Success of intracoelomic radiotransmitter implantation in the treefrog (*Hyla versicolor*)

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Concern about the effects of habitat modification on the natural movements and behavior of amphibians has led researchers to devise several methods to follow individual animals as they migrate among seasonal resources; one such method is the surgical insertion of radiotransmitters. Intracoelomic implantation has come into general use despite the relative lack of specific procedural guidelines and information about the effects of transmitter insertion and removal. The author outlines the surgical implantation procedures used on a small amphibian (gray treefrog) and provides insight into the effects of multiple major surgical invasions on the long-term survival of amphibians. Multiple survival surgeries were successful in this study and may be an alternative to euthanasia in some instances.

Concern about the status of amphibian populations across the globe has led to an increase in the need for information about the location and habits of individuals throughout their life cycle. Determination of terrestrial habitat requirements (*i.e.*, space-use estimates) and quantification of migration and dispersal distances are crucial pieces of information when the goal is to ensure population stability despite increasing habitat modification. These data are particularly difficult to obtain for many amphibians because of their cryptic life-style. Although amphibians may be easily located and studied during the breeding season, when they are congregated in large numbers, they spend the majority of their time in the terrestrial habitat, where they are typically secretive and difficult to find. Of the many methods that have been developed for tracking amphibians as they emigrate from breeding aggregations (*e.g.*, thread trailing, radioactive tagging, fluorescent powders), the most successful method for elucidating amphibian activities at distances from breeding sites has been radiotelemetry.

For amphibians, radiotelemetry requires that individuals have transmitters implanted intracoelomically[1–3] or subcutaneously[4], attached externally via backpacks[5,6] or waistbands[7,8], or contained within the gastric cavity following forced ingestion[9]. The most appropriate method of attachment depends on the morphology of the target species and the particular habitat in which tracking will occur. To study the movement and activity patterns of the gray treefrog (*Hyla versicolor*) during the nonbreeding season, the author surgically implanted radiotransmitters into the coelomic cavity. Implantation was preferable because of concern that backpacks would hinder the ability of the animals to maneuver in tight spaces (*e.g.*, treeholes) and waistbands would not remain attached to the slender treefrogs. Although ingestion of transmitters has been shown not to influence feeding behavior of larger amphibian species[9] or snakes[10], the large size of the transmitter compared with the size of the treefrog suggested that forced ingestion may be inappropriate.

Several workers have reported that implantation of foreign elements into the body cavity has in some instances pathological and behavioral effects on birds[11] and mammals[12], but limited information exists about the effects of implantation in amphibians[13]. In fact, only a few investigators have reported procedural techniques for insertion of transmitters (or dataloggers) into the body cavity of amphibians[1–3,14,15], despite a preference for the method for the relocation of marked animals in a variety of habitats. Although descriptions of general surgical techniques for amphibians have appeared in the literature[16,17], this report details the

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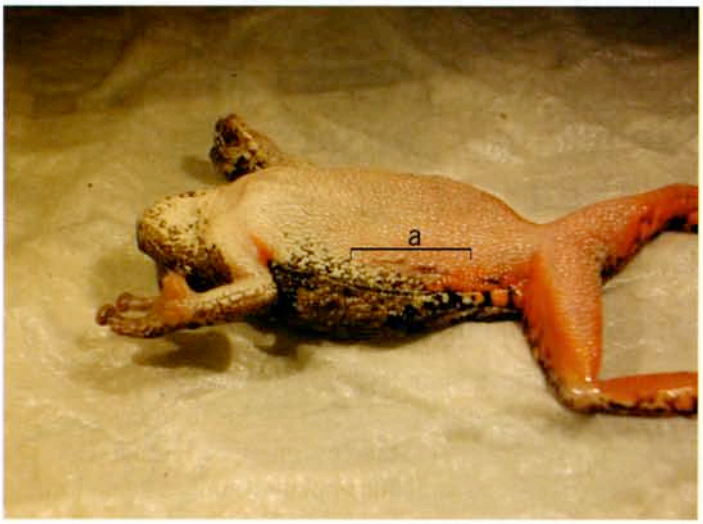

FIGURE 1 | Individual no. 0895 showing positioning for surgery, and the location/length of incision (**a**).

surgical procedures used for implantation and subsequent removal of transmitters in gray treefrogs, and provides insight into the fate of individuals during the weeks following the implantation surgery and transmitter removal 1 year later in a second survival surgery.

METHODS

Animals and radiotransmitters

The University of Missouri Animal Care and Use Committee (Protocol 3950) approved all of the procedures.

