2008

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ABSTRACT -- The Texas horned lizard (*Phrynosoma cornutum*) is a Texas state-threatened species and acquisition of data related to the species' ecology is essential. To accomplish this task individual animals need to be marked. Many marking techniques are available for lizards, however the majority of techniques have been tested on adults only. Studies involving hatchling and juvenile horned lizards are scarce due to problems associated with marking and relocating individuals in these age classes. I demonstrated that injection of passive integrated transponders (PIT's) can safely be used as a marking method in young Texas horned lizards. Thirty-two captive bred hatchling lizards were used. Hatchlings were allowed to grow to 20 mm snout-vent length (SVL) before PIT's were inserted into 16 hatchlings while the other 16 hatchlings were used as control animals. Hatchlings were measured and weighed weekly for 14 weeks and blood samples obtained weekly for 3 weeks to assess if PIT's affected lizard growth and health. No differences were noted in treatment effects for SVL ($F_{1,450} = 0.85$, $P = 0.37$), weight ($F_{1,450} = 1.60$, $P = 0.22$), or white blood cell and differential counts ($F_{1,30} < 1.47$, $P > 0.23$). However, an interaction between treatment and week occurred ($F_{14,450} = 1.79$, $P = 0.04$) for SVL. Texas horned lizard hatchlings that were PIT-tagged were larger in SVL during weeks 3, 7 to 10, and 12 than control lizards. No differences in SVL were noted between treatment groups during the remaining weeks. A duration effect was noted for both SVL ($F_{14,450} = 7654.0$, $P = 0.0001$) and mass ($F_{14,450} = 1595.1$, $P = 0.0001$). Snout-vent length for both PIT-tagged and control lizards increased weekly until week 12 when growth rate began to slow down. Mass gain for both groups of lizards was similar, with weekly spurts occurring except between weeks 0 and 1, 7 and 8, 12 and 13, and 13 and 14. Passive integrated transponders did not interfere with the growth or health of young Texas horned lizards; therefore, they can be safely used as a marking tool.

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Key words: hatchling, marking device, passive integrated transponder, *Phrynosoma cornutum*, reptile, Texas horned lizard.

The Texas horned lizard (*Phrynosoma cornutum*) was once widespread and abundant throughout Texas (Donaldson et al. 1994), however its population has experienced a dramatic decline (Henke 2003). Because of this, the Texas horned lizard is listed as a threatened species by the state of Texas (Texas Parks and Wildlife Code 1987) and as a Species of Concern by the United States Fish and Wildlife Service, federal category C2 (http://www.tpwd.state.tx.us/huntwild/wild/species/thlizard/).

Knowledge of individual movements is essential to understand a species' ecology, however animals must be individually marked to obtain such information. Marking methods for horned lizards have included hot branding (Clark 1971), toe clipping (Ferner 1979, Henke and Montemayor 1998), body tags (Fisher and Muth 1989), fluorescent powder (Stark and Fox 2000), radioactive markers (O'Brien et al. 1965), and transponders (Camper and Dixon 1988, Henke and Montemayor 1998). Problems associated with each marking method have been espoused (Ferner 1979). For example, hot branding can cause abnormal behavior and greater mortality due to infection (Nietfeld et al. 1994), toe clipping is not favored by many Institutional Animal Care and Use committees because it is a mutilation technique (S. E. Henke, Texas A&M University-Kingsville, personal observation), body tags can get caught in vegetation and debris entangling the animal (Nietfeld et al. 1994), radioactive markers can harm or kill animals carrying them (Nietfeld et al. 1994), and fluorescent powder and dyes potentially can make lizards visible to predators thus increasing their mortality, however to my knowledge this has not been quantified for lizards. Passive integrated transponders (PITs) have been used safely and successfully in adult Texas horned lizards (Camper and Dixon 1988), but have yet to be evaluated for safe use in hatchling and juvenile horned lizards. I evaluate the use of PIT's in hatchling (i.e., 3 to 14 week old) Texas horned lizards.

