TEST AND EVALUATION OF VARIOUS TECHNIQUES TO STUDY REFUGED LIZARDS IN THE FIELD

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ABSTRACT—We report the use and evaluation of various techniques to locate, identify, and observe our target species, the collared lizard (*Crotaphytus collaris*), while refuged inside rock crevices. We tested the use of radiotelemetry, visual observation via an articulating borescope, remote monitoring of passive integrated transponder tags implanted in subjects as they entered and left refuges, and the measurement of body temperatures of subjects inside refuges. The combined set of techniques provides reliable results and has the potential to uncover novel behavior and social interactions of occupants of otherwise inaccessible refuges or burrows for a host of wild animals.

RESUMEN—Reportamos el uso y evaluación de varias técnicas para localizar, identificar y observar a nuestra especie focal, la Lagartija de Collar (*Crotaphytus collaris*), mientras se encontraba refugiada en grietas de rocas. Probamos el uso de radiotelemetría, observación visual vía un boroscopio flexible, monitoreo remoto de marcas PIT implantadas en sujetos mientras entraban y salían de refugios, y la medida de temperaturas corporales de sujetos dentro de refugios. El juego completo de técnicas proporciona resultados fiables y tiene la posibilidad de revelar nuevo comportamiento desconocido e interacciones sociales de ocupantes de refugios o madrigueras de otros modos no accesibles en muchos tipos de animales silvestres.

Research in animal ecology often requires locating specific subjects and observing them with minimal disturbance. Direct observation of subjects that are refuged is particularly challenging, prompting researchers to develop alternative techniques. One such alternative technique is radiotelemetry. Telemetry allows subsequent monitoring of individuals even when they enter refuges in dens, deep narrow cracks, or rock crevices (Smith, 2009; Santoyo-Brito and Fox, 2012). Another alternative technique is fiber-optic technology, which allows accurate inspection of refuged animals (Markwell, 1997; Purcell, 1997; Jones et al., 2003), and yields clear imagery of narrow, poorly lit cavities (Boland and Phillips, 2005). Passive integrated transponders (PIT tags) can be used to permanently mark individuals (Rove and Kelly, 2005; Henke, 2008; Plummer and Ferner, 2012) and can be used with applications that allow for remote monitoring without the need to physically recapture the animal to record temporal and spatial information (Gibbons and Andrews, 2004; Gruber, 2004; Rehmeir et al., 2006). To measure and record body temperature, researchers sometimes use thermocouples, usually inserted into the cloaca. Body temperature of refuged individuals can be recorded by simply touching the skin with the tip of the thermocouple. Cloacal and abdominal temperature readings yield similar results (Paranjpe et al., 2012).

The objectives of our study were to test and evaluate the efficacy of the combination of techniques reviewed above to study the behavioral ecology of refuged lizards, specifically 1) internal radio microtransmitters, 2) articulating borescope, 3) thermocouple attached to the borescope to measure body temperature, and 4) remote interrogation of implanted PIT tags.

MATERIALS AND METHODS—Our subjects were *Crotaphytus collaris* inhabiting a 1,200-m riprap dam at Sooner Lake, Pawnee County, Oklahoma. Lizards used the numerous crevices between and underneath rocks for refuge during the day and to spend the night. This study was a part of a larger one in which we had marked all the lizards along the entire length of the dam. For this study we focused on a 13- × 400-m stretch of the rocks, inner face of the dam along which there were 27 marked lizards, each implanted with a 8.5- × 2.12-mm PIT tag (Biomark, Inc., Boise, Idaho).

Attaching transmitters to the outside of the body does not work for lizards that squeeze into tight rock crevices because the transmitters are quickly scraped off (S.F.F., pers. observ.). Also, in pilot laboratory trials when we tried taping a transmitter to the base of the tail, the lizards were obviously bothered by the transmitter; they assumed a stiff, upright, standing position and bit at the radio. Because this study was of short duration and designed to test various field techniques used to study lizards that refuge into rock cracks and crevices, we force fed 2-g radio microtransmitters into subjects (Santoyo-Brito and Fox, 2012) instead of surgically implanting them. We located telemetered lizards before dawn while subjects were still inside their nocturnal refuges and verified the occupancy of the refuge.
visually via a 1.6-m medical borescope (Olympus Model CLV-10). During two field sessions (21–25 July and 29 July–1 August 2009) we tested this technique and searched for telemetered lizards a total of 18 times ($N = 2$ males and 4 females; one male and one female were used in both sessions). We measured the external temperatures of these lizards using a thermocouple attached to the tip of the borescope by pressing it against the side of the subject (while viewing the thermocouple and lizard through the borescope), and also measured the ambient air temperature inside and outside the refuge using the same thermocouple. Temperature readings were recorded with the light of the borescope turned off and after temperatures stabilized. We also tested the greatest distance at which a radio placed into a natural refuge 10–15 cm deep could be detected ($N = 20$), and to obtain data from even deeper refuges, we measured the greatest detection distance of a radio placed 50 cm inside a pile of rocks compared with the same radio placed on top of the rock pile ($N = 10$ pairs).

We conducted trials with six subjects (three males and three females) to relate external and internal body temperatures across a range of ambient temperatures. We used three thin thermocouples, one inside the cloaca, one on the abdomen, and a third inside a chamber (plastic box with mesh top, 12.0 x 12.0 x 9.4 cm tall). We ran one trial per lizard on either 19 July or 24 July when conditions were clear and sunny. We first allowed subjects to acclimate to the experimental conditions (one subject per chamber) for 15 min. Then we read the temperature of the three thermocouples every 15 min from 0700h to 1400h while chambers were left outdoors in the shade. This allowed lizard body temperature to equilibrate with the ambient temperatures, which rose slowly from 23 to 31°C. In addition, while still inside trial chambers, lizards were placed in an incubator set at 37°C (after first bringing the lizard back to room temperature) for 1 h until body temperature had stabilized. We regressed temperatures taken in the cloaca against skin temperature to test whether or not external body temperature was a strong predictor of internal temperature.

