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Coelomic Response and Signal Range of Implant Transmitters in *Bufo cognatus*

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Radio telemetry is a useful technique in ecological studies to determine habitat use, survival, and dispersal of organisms (Faccio 2003; Lamoureux et al. 2002; Millspaugh and Marzluff 2001). For amphibians, radio transmitters are attached externally using a belt harness or implanted into the coelomic cavity (Muths 2003; Werner 1991). External attachment of transmitters may affect amphibian dispersal or other life cycle events (e.g., breeding, feeding; Richards et al. 1994). Also, external transmitters are not feasible for amphibians that estivate underground, because they could interfere with digging and concealment. Alternatively, implanting transmitters requires invasive surgery that might affect body condition and survival (Madison and Farrand 1998), or damage vital organs within the coelomic cavity. Also, information on aboveground signal range of transmitters after implantation has not been published. Our goal was to describe the general effects of implant transmitters on coelomic response of a common amphibian (*Bufo cognatus*) in the Great Plains (Gray et al. 2004), and measure aboveground signal distance over four months, which was the expected battery life of the transmitters.

Materials and Methods.—We collected 14 Great Plains Toads

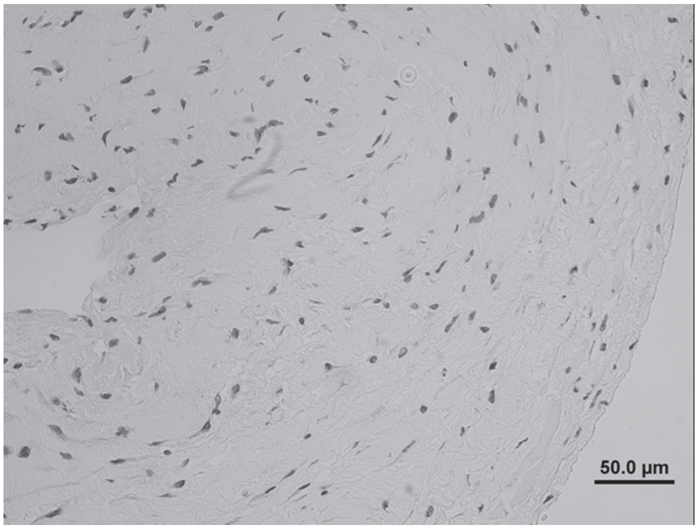

FIG. 1. Four months post-surgery microscopic view of the connective tissue encapsulating transmitters implanted in the coelomic cavity of 9 *Bufo cognatus*.

(*Bufo cognatus*) along roads at night during intense rain on 12 May 2001 northwest of Lubbock, Texas (33˚39'–33˚51'N, 101˚49'–102˚10'W) in the Southern High Plains. We placed all toads in separate 38-liter aquariums and fed them native crickets and beetles *ad libitum* for the duration of the study. Each aquarium contained 10 cm of soil substrate and a 500 cm^3 plastic water reservoir that was flush with the substrate surface to allow natural burrowing and water absorption by the toads.

We used SM1-H transmitters manufactured by AVM Instrument Company. These transmitters were single-staged (150 MHz) and powered by a CR2040 lithium manganese dioxide cell with an internal-loop antenna that encircled the battery. Signal strength and pulse rate of our transmitters at the beginning of the study were between -21 and -25 dBm and 58 and 68 BPM, respectively. The expected battery life given the power output (0.065 mA) was 4 months. The transmitter, battery, and antenna were encapsulated in RTV plastic and coated with clear dental acrylic. The final mass and volume of the transmitter package were 6.63 g (5–10% of individual toad body mass, 60–118 g) and 2.45 cm^3, respectively.