METHODS

Five adult Texas horned lizards (2M:3F) were obtained from Texas Parks and Wildlife Department biologists, who confiscated them from individuals who illegally collected them in Texas. Because the collection location was unknown, lizards were not released. Instead the adults were allowed to be maintained in captivity for educational purposes via permit No. SPR0890-274, which is held by the Horned Lizard Conservation Society.

Adults were held in a 227-liter aquarium equipped with a 13-cm layer of sandy soil, heat lamp, and refugia. They were given food (i.e., harvester ants (*Pogonomrymex* sp.) and crickets (Family Gryllidae)) and water *ad libitum*.

From these adults, two clutches of hatchlings were born in captivity. Clutch 1, which consisted of 14 hatchlings (7M:7F), emerged on 13 July 2001, while Clutch 2, which consisted of 18 hatchlings (8M:10F), emerged on 17 July 2001. Upon emergence, hatchlings were measured for snout-vent length (SVL), weighed, and sexed. Sex determination was verified after they were greater than 1 year old. Sex determination of individuals at hatching was accurate. Because hatchlings at emergence measured 10 to 11 mm SVL and weighed less than 1 gram, they were allowed three weeks growth to be at least 20 mm SVL. This growth time was needed because many hatchlings at emergence were smaller than the length of a PIT.

Hatchlings were divided into two groups. Group 1 received a PIT and group 2 was the control group. Group 1 consisted of seven hatchlings from clutch 1 (4M:3F) and nine hatchlings from clutch 2 (5M:4F). Group 2 consisted of seven hatchlings from clutch 1 (3M:4F) and nine hatchlings from clutch 2 (3M:6F). All hatchlings were marked individually by toe-clipping (Cagle 1939) and in addition, members of group 1 received a PIT (AVID Microchip ID Systems, Mandeville, Louisiana). Passive integrated transponders, which measured 11 x 2 mm and weighed 0.08 g, were injected intraperitoneally in the abdominal region via a 16-gauge needle. Hatchlings were maintained in eight 76-liter aquaria, which were set up as previously described. Four hatchlings, two from groups 1 and 2, respectively, were maintained per aquarium. Food and water were provided *ad libitum*. Hatchlings were monitored weekly to assess their health for three weeks after implanted with a PIT. About 0.2 mL of blood were collected in heparinized syringes via ventral tail caudal vein puncture (Powell and Knesel 1992). White blood cells (WBC) were counted by using Isoton II solution and lytic reagent in a Z1 particle counter (Beckman-Coulter, Inc., Fullerton, California). Thin blood smears were prepared at the time of collection and stained with a Wrights-Giemsa stain within 24 hr. Differential WBC counts were conducted by counting 200 leukocytes at 1,000X magnification. Hatchlings also were weighed to the nearest 0.1 g and measured (SVL) weekly until they entered hibernation. Individuals again were monitored at time of spring emergence. In addition, hatchlings were observed throughout the study to subjectively determine if PIT's adversely affected movements or caused obvious abnormal behaviors.

I used a repeated measures design with treatment (PIT-tagged and control), sex (male and female), and week (0 = initial through 14) as main effects and hatchlings (N = 32) as replications. The distribution of residual errors was tested to verify normality with the Shapiro-Wilk test (PROC UNIVARIATE procedure; SAS 1989). Homogeneity of variances among treatments was evaluated with Bartlett's test (Steel and Torrie 1980). A general linear analysis of variance (PROC GLM; SAS 1989) was used to test the main and interactive effects on the growth and health of the hatchlings. Because

the effect of sex and its corresponding interactive effects were not significant ($F_{1,11} < 1.48$; $P > 0.44$), hatchlings were pooled irrespective of sex and re-analyzed with the main and interactive effects of treatment and weeks. Multiple comparisons were made by using Tukey's studentized range (HSD) test when a significant F-test was noted (Cochran and Cox 1957). Single variants of the interaction were analyzed separately within each grouping of the other main effects when a significant interaction was noted. Statistical significance was inferred at $P < 0.05$.