In the laboratory, we recorded movement of known individuals into and out of a rock refuge 10–15 cm deep ranged from 12.2 to 69.3 m ($t = 10.48, N = 5, P < 0.001$). Most of the lizards measured at the same time in a different refuge was 26.6 m, whereas the air temperature outside the refuge was 20.6°C (and a refuged lizard measured at the same time in a different refuge was 26.5°C). Regressions predicting internal body temperature from external (skin) temperature in trial chambers across a wide range of ambient temperatures were significant for all six trials (each regression with 31 points except 1 with 30 points; all $t > 0.992$, all $P < 0.001$). In fact, regressions hardly differed from a slope of 1.0: in only one case was there a significant difference ($t = 3.86, 29 df, P < 0.001$; all other $P > 0.05$). Pooling all points for all six lizards resulted in the highly significant regression equation of cloacal temperature $= 0.981 \times$ skin temperature $+ 0.188$ ($r^2 = 0.994, F_{1,183} = 29.058.1, P < 0.001$; Fig. 1), and this slope differed significantly from 1.0 ($t = 3.17, 183 df, P < 0.002$). Cloacal temperatures were slightly lower than skin temperatures, particularly at higher temperatures, but this difference was biologically negligible at realistic temperatures <40°C.

Maximum detection distances of transmitters placed into natural rock refuges 10–15 cm deep ranged from 12.2 to 69.3 m ($x = 35.8 \pm 14.7 m, N = 28$), whereas those placed deeper (50 cm) into rock piles were detected at 11.2 to 40.0 m ($x = 20.5 \pm 7.5 m, N = 10$). Using a paired $t$-test, we determined that a radio on top of the rock pile was detected at a significantly longer distance ($x = 10.5 m$...
longer) than the same one inside the refuge ($t = 4.207, 9 \text{ df}, P = 0.002$).

During the 4 days of laboratory monitoring of the seven subjects with PIT tags, we recorded 114 passages through the antenna and all seven lizards used the refuge, with many instances of cohabitation. Average residency inside the refuge during daylight hours after lizards emerged for activity was $36.4 \pm 47.3 \text{ min (} N = 47\text{), and during overnight hours residency was } 15.8 \pm 1.1 \text{ h (} N = 10\text{). Two (one male, one female), three (two males, one female), and four lizards (three males, one female) stayed overnight in the same refuge together for the three nights, respectively. Unfortunately, we did not have the antenna available for fieldwork until quite late in the season and lizards were not moving about very much. Most were not active above ground. Nevertheless, we recorded multiple passages of three different lizards through the antenna over the 2 days. Average residency during daylight hours after lizards emerged for activity was $33.1 \pm 36.7 \text{ min (} N = 6\text{), and during overnight residency was } 20.4 \pm 1.9 \text{ h (} N = 3\text{). All three lizards stayed overnight in the same refuge together (all juveniles: two males, one female), and also cohabited the refuge for large parts of the daylight hours.}

**DISCUSSION**—We report the results of various techniques to locate, observe, and identify free-ranging collared lizards, *Crotaphytus collaris*, while they were refuged inside rock crevices. Radiotelemetry worked well to detect collared lizards refuged inside rock crevices. The signal of these rather small, internal-antenna transmitters positioned 10–15 cm inside a crevice could be detected at appreciable distances above ground, up to nearly 70 m. The same transmitters 50 cm deep were detected at shorter maximal distances, but still up to 40 m. The radio signal from within rock crevices was attenuated, but not so much to limit the efficacy of the technology for field studies.

We implanted PIT tags into subjects so that we could test the method of remote monitoring as subjects entered and left refuges through a small ring antenna. The combined technology of permanently marking individuals utilizing PIT tags and monitoring their refuge occupancy via a custom-made antenna allows investigators to gather data on the ecology of refuge use in situations otherwise difficult for observations. Merely testing this combined technology revealed that different collared lizard juveniles of both sexes cohabited the same refuges, despite the finding that male juveniles active in the field in the fall are territorial and do not allow proximity of other males (S.F.F. and E.S.-B., pers. observ.). Perhaps this tolerance of other males resulted from the trial being run late in the season when juveniles were about to enter hibernacula (adults were no longer surface active). Communal hibernation has been reported in two different populations of collared lizards where potential hibernacula were abundant (Legler and Fitch, 1957; Curtis and Baird, 2007). The hibernacula discovered by Curtis and Baird (2007) contained multiple subjects of both sexes and different ages.

The techniques that we tested and evaluated to locate and observe lizard subjects are reliable and minimally invasive to both the subjects and their microhabitats, and it seems likely that any rock-refuging or burrowing species can be studied using these methods. The tip of the borescope that we used was flexible such that it could be manipulated and positioned to touch the thermocouple to different parts of the body as determined by viewing through the borescope. The mounted thermocouple was easily used to record microhabitat temperature deep inside the crevice. The technology did not affect the behavior of individuals, and can provide a broader insight of behavior that has not been thoroughly studied before, possibly revealing new information on refuge cohabitation and social interactions inside refuges (Boland, 2004; Smith, 2009).

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Literature Cited


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