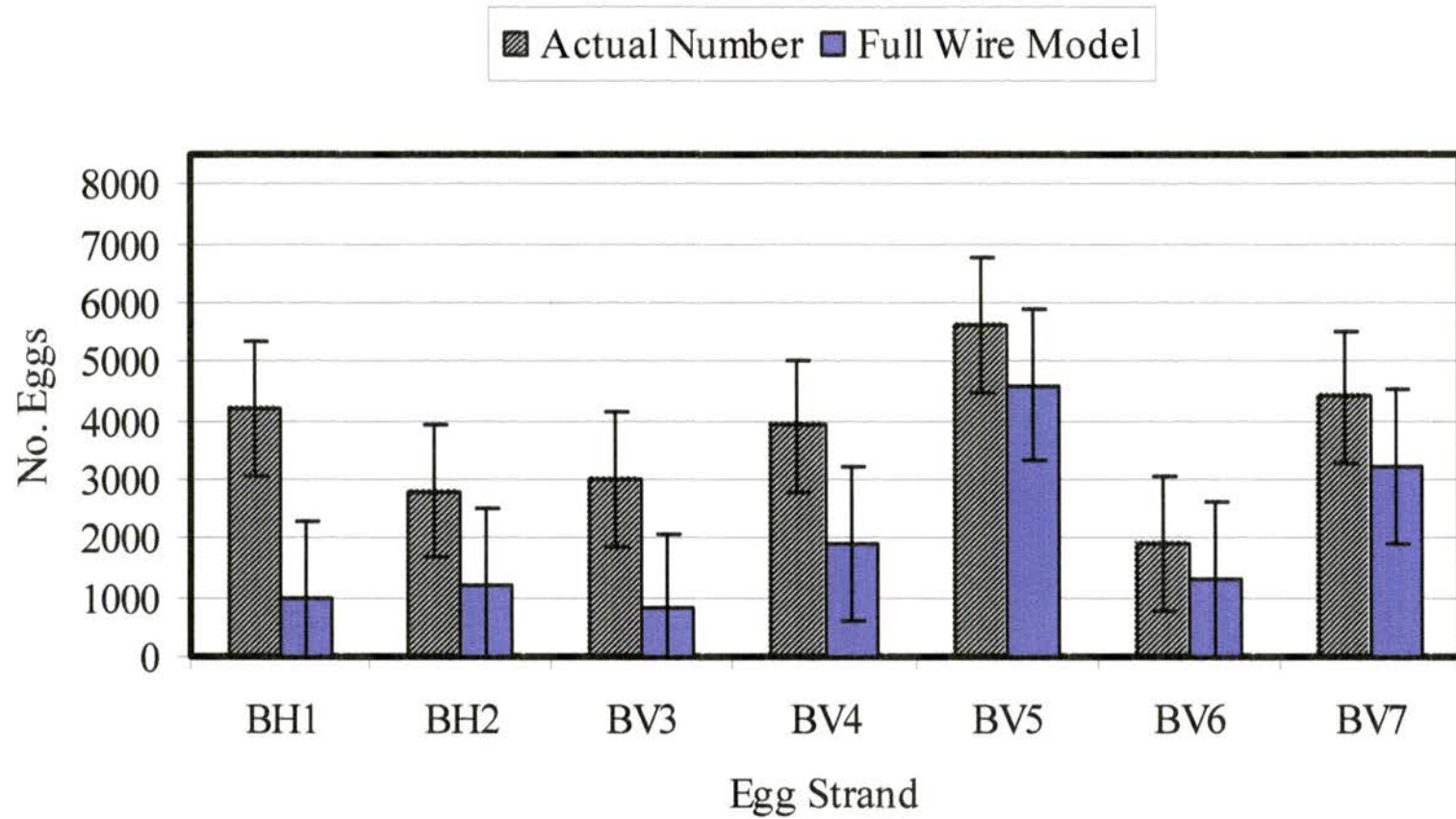


Figure 2a. Average Houston and Gulf Coast toad egg number (No. egg/strand) estimated by the full wire model and compared to the actual egg number of each strand. BH1 and BH2 are *B. houstonensis* egg strands. BV3 through BV7 are *B. valliceps* egg strands. $P = 0.03$, Significant difference between actual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

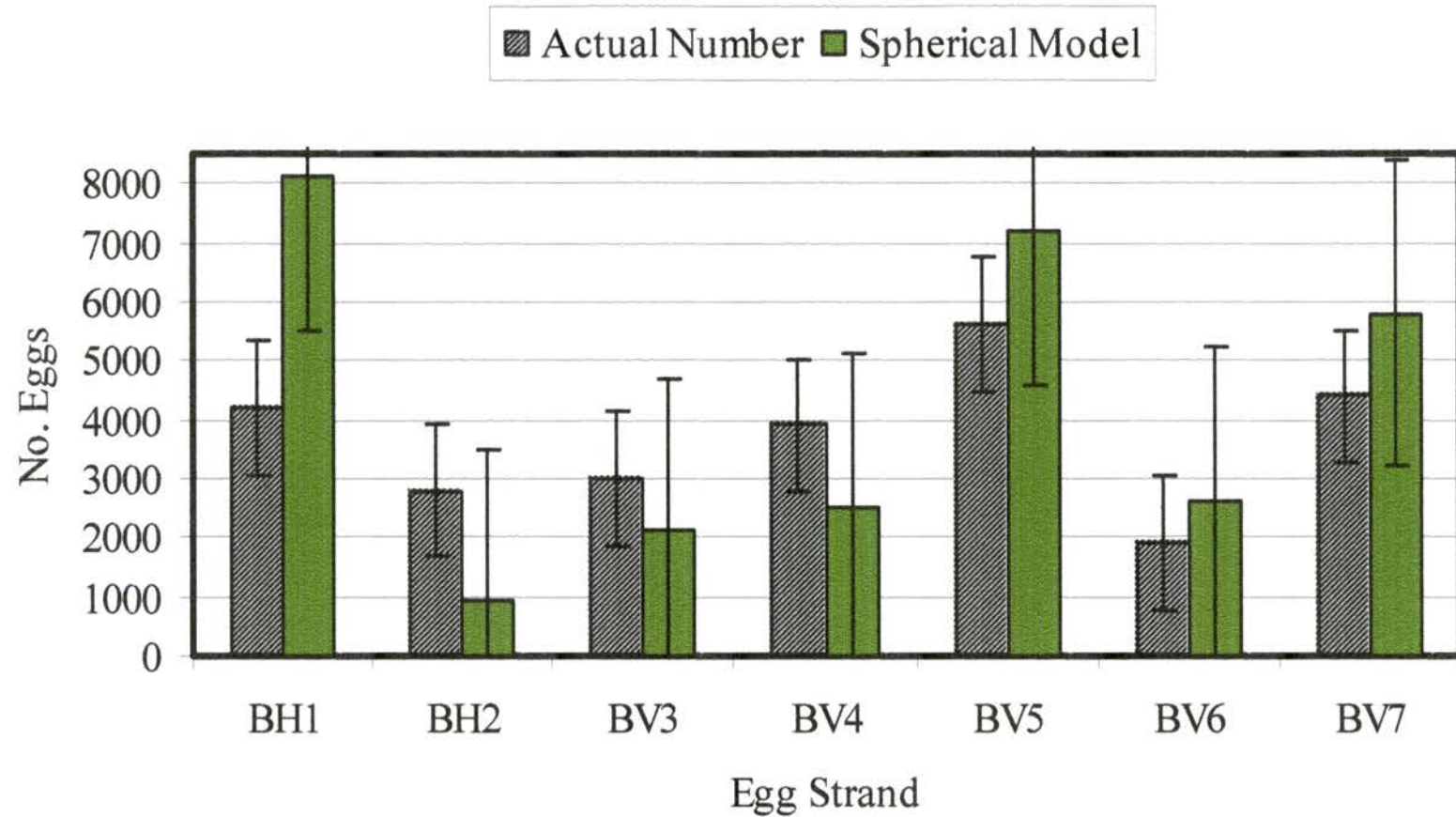


Figure 2c. Average Houston and Gulf Coast toad egg number (No. egg/strand) estimated by the spherical model and compared to the actual egg number of each strand. BH1 and BH2 are *B. houstonensis* egg strands. BV3 through BV7 are *B. valliceps* egg strands. $P = 0.68$, No significant difference between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

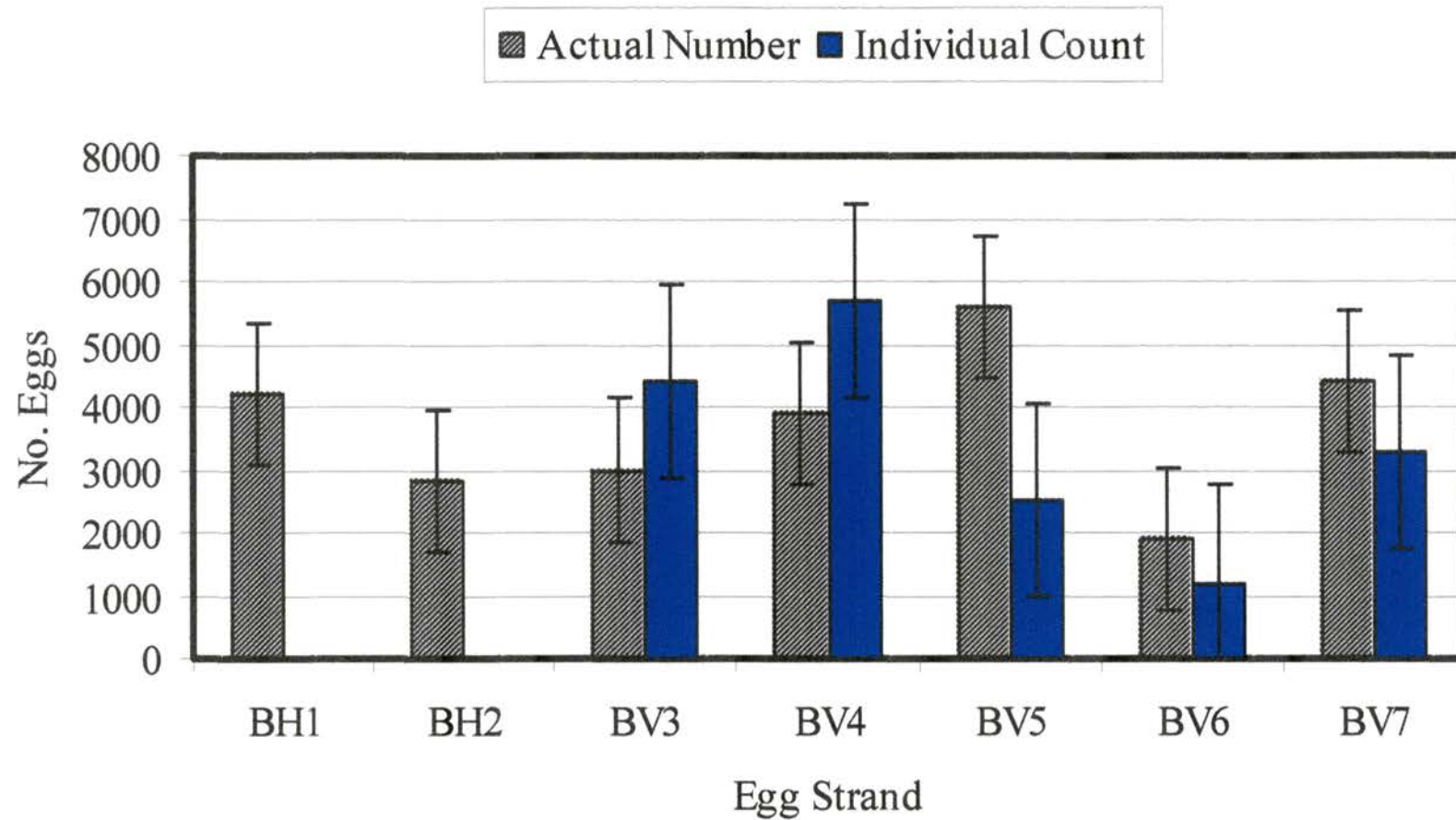


Figure 2d. Average Houston and Gulf Coast toad egg number (No. egg/strand) estimated by the individual count method and compared to the actual egg number of each strand. BH1 and BH2 are *B. houstonensis* egg strands. BV3 through BV7 are *B. valliceps* egg strands. This method was not used on the Houston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

Table 1. Technique difficulty on a scale of 1 to 5, 5 being the most labor intensive to estimate. The amount of time for each technique is given in minutes. The spherical model was the least difficult to estimate as well as the least time consuming. The individual model was the most difficult as well as the most time consuming.

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

Table 2. Percent survival of *B. houstonensis* & *B. valliceps* individuals from egg to metamorph. Egg strand number, species, location, estimated cohort egg number, actual cohort egg number, total juveniles emerged, percent survivorship of the actual number, percent survivorship of estimated techniques, percent survivorship of the full wire model, the wire section model, the spherical model, and the individual count method are shown.

Strand No.	Species	Location	Actual Egg No.	Full Wire Model Egg No.	Wire Section Model Egg No.	Spherical Model Egg No.	Individual Count Egg No.	Total Juveniles Emerged
BH1	<i>B. houstonensis</i>	Wild	4211	980	5560	8100		332
BH2	<i>B. houstonensis</i>	Wild	2807	1230	2070	900		
BV3	<i>B. valliceps</i>	Enclosure 1	2991	850	2700	2100	4437	83
BV4	<i>B. valliceps</i>	Enclosure 1	3932	1920	4230	2500	5761	
BV5	<i>B. valliceps</i>	Enclosure 2	5614	4610	2350	7200	2538	76
BV6	<i>B. valliceps</i>	Enclosure 2	1887	1320	1750	2600	1209	
BV7	<i>B. valliceps</i>	Enclosure 2	4420	3190	4190	5800	3326	

Strand No.	Species	Location	Full Wire Model Avg. Survivorship	Wire Section Model Avg. Survivorship	Spherical Model Avg. Survivorship	Individual Count Avg. Survivorship	Actual Count Avg. Survivorship	All Techniques Avg. Survivorship
BH1	<i>B. houstonensis</i>	Wild	15.02%	4.35%	3.69%	-	4.73%	5.29%
BH2	<i>B. houstonensis</i>	Wild						
BV3	<i>B. valliceps</i>	Enclosure 1	3.00%	1.20%	1.80%	0.81%	1.20%	1.36%
BV4	<i>B. valliceps</i>	Enclosure 1						
BV5	<i>B. valliceps</i>	Enclosure 2						
BV6	<i>B. valliceps</i>	Enclosure 2	0.83%	0.92%	0.49%	1.07%	0.64%	0.76%
BV7	<i>B. valliceps</i>	Enclosure 2						

DISCUSSION

Given the importance of maintaining the integrity of fragile eggs especially for endangered taxa, these estimation techniques are vital in avoiding physical displacement or manipulation. Precision was increased after several egg strand trials. Precision, not accuracy, is needed for these techniques to work, as accuracy is probably unrealistic.

Choosing the best estimation technique requires the evaluation of several factors. In terms of practical use and application of these techniques, it is best to have a less modeling time and a low degree of difficulty. However, difficulty and time varied among the 4 techniques, as did their relative precision.

For example, while the spherical model was the fastest estimate and the degree of difficulty was very low, the deviation in estimation was extremely high ($SD = 2098$). Individually counting the eggs was extremely difficult and time consuming; therefore, it is not recommended. The full wire technique had a moderate time period and was more strenuous to model. The complications associated with modeling an entire egg strand all at one time make this technique sub-optimal.

The wire section technique had a moderate degree of difficulty and low effort time. This technique also had the lowest deviation from the actual number of eggs ($SD = \pm 1279$). Given the model's flexibility in technique and realistic time consumption, the wire section model is the technique most likely to be used for practical applications. The technique allows for quick maneuverability and is simple in concept. By using this method, it is quite possible to create estimates for dozens of egg strands within the short period between deposition and hatching.

There are two possible explanations for the lower *B. valliceps* survivorship. The 2 *B. houstonensis* cohorts were in optimal conditions for survival and the 5 *B. valliceps* were not. Shade, confinement, and manipulation could have played a role in *B. valliceps* survivorship as it is well documented that lower pond temperature (Wilbur and Collins 1973, Semlitsch 2000a) and increased density (Smith 1983, Petranka and Sih 1987, Petranka 1989, Scott 1990, Van Buskirk and Smith 1991, Scott 1994) in larval populations not only decrease body size upon emergence, but survivorship as well (Wilbur 1972, Wilbur and Collins 1973, Wilbur 1976, 1977a, 1977b, Smith-Gill and Gill 1978, Smith-Gill and Berven 1979, Pough and Kamel 1984, Goater et al. 1993). The other possibility is that accuracy of estimation increased over time and the five *B. valliceps* cohort estimations are more representative of realistic survivorship numbers for successful egg strands in the wild.

Lower *B. valliceps* survivorship was most likely due to manipulation and confinement. However, the amount of practice involved with *B. valliceps* strands was greatly increased and, therefore, techniques became more precise over time. Because *B. houstonensis* estimation received little practice, *B. valliceps* is a resilient species, and yet *B. valliceps*' survivorship was relatively low, the endangered *B. houstonensis* actual survivorship was probably closer to that of *B. valliceps*' survivorship. However, It is not known how close.

Regardless of when estimation techniques were perfected and which species benefited from better precision, there was a decrease in *B. houstonensis* survivorship over the course of 13 weeks. Using PVA simulations, Hatfield et al. (in press) determined that if juvenile survivorship for the first 52 weeks was between 1% and 2%, then there

was a high probability of extinction. Metamorphic survivorship was 4.73% and juvenile survivorship at 13 weeks was 4.0%, indicating a 15% decline. If a 15% decline were consistent every quarter until a year, then juvenile survivorship would be much less than 1% (0.0001%). This decline is indicative of serious problems for the species given current PVA models. More research is needed to determine the last missing link in the survivorship chain: survival of older juvenile (> 13 weeks) to adult.

By identifying these techniques and making them applicable for realistic situations, more precise survivorship numbers can be calculated. Monitoring of Houston toad populations can use these methods to aid data collection related to population dynamics. More accurate survivorship calculations for the remaining subpopulations will give a better estimate of the metapopulation trend for this endangered species.