RESULTS and DISCUSSION

Passive integrated transponders did not affect the growth (Table 1) or health (Table 2) of hatchlings. No mortalities or abnormal behaviors and movements were noted for any individual. White blood cell and differential counts were similar ($F_{1,30} < 1.47$, $P > 0.23$) between PIT-tagged and control lizards (Table 2). Also, differences were not observed ($F_{2,60} < 0.78$, $P > 0.46$) in week or interactive effects for blood counts. No differences were noted in treatment effects for SVL ($F_{1,450} = 0.85$, $P = 0.37$), mass ($F_{1,450} = 1.60$, $P = 0.22$), or in the interactive effect for mass ($F_{14,450} = 1.26$, $P = 0.23$). However, an interaction between treatment and week occurred ($F_{14,450} = 1.79$, $P = 0.04$) for SVL. Hatchlings that were PIT-tagged were larger in SVL during weeks 3, 7, 8, 9, 10, and 12 than control hatchlings (Table 1). No differences in SVL were noted between treatment groups during the remaining weeks. A duration effect was noted for both SVL ($F_{14,450} = 7654.0$, $P = 0.0001$) and mass ($F_{14,450} = 1595.1$, $P = 0.0001$). Snout-vent length for both PIT-tagged and control hatchlings increased weekly until week 12 when growth rate began to slow down (Table 1). Mass gain for both treatment groups was similar (Table 1), with weekly gains occurring except between weeks 0 and 1, 7 and 8, 12 and 13, and 13 and 14 (Table 1). Average growth rate for hatchlings was 0.518 ± 0.01 mm/d during the first 11 weeks; whereas average growth rate declined to 0.171 ± 0.01 mm/d during the 3 weeks prior to hibernation. Hibernation occurred between 16 November and 1 December 2001 and re-emergence after hibernation occurred between 15 and 21 March 2002. Average hatchling growth during hibernation was 0.011 ± 0.001 mm/d. Mass loss after re-emergence was similar ($F_{1,30} = 1.3$, $P = 0.27$) between hatchlings that received a PIT (5.6 ± 0.2 g; $\bar{x} \pm SE$) and the control hatchlings (5.1 ± 0.3 g).

Passive integrated transponders can be safely used to individually mark Texas horned lizards that are at least 20 mm SVL. Transponders did not cause mortality, affect health, obstruct movements, or affect the growth of the hatchlings. Mean growth of hatchlings with and without PIT's was similar to that reported for juvenile Texas horned lizards (Henke and Montemayor 1997). In addition, PIT's did not appear to alter hatchling behavior such as feeding, digging, and basking.

Table 1. Weekly growth of PIT-tagged (treatment; N = 16) and non-tagged (control; N = 16) hatchling Texas horned lizards maintained in captivity from July 2001 to March 2002.

	Snout vent length (mm)				Mass (g)			
	Control		Treatment		Control		Treatment	
Weeks	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
0	21.3 Aa[1,2]	0.2	21.4 Aa	0.2	1.6 Aa	0.1	1.8 Aa	0.1
1	25.4 Ab	0.2	25.2 Ab	0.2	2.4 Aa	0.2	2.4 Aa	0.1
2	29.4 Ac	0.3	29.2 Ac	0.3	4.9 Ab	0.2	4.4 Ab	0.2
3	33.1 Ad	0.4	33.8 Bd	0.2	7.4 Ac	0.2	7.4 Ac	0.2
4	36.6 Ae	0.4	37.5 Ae	0.2	10.6 Ad	0.2	10.4 Ad	0.2
5	40.2 Af	0.4	40.9 Af	0.3	13.8 Ae	0.3	13.7 Ae	0.2
6	43.8 Ag	0.4	44.6 Ag	0.3	17.4 Af	0.3	17.0 Af	0.3
7	47.2 Ah	0.4	48.4 Bh	0.2	20.8 Ag	0.3	21.5 Ag	0.4
8	50.7 Ai	0.4	51.8 Bi	0.2	21.9 Ag	0.3	22.8 Ag	0.4
9	54.0 Aj	0.2	55.2 Bj	0.2	23.9 Ah	0.3	24.5 Ah	0.4
10	57.8 Ak	0.2	58.4 Bk	0.2	26.1 Ai	0.3	25.8 Ai	0.3
11	61.2 Al	0.3	61.3 Al	0.2	27.9 Aj	0.4	27.8 Aj	0.3
12	63.1 Am	0.3	63.7 Bm	0.2	29.5 Ak	0.5	30.1 Ak	0.3
13	64.0 Amn	0.3	64.5 Amn	0.2	30.2 Akl	0.5	31.4 Akl	0.3
14	64.6 An	0.3	65.1 An	0.2	30.4 Al	0.7	32.2 Al	0.3
RE[3]	66.1 Ao	0.4	66.2 Ao	0.1	25.3 A[4]	0.7	26.6 A	0.5