We implanted transmitters into 10 of the 14 toads on 2 June 2001. The four remaining toads did not receive transmitters and were maintained in captivity simultaneously. These toads were used as controls so we could compare histological responses to the transmitters at the end of the study. Our surgical procedures were similar to Madison (1997) and Seebacher and Alford (1999). We soaked transmitters and surgical instruments ≥ 30 minutes in chlorhexadine then rinsed them with sterile water. Toads were anesthetized by placing them into a solution of distilled water and tricaine methanesulfonate (1000 mg/L, Faccio 2003); sedation occurred within 10–32 minutes (mean = 18.5, SD = 6.8). After anesthetization, we made a 20-mm skin incision in the left caudoventrolateral quadrant of the abdomen with a sterile #20 scalpel. Next, we punctured through the abdominal wall and into the coelomic cavity with closed pointed surgical scissors. After puncturing the wall, the scissors were opened to separate the muscular fibers creating an opening into the coelomic cavity. The opening was maintained with thumb forceps then the transmitter was placed into the cavity. The incision was closed in two layers with 3-0 absorbable suture material. First, we sutured the abdominal wall with 3 stitches 5 mm apart in a simple interrupted pattern. Next, we closed the skin in a similar fashion. The incision was dried using sterile 4 × 4 gauze sponges. Finally, we applied surgical glue along the entire length of the incision to provide a temporary sealant. The entire surgical procedure lasted 8–10 minutes. Toads were then placed in distilled water containing antibacterial tetracaine powder (1% solution) for recovery (mean = 40.6, SD = 11.7 minutes). After anesthetic recovery, we returned toads to aquariums.

We measured aboveground signal distance (m) and body mass (g – transmitter package mass) twice per month from 2 June to 2 October 2001. We used a fully charged R2100 Advanced Telemetry Systems receiver and a 3-element AF Antronics Yagi antenna (model #F152-3FB) tuned specifically for 150–152 MHz to measure signal distance. Aboveground distance was determined by placing each toad in a plastic bucket and traversing a linear transect until the signal was not received. We considered these measure-

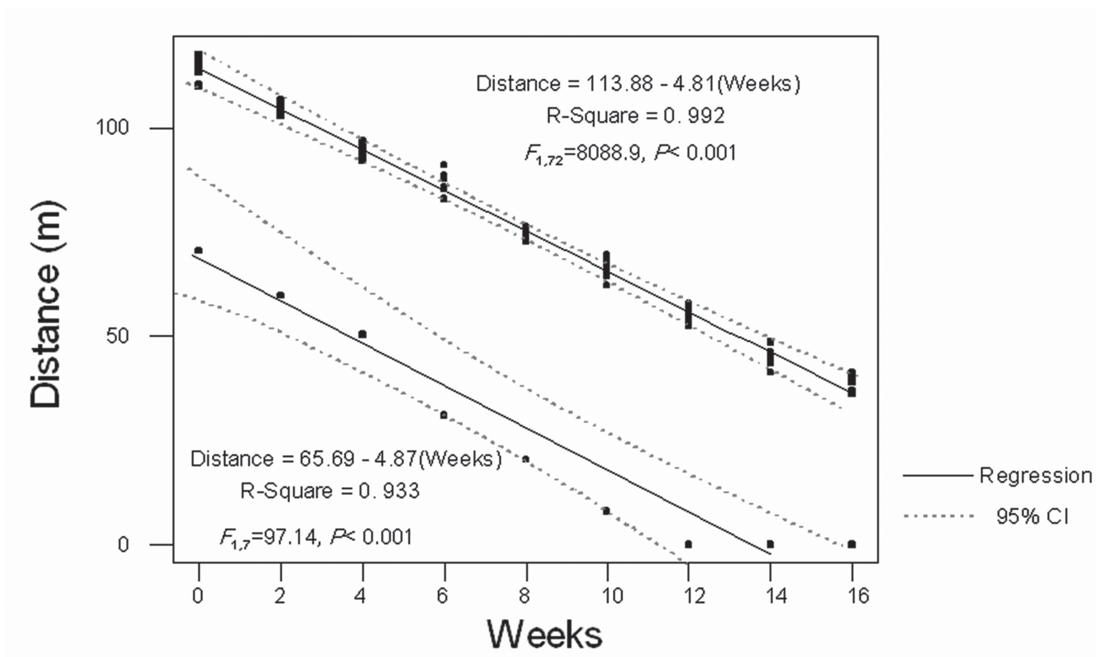

FIG. 2. Aboveground signal distance in two-week increments (2 June [week = 0] – 2 October 2001 [week = 16]) of SM1-H internal-loop antenna transmitters implanted into 10 Great Plains Toads (*Bufo cognatus*). The bottom regression is for a prototype transmitter that lost battery strength prior to the study.

ments maximum signal distances under ideal conditions because toads were aboveground and no obstructions existed between them and the receiver. Individual toad survival also was recorded.