CHAPTER 3

POSTMETAMORPHIC BIOECOLOGY OF THE JUVENILE HOUSTON TOAD, *BUFO HOUSTONENSIS*

INTRODUCTION

Buffer zones provide protection against anthropogenic activities which impact wetlands and surrounding habitat. The habitat surrounding wetlands are not merely buffer zones, but they provide core habitat for many semi-aquatic species. Buffer zones are, therefore, essential for the survival of myriad species and for the preservation of biological diversity (Semlitsch and Jensen 2001). Biologists who study semi-aquatic species have long understood the importance of uplands immediately adjacent to wetlands for the survival of myriad species of toads, turtles, salamanders, and other organisms (Semlitsch 1998).

The endangered species, *Bufo houstonensis*, also requires upland habitat adjacent to its wetland ponds for survival. Anthropogenic activities around breeding ponds and core habitat appear to play an important role in the trend toward extinction of *B. houstonensis* (Brown 1975). Designated protection of appropriate buffer zones around native ponds would greatly reduce these activities. Information from all stages of the

toad's life cycle, especially the juvenile stage, could help define Houston toad core habitat characteristics.

The habitat, used by the *B. houstonensis* juvenile stage, needs to be a part of the definition of "core habitat" for a species. Likewise, juvenile dispersal helps to determine the configuration of a buffer zone. Juvenile dispersal is among the most important life-history movements as it connects populations and can thus mitigate against the deleterious effects of genetic isolation (Gill 1978, Berven and Grudzien 1990, DeMaynadier and Hunter 1999). When migrating to other ponds, amphibian juveniles tend to travel greater distances than adults (Breden 1987), sometimes extending to 200 m or beyond (Semlitsch 1998). Therefore, juveniles can actually use more of the upland habitat surrounding wetland ponds than adults and can thus be better indicators of required habitat.

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

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

Contrary to the rapidity of juvenile growth, morphological characteristics develop slower in bufonids. Juveniles of the genus *Bufo* are reported to be morphologically indistinguishable until maturity (Blair 1972). This ambiguity has caused concern as *B. houstonensis* can be confused with *B. valliceps*, *B. woodhousei*, and other congeners as juveniles. In terms of conservation, detection of species' differentiation earlier than maturity would be beneficial. Identification of juvenile toads would provide an opportunity to better document survival through better estimation measures. Earlier detection could also enhance Houston toad population surveys if male audio calls, amplexus, or egg strands were not directly observed. Therefore, it is beneficial to empirically evaluate the point at which morphological differentiation among bufonids sympatric with *B. houstonensis* occurs.

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

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

METHODS

STUDY SYSTEM

The study site is located on the Griffith League Ranch (1,962-hectacre; 4,848-acre) in Bastrop County owned by the Boy Scouts of America. The Griffith League Ranch (GLR) serves as an ideal site for the study of juvenile Houston toads consequent of numerous ponds, favorable habitat conditions, and documented Houston toad populations. Multiple ponds were surveyed for juveniles. Pond 2 was used as the main

research site. Pond 11 and a 24-artificial-pond array were subsidiary sites on the GLR that were used in various comparisons.

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

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

PROJECT DESIGN

Collection Dates

Data were collected from 26 May 2001 – 17 June 2001, 18 April 2002 – 25 June 2002, and 23 April 2003 – 15 August 2003. The 2001 data were provided from a feasibility study by M. Gaston and M. R. J. Forstner.

Research Site — Native Pond

Seventeen ponds on the GLR have had Houston toad choruses and 7 of these are known Houston toad breeding sites. One pond was used for this project (Pond 2).

Abundance of Houston toads, characteristic habitat of carrizo sand and mixed hardwood/pine forest, and easy access made it an optimal research site.

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

Egg mass surveys were conducted to determine how many egg masses were in the pond. Sets of elliptical-shaped traps (Figure 1) were arranged at the pond's edge around *B. houstonensis* egg strings. The ellipses were placed 2 m, 5 m, and 8 m from the egg strings. Each end of the aluminum flashing was placed 3 m away from the previous end and 1 m into the water. This layout helped determine in what direction and at what rate the juveniles emerged from the natural pond. Emerging juveniles caught by the first aluminum drift fence were marked and released on the opposite side of the flashing.

A flagged grid pattern encircled Pond 2. The grid consisted of 13 lines with flags placed every 5 m up to 50 m in 2002 and 250 m in 2003. The grid pattern was used to randomly select sampling. Quadrat plots were used in 2002 to randomly sample for *B. houstonensis* juveniles throughout the adjacent upland habitat surrounding Pond 2. PVC pipes were cut to form one 5-m² plot. This plot was randomly sampled 100 times in a flagged grid encircling the pond and extending 50 m into the surrounding upland habitat. A 1-m² plot was used to sample only the pond's immediate edge in 2002.

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

In 2003, to further increase efficiency in sampling, there was a change in the methodology. Quadrat plots and refugia were combined into smaller refugia that sampled more of the habitat. Fifty 1-m² refugia were randomly dug into the flagged grid, which now extended 250 m into the upland habitat. Because refugia were randomly placed, they took the place of the previous quadrat plots and large refugia.

By using all three methods at the natural pond, a variety of habitat types were sampled. Five categories were used to determine habitat preference: (1) habitat type: forest vs. pasture; (2) vegetation type: loblolly pine vs. mixed hardwood-juniper vegetation; (3) soil type: carrizo sand vs. red clay subsoil; (4) soil moisture: moist vs. dry; and (5) sun exposure: shade vs. sunlight. At the time of capture, habitat condition was recorded.

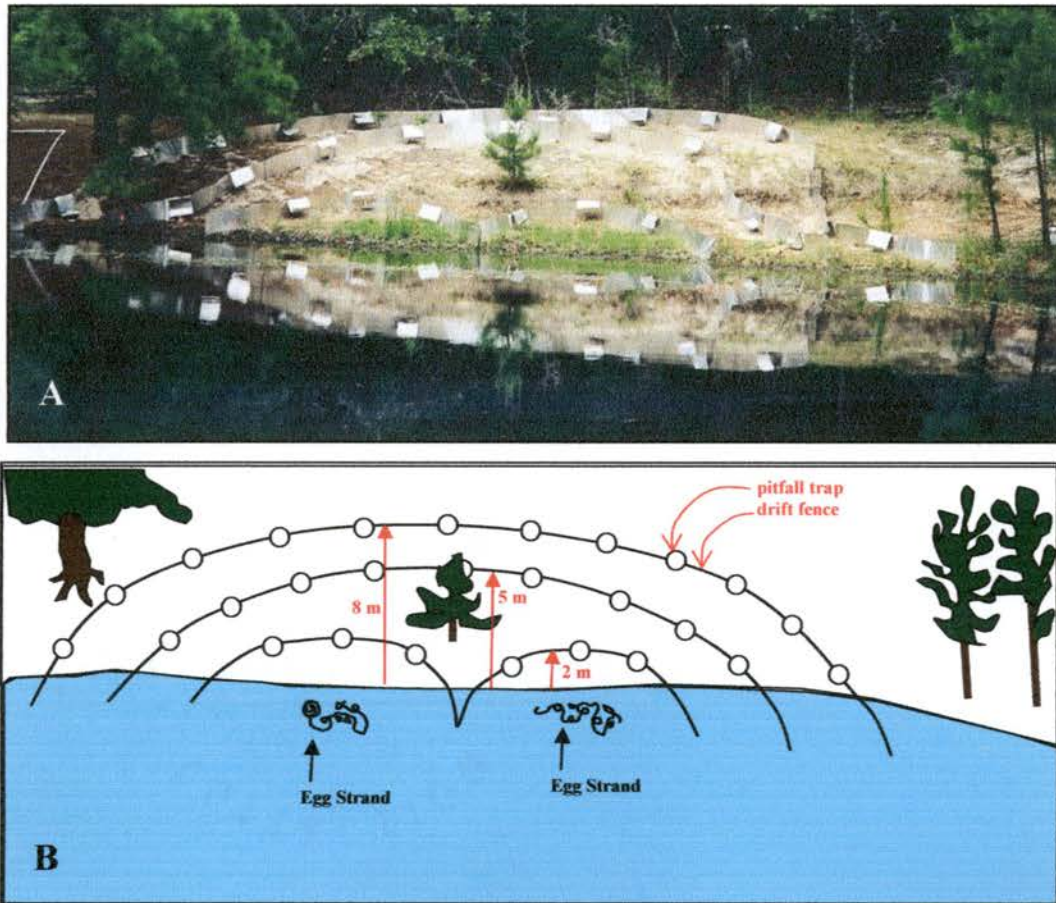


Figure 1. Elliptical aluminum arrays in (A) 2002 where the arrays curve around two *B. houstonensis* egg strands, and (B) an illustration of these arrays. The semicircles represent the elliptical aluminum arrays around an egg strand (shown in black). The arrays were placed at 2 m, 5 m, and 8 m away from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



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

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

Research Site — Artificial Ponds

In addition to sampling at the natural pond, twenty-four artificial ponds used to examine biotic factors effecting Houston toad tadpole survival provided data for comparison with results from natural ponds. In 2002 and 2003, pitfall traps positioned around artificial ponds captured juveniles as they emerged from ponds. Juveniles were marked and followed throughout the habitat. There were 2 sets of traps: (1) around each individual pond, and (2) around the perimeter of the entire 24-pond array.

Aluminum flashing bordered each pond at one meter. Two 2.5-quart paint buckets were positioned as pitfall traps for each individual pond border. An outer perimeter of the pond array was placed 10 m on the north and south sides and 20 m on the east and west sides away from the outer ponds. These traps consisted of two 30.5 m and two 45.7 m linear pitfall arrays. The 5-gallon pitfall buckets were buried in the ground flush with the surface. Once a juvenile was captured in a trap, it was released outside of the 24-pond array detect movement to the outer perimeter. All juvenile Houston toads were quantified and marked as at the natural pond site.

Additional Pond Sampling

In an attempt to extrapolate results on the GLR to a general case across Bastrop County, several additional ponds were sampled in 2003 outside of the main research sites (details can be found in APPENDIX 5). Two privately owned ponds with dispersing bufonid juveniles were surveyed. Pond 11 on the GLR had juveniles dispersing in early summer and was included as a third additional pond. At least 15 measurements and tissue samples were taken from each of the 3 locations. These samples were collected to compare juvenile data from the main study site to those in the surrounding area and to evaluate differences in growth patterns at multiple locations across the landscape.

MOLECULAR IDENTIFICATION

Bufo sp. juveniles are phenotypically indistinguishable at the species level. Several sympatric species of *Bufo* inhabit the GLR, including *B. valliceps*, *B. woodhouseii*, and *B. houstonensis*. To verify the data on each juvenile, a polymerase chain reaction (PCR) marker system was used to distinguish the different species. This test positively identified juvenile *B. houstonensis* by using species-specific primers designed in our laboratory. A positive band (successful amplification) identified Houston toads to the exclusivity of other taxa. Samples from each of the cohorts were sequenced. DNA was extracted from tissue obtained by clipping toes of captured juveniles using the DNeasy protocol for Animal Tissues (Quiagen #69506). The DNA was amplified by PCR using the primers BHCB primers sequence and BHDLR2 primers sequence. The PCR product was analyzed using agarose gel electrophoresis to determine the species of

the tissue donor. This identified species by the presence and relative size of the resolved PCR product on the electrophoresed gel.

STATISTICAL ANALYSIS

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

RESULTS

GROWTH & DEVELOPMENT

Evaluation of 2001 – 2003 Data

General pond information about initial juvenile captures, and days sampled can be found in Table 1. The number of ponds monitored increased over the 3-year period. Sites were not consistent in the number of juveniles caught nor the number of days of observation. A t-test indicated a significant difference in abundance and days observed ($P = 0.040$), confirming that multiple juveniles were captured some days and few on others. For a detailed description of the mark/recapture data, refer to APPENDICES 1 – 3.

Molecular identification confirmed that juveniles expected to be *B. houstonensis* were indeed *B. houstonensis*. There were several juvenile tissue samples taken later in the summer when cohort marking was already completed that were not expected to be *B. houstonensis*. These specimens were confirmed to be the genus *Bufo* but not *B. houstonensis*. These juveniles were most likely *B. valliceps* as this species' breeding season is later than *B. houstonensis*. *B. valliceps* juveniles emerged later but grew more quickly than *B. houstonensis* juveniles and could look similar to *B. houstonensis* juveniles at emergence. Figure 3 provides exemplar PCR results of *Bufo* tissue samples taken. For a complete record of PCR results from *Bufo* tissue, refer to APPENDIX 4. For a detailed description of each sample taken, refer to APPENDIX 5.

Initial *Bufo* juvenile mass for all 3 years is illustrated in Figure 4. Mass increased more rapidly in 2003 than in preceding years. This analysis excluded recapture data for 2001. There was no significant difference in initially-captured juvenile mass ($P = 0.663$) for the three years.

Evaluation of 2002 & 2003 Data

Juvenile mass data from this study was compared to the mass data reported in the captive study of Quinn and Mengden (1984). A significant difference was found when the 2002 data and 1984 data were compared ($P = 0.04$). The comparison of the 2003 data to the 1984 data ($P < 0.01$) also was significantly different. When comparing the raw

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

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

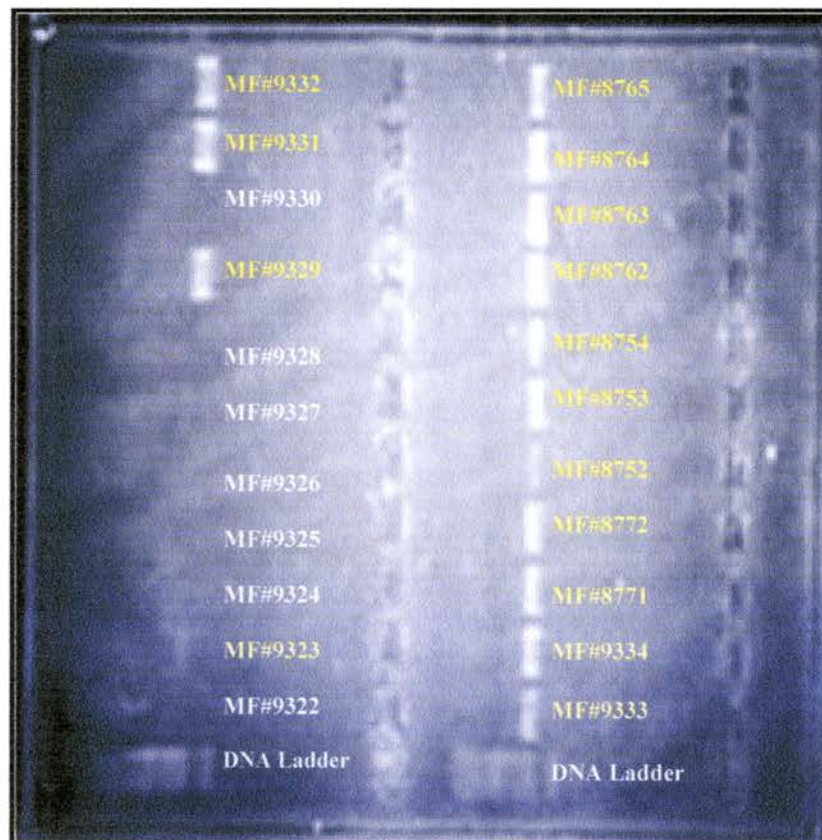


Figure 3. Example of PCR Molecular identification results of juvenile *Bufo* tissue samples from MF#9322 - MF#9334 and from MF#8752– MF#8772 using a 1% Agarose gel and *Bufo houstonensis* specific primers, BHCB and BHDLR2. Samples in yellow are *B. houstonensis* positive. Samples in white are inconclusive.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

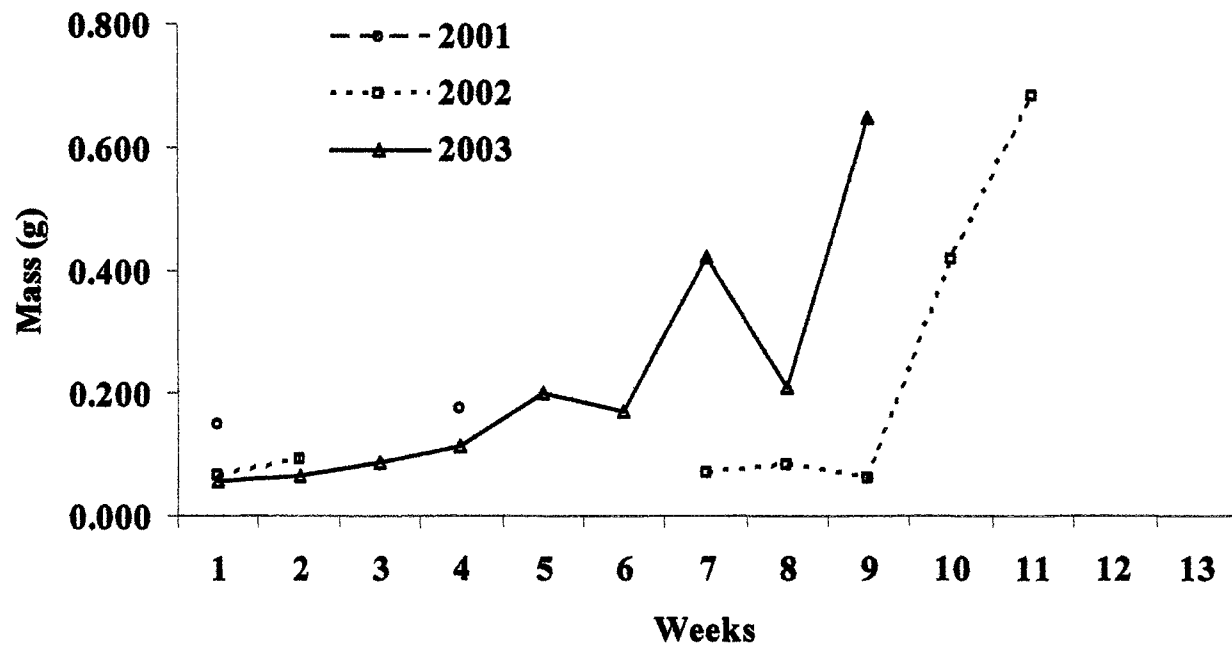


Figure 4. Initial mass of *B. houstonensis* juveniles for 13 weeks during the spring of 2001, 2002, and 2003 post-metamorphosis. The 13-week interval that began the last week of April and ended the second to last week of July was the same for all three years. Initial-capture mass was used for all three years in this analysis due to the lack of recapture data in 2001.

data among all 3 years (1984, 2002, & 2003), a significant difference was also found ($P < 0.01$). The regression lines indicate a strong linear relationship in the 2003 (81%) and 1984 (99%) datasets. The 2002 data was weakly correlated with a linear relationship (34%). A quadratic relationship was a better fit for the 2002 dataset (85%). However, if more data could have been gathered in 2002, then the data would have exhibited a stronger linear relationship.