We captured a total of 22 adult (10 male and 12 female) gray treefrogs in experimental arboreal retreats constructed from acrylonitrile butadiene styrene conduit and marked them by toe excision during the course of a separate study. The timing of capture (~25 d before the first 0° C night) and body mass determined the selection of frogs for surgery. We captured 10 frogs from 2 October to 21 October 2003 and 12 frogs from 27 September to 14 October 2004. Transmitters were not to exceed 10% of an individual's mass[18], and we included in the study only frogs weighing more than 8.5 g. At the time of capture, the average length of individuals was 50.8 mm (±1.2 mm s.e.) from snout to vent, and the average weight was 11.5 g (±0.9 g s.e.). Individuals were kept overnight in individual ventilated plastic containers (23.5 × 15.2 × 16.5 cm) with several wetted paper towels until surgery the following day.

Holohil Systems Ltd. (Carp, Ontario, Canada) supplied 12 BD2 radiotransmitters (weight 0.85 g, dimensions 14 × 6.5 × 3.5 mm) with internal helical antennae and an expected battery life of ~28 d in 2003 and 25 d in 2004. We soaked radio transmitters that had been encapsulated in an inert waterproof expoxy in ethyl alcohol and then rinsed them with sterile water immediately before implanting them.

Implantation procedure

We performed all surgical procedures in a facility approved by the University of Missouri Animal Care and Use Committee. Before surgery, we sterilized a laboratory table with a 10% bleach solution followed by ethyl alcohol. Surgeries took place on new paper towels moistened with sterilized water, under a dissecting microscope with supplemental fiber optic light source, with the surgeon wearing latex examination gloves throughout the surgical procedures.

Before surgery we weighed each frog with a digital scale and measured it with a digital caliper, then submerged the frog in a 0.2% (in 2003) or 0.4% (in 2004) aqueous solution of ethyl-*m*-aminobenzoate methanosulfonate (MS-222, Sigma-Aldrich Co., St. Louis, MO) buffered to pH ~7 with sodium bicarbonate[17]. MS-222 is in common use for anesthesia of fish and amphibians[19]. We kept the frogs in the anesthetic solution until a loss of righting behavior and lack of response to toe pinching ($\bar{x}$ = 6 min, ±2.5 min s.e.) indicated deep sedation. Immediately after anesthesia we rinsed frogs with sterile water to remove residual anesthetic[17], although other reports of amphibian surgery suggest that individuals be held in the anesthetic solution for the duration of the procedure to maintain hydration and unconsciousness. No breathing or buccopharyngeal movement was detectable in anesthetized frogs, but the heartbeat remained visible under the dissecting microscope when dorsally recumbent. Following removal from the MS-222 solution, frogs remained immobile for a little less than 30 min. Obvious breathing and gulping behavior preceded awakening. Supplemental drops of anesthesia solution occasionally administered to the throat and chest region maintained sedation and prevented desiccation. Individuals righted themselves ~20 min after surgery.

Radiotransmitter implantation consisted of a 6-mm incision in the ventrolateral abdominal musculature lateral from and parallel to the ventral midline and anterior to the right hindlimb (**Fig. 1**). We positioned the incision site in such a way as to avoid major veins and minimize rubbing of the wound by the hindlimbs and substrate. We inserted the transmitters into the coelomic cavity, positioning them parallel to the intestines and gonads[1]. Between five and six sutures closed the musculature and the epidermis separately[16] with absorbable 4-0 (in 2003) or 5-0 (in 2004) chromic gut monofilament (Ethicon, Inc., Somerville, NJ). VetBond tissue adhesive (3M Animal Care Products, St. Paul, MN) sealed the incision site; after the VetBond had dried we applied a

minimal amount of the topical analgesic lidocaine (2%; Akorn Inc. Buffalo Grove, IL).

Within 24 h after implantation we released the frogs at the point of capture and tracked them for the duration of the transmitter battery life (~25 d).

Radiotransmitter removal

Before the batteries failed, we removed the radiotransmitters and allowed the animals to recover in clean plastic containers with newly moistened paper towels for at least 24 h before returning them to the field.

We used sterilized forceps[20] to remove the radiotransmitters from anesthetized frogs via a slightly larger incision (7 mm) in a similarly lateral position on the opposite side from the initial surgical site. After closing the extrication incisions with six to seven stitches in the muscle and between seven and eight stitches in the epidermis, we applied VetBond and lidocaine on the surface of the wound as described earlier for implantation procedures. Frogs recovered from their second surgery in individual plastic containers with moistened paper towels for several days (depending on the outside temperature) before being permanently released.

Before release, the behavior (*i.e.*, body posture, avoidance behavior) of each animal had returned to normal. Monitoring of experimental arboreal refugia continued through the spring of 2005. We recorded the recapture of any animals after surgical procedures occurred.