At completion of the study, we sent all live toads (controls and ones with transmitters) to the University of Georgia Tifton Veterinary Diagnostic and Investigational Laboratory where they were euthanized and necropsied. Macroscopic inspections included surgical site condition, coelomic response to transmitters, and general organ condition. Tissue sections of all organs (including skin) were collected, processed, and examined microscopically.

Aboveground signal distance and toad body mass were regressed against post-surgery duration in two-week increments from the initiation until the end of the study (0, 2, 4, …, 16 weeks) to compare signal range and body mass through time. We performed two separate regressions for signal distance because maximum distance of one transmitter was markedly less throughout the study compared to the other nine transmitters. This transmitter was sent as a prototype six months prior to the study and used twice; all other transmitters had batteries at full strength at the beginning of the study. Also, one toad died during the study on 5 July 2001 thus its signal distance was not measured afterward nor used in the regression after week 4. Proportion of toads surviving until the end of the study was tested against a constant proportion ($p_0 = 0.5$) using a one-sample binomial test to determine if the overall survival rate differed ($\alpha = 0.05$) from the mortality rate (Milton and Arnold 1995:322). Necropsy results are presented as summary statistics.

Results.—Pathological examinations were performed on 9 of the 10 toads with transmitters and the 4 control toads. The deceased toad could not be analyzed because of advanced autolysis. Surgical sites of all toads were healed with minimal scarring. Granulation tissue existed at the surgical site of all toads and few mixed inflammatory cells (heterophils, macrophages, and lymphocytes) were present in one toad (11.1%). All transmitters were in the right caudoventrolateral coelome except one, which was in the left caudoventrolateral coelome. Each transmitter was encapsulated in a smooth, transparent connective tissue <1 mm thick (Fig. 1); few inflammatory cells and occasional vascularization were noted in the capsule of one transmitter. Six of 9 transmitters (67%) were adhered to the coelomic wall only or to the coelomic wall and 1–2 nearby organs by a strand of fibrous tissue (hereafter adhesion) that was ≤ 1 mm diameter × 1–4 mm in length. Organs of attachment included the liver, ovary, and mesentery. Histological analyses and comparison with control toads revealed no damage to the organs of attachment or other organs (i.e., lungs, spleen, kidneys, large and small intestines) in the coelomic cavity.

Aboveground signal distance decreased predictably through the study (Fig. 2). Mean aboveground signal distance at the initiation and completion of the study for the 9 transmitters was 114.2 m (SD = 2.7) and 39.4 m (SD = 1.9), respectively. The maximum distance of the prototype transmitter at the initiation of the study was 70.4 m with no signal by week 12. Toad body mass did not change ($F_{1,7} = 0.03–5.10$, $P = 0.06–0.86$) throughout the study. The proportion of toads surviving was 0.90, which differed ($P = 0.011$) from $p_0 = 0.5$. Mortality of the one toad was due to post-surgery complications; one suture pulled through the skin causing secondary infection.

Discussion.—Our results suggest that intracoelomic placement of radio transmitters does not negatively affect survival, body mass, and coelomic condition of amphibians. Post-surgery survival was 90% and body mass of all individuals was stable for the duration of the study. Previous studies also have suggested that survival and body mass are unaffected by implant transmitters (Madison 1997; Madison and Farrand 1998; Werner 1991).