There was a significant correlation between mass and snout-to-urostyle length as was expected in both 2002 and 2003. This 2003 correlation is shown by the Pearson Correlation Coefficient ($r = 0.93$) and the Spearman Correlation Coefficient ($r_s = 0.66$). The Spearman coefficient is more conservative than the Pearson coefficient, but it still indicates a positive correlation. Figure 6 depicts the correlation of SUL and mass in 2003. The linear equation is $y = 10.222x + 7.551$.

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

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

Two sites, the natural and artificial settings, were compared for 2002 and 2003. No juveniles were recaptured in the artificial pond setting in either year. In 2002, there

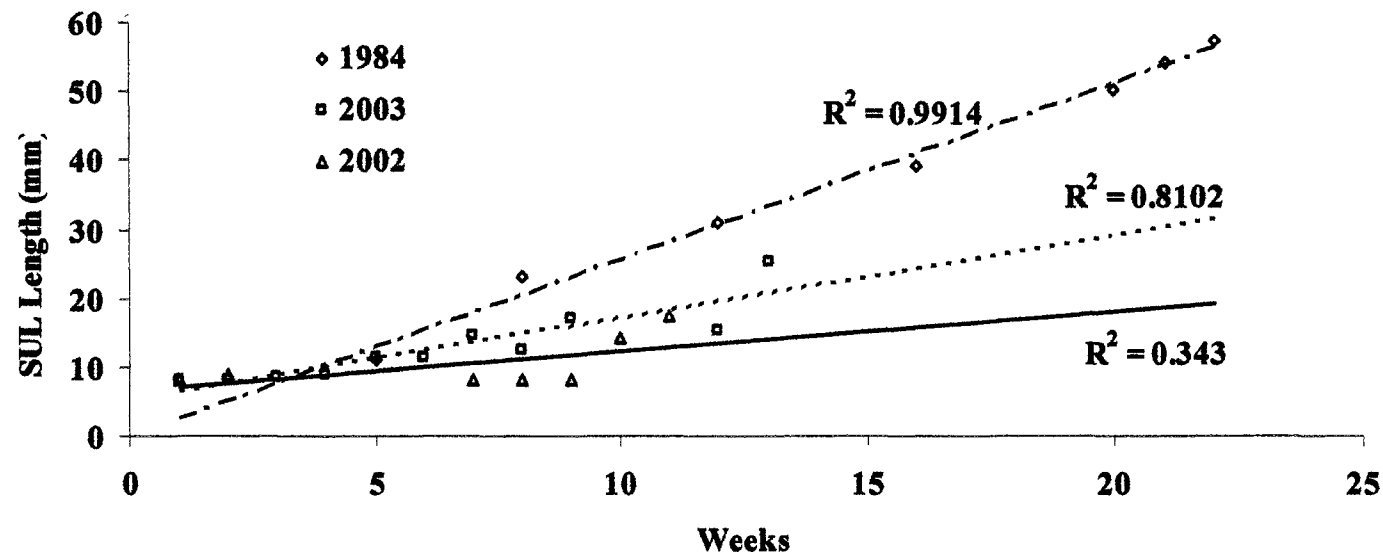


Figure 5. Snout-to-Urostyle length comparison among 1984 captive data and this study's 2002 and 2003 wild-caught data. Comparison was categorized by weeks, from the last week of April to the first week in October. 1984 Quinn & Mengden had an R^2 value of 0.99. The 2002 data had an R^2 value of 0.34. The 2003 data had an R^2 value of 0.81. Comparison of all three years was significantly different ($P < 0.01$). Comparison of 2002 to 1984 data was significantly different ($P = 0.04$). Comparison of 2003 to 1984 data was significantly different ($P < 0.01$).

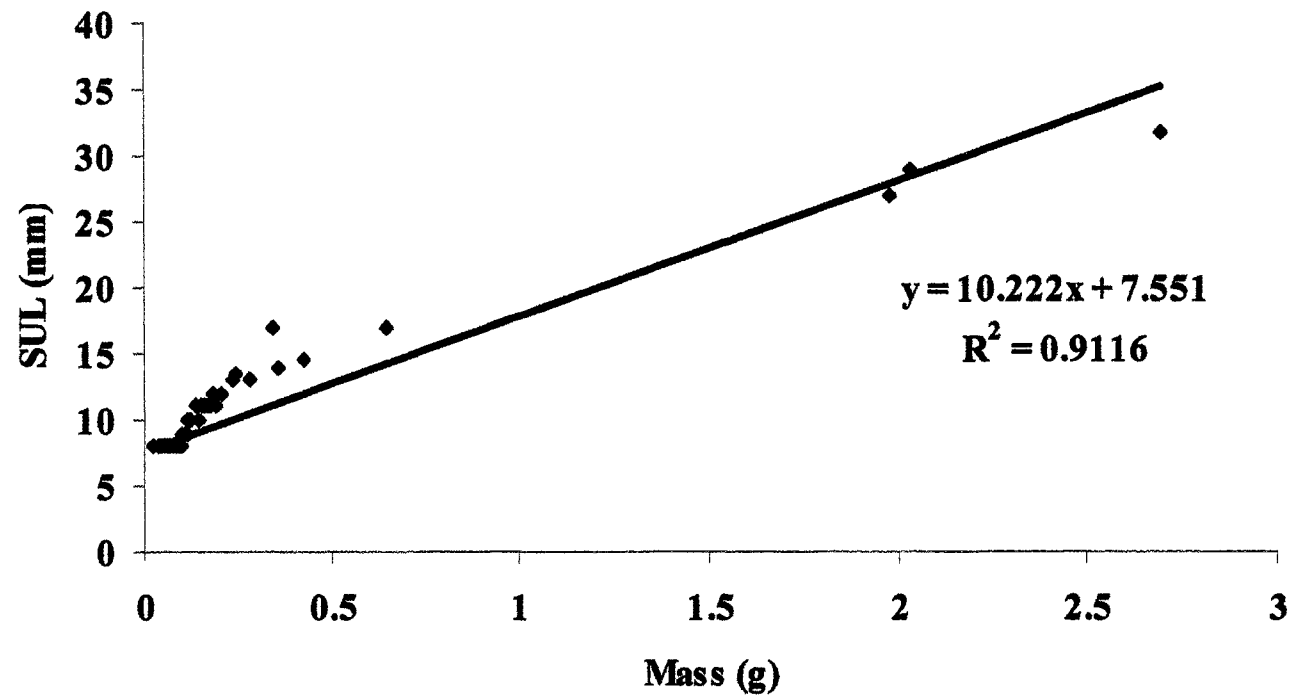


Figure 6. 2003 correlation of mass (g) to snout-to-urostyle length (mm). $R^2 = 0.91$ indicating that 91% of the variation in the dataset is explained by the line, $y = 10.222x + 7.551$.

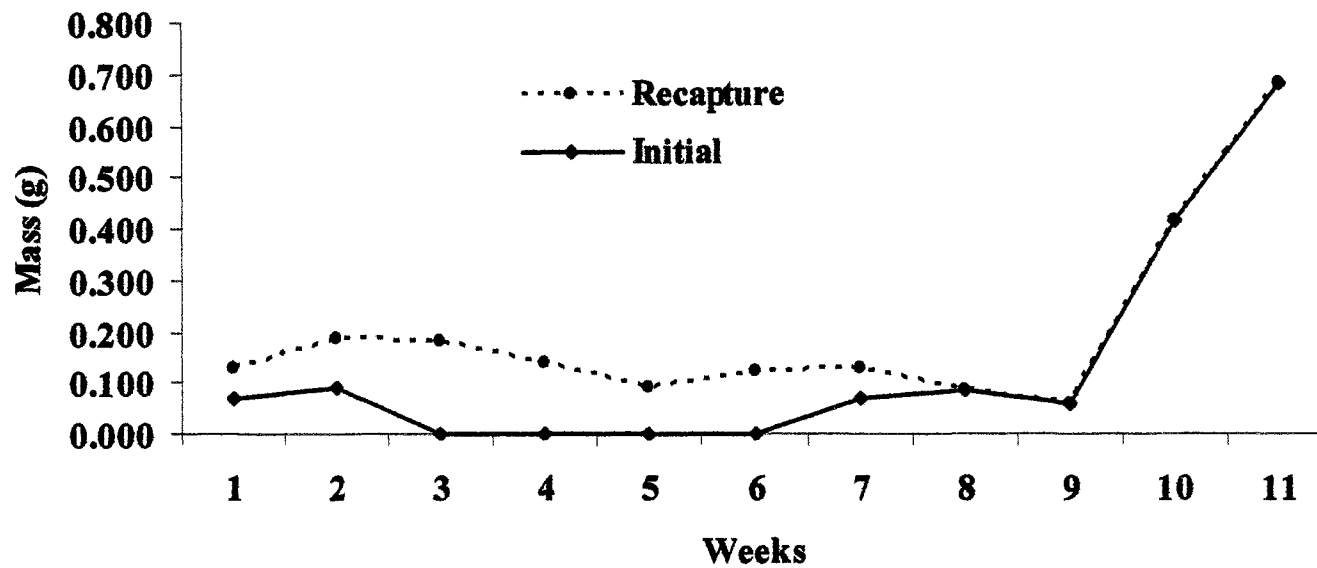


Figure 7. Initial and recapture mass of postmetamorphic *B. houstonensis* juveniles at Pond 2 for 11 weeks in 2002. The 11-week interval that began the last week of April and ended the second week of July was the same for all three years. The blue triangle represents the initial average mass per week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

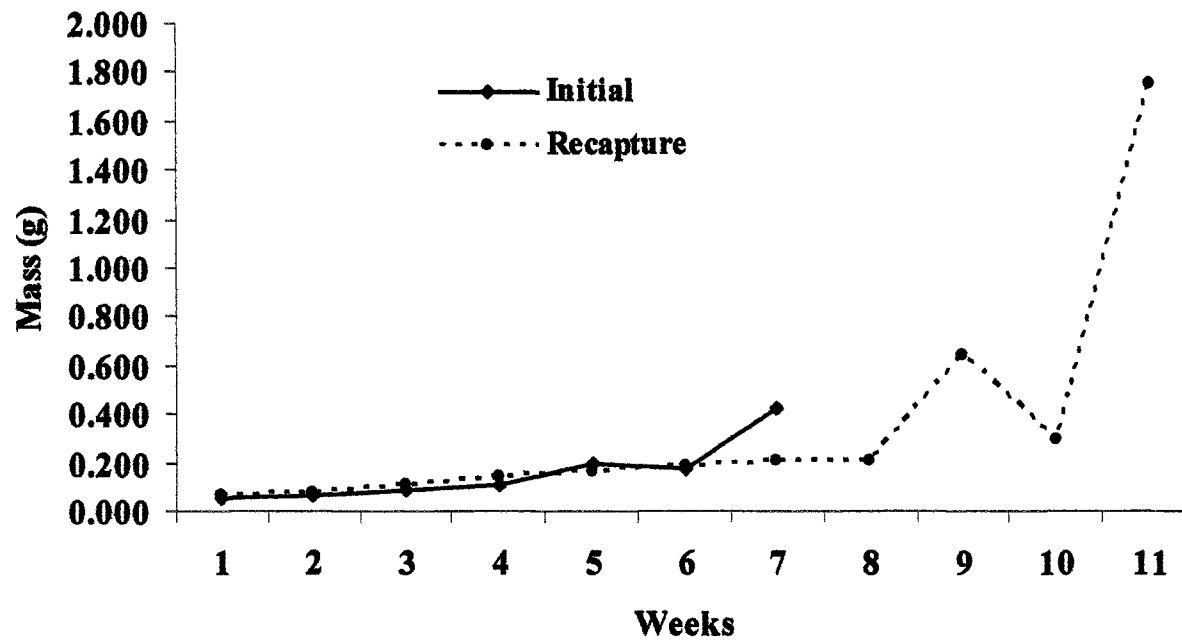


Figure 8. Initial and recapture mass of postmetamorphic *B. houstonensis* juveniles at Pond 2 for 11 weeks in 2003. The 11-week interval that began the last week of April and ended the second week of July was the same for all three years. The blue triangle represents the initial average mass per week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

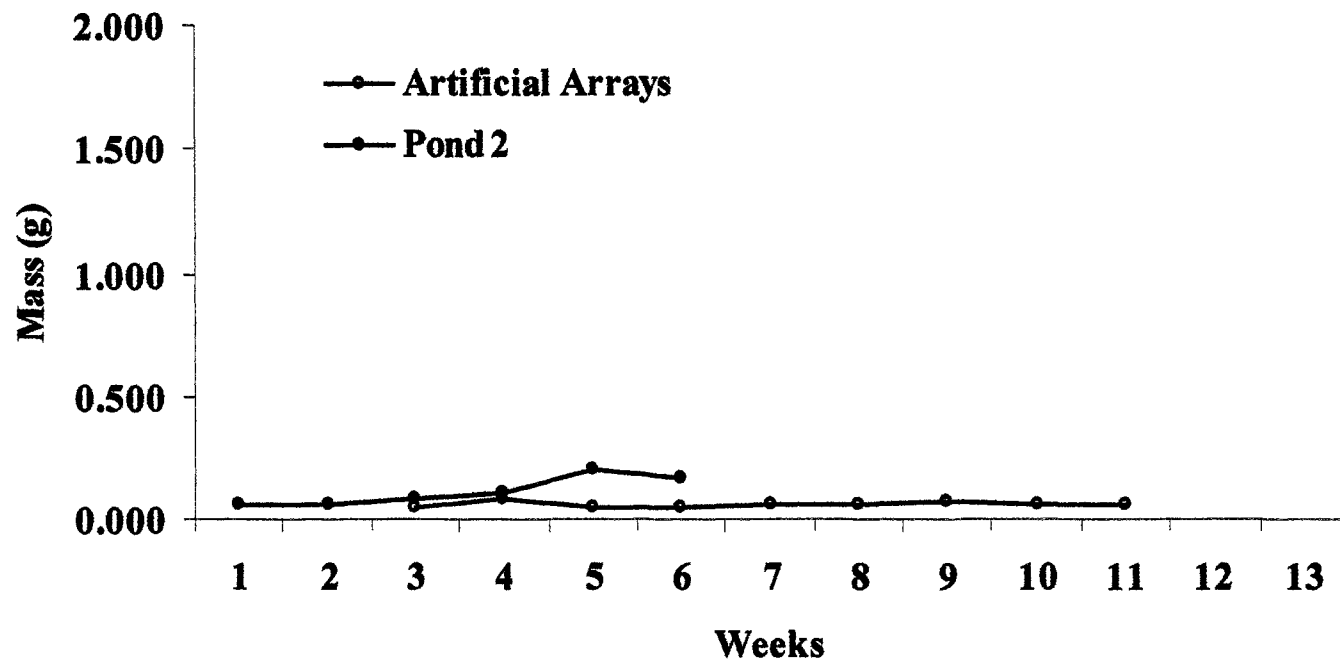


Figure 9. Initial mass of postmetamorphic *B. houstonensis* juveniles from one cohort in 2002. Data shown is average mass/day from 18 April 2002 to 25 June 2002. Half of the cohort was reared in a natural pond setting and the other half was reared in 24 artificial ponds. The solid blue diamond represents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

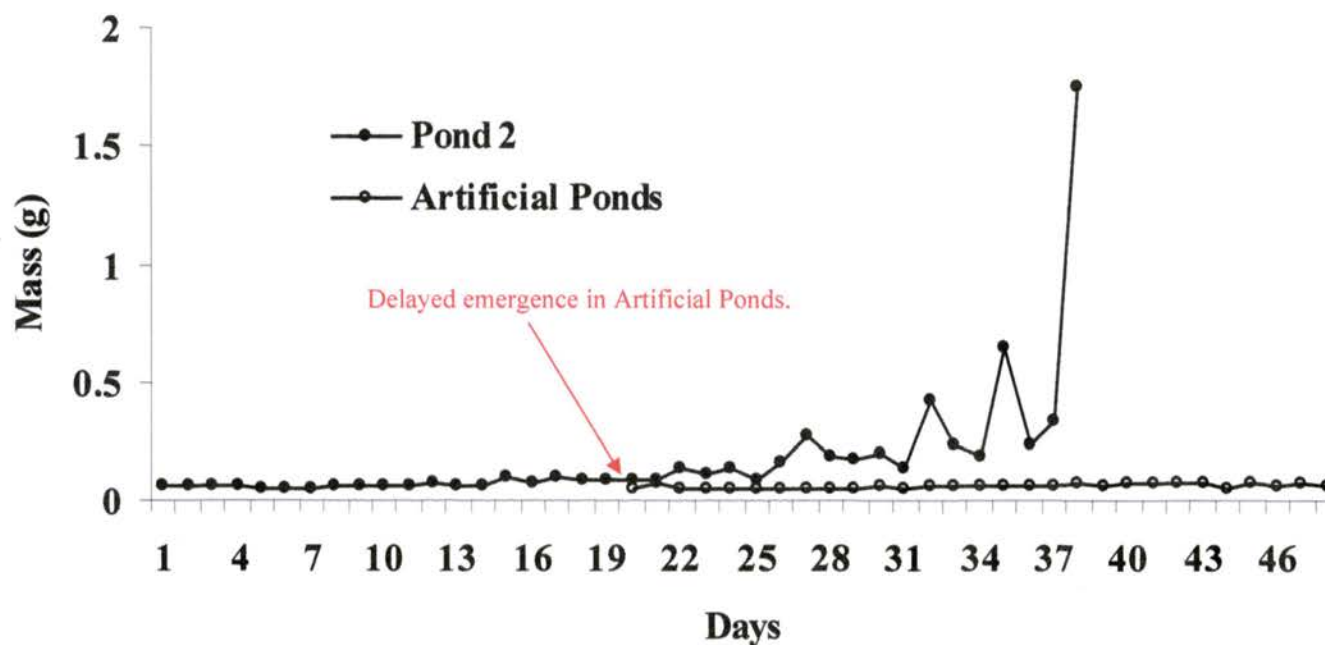


Figure 10. Initial mass of postmetamorphic *B. houstonensis* juveniles from one cohort in 2003. Data shown is average mass/day from 23 April 2003 to 20 July 2003. Half of the cohort was reared in a natural pond setting and the other half was reared in 24 artificial ponds. Juveniles were still found emerging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

was not a significant difference ($P = 0.12$) between Pond 2 and the artificial pond (Figure 9) initial masses. However, in 2003, there was a significant difference ($P = 0.03$) in the data between Pond 2 and the artificial pond (Figure 10) initial masses. This was a result from a modification in the artificial pond experiment during the second year (2003). In 2003, the artificial ponds were covered by a mesh lining that resulted in very different results. Emergence from the artificial array did not begin until almost 3 weeks after those in the natural pond in 2003. In 2002, juveniles began emerging from artificial ponds almost a week and a half after Pond 2 juveniles ceased to be found at all.

