ability to extract usable DNA from claw clippings may prove extremely useful and aid in future molecular investigations of turtles and other reptiles.

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Assessment of Intracoelomic Implantation and Waistband Harness Radiotransmitter Attachment on Wood Frogs (*Lithobates sylvaticus*) and Boreal Toads (*Anaxyrus boreas boreas*)

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Radiotelemetry has proven to be a valuable tool for determining amphibian activity, dispersal and migration patters because amphibians are often secretive, nocturnal and sometimes move long distances in short periods of time (Madison et al. 2010; Richards et al. 1994). Despite this success, transmitter attachment is often problematic for amphibians, especially anurans. Two techniques for transmitter attachment are available: "harness attachment" (also called belt or backpack) where radios are secured externally via a waist belt, and "implantation" where radios are inserted intracoelomically (sometimes subcutaneously) via surgery through the abdominal wall.

Harness attachment is most commonly used for anurans, because fitting harnesses *in situ* is relatively quick (5–10 min) and external transmitters with whip-like antennae are detectable over great distances (> 200 m), reducing the likelihood of losing subjects. If harnesses break or are fitted too loosely animals can shed transmitters; if fitted too tightly harnesses can restrict animal mobility and cause blood pooling in extremities (Bartelt and Peterson 2000). Skin lesions are often associated with external harnesses as well (Goldberg et al. 2002; Weick et al. 2005), which can cut through muscle if left unchecked. Skin lesions can also produce infections. A wide variety of harness materials have been used in past studies in an attempt to maximize transmitter retention while minimizing harm to animals (Goldberg et al. 2002), but injuries are still common. Given these possibilities and associated behavioral changes, external transmitters can produce conclusions on activity patterns that are not representative of the species as a whole (Bartelt and Peterson 2000; Johnson 2006).

To avoid these issues, many researchers favor internal transmitters. The acute stress associated with surgical implantation of radiotransmitters may be less taxing on animals than the chronic stress associated with harnesses, particularly for prolonged studies (Johnson 2006). Intracoelomic and subcutaneous surgical implantation techniques have been used on a variety of amphibian taxa (Eggert 2002; Johnson 2006; Stouffer et al. 1983). Surgical incisions from transmitter implantation in both laboratory and field settings have healed well in previous studies (Goldberg et al. 2002; Johnson 2006; Weick et al. 2005). Disadvantages of internal transmitters include a smaller detection radius (20–50 m) of the helical coiled antennae, increasing the likelihood of losing subjects that move greater distances over short periods of times. There is also the potential for mortality either directly from surgical error or in response to anesthesia. For example, the most problematic aspect of implantation surgery on the Barking Frog (*Eleutherodactylus augusti*) was determining the appropriate level of anesthesia to use (Goldberg et al. 2002), which can vary with water temperature (ASIH et al. 1987). Infections are also possible.

Investigations into the relative impacts of radiotransmitter attachment techniques are necessary because of these variables. Generalizations might be made for species within the same genus or family, provided that the species in question share similar physiologies, habitat requirements, life-history traits and behavior. However, species-specific responses are known to exist (Rowley and Alford 2007) and rigorous assessments of data quality and methodology are scarce. Therefore, the objective of this study was to assess the suitability of waistband-harnessed and intracoelomic implantation radiotransmitter attachment techniques for use on Wood Frogs (*Lithobates sylvaticus*) and Boreal Toads (*Anaxyrus boreas boreas*). Adult Wood Frogs are relatively small (40–57 mm snout–vent length [SVL]) semi-aquatic anurans with thin, smooth skin. Boreal Toads are larger (63–78 mm SVL) terrestrial anurans with thick, rough skin.

Methods

Radiotelemetry was conducted from May through August in 2007 and 2008, on 111 Wood Frogs (62 external and 49 internal transmitters) and 38 Boreal Toads (22 external and 16 internal transmitters) in four wetland areas on the Canadian Boreal Plain near Whitecourt, Alberta. Animals were collected by hand, using a D-frame aquatic net, or in pitfall traps maintained by an amphibian monitoring study (the Study of Forestry and Amphibians [SOFA] project) established at the same sites. All animals (regardless of intended radiotransmitter attachment method) were transported to a base camp where body weight and SVL were recorded and radiotransmitters were attached. For Wood Frogs, 0.7 g BD-2 (external) and 0.8 g BD-2H (internal) transmitters (3 week runtime) were used. For Boreal Toads, 1.4 g BD-2 and 1.5 g BD-2H transmitters (9-week runtime) were used in 2007, and 1.6 g BD-2 and 1.7 g BD-2H transmitters (11-week runtime) were used in 2008. Transmitters were obtained from Holohil Systems Ltd. (Carp, Ontario).