RESULTS AND DISCUSSION

Previous research indicated that chromic gut sutures were a source of irritation in several taxa, including reptiles, but gave no mention of effects on amphibians[20]. In this study the sutures did not appear to cause any irritation. Absorbable sutures have been reported to dissolve too rapidly in moist amphibian habitats, and some have suggested the use of nonabsorbable monofilament sutures for the epidermis[17]. My results support this contention when using 5-0 sutures, because surgeries performed in 2004 had a higher occurrence of dehiscence than in 2003, but 4-0 sutures appeared to be sufficiently large to persist until wounds had healed. The arboreal habits of adult gray treefrogs may allow absorbable sutures to persist long enough for the wounds to heal. Furthermore, the sealing of incision sites with tissue adhesive provided additional strength to the sutures. There has been poor documentation of the proper dosages of analgesics for amphibians[17], so lidocaine was used sparingly. Lidocaine produced temporary redness in some instances.

Of the 22 frogs carrying radiotransmitters, 7 disappeared during the course of the two study periods (2003 and 2004) or remained missing after the termination of the study. At least one disappearance was the result of predation, and the rest were probably due to battery failure before recapture for transmitter extraction. Some wounds healed more rapidly than others, but all insertion wounds

TABLE 1. Surgery and last recapture dates for all animals tracked during 2003 and 2004 that underwent survival transmitter removal[a]

Animal ID	Sex	Extraction surgery date	Initial recapture date	Number of days until first recapture	Final recapture date	Minimum days surviving
0330	M	7 Dec 2004	13 Apr 2005	127	13 Apr 2005	127
0736	F	19 Nov 2003	16 Apr 2004	149	22 May 2004	185
0745	M	31 Oct 2003	23 Apr 2004	175	13 May 2004	195
0769	F	2 Nov 2003	9 Apr 2004	159	10 Apr 2005	543
0837	M	3 Nov 2003	N/A	N/A	N/A	N/A
0881	F	6 Dec 2004	5 Apr 2005	120	5 Apr 2005	120
0884	F	18 Nov 2003	23 Apr 2004	157	19 Aug 2004	275
0888	M	30 Oct 2003	N/A	N/A	N/A	N/A
0895	F	5 Nov 2004	N/A	N/A	N/A	N/A
1026	M	3 Oct 2003[b]	23 Apr 2004	203	23 Apr 2004	203
1161	F	6 Dec 2004	N/A	N/A	N/A	N/A
1720	M	6 Dec 2004	N/A	N/A	N/A	N/A
2838	F	16 Nov 2003	21 Apr 2004	157	21 Apr 2004	157
4032	F	8 Oct 2004[b]	5 Apr 2005	179	5 Apr 2005	179
4070	F	8 Oct 2004[b]	13 Apr 2005	187	13 Apr 2005	187
4074	F	3 Nov 2004	N/A	N/A	N/A	N/A
4080	M	6 Dec 2004	N/A	N/A	N/A	N/A
4087	M	6 Dec 2004	N/A	N/A	N/A	N/A
$\bar{\chi}$				149[c]		229[c]

[a]All recaptures were recorded in experimental arboreal refugia.
[b]Implantation surgery.
[c]Excluding implantation surgery dates.

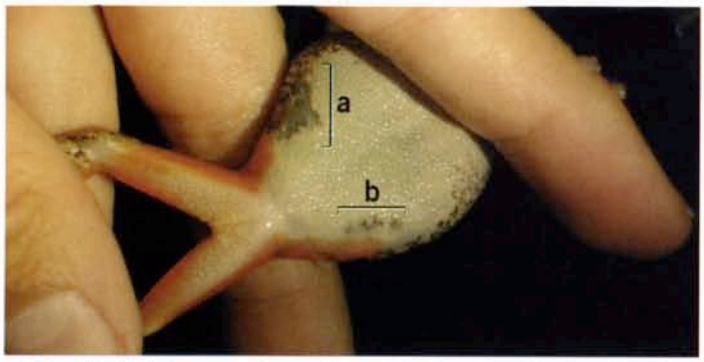

FIGURE 2 | Individual no. 0769 showing (**a**) scarring of early surgery with insufficient epidermal stitches 204 days after surgery, and (**b**) scarring of transmitter removal surgery with use of nine epidermal stitches and VetBond 173 days after surgery.

healed before the extraction procedures were performed. All sutures in muscle tissue maintained integrity, but early surgeries with fewer epidermal sutures (two to three) did show some evidence of minor dehiscence of the skin incision. Upon recapture, these frogs appeared to remain healthy and free from infection.

Of the five individuals lost because of presumed battery failure, we recaptured three (frogs no. 1026, no. 4032 and no. 4070) in arboreal retreats ~6 months after implantation and identified them by their unique toe clip markings. A larger amount of fibrous tissue had accumulated around the surface of the transmitter implanted in no. 1026 than around those that had been removed after the typical 25-d period, and that animal expired several days following removal surgery. Excess tissue was not apparent in frogs no. 4032 and no. 4070, and the extraction surgeries were successful. No other direct mortality occurred from surgical procedures. There was no evidence of infection at or around the incision sites; however, individual no. 0895 developed swelling in the right hindlimb during the course of tracking and died several days after the transmitter removal surgery.