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

Evaluation of 2003 Data

Various ponds were surveyed for *B. houstonensis* juveniles in addition to the 2 main research sites (natural and artificial ponds). One privately owned pond (Ponderosa Dr., Bastrop County) had over 60 juveniles dispersing from a recently excavated pond. Sixty-nine measurements and tissue samples were taken from this site. Another privately owned pond near HWY 290 in Bastrop County was inspected. This pond yielded 18 juvenile tissue samples and measurements. Pond 11 on the GLR also had dispersing juveniles. Sixteen samples were taken from this site. The samples were determined to be



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

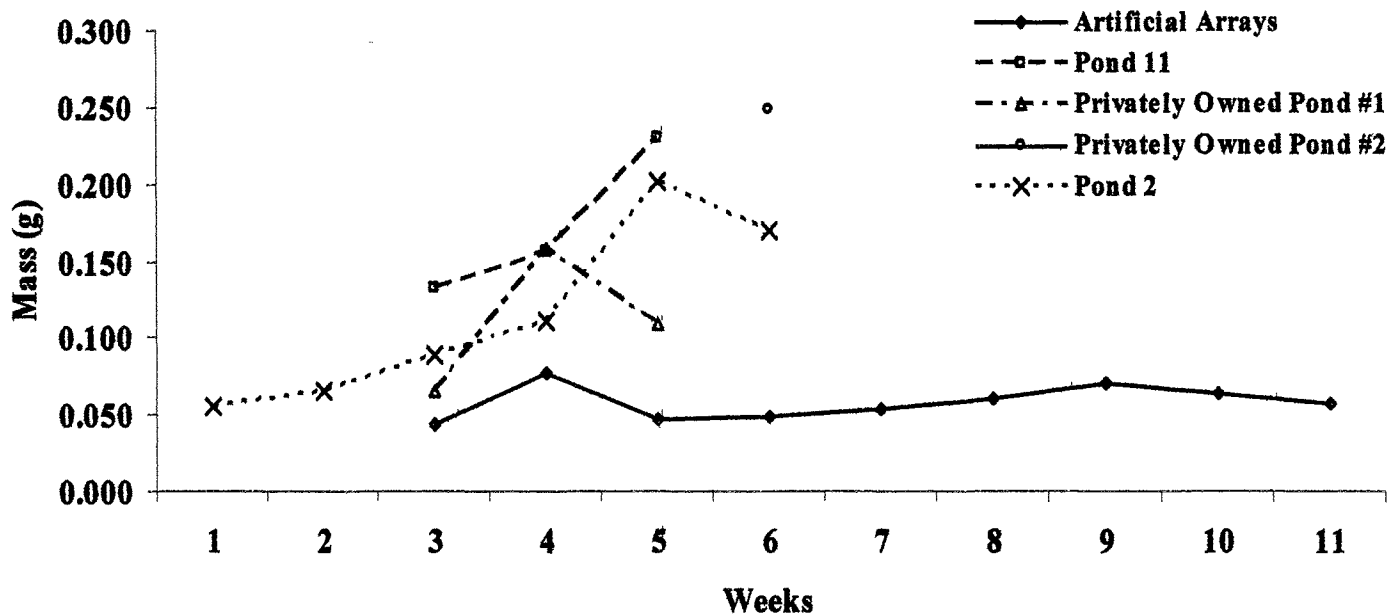


Figure 12. Initial mass of postmetamorphic *B. houstonensis* juveniles for 11 weeks in five ponds sites in Bastrop County. All pond sites used the same 11-week interval that began the last week of April and ended the second week of July. The solid blue diamond represents data collected from the artificial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

B. houstonensis tissue (APPENDIX 4) and a detailed description of each sample can be found in APPENDIX 5. The 5 different pond sites in Bastrop County for 2003 are compared in Figure 12. Growth from all but 1 pond shows a similar pattern. Growth for the artificial ponds was slow and inconsistent with the other 4 pond sites. Pond 2 had the highest average growth for each week. There was no significant difference among the 5 ponds ($P = 0.36$). The 2 privately owned ponds were compared and had no significant difference ($P = 0.12$).

MORPHOLOGICAL IDENTIFICATION

Assumptions have been made that species-discriminant morphological differentiation among bufonids cannot be identified until adulthood (Blair, 1972). However, morphological differences of *B. houstonensis* and sympatric *B. valliceps* were detected by 13 weeks after emergence. Figure 10 illustrates morphological distinction in juvenile *B. houstonensis* and *B. valliceps*. These photos were taken on 20 July 2003 in week 13 of the *B. houstonensis* post metamorphosis.

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

Morphological characteristics that distinguish *B. houstonensis* from *B. valliceps* were the presence of: continuous dorsal spots of light color and blotchiness, lack of a

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

PCR positive bands for *B. houstonensis* only primers and genus *Bufo* primers confirmed these morphological discriminations. Figure 14 illustrates the confirmation the predicted *B. houstonensis* tissue. All characterized to be *B. houstonensis* were confirmed. All those thought to be *B. valliceps* were negative on the gel using *B. houstonensis*-specific primers and were positive on *Bufo* genus-specific primers.

DISPERSAL PATTERNS

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

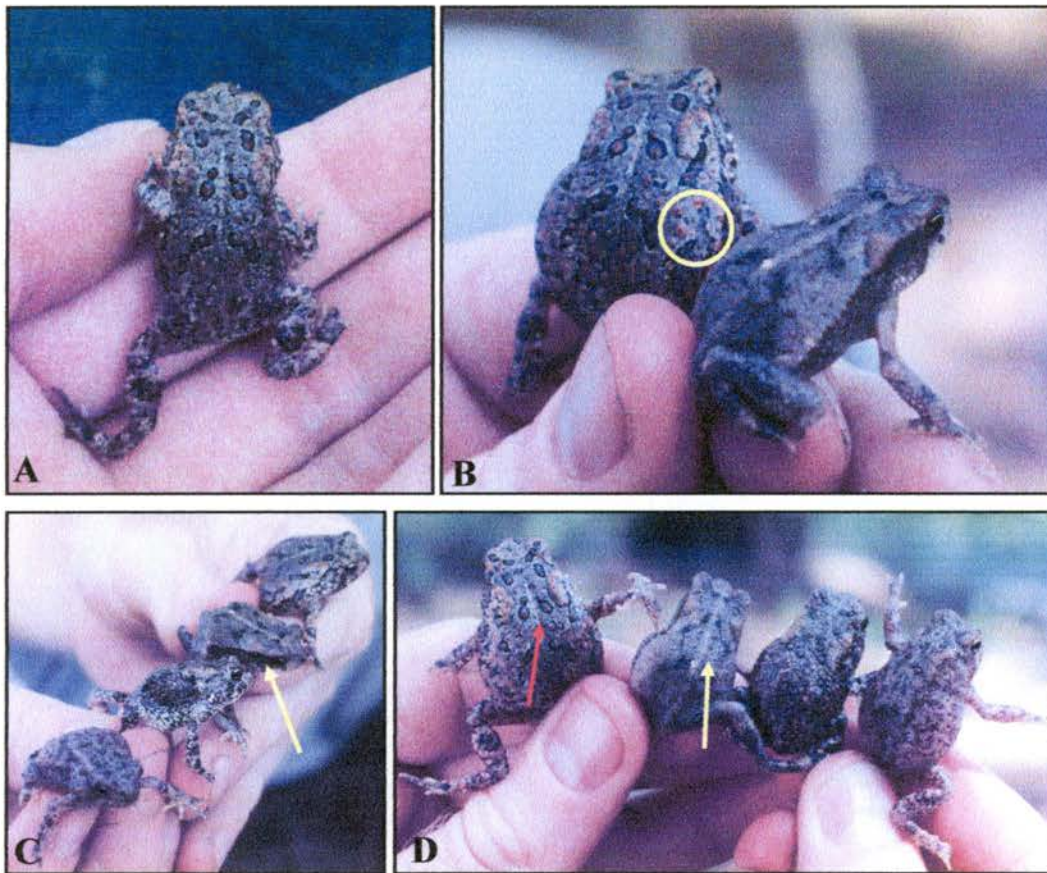


Figure 13. Four illustrations of morphological distinction. These photos were taken on 20 July 2003 that is week 13 of the 2003 *B. houstonensis* postmetamorphic emergence. *Bufo* juvenile illustrations depict (A) a *B. houstonensis* juvenile alone (wt=2.696 g), (B) the same *B. houstonensis* juvenile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

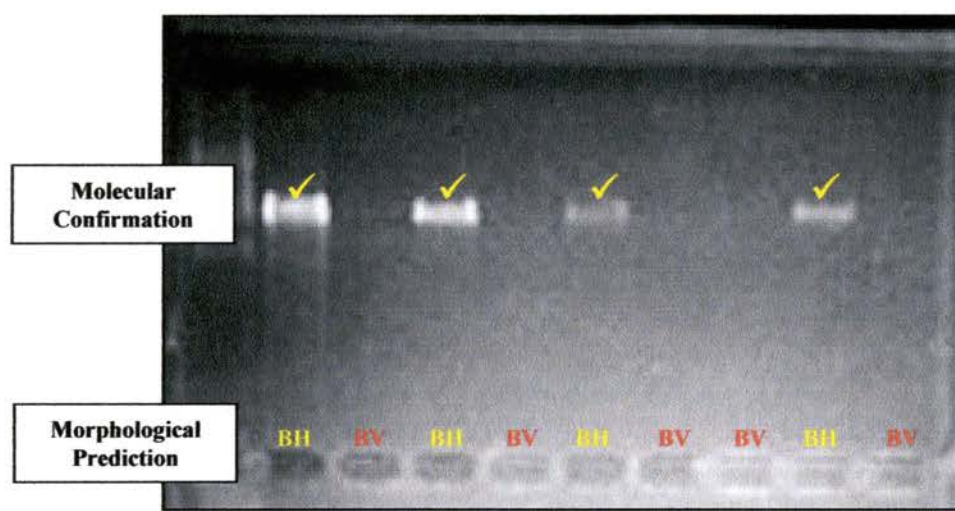


Figure 14. PCR results on a 1% Agarose gel using primers BHCB and BHDLR2. The row of morphological predictions represents the field assessment of morphological differences and the educated deduction as to which species the juvenile was. The molecular confirmation represents which tissue donor was *B.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

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

Figure 15 provides a topographical location of Pond 2, the breeding pond selected for this research and, the distribution of juvenile dispersal patterns within the first month of post emergence. Color is used to demonstrate correlation between time and the maximum scale of dispersal distance over which *Bufo* juveniles were found. By 5 days post-emergence, 116 juveniles were found dispersing up to 5 m away. By 10 days post-emergence, 104 juveniles were found dispersing up to 8 m away. By 15 days post-emergence, 55 juveniles were found dispersing up to 12 m away. By day 20 post-emergence, 31 juveniles were found dispersing up to 18 meters away. And by day 30 post-emergence, 11 juveniles were found up to 35 m away.

Beyond 30 days or 1 month, five *Bufo* juveniles were found in the large refugia 20 m - 35 m away from the pond in 2002 by late June (8 weeks post-emergence). Four juveniles were found in the small refugia up to 50 m away from Pond 2 in 2003 by late July (11 weeks post-emergence). Eighteen *Bufo* juveniles were found throughout the GLR in 2003, immediately only after significant precipitation occurred.

A correlation was found when comparing dispersal distance to juvenile mass. A greater correlation was recognized in 2002 ($r = 0.67$) than in 2003 ($r = 0.51$). However,

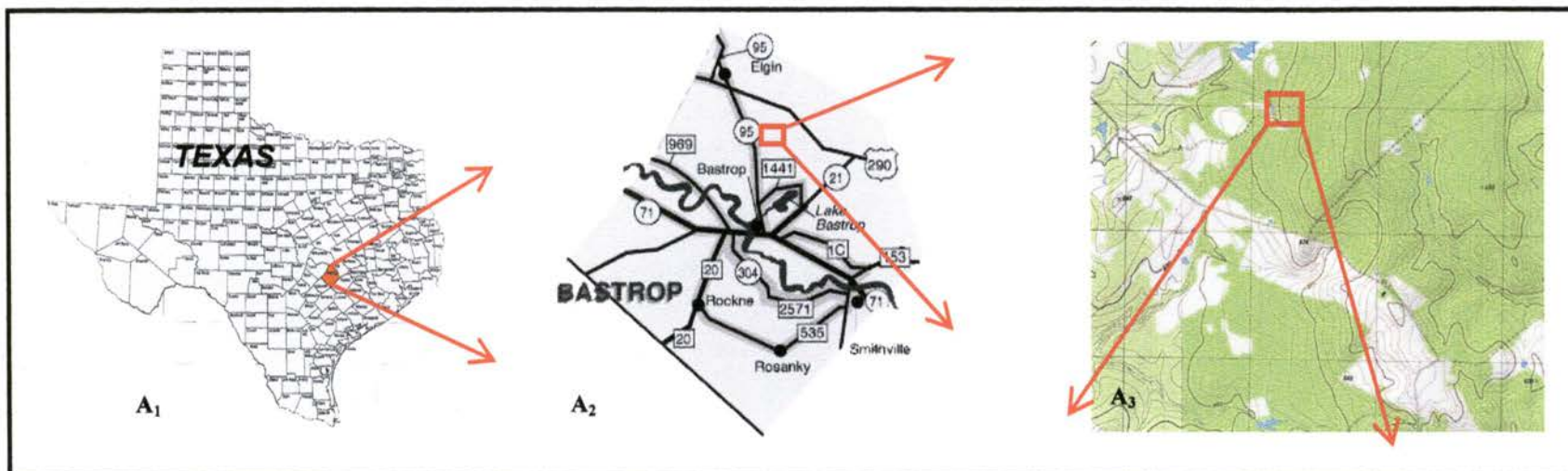
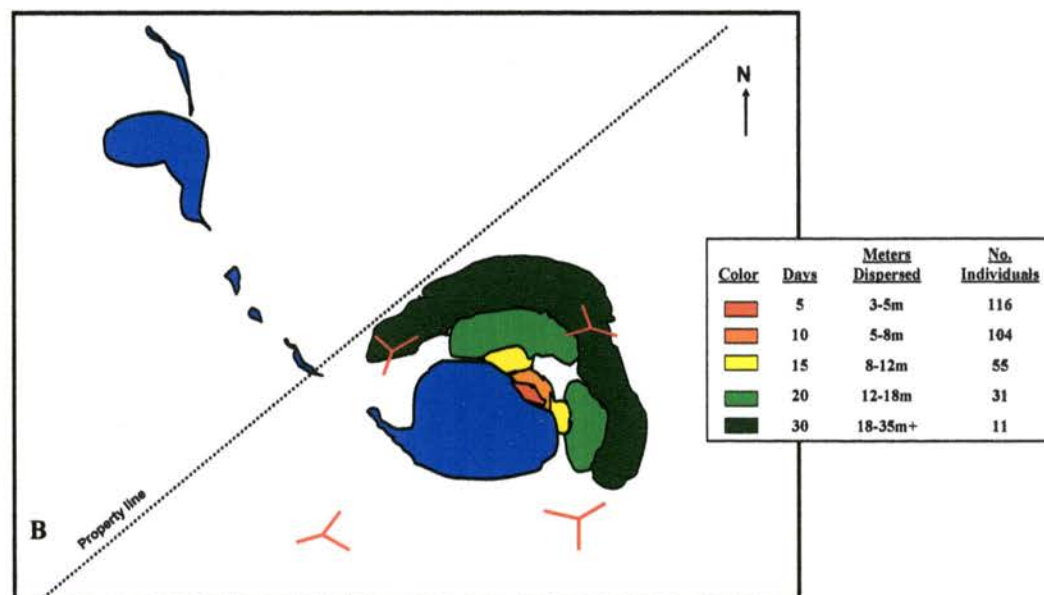


Figure 15. Topographical location of (A₁) Bastrop County, (A₂) Griffith League Ranch, and (A₃) Pond 2, the breeding pond selected for this research and, (B) enlargement of the red box in A₃ to show the distribution of juvenile dispersal patterns within the first month of post-emergence. The colors indicated the distribution (B) are explained in the adjacent chart where red represents the average dispersal of 3-5m at day 5, orange represents the average dispersal of 5-8m at day 10, yellow represents the average dispersal of 8-12m at day 15, light green represents the average dispersal of 12-18m at day 20, and dark green represents the average dispersal of 18-35m+ at day 30. The y-shaped permanent arrays are represented in red.



both years indicated a positive correlation. This positive relationship signified that larger juveniles disperse farther away from breeding ponds. Juvenile habitat preference was addressed. Because all sampling covered a large array of habitat, preference was examined during quadrat sampling. Four factors were addressed: soil moisture (moist vs. dry environment), habitat shading (shade vs. open habitat), soil type (sand vs. clay soils), and vegetation type (pine vs. mixed oak-juniper vegetation). If soil was damp to the touch, then soil was considered moist. Although incident sunlight was not quantified, shading can be described as less than 20% incident sunlight total on any part of a 1 meter grid. The soil was described as one of two types, either Carizzo sand or red clay subsoil. Although vegetation was not quantified, the vegetation type was noted during the quadrat surveys and categorized into either a loblolly pine forest or a mixed oak-juniper forest.