Transmitter attachment and radiotelemetry procedures were approved by the Lakehead University Animal Care Committee. External transmitters were attached via waistband harnesses constructed from stretch bead cord following Baldwin et al. (2006). The surgical procedure employed for radiotransmitter implantation was similar to that described in Goldberg et al. (2002). Subjects were anaesthetized in 0.01% solution of MS-222. Time to suitable anesthesia was highly variable, but generally was 10–30 min. Boreal Toads took longer to anesthetize. Lateral

Table 1. Summary of tracking periods.

Tracking Period (Days)	Boreal Toad (N)	Wood Frog (N)
1–9	4	10
10–19	9	57
20–29	3	22
30–39	6	9
40–49	8	7
*	5	3
70–89	3	

* 50–69 for Boreal Toads; 50–75 for Wood Frog

incisions (12–15 mm) were made first though the skin and then through the muscle layer (rectus abdominus) along the right side near the ventral midline. Transmitters were inserted within the coelomic cavity along the muscle wall. Muscle and cutaneous layers were sutured separately using absorbable suture material (4/0 chromic gut in 2007 and 4/0 polydioxanone suture in 2008). Three to five simple, interrupted sutures were used to close each tissue layer. Tissue glue (Dermabond© in 2007; Vetbond© in 2008) was applied to sutures and surrounding skin to seal the cutaneous incision. Each surgical procedure took an average of 20–30 min. Animals were revived in local stream water and held overnight for observation before being released at the site of their capture the following morning.

The 3-week runtime of the Wood Frog radiotransmitters required that transmitters approaching the end of their battery lives be exchanged for fresh ones. Individuals with external transmitters were refitted with new transmitters only if skin lesions were absent. Re-implantation of coelomic transmitters was performed in 2007 but not in 2008.

Animals were located once every 24–48 h by using R-1000 receivers (Communication Specialists Inc., Orange, California) with a 3-element yagi-style antennae. Each animal location was marked using GPS. Animals were examined for presence of injuries or infection associated directly with radiotransmitter attachment, or notable weight loss. Animals were recaptured for transmitter removal on the final day of the average runtime of their respective transmitters. Final weights of recaptured animal were recorded after radiotransmitters were removed.

Home range is likely to be positively related to observation period for species that do not actively defend territorial home ranges; the longer an animal is followed, the farther it moves. Both species in this study were expected to exhibit this relationship. Three simple movement parameters were derived to assess the suitability of both attachment techniques for collecting geospatial data. Home range area (HR, m^2) and linear distance travelled (LD, m) describe seasonal movement patterns. Mean patch spacing (MPS, m) describes movement behavior over a shorter timeframe (24–48 h).

Univariate analysis of variance was used to describe the relationships between transmitter attachment, tracking period (the length of time data were collected on an individual), animal condition (change in body weight and the presence of injuries), and movement parameters. Movement parameters were $\log_{10}$-

TABLE 2. Summary of animal fates (pooled 2007 and 2008): HR = home range, MPS = mean patch spacing, LD = linear distance, SE = standard error.

Species	Transmitter	Animals (N)	Injuries (N)	Deaths (N)	Lost (N)	Tracking Period (days) Mean (min–max)	HR Mean (min–max) (m^2)	MPS Mean ± SE (m)	LD Mean ± SE (m)
Wood Frog	External	62	24	*7	19	20 (1–74)	175 ± 13	15 ± 2	51 ± 5
	Internal	49	9	11	7	21 (3–55)	192 ± 17	16 ± 2	54 ± 6
Boreal Toad	External	22	9	*5	7	41 (6–79)	5839 ± 623	58 ± 9	302 ± 61
	Internal	16	0	5	9	28 (1–88)	3908 ± 675	73 ± 15	223 ± 41

The difference between the total number of animals and those that died or were lost represents animals that were successfully released.

* 1 confirmed case of predation.

transformed for analysis. Species were considered separately. Tracking period was divided into six categories for Wood Frogs, and seven categories for Boreal Toads (Table 1). Animals with single observations were omitted from analysis. A critical alpha value of 0.05 was employed.

RESULTS

Tracking period and movement.—Transmitter attachment method had no effect on Wood Frog tracking period or movement parameters ($P > 0.87$). Boreal Toad tracking period ($F_{1,36} = 4.08$, $P = 0.05$) and HR ($F_{1,36} = 4.25$, $P = 0.05$) were greater with external transmitters, but MPS and LD did not differ between transmitter types ($P > 0.47$) (Table 2). Tracking period interacted with Wood Frog HR ($F_{1,104} = 18.32$, $P << 0.01$) and LD ($F_{1,104} = 2.51$, $P = 0.03$), as well as Boreal Toad HR ($F_{1,36} = 7.12$, $P << 0.01$). All other movement parameters did not interact with tracking period ($P > 0.72$).

Mortality due to surgery.—Six animals did not recover from anesthesia (1 Wood Frog and 5 Boreal Toads, not included in the 149 animals used for our analyses). We do not know why these animals died; the general surgical methodology was consistent throughout, as was the concentration of MS-222 used.