Intracoelomic placement of transmitters was within the left caudoventrolateral quadrant, but at postmortem examination, 8 of 9 transmitters were found in the right caudoventrolateral quadrant. Although the exact mechanism for transmitter displacement is unknown, it may have been a consequence of stomach expansion. The stomach is located within the left middle coelome (Duellman and Trueb 1994). During feeding and subsequent stomach expansion, the stomach may have extended into the left caudal region, displacing the transmitter to the right. Once displaced, the transmitter may not have returned to the left caudal position after gastric emptying.

Coelomic response to the transmitters was similar for all toads. A thin transparent connective tissue capsule formed around all transmitters, which is a typical histological response to implants (Butler et al. 1997; Eltze et al. 2003; Kellar et al. 2002; Laitung et al. 1987; Shannon et al. 1997; Walboomers et al. 1998; Walboomers and Jansen 2000; Zhao et al. 2000). One capsule had minimal vascularization and inflammatory cell infiltrates, which was consistent with local irritation. Most toads developed one or more fibrous adhesions that resulted in anchoring the transmitters within the coelome. Adhesions are common surgical complications in all species, and they result from local trauma as well as constant irritation (Alimoglu et al. 2003; Goodwin and Grizzle 1991; Herzog et al. 1970; Montz et al. 1991). In general, adhesions are not considered beneficial structures; however in our toads, the adhesions may have aided in preventing mechanical trauma to nearby organs or preventing cranial migration during normal body movements by anchoring the transmitters locally.

Coelomic and body mass response to implants likely would be similar for other large anurans (e.g., > 60 g in body mass). Mortality of one toad occurred secondary to dehiscence of the surgical site, which has been documented previously (Werner 1991). Thus, we recommend external sutures encompass > 2 mm of skin on each side of the incision. This may be especially important for species that are fossorial such as *Bufo cognatus*.

Average aboveground signal distance at the beginning of the study was 114 m and decreased linearly over the 4 months to 40 m. This initial signal distance was 23% of the manufacturer's claim of 500 m. Only 1 of 10 transmitters lost signal in < 4 months (the expected battery life); this transmitter was the prototype and may have been operating at a reduced power capacity at the initiation of the study.

Considering that average aboveground signal distance of our implant transmitters was approximately 100 m, researchers may need to monitor amphibians frequently (1–2 times/day) in the field so they are not lost. Also, implant transmitters might not be reasonable for amphibian species that disperse long distances over short duration. New multi-staged transmitters (e.g., G3-1V) may have greater signal range than the SM1-H transmitters used in our study (B. Corbett-Kermeen, AVM Instrument Company, pers. comm.). Signal distance also might be reduced by environmental variables that we did not measure, such as aboveground vegetation and substrate (if amphibians become fossorial, Madison 1997). Thus, our distance estimates likely represent best-case scenarios of signal range for SM1-H implant transmitters.

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Observations on Problems with Using Funnel Traps to Sample Semi-Aquatic Snakes

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Funnel traps have been shown to be an effective method for sampling snakes in many different aquatic habitats (Fitch 1999; Greene et al. 1999; Karns et al. 2000; Keck 1994). However, any technique can have biases which need to be considered when choosing an appropriate sampling method. In this note I report problems encountered using funnel traps to sample snakes. These problems include: 1) predation upon trapped Banded Watersnakes (*Nerodia fasciata fasciata*) by Eastern Cottonmouths (*Agkistrodon piscivorus piscivorus*); 2) potentially biased sex ratios of *N. fasciata* and *A. piscivorus*; and 3) predation upon trapped snakes by red imported fire ants (*Solenopsis invicta*).

I used both commercially available minnow traps of dimensions 42 cm long by 22 cm in diameter (Cuba Specialty Manufacturing Co., Filmore, New York; funnel openings enlarged to 3 cm with a rake handle), and funnel traps made from hardware cloth that were 41 x 22 cm with 5-cm funnel openings (Fitch 1987) to sample *N. fasciata* and *A. piscivorus.* The study site was the Pee Dee Research and Education Center (PDREC), a 972-ha experimental farm owned by Clemson University, located in the upper coastal plain of Darlington County, South Carolina, USA. Traps were