While no explicit statistical evaluation was made, several assumptions could be deduced from the data. All four habitat factors were identified during the extensive sampling around Pond 2. From the quadrat and refugia sampling, juveniles were present in areas of moisture and shade for the first 13 weeks post-emergence. Juveniles were not found in unshaded (> 80% incident sunlight) areas or in dry habitat. Juveniles were found in both sandy and clay soils and both vegetation types. Hence, soil and vegetation type were probably not factors in habitat choice. Consequently, an assumption that juveniles are most likely going to be found in moist, shaded habitat can be made.

DISCUSSION

GROWTH & DEVELOPMENT

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

Although significantly different from a captive bred study, those in the field are similar. This study had an average of 8.2 mm SUL and 0.065 g in 2002 and 8.0 mm SUL and 0.056 g in 2003 just after metamorphosis. These values are close to data obtained in three other studies that have measured the growth of post-metamorphic bufonids under field conditions (7.5 – 9.9 mm SUL for *B. woodhousei fowleri*: Clarke 1974, 0.050 g for *B. bufo*: Goater 1994; 7 – 9 mm SUL for *B. houstonensis*: Hillis et al. 1984) but is somewhat lower than that reported from a third study (10 mm SUL for *B. marinus*: Werner 1986).

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

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

The comparison of initial and recapture measurements for 2002 and 2003 did not indicate a significant difference. This result should not be surprising as these data are from the same individuals of a cohort. Figure 6 and Figure 7 indicate the precision in measurements of these data.

As expected, growth was rapid in the first weeks of life. Development can be inconsistent at this time, even among individuals from the same cohort. When 2 halves of the same cohort were divided into natural and artificial pond settings, results were not as expected (Figure 8). In 2002, mass from juveniles emerging from the artificial ponds was comparable to the natural pond and showed no significant difference ($P = 0.12$).

However, in 2003 there was a significant difference in the mass of juveniles emerging from artificial ponds and those emerging from the natural pond ($P = 0.03$). In 2003, black mesh screens were made to cover ponds and prevent non-experimental individuals from breeding. Temperature and incident light were reduced by 50% due to the screens. Consequently, the development of larval *B. houstonensis* was delayed. This delay extended metamorphosis to beyond 3 weeks after juveniles had emerged from the natural pond. Emergence was slow; only 1 or 2 juveniles were captured per day. In fact, emergence continued for at least 5 weeks after most individuals finished metamorphosis in the natural pond.

These results concur with a similar growth study on bufonid juveniles. Goater (1994) compared the size difference among juveniles of varied density. Goater (1994) stated that juveniles in the wild will grow larger due to the lack of confined space than those in a contained environment. Extreme resource limitation resulting in very low growth in size beyond emergence was also observed (Goater 1994). The limitation of resources in addition to the lower temperatures in the artificial ponds could have slowed metamorphosis in *B. houstonensis*.

Individuals from the artificial ponds not only encountered delayed metamorphosis, but were never recaptured again in either year. This absence was most likely related to unfavorable habitat conditions. The artificial ponds were located in an open field and received no shade or no protection for juveniles analogous to the forest cover at the other sites. This lack of protection increased chances for dessication. In addition, *Solenopsis invicta*, the imported fire ant, a known predator, was present in large numbers in this pasture. Ant mounds were observed daily around artificial arrays. Ants

were observed surrounding the water's edge when *B. houstonensis* juveniles were metamorphosing and emerging.

This absence of juveniles in an open field is also supported by the 2002 and 2003 natural pond site data where no juvenile was found in a sunlit and/or dry environment. This is important because the 100% absence of capture in an open field may imply that *B. houstonensis* metamorphic success in open fields or pastures is not likely. This is consistent with the fact that adult Houston toads have yet to be caught in the middle of an open field on the GLR (M. R. J. Forstner & T. M. Swannack, unpubl. data). Adults were caught along the perimeter of several open fields, but none were found in the center of an unshaded open field (M. R. J. Forstner & T. M. Swannack, unpubl. data).

Other research also supports these data. Although Rothermel (in press) had some juvenile migratory success in *B. americanus*, *Ambystoma texanum*, and *A. maculatum* (< 15% survivorship) in an open field, the impact open habitat had on multiple amphibians was determined to be negative. In a field/forest comparison study, *B. americanus* juveniles tended to avoid open-canopy habitat and recapture rates were much lower in a field setting (Rothermel and Semlitsch 2002).

Lower survival in fields implies that forest fragmentation is likely to reduce dispersal rates between local populations, with negative consequences for population persistence of the species tested. Decreased juvenile survival during emigration (Rothermel, in press) indicate that pastures or grasslands could act as Houston toad population sinks. The implication that open fields are detrimental to *B. houstonensis* juveniles and adults could lend support to conservation efforts, perhaps in the conservation/restoration of Houston toad corridors. Habitat alteration resulting in

continuous open grassy areas with no canopy cover should be considered detrimental to Houston toad juvenile dispersal and ultimately, its survival.

MORPHOLOGICAL IDENTIFICATION

Bufo juvenile species can be correctly identified using morphology at 13 weeks of age. However, this distinction has only been confirmed in 2 sympatric species, *B. houstonensis* and *B. valliceps*. Comparisons of *B. houstonensis* with other juveniles of congeners, such as *B. woodhousei* and *B. americanus*, should also be explored due to their potentially less distinct juvenile morphological differences. Therefore, more information must be gathered before this early differentiation can become reliable. However, these data are immediately useful in monitoring in Bastrop County and can be supplemented by DNA identification (APPENDIX 4).

Distinguishing the species morphologically at such an early age will aid surveyors of Houston toad habitat. Early detection, in addition to typical surveying procedures, might increase the accuracy of estimating populations and improve density estimates.

Emerging juveniles linger near the pond's edge for the first 3 weeks, providing time to survey for Houston toad habitat and establish its potential as a breeding pond. Surveyors performing night surveys may not detect Houston toad chorusing at a pond due to a variety of circumstances (weather conditions, time of night, the presence of a predator near a pond), but finding emerging juveniles documents the species' presence. By 13 weeks, species differences can be determined and can aid in proper identification of Houston toad, and viable habitats.

DISPERSAL PATTERNS

Results from monitoring dispersal and movement patterns show a juvenile presence in moist, shaded habitat. Juveniles immediately sought shade and cover and were most abundant in thick leaf litter. Either juveniles had a preference towards shadier, moist areas or they simply died in areas lacking in these qualities. Soil and vegetation type were neither preferred nor avoided as juveniles occurred in 2 types of soil and 2 types of vegetation (carrizo sand, red clay subsoil, pine, or oak-juniper forests). A preference for forest habitat (Semlitsch 2000a) is found in many amphibians and thus, supports these observations of shade preference. *B. americanus*, *A. texanum*, and *A. maculatum* all had difficulty migrating from an open field to a forest due to the dry, open conditions (Rothermel, in press). The migratory success of juvenile *B. houstonensis* was quite likely exacerbated by *Solenopsis invicta*.

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

These data show that the few survivors found used damp, protected areas. Refugia were useful in demonstrating that juveniles could be found in moist habitats. The small numbers of individuals that were found in these refugia may seem inconsequential, but compared to the absence of juveniles found when doing the quadrat surveys, these data are invaluable. This environment may also be a suitable habitat for a

more permanent refuge throughout the year and could be used as a potential habitat supplement for the conservation of isolated subpopulations.

Juveniles remained in the adjacent upland habitat for at least 13 weeks, with juveniles still caught within a 50 m radius surrounding the pond of emergence. Thomas and Allen (1997) observed *B. houstonensis* up to 40 m away but could only make assumptions as no mark-recapture methods were used. Hillis et al. (1984) observed *B. houstonensis* juveniles as far as 100 m from the ponds, but again, no mark-recapture techniques were used to confirm this. The migratory distances found in this study resulted in hard evidence that critical juvenile habitat extends beyond the immediate pond's edge.

The known radius (50 m) around the breeding pond can aid in the design of a buffer zone, where little to no human interaction in that zone is permitted. The buffer zone should be enforced while *B. houstonensis* juveniles are still highly concentrated around the pond and have yet to disperse fully. Beyond 12 weeks, juveniles are much less dense so there is less potential for the majority of juveniles to die from human or animal presence. Therefore, preventing these interactions would reduce impacts to habitat that juveniles use as shelter and protection.

Buffer zones in general have become beneficial to myriad species. Scientists studied nesting and egg deposition of three species of freshwater turtles (mud turtle, *Kinosternon subrubrum*; Florida cooter, *Pseudemys floridana*; and yellow-belly slider, *Trachemys scripta*) to determine core buffer zone habitat and concluded that 90% of individuals remain in a 73 m zone (Burke and Gibbons 1995). Another study involved 6 species of pond-breeding salamanders (Jefferson salamander, *Ambystoma jeffersonianum*;

spotted salamander, *A. maculatum*; marbled salamander, *A. opacum*; mole salamander, *A. talpoideum*; small-mouthed salamander, *A. texanum*; and tiger salamander, *A. tigrinum*) determined that juveniles dispersed as far as 247 m from the natal wetland pond (Semlitsch 1998). On average, 164 m would encompass 95% of the sample population. As a result, these species were protected and population viability was maintained.

This study's results give support for the contention of buffer zones for *B. houstonensis*. The 50 m layer of protection would encompass up to 95% of the sampled population. However, juvenile dispersal distances have not been determined beyond 13 weeks. A larger buffer zone should be then implemented to include the majority of the population beyond 13 weeks. Buffer zones serve to shelter critical habitat vital for feeding, growth, maturation, and maintenance of the entire juvenile and adult breeding population (Gill 1978, Scott 1994, Semlitsch et al. 1998) of *B. houstonensis*. A buffer zone would then serve to further protect populations by reducing the potential for edge effects that may penetrate into critical habitats (DeMaynadier and Hunter 1996).

Houston toad populations could then be given a better chance at survival and persistence.

CHAPTER 4

CONSERVATION IMPLICATIONS OF SURVIVORSHIP TECHNIQUES AND JUVENILE ECOLOGY IN THE HOUSTON TOAD, *BUFO HOUSTONENSIS*

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

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

include actions to ensure a high probability of juvenile survival (e.g., protection of critical habitat adjacent to the pond or wetland), will help maintain local species populations and provide dispersers for recolonization (Semlitsch 2000b).

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

Hatfield et al. (in press) conducted several Population Viability Analysis (PVA) computer simulations for *B. houstonensis* to estimate survival rates under a variety of circumstances. After multiple simulations, this study recognized that populations with 2% juvenile survival usually have a low probability of extinction. However, if juvenile survival is 1%, populations usually have a high probability of extinction.

Hatfield et al. (in press) specifically recommended determining whether the simulations performed were representative of low or high juvenile survival scenarios. Additional recommendations such as actual estimation of the number of eggs laid, estimation of the number of metamorphs, and marking/recapturing of juveniles should be completed. With these recommendations, the importance of juvenile data in assessing population dynamics is finally recognized.

Previous unpublished research (Forstner, M.R.J. and Swannack, T., pers. comm.) has estimated that by the first year of adulthood, 1 out of every 200 (0.05% survivorship) Houston toads survive. This percentage is much lower from the PHVA estimated 1%

survival rate where populations have a high probability of extinction (Hatfield et al. in press), indicating that Houston toads could possibly have an even higher extinction probability.

My research is similar to these studies as *B. houstonensis* survival from egg to metamorph was 4.7% (Chapter 2). There were 15% fewer juveniles estimated to be living by week 13 (1 quarter of a year) with a survivorship of 4.0%. Therefore, if a 15% decline occurs in *B. houstonensis* juveniles every 13 weeks, juveniles from the 2003 egg strands in Pond 2 will be almost completely lost by maturity (year 1) with an estimated survivorship of 0.03%, an even lower prediction than the 0.05% survival estimated from unpublished data (Forstner, M.R.J. and Swannack, T., pers. comm.).

Not only is the 15% decline most likely overestimated due to high egg number estimates, but Pond 2 is a best-case scenario where most other ponds had little or no productivity. These are just two factors that indicate the population is serious risk of extinction. Survivorship data from older juvenile (> 13 weeks) to adult is greatly needed to fill the gap in survivorship estimation. However, with this study's juvenile data, baseline survivorship data has been initiated where there was no prior research.

The estimation techniques developed for use in determining survivorship data (Chapter 2) are a vital addition in censusing Houston toad populations. The Wire Section model is the technique recommended for practical application. This technique yields high precision, quick maneuverability and simplicity. The model uses wires that are cut to the average length of 50 eggs to estimate the number of eggs in a cohort. Estimation time is 30 to 40 minutes. Conservationists and/or endangered species surveyors can use

this method to estimate egg numbers without displacement or manipulation that could cause detrimental effects, such as abnormalities or even mortality.

An understanding of the multiple aspects of juvenile ecology (Chapter 3) will impact the management of the Houston toad. Analysis of juvenile growth and developmental patterns are essential because it contributes to an understanding of the population processes of the species (Clarke 1974). Juvenile growth in the Houston toad was found to be both rapid and variable. When comparing this study's wild-caught data to that of previously published captive-bred data, it was not surprising that the wild-caught data would show lower growth rates. However, what could be beneficial management efforts is not that growth rates were lower, but that this study's growth data was comparable to other bufonid growth rates in 3 similar studies. Therefore, wild-caught juveniles, at least the ones that survive, are growing as well as can be expected considering multiple stressors involved.

It is important to note the correlation between SUL and mass because with such a high correlation, measurement assumptions can be made when there are time constraints in the field. By using the simple linear equation, $y = 10.2x + 7.5$, where $y = \text{SUL (mm)}$ and $x = \text{mass (g)}$, field biologists can then use just 1 measurement and calculate the other. Variability in years and cohorts due to climate conditions, habitat conditions around breeding ponds, and myriad other factors should be taken into consideration. A large sample should always be taken.

The juveniles observed were only found in areas of canopy-cover and moisture. Therefore, it is recommended that conservation efforts should focus on reducing large areas of open fields near and/or around Houston toad habitat or breeding ponds. If open

areas are already present near and/or around Houston toad habitat or breeding ponds, then vegetative reconstruction of native flora for canopy cover should be implemented when possible. In 2002, the Boy Scouts of America planted vegetation on the Griffith League Ranch to connect two habitats previously separated by a cleared field. They planted native flora along a natural drainage. 82% vegetation survival occurred and is an appropriate remedy to open fields.

Corridors could also be used to increase connectivity and provide canopy cover to nearby ponds (Beier and Noss 1998). However, corridors are controversial in that they can introduce invasive species, diseases, spread parasites, fire and other problems (Noss 1987, Beier and Loe 1992). In this case, the benefits out-weigh any negative factors because varied dispersal through suboptimal habitat is better than little or no dispersal.

In 2003, delayed growth of a cohort was discovered when tarps were placed over artificial ponds and juveniles emerging from these ponds took 3 weeks longer to metamorphose. The constant shade decreased the light exposure and pond temperature and, consequently, delayed emergence. The lack of required resources such as food or shelter could also have influenced the metamorphic delay. Together, temperature decrease and resource limitations created a suboptimal environment which ultimately prolonged metamorphosis.

Due to the relative density of water and the presence of aquatic vegetation, a longer amount of time is needed to heat up a pond than a terrestrial environment. Tadpoles need an optimum aquatic environment for normal metamorphosis to occur (Breven and Chadra 1988). Sun exposure above a pond will allow the pond to warm and speed up metamorphosis. Once tadpoles metamorphose into the harsh terrestrial

environment, shade is required for protection. Therefore, there should be little to no canopy cover above the pond, but rather around the pond, thus supporting relatively larger ponds than the ephemeral puddles often implied as suitable to the toad (Hillis et al. 1984). Relatively dense ground vegetation should either be planted or retained around the pond to prevent desiccation upon emergence.

B. houstonensis morphology, although thought to be slow in development (Blair 1972), will allow identification to the species level at 13 weeks post emergence for using these characteristics: the presence of continuous dorsal spots of light reddish color and blotchiness, lack of a conspicuous mid-dorsal line, and lack of dark lateral coloration. Molecular identification can also be used to distinguish bufonid juveniles using the polymerase chain reaction (PCR) marker system.

Both identification methods have their advantages and disadvantages. Morphological identification is simple, fast, cheap, and needs minimal training. The downside to morphology is that this identification can be unreliable and juveniles must develop characteristics before identification is possible (13-weeks). Molecular identification is much more accurate, reliable, and can be used immediately starting at the tadpole stage. However, extensive training and expenses are involved and the process is time consuming.

This study focused more so on morphological identification and solely used molecular analyses to confirm morphological identification predictions. To distinguish *Bufo* juveniles at the earliest age possible (save molecular analyses) would be beneficial to conservation managers when surveying Houston toad habitat. In addition to typical

surveying procedures, this earlier detection might increase the accuracy of estimating population persistence and yearly survival.

Juvenile dispersal is one of the key factors in determining species range in the critical metamorphosing period. The radius around a breeding pond in which most juveniles ($> 75\%$) inhabit would improve management practices. The radius around a breeding pond can act as a guide for the implementation of a buffer zone, where little to no human interaction is permitted. This implementation does not have to be permanent. However, it is preferable that this buffer zone is enforced during the time juveniles are concentrated around the breeding pond.

This would significantly decrease anthropogenic activities such as hunting, fishing, and cattle ranching. Houston toad breeding ponds can be associated with water tanks used for cattle. The reduction of cattle around breeding ponds would be a major improvement as incidental juvenile deaths due to cattle presence near breeding ponds would decrease. The reduction of human interaction with Houston toad breeding ponds during juvenile emergence and early dispersal would also prevent habitat damage and unnecessary mortality. Based on the results of this study, it is recommended that a buffer zone be implemented around known Houston toad breeding ponds at least 75 m in radius when in suitable Houston toad habitat between May and July. 75 m is recommended rather than the observed 50 m because the dispersal patterns beyond 13 weeks are still unknown. When patchy habitat borders Houston toad breeding ponds, the best scenario possible should be implemented, whether it is a partial buffer zone, a corridor, or varied vegetation reconstruction. Variation among breeding seasons and habitat should always be considered on an site-specific basis.