[1]Means with the same upper case letter are not different ($P > 0.05$) between treatments.
[2]Means with the same lower case letter are not different ($P > 0.05$) between weeks within the same treatment.
[3]RI = Re-emergence after hibernation occurred between 15 to 21 March 2002.
[4]Mass loss after re-emergence was not analyzed within weekly growth.

Table 2. Weekly white blood cell (WBC) and differential counts of PIT-tagged (treatment) and non-tagged (control) hatchling Texas horned lizards maintained in captivity from July 2001 to March 2002.

	Control (N=16)						Treatment (N=16)					
	Week 1		Week 2		Week 3		Week 1		Week 2		Week 3	
Blood	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
WBC ($x10^9$/l)	27.5	10.3	29.3	9.7	29.9	6.7	33.4	11.7	29.4	6.8	31.9	2.8
Lymphocyte (%)	25.3	7.8	28.2	8.3	24.4	6.1	26.2	5.7	28.5	3.3	29.3	1.6
Heterophils (%)	62.2	12.2	58.1	11.7	54.4	5.8	64.5	4.7	61.3	2.8	58.1	5.4
Eosinophils (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.2	1.0	0.3
Basophils (%)	1.0	0.3	0.5	0.1	1.8	0.2	0.0	0.0	0.5	0.1	0.5	0.1
Monocytes (%)	11.5	1.2	13.2	1.7	19.4	2.5	9.3	2.0	8.0	0.3	11.1	2.1

Passive integrated transponders during my study did not fail. Camper and Dixon (1988) reported a 1% failure of PIT's implanted in reptiles and amphibians. My study can be used to satisfy animal welfare organizations (i.e., Institutional Animal Care and Use Committees) as to the safety of PIT's for use in hatchling and juvenile lizards.

Long-term population and ecological studies are needed in field herpetology, however herpetologists might be reluctant to use permanent marking techniques until such techniques are shown to be successful. I illustrated that a permanent marking technique can be used for rare and conservation-sensitive species without fear of harming individuals in a population. Permanently marking Texas horned lizards has allowed us to gather information such as dispersal from natal areas, movements, longevity, and potential causes of mortality. By conducting repeated searches during several years, we have been able to map Texas horned lizard locations through time, calculate survival rates, and growth rates (Henke and Montemayor 1997). Also, I have surmised Texas horned lizard mortality from PIT's found inside domestic cat scats, owl pellets, and raptor casts. Such information could be collected via radio telemetry, however, telemetry studies can be expensive and transmitters would require replacement for long-term studies. If budget is a concern and researchers are available, conducting searches for permanently marked Texas horned lizards can yield valuable data (Fair and Henke 1997, Henke and Montemayor 1998).

ACKNOWLEDGMENTS

I thank the Horned Lizard Conservation Society and Texas Parks and Wildlife Department for allowing me to raise Texas horned lizards in captivity. I thank A. M. Fedynich, B. Ballard, D. Fogell, and two anonymous reviewers for reviewing an earlier draft of this manuscript. Funding was provided by the Caesar Kleberg Wildlife Research Institute. This paper is publication number 03-123 of the Caesar Kleberg Wildlife Research Institute.

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Received: 18 August 2007 *Accepted: 4 January 2009*

Associate Editor for Herpetology: Dan Fogell