Post-operatively, most sutures healed completely and suture material disappeared after three weeks. In 2008, there were four instances where Wood Frogs either developed lesions around sutures, or suture material did not fully dissolve by the time transmitters were removed. Nevertheless, these individuals were still active in the field. There were no confirmed cases of infection associated with surgical incisions or harness lesions. Multiple implantation surgeries had no observable impact on animals in 2007, with the exception of one Wood Frog that exhibited severe weight loss.

Weight loss.—Transmitter attachment method was associated with changes in body weight in Wood Frogs ($F_{1,43} = 6.43$, $P = 0.02$). More animals gained weight with internal transmitters than with external transmitters, although individuals from both categories exhibited weight loss (Table 3). All Boreal Toads observed until the end of their transmitter runtimes exhibited weight gain regardless of transmitter attachment method (Table 3).

Injuries.—Injuries were associated most commonly with external transmitters (Wood Frog: $F_{1,104} = 8.48$, $P < 0.01$; Boreal Toad: $F_{1,36} = 8.11$, $P < 0.01$) (Table 2). Injuries interacted with tracking period for Boreal Toads ($F_{1,36} = 13.51$, $P < 0.01$), but not Wood Frogs ($F_{1,104} = 3.14$, $P = 0.08$). The presence of injuries did not influence the movement parameters of either species (Wood Frog: $P > 0.39$; Boreal Toad: $P > 0.11$). Injuries were most common and developed earlier among Wood Frogs.

Susceptibility to predation.—There were only two confirmed cases of predation (Table 2), both associated with external transmitters and attributed to avian predators.

Reproduction.—The presence of external transmitters did not prevent amplexus. Grasping males would sometimes shed transmitters fitted to gravid females. Amplexus was not observed in animals fitted with internal transmitters, but there was no evidence suggesting implantation interfered with reproduction (but see Heemeyer et al. 2010).

DISCUSSION

Transmitter attachment method had mixed effects between Wood Frogs and Boreal Toads. Both transmitter types were able to capture Wood Frog movements to the same degree. However, Boreal Toad home ranges were larger with the use of external transmitters. The toads were capable of pulse movements that far

TABLE 3. Summary of animal weight change observed during 2008 for 43 Wood Frogs and 6 Boreal Toads.

Species	Transmitter Type	Weight Loss (N)	Weight Gain (N)	Proportion of Animals Exhibiting Weight Loss (%)	Tracking Period [Days] Mean (min–max)
Wood Frog	External	18	3	86	19 (7–40)
	Internal	12	10	55	18 (13–22)
Boreal Toad	External	0	3	0	54 (34–79)
	Internal	0	3	0	68 (53–87)

exceeded the detection radius of internal transmitters, and were easily lost during these periods. This also accounts for the shorter tracking periods reported for Boreal Toads fitted with internal transmitters. HR values for both species and Wood Frog LD expressed a positive relationship with tracking period, as expect for species without fixed, defended territorial home ranges. MPS was not affected by tracking period, which reflects the relative scales of the three movement parameters. Home range and LD reflect areas exploited and distance traveled over the entire tracking period, whereas MPS reflects the distance an animal can travel in a single tracking interval (24–48 h).

Internal transmitters appear to be most appropriate for longer studies, since the chance of developing lesions as the result of chronic harness exposure increases with time. More Wood Frogs gained weight when fitted with internal transmitters than those with external transmitters. Although amphibian weights are known to be extremely variable and are often linked to dehydration (Stebbins and Cohen 1995), the weight changes observed corroborate our data on abrasion injuries which demonstrates that transmitter implantation is in fact less harmful to anurans. While there are health risks associated with either radiotransmitter attachment technique (Goldberg et al. 2002; Weick et al. 2005), the chronic distress associated with belt abrasion appears to be greater than the short-term and admittedly intense strain imposed by radiotransmitter implantation.

Boreal Toads appear to be better than Wood Frogs at withstanding the perturbations associated with transmitters. In the case of harness attachments, this could be attributed to skin structure (thickness and irregularity) and body size, but might also reflect the relative transmitter-to-animal weight ratio. The transmitter percent of body weight in Wood Frogs (approaching 10%) was greater than that for Boreal Toads (under 5%). Our findings support the recently recommended maximum ratio of 5% (Goldberg et al. 2002).

Our data also support the notion of species-specific transmitter effects (Rowley and Alford 2007), although generalizations can be made. Internal transmitters are recommended for smaller-bodied anuran species with semi-aquatic life-history traits, like Wood Frogs and many other species belonging to the genera *Rana* and *Lithobates*. Larger species, and those sharing more terrestrial or semi-arid life-history traits (e.g., *Bufo* and *Anaxyrus*) are likely better able to withstand waistband harnesses. It is also necessary to consider a species' behavior. Internal transmitters were capable of capturing the movements of Wood Frogs as accurately as external transmitters. However, external transmitters were better able to capture the longer-distance pulse movements of Boreal Toads. We generally recommend internal transmitters whenever appropriate.

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