Given the survivorship results of this study, the risk of extinction is now reinforced. Immediate integration of these improved techniques into monitoring the remaining populations should occur. The next step is to improve the overall management strategy for long-term recovery. These results demonstrate the importance of the juvenile ecology in proper management techniques. Juvenile factors such as growth and development, morphology, and dispersal patterns are all equally important and ultimately help define population survivorship. Seven conservation recommendations are outlined in Table 2. These improvements seek to enhance the efficiency and productivity of Houston toad management practices. However, more juvenile data remains to be gathered. But with this initial baseline data, a better assessment of the current Houston toad population and where current management practices need to be is finally begun.

Table 1. Recommendations and helpful suggestions for the improvement of field techniques and management of the endangered Houston toad, *Bufo houstonensis*.

Recommendations For Management Improvements of the Houston toad, <i>Bufo houstonensis</i>
1. Prevent large, open fields in or near Houston toad habitat or breeding ponds. 2. If open fields exist, then conduct vegetative reconstruction for habitat rehabilitation. 3. Implement a protected 75 m buffer zone radius around Houston toad breeding ponds 4. If optimal habitat bordering breeding ponds is unavailable, the biologist's judgement must be used to determine the best scenario possible. 5. The buffer zone should remain implemented for the duration of May through July for juveniles and February to May for breeding. 6. For future construction of ponds, at least half of the pond should be unshaded by canopy cover. To compensate for excessive sunlight exposure, low ground vegetation or structures should be either planted or retained.
Suggestions in Improving Field Techniques
1. For egg number estimation used in survivorship calculations, use the Wire Section Model as it is efficient, simple in concept, and disturbance-free. 2. When in the field, morphological identification will be efficient as long as characteristics of at least 13-week-old juveniles are used. 3. If time constraints prevent complete measurement of juvenile, either measure mass (g) or SUL (mm) and use equation to determine missing measurement: $y = 10.2x + 7.5$.

APPENDIX 1

2001 Houston toad juvenile mark and recapture data, maximum temperature (°C), minimum temperature (°C), dew point (°C), precipitation in cm, and percent moon cover for the observation dates.

Date, <i>t</i>	Number of juveniles caught* C_t	Number of recaptures R_t	Number of newly marked	Marked juveniles at large M_t	Max Temp (°C)	Min Temp (°C)	Dew Point (°C)	Precip. (cm)	Moon %
6/11/2001	3	0	3	0	33.9	19.4	20.5	0	72
6/13/2001	3	0	3	3	35.6	25	23.3	0	54
6/16/2001	2	0	2	6	33.9	20	21.2	0	25
6/17/2001	4	0	4	9	33.9	21.1	20.6	0	17
7/3/2001	1	0	1	12	32.2	19	20.7	0	97
7/7/2001	1	0	1	13	35	21.7	20.8	0	96
7/8/2001	1	0	1	14	34.4	20.6	31	0	91
Totals	15	0	15	15					

*This data was collected by M. Gaston

APPENDIX 2

2002 Houston toad juvenile mark and recapture data, maximum temperature (°C), minimum temperature (°C), dew point (°C), precipitation in cm, and percent moon cover for the observation dates.

Date, <i>t</i>	Number of juveniles caught* C_t	Number of recaptures R_t	Number of newly marked	Marked juveniles at large M_t	Max Temp (°C)	Min Temp (°C)	Dew Point (°C)	Precip. (cm)	Moon %
4/18/2002	30	0	30	0	30	21	20.6	0	31
4/19/2002	8	0	8	30	30	21	20.2	0	42
4/20/2002	38	4	34	38	31	22	19.9	0	53
4/21/2002	60	0	60	72	29	20	20.9	0	64
4/22/2002	116	54	62	132	31	19	20.2	0	74
4/23/2002	104	71	33	194	31	20	20	0	84
4/24/2002	111	80	31	227	32	20	20.4	0	92
4/25/2002	71	53	18	258	28	18	19.3	0	97
4/26/2002	76	54	22	276	31	20	19.8	0	100
4/27/2002	28	24	4	298	32	22	20.9	0	99
4/29/2002	30	21	9	302	34	22	21.4	0	91
4/30/2002	19	18	1	311	34	21	20.4	0	83
5/1/2002	8	7	1	312	35	22	20.6	0	75
5/2/2002	18	18	0	313	32	22	20.2	0	65
5/3/2002	22	22	0	313	28	17	17.6	0	55
5/4/2002	20	20	0	313	31	16	20.9	0	46
5/5/2002	15	15	0	313	34	23	22.1	0	36
5/6/2002	7	7	0	313	33	23	21.2	0	27
5/7/2002	9	9	0	313	32	24	22.4	0	19
5/8/2002	4	4	0	313	33	23	21.3	0	13
5/9/2002	6	6	0	313	33	22	22.3	0	7
5/10/2002	6	6	0	313	33	23	21	0	3
5/11/2002	5	5	0	313	34	24	20.9	0	0
5/12/2002	3	3	0	313	33	24	21.5	0	0
5/13/2002	4	4	0	313	27	14	11.2	0	2
5/14/2002	3	3	0	313	27	11	10.5	0	5
5/17/2002	1	1	0	313	32	20	19.4	0	28
5/26/2002	2	2	0	313	33	19	19.3	0	100
5/27/2002	2	2	0	313	33	17	18.6	0	98
5/29/2002	3	1	2	313	30	19	20	0	87
6/10/2002	1	0	1	315	36	25	21.8	0	0
6/15/2002	1	0	1	316	33	21	17.6	0	25
6/16/2002	1	0	1	317	31	19	20	3	36
6/19/2002	2	0	2	318	36	22	20	0	69
6/24/2002	2	0	2	320	35	18	18.1	0	100
6/25/2002	2	0	2	322	36	19	20.8	0	99
Totals	838	514	324	324					

APPENDIX 3

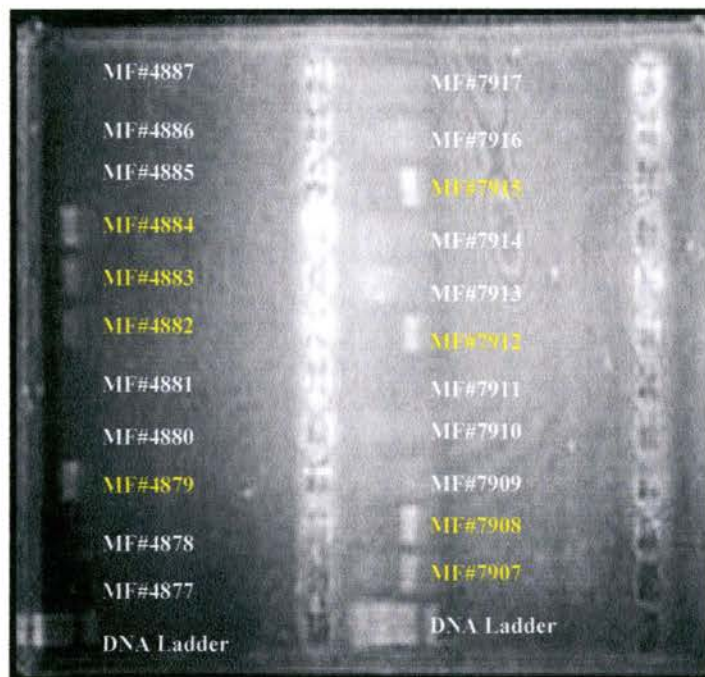
2003 Houston toad juvenile mark and recapture data, maximum temperature (°C), minimum temperature (°C), dew point (°C), average percent humidity, precipitation in cm, and percent moon cover for the observation dates.

Date, <i>t</i>	Number of juveniles caught C_t	Number of recaptures R_t	Number of newly marked	Marked juveniles at large M_t	Max Temp (°C)	Min Temp (°C)	Dew Point (°C)	Average Humidity %	Precip. (cm)	Moon %
4/23/2003	7	0	7	0	27	18	19.1	80	0	48
4/24/2003	5	0	5	7	34	11	14.2	51	0	37
4/25/2003	18	5	13	12	31	10	9	37	0	28
4/26/2003	92	21	71	25	32	19	12.3	56	0	19
4/27/2003	71	51	20	96	32	13	14.8	64	0	12
4/28/2003	62	37	25	116	31	17	17.5	67	0	7
4/29/2003	53	17	36	141	28	17	19.3	78	0	3
4/30/2003	40	31	9	177	30	20	20.8	79	0	1
5/1/2003	47	29	18	186	31	21	21.3	72	0	0
5/2/2003	37	21	16	204	31	22	21.7	72	0	1
5/3/2003	24	16	8	220	31	22	21.6	72	0	4
5/4/2003	28	20	8	228	33	23	21.9	72	0	9
5/5/2003	33	14	19	236	33	24	23.5	75	0	16
5/6/2003	27	17	10	255	29	24	23.4	84	0	23
5/7/2003	21	11	10	265	33	24	23.4	75	0	32
5/8/2003	16	7	9	275	34	24	22.8	66	0	42
5/9/2003	12	6	6	284	33	23	22.3	67	0	53
5/10/2003	15	7	8	290	32	24	22.8	72	0	64
5/11/2003	12	7	5	298	29	19	17.7	61	0	74
5/12/2003	8	5	3	303	23	17	15.9	67	1	83
5/13/2003	4	4	0	306	33	19	20.5	70	0	91
5/14/2003	7	5	2	306	36	22	21.6	65	0	97
5/15/2003	7	7	0	308	36	23	22.3	63	0	100
5/16/2003	7	7	0	167	36	24	21.9	58	0	99
5/17/2003	6	4	2	308	34	17	15.7	45	0	96
5/18/2003	4	3	1	310	34	16	18	62	0	90
5/19/2003	7	5	2	311	36	19	21.7	65	0	82
5/20/2003	4	3	1	313	27	17	20	71	0	73
5/21/2003	5	4	1	314	27	17	16.2	71	0	63
5/22/2003	6	4	2	315	29	18	17.8	69	0	53
5/23/2003	4	4	0	317	33	16	18	64	0	43
5/24/2003	5	5	0	317	34	19	19.5	63	0	33
5/25/2003	6	6	0	317	32	19	19.4	64	0	25
5/26/2003	3	3	0	317	30	19	19.2	71	0	17
5/27/2003	6	5	1	317	31	20	18.4	64	0	10
5/28/2003	7	7	0	318	31	13	13.2	53	0	5
5/29/2003	5	5	0	318	36	12	14.4	54	0	2
5/30/2003	5	4	1	318	38	17	16.5	44	0	0
5/31/2003	3	3	0	319	37	19	17	45	0	0
6/1/2003	9	9	0	319	36	18	20.5	63	0	2
6/2/2003	9	8	1	319	34	21	21.3	64	0	6
6/3/2003	7	7	0	320	35	19	21.6	66	0	12
6/4/2003	8	8	0	320	33	20	20.3	70	2	19
6/5/2003	5	5	0	320	28	19	21.7	79	2	28

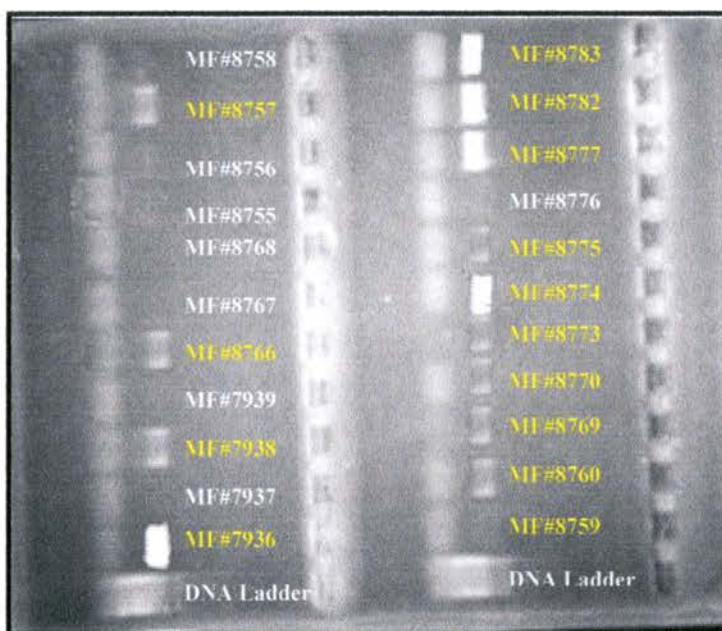
6/6/2003	3	2	1	320	30	18	18.7	71	0	38
6/7/2003	1	1	0	321	32	15	17.6	67	0	49
6/11/2003	1	1	0	321	36	23	23.3	70	0	89
6/15/2003	1	1	0	321	29	20	20.8	73	1	98
6/19/2003	1	1	0	321	34	19	19.5	60	0	68
7/9/2003	1	1	0	321	32	22	23.1	73	0	79
7/15/2003	1	1	0	321	30	21	21.4	65	0	95
7/20/2003	4	0	4	321	34	21	22	75	0	55
Totals	780	455	325	325						

APPENDIX 4

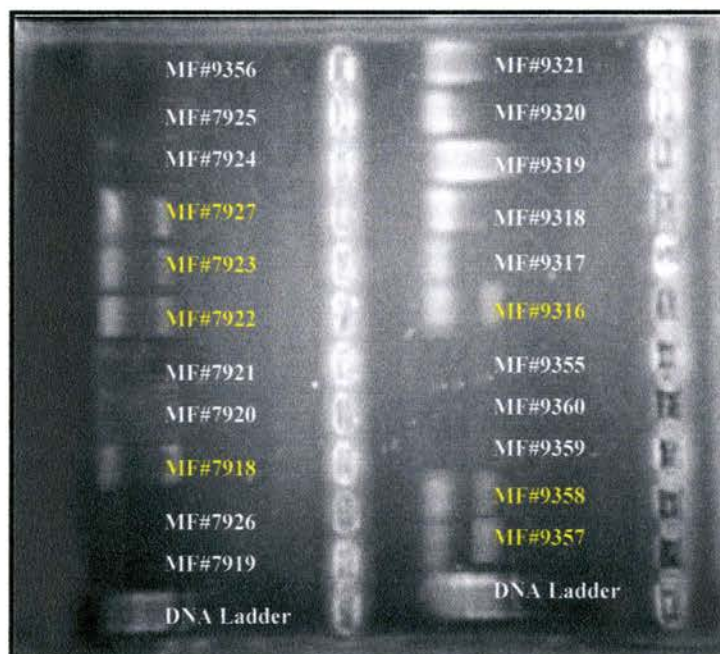
PCR Molecular identification results of juvenile *Bufo* tissue samples ranging from MF#4867 - MF#9016 using a 1% Agarose gel and *Bufo houstonensis* specific primers, BHCB and BHDLR2. Samples in yellow are *B. houstonensis* positive. Samples in white are inconclusive. These samples were run again and were attempted to be identified a second time.



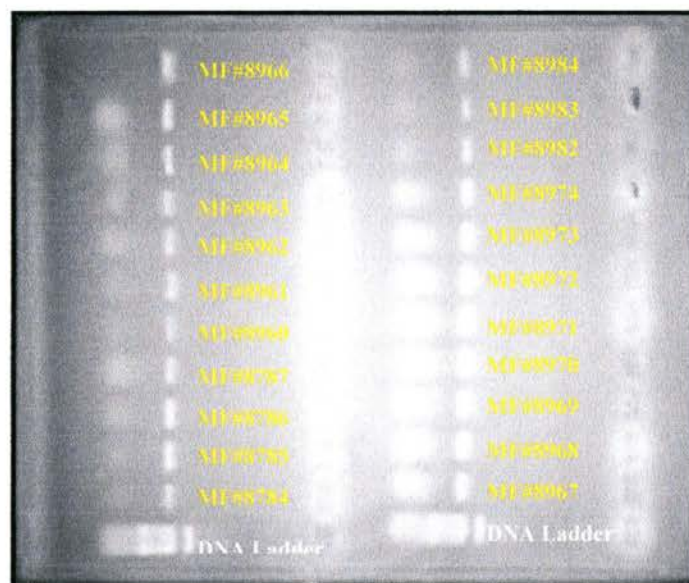
Bufo tissue samples from MF#4877 - MF#4887 and from MF#7907 - MF#7917.