During 2004 we recaptured six of the seven frogs that underwent transmitter removal surgery in 2003 (**Table 1**) and identified them by their toe clip markings. These individuals showed varying degrees of scarring that appeared to be a corollary of the investigator's improvement in surgical proficiency (**Fig. 2**). All six recaptured animals were in the same experimental arboreal refuge in which they were initially captured (before surgery) and appeared to be behaving normally. We observed one female (no. 0769) in amplexus at a breeding site; the frog traveled 100 m back to its diurnal refuge site within a 48-h period. During brief monitoring in the spring of 2005 (5 April–14 April), we also recaptured four individuals that we had tracked in 2004 (**Table 1**). In total, we recaptured 10 of the 18 individuals that underwent transmitter removal surgery, and each survived for at least 120–543 days (**Table 1**). Overall, individuals weighed an average of 1.1 g (9%) less directly after the removal of the radiotransmitter than they did directly before implantation (**Table 2**).

Intraperitoneal implantation of radiotransmitters did not adversely affect gray treefrog behavior either while implanted or after removal. We recaptured the vast majority of individuals within their presumed nonbreeding season foraging grounds between 120 and 543 days after their final surgeries. Similarly, we also recaptured frogs that were not subject to surgical procedures within their fall foraging sites during spring monitoring of diurnal refugia. All recaptured animals that had had surgery appeared to be in good health, were active, and did not display any external malformations as a result of two major survival surgeries.

Although the procedures outlined subject individuals to stress, these data suggest that normal behavior resumes quite rapidly and long-term effects are minimal. Other studies that have used less invasive external transmitter

TABLE 2. Weight loss during the interval of transmitter implantation for all frogs tracked during 2004 that underwent transmitter removal

Animal ID	Sex	Mass (g) before implantation	Mass (g) following extraction	Weight change (g)	Percent weight loss	Days with implant
0330	M	9.90	8.49	−1.41	14.24	28
0881	F	14.93	12.13	−2.80	18.75	15
0895	F	14.46	14.35	−0.11	0.76	26
1161	F	12.20	10.34	−1.86	15.25	23
1720	M	9.19	9.17	−0.02	0.22	30
4074	F	13.58	12.30	−1.28	9.43	27
4080	F	11.60	10.93	−0.67	5.78	30
4087	M	11.19	10.32	−0.87	7.77	25
$\bar{X}$		12.13	11.00	−1.13	9.02	25.50

attachment procedures (*e.g.*, waistbands) have reported problems with external lesions on the skin after prolonged attachment of transmitters. Certainly, these irritations can pose a great stress to individuals due to the prolonged duration of the irritation and, as a result, may bias any movement data collected. Similarly, individuals no. 1026, no. 4032, and no. 4070 demonstrate that the extended presence of a transmitter within the body cavity does not prevent successful overwintering but may lead to complications during extraction. The duration of any study involving tracking of amphibians should receive careful consideration in light of these limitations.

Most, if not all, of the animals used in this study were able to survive the winter immediately after a second surgical procedure, barring extraneous circumstances. These data suggest that the standard Animal Care and Use Committee protocol, requiring that animals be euthanized instead of undergoing a second major survival surgery, should have a case-specific application. The possible benefits of returning individuals to a population (especially in the case of endangered species) may outweigh the temporary stressor of additional surgery in some instances. The loss of weight during the study is of some concern when one considers that the primary activity of anurans between breeding and hibernation is to forage and restore energy reserves that were used during breeding activities. However, all animals were able to survive the winter and to resume foraging the following spring, and no. 0769 was healthy enough to produce a clutch of eggs during the breeding season after two major surgeries (**Fig. 2**). Without information about typical patterns of weight loss (or gain) of amphibians after a prolonged breeding season, the observed weight loss is difficult to put into context. However, during a study of the effect of desiccation on body mass of gray treefrogs, individuals were able to recover from weight loss of as much as 30% and typically varied by 5% in the control group (unpublished data).

To the author's knowledge no other study has reported wild recaptures of radiotracked individuals after removal of internally housed transmitters. Despite the use of such surgical implantations in the past[1,3,13,21], little information exists about the effects of surgical procedures following multiple major survival surgeries. Multiple surgeries did not have any obvious adverse effects on individuals, and when done properly, may be appropriate for use on critically imperiled species. When followed, the methods outlined in this study will help to generate crucial information about the movements and activities of amphibians that are moderate to large in size.

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COMPETING INTERESTS STATEMENT

The author declares that he has no competing financial interests.

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