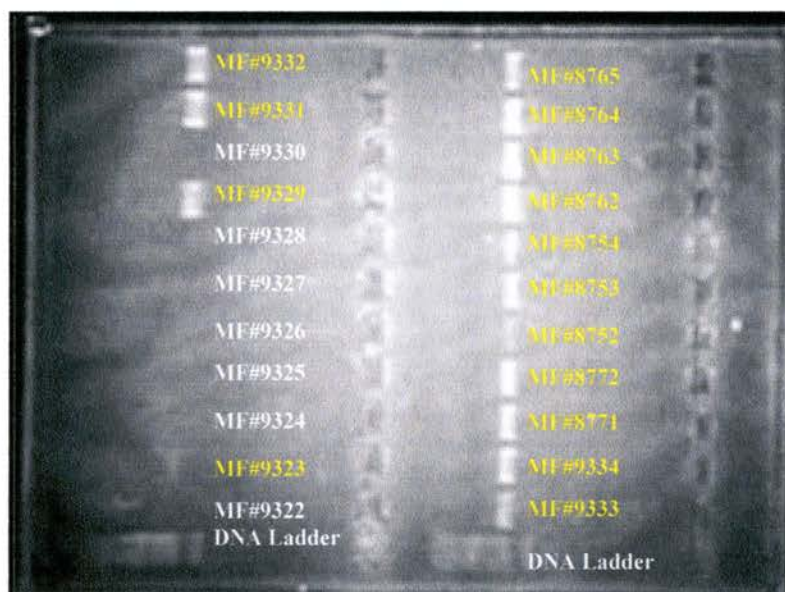
Bufo tissue samples from MF#7936 - MF#7939, from MF#8766 - MF#8760, and from MF#8769 - MF#8783



Bufo tissue samples from MF#7919 - MF#7927, from MF#9355– MF#9360, and from MF#9316 - MF#9321



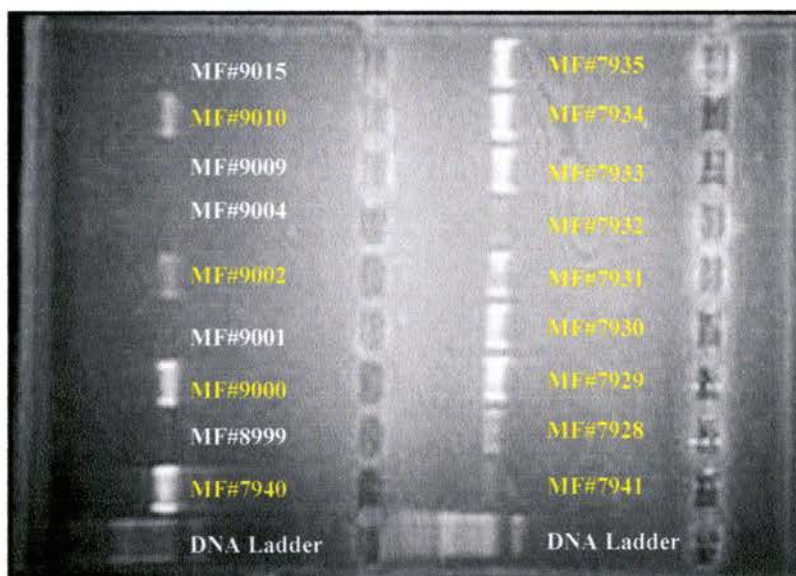
Bufo tissue samples from MF#8784 - MF#8787 and from MF#8960– MF#



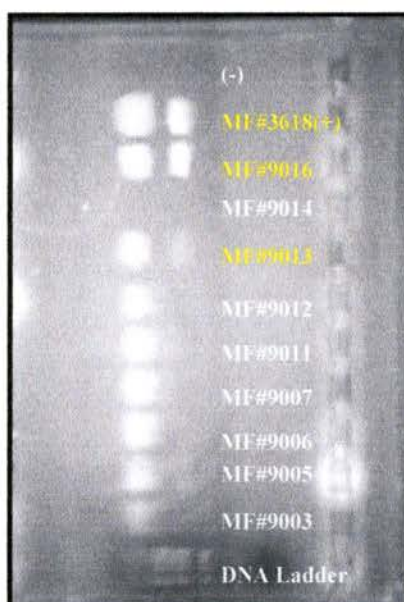
Bufo tissue samples from MF#9322 - MF#9334 and from MF#8752– MF#8772



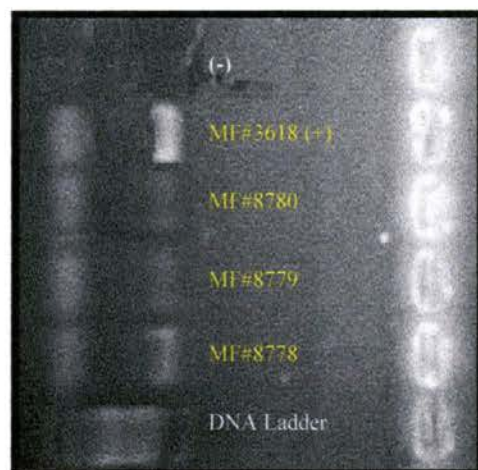
Bufo tissue samples from MF#8985 - MF#8997 and from MF#8784– MF#8788



Bufo tissue samples from MF#7928 - MF#7940, and from MF#8999 - MF#9015



Bufo tissue samples from MF#9003 - MF#9016



Bufo tissue samples from MF#8778 - MF#8780



Bufo tissue samples from MF#8789 - MF#8792

APPENDIX 5

Juvenile Houston toad tissue catalog data showing the date collected, catalog number (MF), Genus, Species, specific locality, additional data, PCR status, and species results. A presence (+) on the PCR gel indicates the tissue was molecularly identified as *B. houstonensis*. The absence of a band (-) indicates that the tissue was either not *B. houstonensis* or the procedure for PCR was not used correctly. The number adjacent to a “-” indicates how many PCRs were performed.

Date Collected	MF #	Genus	Species	Specific Locality	Additional Data	PCR Status*
6/11/2001	MF# 4867	<i>Bufo</i>	<i>species</i>	GLR	collected by M. Gaston, 2001 tDNA only	-
6/11/2001	MF# 4868	<i>Bufo</i>	<i>species</i>	GLR	"	-
6/11/2001	MF# 4869	<i>Bufo</i>	<i>species</i>	GLR	"	-
6/11/2001	MF# 4870	<i>Bufo</i>	<i>species</i>	GLR	"	-
6/11/2001	MF# 4871	<i>Bufo</i>	<i>species</i>	GLR	"	-
6/11/2001	MF# 4872	<i>Bufo</i>	<i>species</i>	GLR	"	-
6/11/2001	MF# 4873	<i>Bufo</i>	<i>species</i>	GLR	"	-
6/11/2001	MF# 4874	<i>Bufo</i>	<i>species</i>	GLR	"	-
6/11/2001	MF# 4875	<i>Bufo</i>	<i>species</i>	GLR	"	-
6/11/2001	MF# 4876	<i>Bufo</i>	<i>species</i>	GLR	"	-
6/11/2001	MF# 4877	<i>Bufo</i>	<i>species</i>	GLR	collected by M. Gaston	-
6/13/2001	MF# 4878	<i>Bufo</i>	<i>species</i>	GLR	"	-
6/13/2001	MF# 4879	<i>Bufo</i>	<i>species</i>	GLR	"	+
6/13/2001	MF# 4880	<i>Bufo</i>	<i>species</i>	GLR	"	-
6/13/2001	MF# 4881	<i>Bufo</i>	<i>species</i>	GLR	"	-
6/13/2001	MF# 4882	<i>Bufo</i>	<i>species</i>	GLR	"	+
6/17/2001	MF# 4883	<i>Bufo</i>	<i>species</i>	GLR	"	+
6/17/2001	MF# 4884	<i>Bufo</i>	<i>species</i>	GLR	"	+
6/17/2001	MF# 4885	<i>Bufo</i>	<i>species</i>	GLR	"	-
6/17/2001	MF# 4886	<i>Bufo</i>	<i>species</i>	GLR	"	-
6/17/2001	MF# 4887	<i>Bufo</i>	<i>species</i>	GLR	"	-
4/18/2002	MF# 7940	<i>Bufo</i>	<i>species</i>	GLR	p2, 1st array emergence	+
4/19/2002	MF# 7928	<i>Bufo</i>	<i>species</i>	GLR	"	+
4/19/2002	MF# 7929	<i>Bufo</i>	<i>species</i>	GLR	"	+
4/19/2002	MF# 7930	<i>Bufo</i>	<i>species</i>	GLR	"	+
4/19/2002	MF# 7931	<i>Bufo</i>	<i>species</i>	GLR	"	+
4/19/2002	MF# 7932	<i>Bufo</i>	<i>species</i>	GLR	"	+
4/19/2002	MF# 7933	<i>Bufo</i>	<i>species</i>	GLR	"	+
4/19/2002	MF# 7934	<i>Bufo</i>	<i>species</i>	GLR	"	+
4/19/2002	MF# 7935	<i>Bufo</i>	<i>species</i>	GLR	"	+
4/19/2002	MF# 7936	<i>Bufo</i>	<i>species</i>	GLR	"	+
4/19/2002	MF# 7937	<i>Bufo</i>	<i>species</i>	GLR	"	2-
4/19/2002	MF# 7938	<i>Bufo</i>	<i>species</i>	GLR	"	+
4/19/2002	MF# 7939	<i>Bufo</i>	<i>species</i>	GLR	"	2-
4/21/2002	MF#9316	<i>Bufo</i>	<i>species</i>	GLR	"	+
4/21/2002	MF#9317	<i>Bufo</i>	<i>species</i>	GLR	"	2-
4/21/2002	MF#9318	<i>Bufo</i>	<i>species</i>	GLR	"	2-
4/21/2002	MF#9319	<i>Bufo</i>	<i>species</i>	GLR	"	2-
4/21/2002	MF#9320	<i>Bufo</i>	<i>species</i>	GLR	"	2-

4/21/2002	MF#9321	Bufo	species	GLR	"	2-
4/21/2002	MF#9322	Bufo	species	GLR	"	2-
4/21/2002	MF#9323	Bufo	species	GLR	"	+
4/21/2002	MF#9324	Bufo	species	GLR	"	2-
4/21/2002	MF#9325	Bufo	species	GLR	"	2-
5/2/2002	MF# 7907	Bufo	species	GLR		+
5/3/2002	MF# 7908	Bufo	species	GLR	pond 15, ants ate fec	+
5/3/2002	MF# 7909	Bufo	species	GLR	pond 15	2-
5/3/2002	MF# 7910	Bufo	species	GLR	"	2-
5/3/2002	MF# 7911	Bufo	species	GLR	"	2-
5/3/2002	MF# 7912	Bufo	species	GLR	pond 15, died of ants	+
5/3/2002	MF# 7913	Bufo	species	GLR	pond 15	2-
5/3/2002	MF# 7914	Bufo	species	GLR	"	2-
5/3/2002	MF# 7915	Bufo	species	GLR	pond 15, died of ants	+
5/3/2002	MF# 7916	Bufo	species	GLR	pond 15	2-
5/3/2002	MF# 7917	Bufo	species	GLR	"	2-
5/3/2002	MF# 7919	Bufo	species	GLR	pond 19	2-
5/26/2002	MF# 7926	Bufo	species	GLR	array F	2-
5/29/2002	MF# 7918	Bufo	species	GLR	array B-F2	+
5/30/2002	MF# 7920	Bufo	species	GLR	array F	2-
6/10/2002	MF# 7921	Bufo	species	GLR	"	-
6/16/2002	MF# 7922	Bufo	species	GLR	F10 Array	+
6/19/2002	MF# 7923	Bufo	species	GLR	refugia south of pond 2	+
6/24/2002	MF# 7927	Bufo	species	GLR	refugia NW of pond 2	+
6/25/2002	MF# 7924	Bufo	species	GLR		2-
6/25/2002	MF# 7925	Bufo	species	GLR		2-
4/24/2003	MF#9356	Bufo	species	GLR	p2, found dead	2-
4/24/2003	MF#9357	Bufo	species	GLR	"	2-
4/24/2003	MF#9358	Bufo	species	GLR	"	+
4/24/2003	MF#9359	Bufo	species	GLR	"	+
4/24/2003	MF#9360	Bufo	species	GLR	"	2-
4/25/2003	MF#9355	Bufo	species	GLR	p2, 1st array emergence	2-
4/26/2003	MF#9326	Bufo	species	GLR	"	2-
4/26/2003	MF#9327	Bufo	species	GLR	"	2-
4/26/2003	MF#9328	Bufo	species	GLR	"	2-
4/26/2003	MF#9329	Bufo	species	GLR	"	+
4/26/2003	MF#9330	Bufo	species	GLR	"	2-
4/26/2003	MF#9331	Bufo	species	GLR	"	+
4/26/2003	MF#9332	Bufo	species	GLR	"	+
4/26/2003	MF#9333	Bufo	species	GLR	"	+
4/26/2003	MF#9334	Bufo	species	GLR	"	+
4/26/2003	MF#9335	Bufo	species	GLR	"	
4/26/2003	MF#9336	Bufo	species	GLR	"	
4/26/2003	MF#9337	Bufo	species	GLR	"	
4/26/2003	MF#9338	Bufo	species	GLR	"	
4/26/2003	MF#9339	Bufo	species	GLR	"	
4/26/2003	MF#9340	Bufo	species	GLR	"	
4/26/2003	MF#9341	Bufo	species	GLR	"	
4/26/2003	MF#9342	Bufo	species	GLR	"	
4/26/2003	MF#9343	Bufo	species	GLR	"	

4/26/2003	MF#9344	<i>Bufo</i>	<i>species</i>	GLR	"	
4/26/2003	MF#9345	<i>Bufo</i>	<i>species</i>	GLR	"	
4/26/2003	MF#9346	<i>Bufo</i>	<i>species</i>	GLR	"	
4/26/2003	MF#9347	<i>Bufo</i>	<i>species</i>	GLR	"	
4/26/2003	MF#9348	<i>Bufo</i>	<i>species</i>	GLR	"	
5/10/2003	MF#8771	<i>Bufo</i>	<i>species</i>	PO#1	additional pond site; privately owned	+
5/10/2003	MF#8772	<i>Bufo</i>	<i>species</i>	PO#1	"	+
5/11/2003	MF#8752	<i>Bufo</i>	<i>species</i>	PO#2	"	+
5/11/2003	MF#8753	<i>Bufo</i>	<i>species</i>	PO#2	"	+
5/11/2003	MF#8754	<i>Bufo</i>	<i>species</i>	PO#2	"	+
5/11/2003	MF#8762	<i>Bufo</i>	<i>species</i>	PO#2	"	+
5/11/2003	MF#8763	<i>Bufo</i>	<i>species</i>	PO#2	"	+
5/11/2003	MF#8764	<i>Bufo</i>	<i>species</i>	PO#2	"	+
5/11/2003	MF#8765	<i>Bufo</i>	<i>species</i>	PO#2	"	+
5/11/2003	MF#8766	<i>Bufo</i>	<i>species</i>	PO#2	"	+
5/11/2003	MF#8767	<i>Bufo</i>	<i>species</i>	PO#2	"	2-
5/11/2003	MF#8768	<i>Bufo</i>	<i>species</i>	PO#2	"	2-
5/13/2003	MF#8755	<i>Bufo</i>	<i>species</i>	PO#2	"	2-
5/13/2003	MF#8756	<i>Bufo</i>	<i>species</i>	PO#2	"	2-
5/13/2003	MF#8757	<i>Bufo</i>	<i>species</i>	PO#2	"	+
5/13/2003	MF#8758	<i>Bufo</i>	<i>species</i>	PO#2	"	2-
5/13/2003	MF#8759	<i>Bufo</i>	<i>species</i>	PO#2	"	2-
5/13/2003	MF#8760	<i>Bufo</i>	<i>species</i>	PO#2	"	+
5/14/2003	MF#8769	<i>Bufo</i>	<i>species</i>	PO#1	"	+
5/14/2003	MF#8770	<i>Bufo</i>	<i>species</i>	PO#1	"	+
5/14/2003	MF#8773	<i>Bufo</i>	<i>species</i>	PO#1	"	+
5/14/2003	MF#8774	<i>Bufo</i>	<i>species</i>	PO#1	"	+
5/15/2003	MF#8775	<i>Bufo</i>	<i>species</i>	PO#1	"	+
5/15/2003	MF#8776	<i>Bufo</i>	<i>species</i>	PO#1	"	2-
5/15/2003	MF#8777	<i>Bufo</i>	<i>species</i>	PO#1	"	+
5/15/2003	MF#8778	<i>Bufo</i>	<i>species</i>	PO#1	"	+
5/15/2003	MF#8779	<i>Bufo</i>	<i>species</i>	PO#1	"	+
5/15/2003	MF#8780	<i>Bufo</i>	<i>species</i>	PO#1	"	+
5/15/2003	MF#8781	<i>Bufo</i>	<i>species</i>	PO#1	"	2-
5/17/2003	MF#8782	<i>Bufo</i>	<i>species</i>	PO#1	"	+
5/17/2003	MF#8783	<i>Bufo</i>	<i>species</i>	PO#1	"	+
5/17/2003	MF#8784	<i>Bufo</i>	<i>species</i>	PO#1	"	2-
5/17/2003	MF#8785	<i>Bufo</i>	<i>species</i>	PO#1	"	+
5/17/2003	MF#8786	<i>Bufo</i>	<i>species</i>	PO#1	"	2-
5/17/2003	MF#8787	<i>Bufo</i>	<i>species</i>	PO#1	"	2-
5/17/2003	MF#8788	<i>Bufo</i>	<i>species</i>	PO#1	"	
5/17/2003	MF#8789	<i>Bufo</i>	<i>species</i>	PO#1	"	
5/17/2003	MF#8790	<i>Bufo</i>	<i>species</i>	PO#1	"	
5/17/2003	MF#8791	<i>Bufo</i>	<i>species</i>	PO#1	"	
5/17/2003	MF#8792	<i>Bufo</i>	<i>species</i>	PO#1	"	
5/17/2003	MF#8793	<i>Bufo</i>	<i>species</i>	PO#1	"	
5/17/2003	MF#8794	<i>Bufo</i>	<i>species</i>	PO#1	"	
5/17/2003	MF#8795	<i>Bufo</i>	<i>species</i>	PO#1	"	
5/17/2003	MF#8796	<i>Bufo</i>	<i>species</i>	PO#1	"	
5/17/2003	MF#8797	<i>Bufo</i>	<i>species</i>	PO#1	"	

5/17/2003	MF#8798	Bufo	species	PO#1	"	
5/17/2003	MF#8799	Bufo	species	PO#1	"	
5/17/2003	MF#8800	Bufo	species	PO#1	"	
5/17/2003	MF#8945	Bufo	species	PO#1	"	
5/21/2003	MF#8946	Bufo	species	PO#1	"	
5/21/2003	MF#8947	Bufo	species	PO#1	"	
5/21/2003	MF#8948	Bufo	species	PO#1	"	
5/21/2003	MF#8949	Bufo	species	PO#1	"	
5/21/2003	MF#8950	Bufo	species	PO#1	"	
5/21/2003	MF#8951	Bufo	species	PO#1	"	
5/21/2003	MF#8952	Bufo	species	PO#1	"	
5/21/2003	MF#8953	Bufo	species	PO#1	"	
5/21/2003	MF#8954	Bufo	species	PO#1	"	
5/21/2003	MF#8955	Bufo	species	PO#1	"	
5/21/2003	MF#8956	Bufo	species	PO#1	"	
5/21/2003	MF#8957	Bufo	species	PO#1	"	
5/21/2003	MF#8958	Bufo	species	PO#1	"	
5/21/2003	MF#8959	Bufo	species	PO#1	"	
5/21/2003	MF#8960	Bufo	species	GLR	pond 11	+
5/21/2003	MF#8961	Bufo	species	GLR	"	+
5/21/2003	MF#8962	Bufo	species	GLR	"	+
5/21/2003	MF#8963	Bufo	species	GLR	"	+
5/21/2003	MF#8964	Bufo	species	GLR	"	+
5/21/2003	MF#8965	Bufo	species	GLR	"	+
5/21/2003	MF#8966	Bufo	species	GLR	"	+
5/21/2003	MF#8967	Bufo	species	GLR	"	+
5/21/2003	MF#8968	Bufo	species	GLR	"	+
5/21/2003	MF#8969	Bufo	species	GLR	"	+
5/21/2003	MF#8970	Bufo	species	GLR	"	+
5/21/2003	MF#8971	Bufo	species	GLR	"	+
5/21/2003	MF#8972	Bufo	species	GLR	"	+
5/21/2003	MF#8973	Bufo	species	GLR	"	+
5/21/2003	MF#8974	Bufo	species	GLR	"	+
5/21/2003	MF#8975	Bufo	species	PO#1	additional pond site; privately owned	
5/21/2003	MF#8976	Bufo	species	PO#1	"	
5/21/2003	MF#8977	Bufo	species	PO#1	"	
5/21/2003	MF#8978	Bufo	species	PO#1	"	
5/21/2003	MF#8979	Bufo	species	PO#1	"	
5/21/2003	MF#8980	Bufo	species	PO#1	"	
5/21/2003	MF#8981	Bufo	species	PO#1	additional pond site; privately owned	
5/27/2003	MF#8982	Bufo	species	GLR	found 30 m N of p2	+
5/27/2003	MF#8983	Bufo	species	GLR		+
5/27/2003	MF#8984	Bufo	species	GLR		+
5/27/2003	MF#8985	Bufo	species	GLR		+
5/27/2003	MF#8986	Bufo	species	GLR		+
5/27/2003	MF#8987	Bufo	species	GLR		+
5/27/2003	MF#8988	Bufo	species	GLR		+
5/27/2003	MF#8989	Bufo	species	GLR		+
5/27/2003	MF#8990	Bufo	species	GLR		+
5/27/2003	MF#8991	Bufo	species	GLR		+
5/27/2003	MF#8992	Bufo	species	GLR		+
5/27/2003	MF#8993	Bufo	species	GLR		+

5/27/2003	MF#8994	<i>Bufo</i>	<i>species</i>	GLR		+
5/27/2003	MF#8995	<i>Bufo</i>	<i>species</i>	GLR		+
5/27/2003	MF#8996	<i>Bufo</i>	<i>species</i>	GLR		+
5/27/2003	MF#8997	<i>Bufo</i>	<i>species</i>	GLR	trap B-F4	+
6/17/2003	MF#9019	<i>Bufo</i>	<i>species</i>	GLR	Art. Array p3	2-
6/18/2003	MF#9017	<i>Bufo</i>	<i>species</i>	GLR	p2, <i>B. houstonensis</i> ?	+
7/4/2003	MF#9008	<i>Bufo</i>	<i>species</i>	GLR	trap 2-E	2-
7/4/2003	MF#9020	<i>Bufo</i>	<i>species</i>	GLR	by trap 2; N30 deg.42.941'; W101deg.31.573'; elev.2453'	2-
7/5/2003	MF#9013	<i>Bufo</i>	<i>species</i>	GLR	<i>B. houstonensis</i> ? A-W	+
7/6/2003	MF#9015	<i>Bufo</i>	<i>valliceps</i>	Wells Branch	<i>B. valliceps</i> ?	2-
7/7/2003	MF#9007	<i>Bufo</i>	<i>species</i>	GLR	<i>B. valliceps</i> ? p12	2-
7/7/2003	MF#9012	<i>Bufo</i>	<i>species</i>	GLR	<i>B. valliceps</i> ?	2-
7/7/2003	MF#9014	<i>Bufo</i>	<i>species</i>	GLR	<i>B. valliceps</i> ? p5	2-
7/8/2003	MF#9009	<i>Bufo</i>	<i>valliceps</i>	GLR	trap 12-3, <i>B. valliceps</i> ?	2-
7/13/2003	MF#9003	<i>Bufo</i>	<i>species</i>	GLR	trap 2-1, <i>B. valliceps</i> ?	2-
7/14/2003	MF#9011	<i>Bufo</i>	<i>species</i>	GLR	<i>B. valliceps</i> ? by trap 2	2-
7/19/2003	MF#8999	<i>Bufo</i>	<i>species</i>	GLR	<i>B. valliceps</i> ?	2-
7/19/2003	MF#9001	<i>Bufo</i>	<i>species</i>	GLR	<i>B. valliceps</i> ?	2-
7/19/2003	MF#9002	<i>Bufo</i>	<i>species</i>	GLR	<i>B. houstonensis</i> ?	+
7/19/2003	MF#9004	<i>Bufo</i>	<i>species</i>	GLR	<i>B. valliceps</i> ?	2-
7/19/2003	MF#9005	<i>Bufo</i>	<i>species</i>	GLR	<i>B. valliceps</i> ?	2-
7/19/2003	MF#9006	<i>Bufo</i>	<i>species</i>	GLR	<i>B. valliceps</i> ?	2-
7/19/2003	MF#9010	<i>Bufo</i>	<i>species</i>	GLR	<i>B. houstonensis</i> ?	+
7/19/2003	MF#9016	<i>Bufo</i>	<i>species</i>	GLR	<i>B. houstonensis</i> ?	+
7/19/2003	MF#9018	<i>Bufo</i>	<i>species</i>	GLR	found dead, <i>B. houstonensis</i> ?	+
7/22/2003	MF#9000	<i>Bufo</i>	<i>species</i>	GLR	<i>B. houstonensis</i> ?	+

* Molecularly identified as *B. houstonensis*; presence of band on PCR gel will be positive (+); absence of band will be negative (-). Number indicates how many PCRs were performed.

LITERATURE CITED

- ANDERSON, J. D., D. D. HASSINGER, AND G. H. DALRYMBLE. 1971. Natural mortality of eggs and larvae of *Ambystoma t. tigrinum*. *Ecology* 52:1107-1112.
- BEIER, P. AND S. LOE. 1992. A Checklist for Evaluating Impacts to Wildlife Movement Corridors. *Wildlife Society Bulletin* 20.
- _____ AND R. F. NOSS. 1998. Do habitat corridors really provide connectivity? *Conservation Biology* 12:1241-1252.
- BERVEN, K. A., AND B. G. CHADRA. 1988. The relationship among egg size, density, and food level on larval development in the wood frog (*Rana sylvatica*). *Oecologia* 75:67-72.
- _____ AND T. A. GRUDZIEN. 1990. Dispersal in the wood frog (*Rana sylvatica*): implications for genetic population structure. *Evolution* 44:2047-2256.
- BLAIR, W. F. 1972. Evolution of the Genus *Bufo*. University of Texas Press, Austin, Texas, USA.
- BRECKENRIDGE, W. J., AND J. R. TESTER. 1961. Growth, local movements, and hibernation of the Manitoba toad, *Bufo hemiophrys*. *Ecology* 42:637-646.
- BREDEN, F. 1987. The effect of post-metamorphic dispersal on the population genetic structure of Fowler's toad, *Bufo woodhousii fowleri*. *Copeia* 1987:386-395.

- BOONE, M. D., C. M. BRIDGES, AND B. B. ROTHERMEL. 2001. Growth and development of larval green frogs (*Rana clamitans*) exposed to multiple doses of an insecticide. *Oecologia* 129:518-524.
- BROWN, L. E. 1971. Natural hybridization and reproductive ecology of two toad species in a disturbed environment. *American Midland Naturalist* 86:78-85.
- _____. 1975. The status of the near-extinct Houston toad (*Bufo houstonensis*) with recommendations for its conservation. *Herpetological Review* 6:37-40.
- _____ AND R. A. THOMAS. 1982. Misconceptions about the endangered Houston toad (*Bufo houstonensis*). *Herpetological Review* 13(2):37.
- BURKE, V. J. AND J. W. GIBBONS. 1995. Terrestrial buffer zones and wetland conservation: A case study of freshwater turtles in a Carolina Bay. *Conservation Biology* 9:1365-1369.
- BRAGG, A. N. 1960. Feeding in the Houston toad. *Southwestern Naturalist* 5:106.
- CLARKE, R. D. 1974. Activity and movement patterns in a population of Fowler's toad, *Bufo woodhousei fowleri*. *The American Midland Naturalist* 92:257-274.
- CLARK, T. W., R. P. READING, AND A. L. CLARKE. 1994. *Endangered Species Recovery: Finding the Lessons, Improving the Process*. Island Press, Washington, D.C.
- CROUCH, W. B. AND P. W. C. PATON. 2000. Using egg-mass counts to monitor wood frog populations. *Wildlife Society Bulletin* 28(4):895-901.
- DAUGHERTY, C. H., AND A. L. SHELDON. 1982. Age-specific movement patterns of the frog *Ascaphus truei*. *Herpetologica* 38:468-474.

- DAYTON, G. H. AND L. A. FITZGERALD. 2001. Competition, predation, and the distributions of four desert anurans. *Oecologia* 129: 430-435.
- DEMAYNADIER, P. G., AND M. L. HUNTER. 1999. Forest canopy closure and juvenile emigration by pool-breeding amphibians in Maine. *Journal of Wildlife Management* 63:441-450.
- FERNER, J.W. 1979. A review of marking techniques for amphibians and reptiles. Society for the Study of Amphibians and Reptiles, Herpetological Circular No.9.
- GILL, D. E. 1978. The metapopulation ecology of the red-spotted newt, *Notophthalmus viridescens* (Rafinesque). *Ecological Monographs* 48:145-166.
- GOATER, C. P. 1994. Growth and survival of postmetamorphic toads: interactions among larval history, density, and parasitism. *Ecology* 75:2264-2274.
- _____, R. D. SEMLITSCH, AND M. V. BERNASCONI. 1993. Effects of body size and parasite infection on the locomotory performance of juvenile toads, *Bufo bufo*. *Oikos* 66:129-136.
- HATFIELD, J. S., A. H. PRICE, D. D. DIAMOND, AND C..D. TRUE. (*In Press*) Houston Toad *Bufo houstonensis* in Bastrop County, Texas. In: Akçakaya et al. (eds.) *Species Conservation and Management: Case Studies*. Oxford University Press.
- HILLIS, D. M., A. M. HILLIS, AND R. F. MARTIN. 1984. Reproductive ecology and hybridization of the endangered Houston toad (*Bufo houstonensis*). *Journal of Herpetology* 18(1):56-72.
- KENNEDY, J. P. 1961. Spawning season and experimental hybridization of the Houston toad, *Bufo houstonensis*. *Herpetologica* 17:239-245.

- JACOBSON, N. L. 1989. Breeding dynamics of the Houston toad. *The Southwestern Naturalist* 34(3):374-380.
- LABANICK, G. M., AND R. A. SCHLUETER. 1976. Growth rates of recently transformed *Bufo woodhousei fowleri*. *Copeia* 1976:824-826.
- LACY, J. AND J. D. SEAL. 1994. Conservation Breeding Specialist Group: Houston toad: population and habitat viability assessment. Austin, TX. Information found at web site: http://www.cbsg.org/reports/exec_sum/houston_toad_phva.pdf
- LICHT, L. E. 1974. Survival of embryos, tadpoles, and adults of the frogs *Rana aurora aurora* and *Rana pretiosa pretiosa* sympatric in southwestern British Columbia. *Canadian Journal of Zoology* 52:613-627.
- MAXWELL, J. M., AND I. G. JAMIESON. 1997. Survival and recruitment of captive-reared and wild-reared Takehe in Fiordland, New Zealand. *Conservation Biology* 11:683-691.
- MINISTRY OF ENVIRONMENT, LANDS, AND PARKS (MELP). 1998. Standardized inventory methodologies for components of British Columbia's biodiversity: pond-breeding amphibians and painted turtle *In* Standards for components of British Columbia's biodiversity 37:1-10. Vancouver, BC.
- PECHMANN, J. H. K., D. E. SCOTT, J. W. GIBBONS, AND R. D. SEMLITSCH. 1989. Influence of wetland hydroperiod on diversity and abundance of metamorphosing juvenile amphibians. *Wetlands Ecology and Management* 1:3-11.
- _____, D. E. SCOTT, R. D. SEMLITSCH, J. P. CALDWELL, L. J. VITT, AND J. W. GIBBONS. 1991. Declining amphibian populations: the problem of separating human impacts from natural fluctuations. *Science* 253:892-895.

- _____ AND R. D. SEMLITSCH. 1986. Diel activity patterns in the breeding migrations of winter-breeding anurans. *Canadian Journal of Zoology* 64:1116-1120.
- PETERS, J. A. 1968. Houston toad - *Bufo houstonensis* Sanders (endangered). Sheet RA-10, in *Rare and Endangered Fish and Wildlife of the United States*. U. S. Dept. of the Interior/Bureau of Sport Fisheries and Wildlife. (Resource Pub. 34).
- PETRANKA, J. W. 1989. Density-dependent growth and survival of larval *Ambystoma*: evidence from whole-pond manipulations. *Ecology* 70:1752-1767.
- _____ AND A. SIH. 1987. Habitat duration, length of larval period, and the evolution of a complex life cycle of a salamander, *Ambystoma texanum*. *Evolution* 50:1351-1358.
- POUGH, F. H., AND S. KAMEL. 1984. Post-metamorphic change in activity metabolism of anurans in relation to life history. *Oecologia* 65:138-144.
- QUINN, H. R., G. FERGUSON, AND S. R. MAYS. 1987. Captive propagation/release and relocation program for the endangered Houston toad, *Bufo houstonensis*. Progress Report Presented to The Texas Parks and Wildlife Department and the US Department of Interior, Fish and Wildlife Service, Office of Endangered Species.
- _____ AND G. MENGDEN. 1984. Reproduction and growth of *Bufo houstonensis* (Bufonidae). *The Southwestern Naturalist* 29:189-195.
- READING, C. J. 1986. Egg production in the Common toad, *Bufo bufo*. *J. Zool. Lond.* 208:99-107.
- ROTHERMEL, B. B. (in press) Migratory success of juveniles: a potential constraint on connectivity for pond-breeding amphibians. *Ecological Applications*.

- _____. AND R. D. SEMLITSCH. 2002. An experimental investigation of landscape resistance of forest versus old-field habitats to emigrating juvenile amphibians. *Conservation Biology* 16(5):1324-1332.
- RUGH, R. 1941. *Experimental embryology: a manual of techniques and procedures*. New York, New York University.
- SCOTT, D. E. 1990. Effects of larval density in *Ambystoma opacum*: an experiment in large scale field enclosures. *Ecology* 71:296-306.
- _____. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum*. *Ecology* 75:1383-1396.
- SEMLITSCH, R. D. 1998. Biological delineation of terrestrial buffer zones for pond-breeding salamanders. *Conservation Biology* 12(5): 1113-1119.
- _____. 2000a. Principles for management of aquatic-breeding amphibians. *Journal of Wildlife Management* 64(3):615-631.
- _____. 2000b. Size does matter: the value of small isolated wetlands. *National Wetlands Newsletter* January-February Issue: 5-13.
- _____. AND J. R. BODIE. 1998. Are small, isolated wetlands expendable? *Conservation Biology* 12(5): 1129-1133.
- _____. AND J. W. GIBBONS. 1990. Effects of egg size on success of larval samanders in complex aquatic environments. *Ecology* 71:1789-1795.
- _____. AND J. B. JENSEN. 2001. Core habitat, not buffer zone. *National Wetlands Newsletter* 23:5-11.

- SHAFFER, M. L. 1990. Population viability analysis. *Conservation Biology* 4:39-40.
- SHIROSE, L. J. AND R. J. BROOKS. 1995. Age structure, mortality, and longevity in syntopic populations of three species of ranid frogs in central Ontario. *Canadian Journal of Zoology* 73:1878-1886.
- SNODGRASS, J. W., M. J. KOMOROSKI, A. L. Bryan, and J. Burger. 2000. Relationships among isolated wetland size, hydroperiod, and amphibian species richness: implications for wetlands regulations. *The Journal of the Society for Conservation Biology* 14:1-10.
- SMITH-GILL, S. J. AND K. A. BERVEN. 1979. Predicting amphibian metamorphosis. *The American Midland Naturalist* 113:563-585.
- _____ AND D. E. GILL. 1978. Curvilinearity in the competition equations : an experiment with ranid frogs. *American Naturalist* 112:557-570.
- SMITH, D. C. 1983. Factors controlling tadpole populations of the chorus frog (*Pseudacris triseriata*) on Isle Royale, Michigan. *Ecology* 64:501-510.
- THOMAS, L. A., AND J. ALLEN. 1997. *Bufo houstonensis* (Houston toad) behavior. *Herpetological Review* 28:40-41.
- U.S. FISH AND WILDLIFE SERVICE. August 1992. Threatened and Endangered Species of Texas. Endangered Species Office, Albuquerque, New Mexico.
- VAN BUSKIRK, J., AND D. C. SMITH. 1991. Density-dependent population regulation in a salamander. *Ecology* 72:1747-1756.

- WALLS, S. C., AND R. ALTIG. 1986. Female reproductive biology and larval life history of *Ambystoma* salamanders: a comparison of egg size, hatchling size, and larval growth. *Herpetologica* 42:334-345.
- WERNER, E. E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *American Naturalist* 128:319-341.
- WILBUR, H. M. 1972. Competition, predation, and the structure of the *Ambystoma-Rana sylvatica* community. *Ecology* 53:3-21.
- _____. 1976. Density-dependent aspects of metamorphosis in *Ambystoma* and *Rana sylvatica*. *Ecology* 57:1289-1296.
- _____. 1977a. Density-dependent aspects of growth and metamorphosis in *Bufo americanus*. *Ecology* 58:196-200.
- _____. 1977b. Interactions of food level and population density in *Rana sylvatica*. *Ecology* 58: 206-209.
- _____. AND J. P. COLLINS. 1973. Ecological aspects of amphibian metamorphosis. *Science* 182:1305-1314.
- WHITE, G. C., AND K. P. BURNHAM. 1999. Program MARK: survival estimation from populations of marked animals. *Bird Study* 46 Supplement:120-138.

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