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Radiotransmitter Attachment Technique for Box Turtles (*Terrapene* spp.)

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Terrestrial chelonians are often difficult to locate and monitor under natural conditions because many species are cryptic in coloration and behavior. For example, the eastern box turtle (*Terrapene carolina carolina*) has a carapace often spotted with yellow or orange, and brown or black, a pattern that easily blends into substrate of leaf litter. In addition, eastern box turtles bury themselves in leaf litter (i.e., in forms) and spend long periods underground in burrows. In the northeastern United States, eastern box turtles may be easily overlooked during visual surveys and marked individuals often cannot be relocated with any regularity (pers. obs.). Radiotelemetry, which allows observers to relocate an individual on demand, has become an essential tool for studying the movements, home range, habitat use, and other characteristics of box turtles and other chelonians that are difficult to locate and observe.

Application of radiotelemetry to the study of small animals, such as terrestrial turtles, can be challenging. Biologists must consider not only the objectives and constraints of the study, but also the welfare of the animals; many universities and research institutions now require that methods used in live-animal studies be reviewed and approved by an institutional animal care and use committee before implementation. Most studies require that a radiotransmitter remain attached to an individual for an extended period of time; however, the radiotransmitter and attachment must not affect the behavior, physiology, reproductive success, or survival of the study animal if accurate observations are to be obtained. In the case of box turtles, the radiotransmitter and attachment must not excessively burden the animal, become entangled in vegetation, increase rate of detection by predators, pose an obstruction to burrowing, or disrupt copulation or other behaviors. Methods of attachment, such as adhesives or hardware, must be non-toxic and should not increase chances of infection or disease, cause significant injury or developmental abnormalities, or produce potentially damaging amounts of heat. Ideally, the method of attachment should allow the radiotransmitter to be replaced or removed as necessary without causing injury or stress to the study animal.

We attached radiotransmitters to adult eastern box turtles (*T. c. carolina*) as part of a study of their habitat use, home range, and movements in southeastern Massachusetts. To attach the radiotransmitters, we modified a method used by Boarman et al. (1998) to attach radiotransmitters to larger adult desert tortoises (*Gopherus agassizii*). Our goals in selecting a radiotransmitter and attachment method were to minimize: 1) percent of the turtle's body mass of the radiotransmitter and its attachment; 2) the profile of the radiotransmitter and attachment against the carapace, 3) the likelihood the radiotransmitter would interfere with copulation; and 4) handling time and duration of captivity required during radiotransmitter attachment and replacement. We also wanted to maximize the range of signal detection and radiotransmitter life. Here we describe our adaptation of Boarman et al.'s (1998) method and report on the results.

Methods. — We used Advanced Telemetry Systems (Isanti, MN) Model 7PN radiotransmitter (164 MHz range with 1/4 wave antenna) modified for use on box turtles. We selected this model, in part, because its dimensions allowed us to attach it to a single pleural scute with little or no overlap of scute sutures. Radiotransmitter length (mean = 40.0 mm, $n = 11$) was similar to the length of a pleural scute of an adult turtle, while width (mean = 18.8 mm, $n = 11$) was much smaller than the width of the scute. The profile of the radiotransmitter was relatively flat (mean height = 9.6 mm, $n = 11$) and would not greatly increase the profile of the animal when attached. Finally, radiotransmitter mass was low (mean = 12.5 g, $n = 30$), and radiotransmitters were guaranteed to emit a signal for 130–260 days.

After locating an adult study turtle, we attached a radiotransmitter to it using one of two modifications made to the method described by Boarman et al. (1998). Our first modification ("epoxy base") was to attach the radiotransmitter by bonding the entire bottom surface of the radiotransmitter to the third pleural scute of the carapace with waterproof epoxy putty (as described by Boarman et al., 1998), then threading the antenna through pieces of aquarium tubing bonded to pleural scutes (Figs. 1 and 2). Our second adaptation ("silicon base") was to attach the radiotransmitter to the carapace by creating two "brackets" of putty on the edges of the radiotransmitter, with a cushion of nontoxic aquarium silicon sealant between the radiotransmitter and the carapace (Figs. 1 and 2). Both attachment methods are described as follows.

A radiotransmitter attachment kit was prepared in anticipation of capturing turtles. This kit contained 2–4 radiotransmitters, nitrile gloves, cotton balls, isopropyl alcohol, non-toxic silicon aquarium sealant, non-toxic adhesive epoxy putty (Power Poxy, Plug n' Patch Epoxy, Plumbers Formula, #40102, Power Poxy Adhesives, Inc., New Berlin, WI), pre-cut 5 and 10 mm pieces of 3 mm internal diameter aquarium tubing, masking tape, a pocket knife, non-toxic markers (Sharpie, Sanford Corp., Bellwood, IL) and paint pens (Speedball Painters, Hunt MFG Co., Statesville, NC), metric ruler, calipers, and a portable electronic balance (Ohaus Model LS 2000). All radiotransmitters were tested before being placed into the kit, and kit materials were replenished as needed.

Upon locating a turtle we put on nitrile gloves. These gloves remained on during the attachment process. Before attaching the radiotransmitter we measured and recorded carapace length, width, turtle mass, and the time of capture.

We used a cotton ball moistened with isopropyl alcohol to remove dirt from the pleural scutes. We then placed the turtle in a cardboard box and allowed the scutes to dry. Once the alcohol dried we covered the sutures of the third pleural scute, typically the left side, with masking tape. The third pleural scute, posterior to the widest part of the carapace, was selected as the attachment location so that vegetation and other materials would pass over the carapace without getting caught on the radiotransmitter.

Our initial attachment method ("epoxy base") was to cover the bottom of the radiotransmitter with a layer of adhesive epoxy putty (Fig. 2). Our second approach ("silicon base") substituted a thick layer of silicon sealant for the adhesive putty (Fig. 2). In both methods the radiotransmitter, with the antenna extending forward, was then positioned on the bottom of the third pleural scute. The radiotransmitter was pressed against the carapace and held in place. When using the silicon base method, we were careful to leave a layer of silicon approximately 4 mm thick between the radiotransmitter and the carapace and not to squeeze the silicon out from under the sides of the radiotransmitter.

We then prepared approximately 1/5 package of nontoxic epoxy putty according to the directions on the packag-

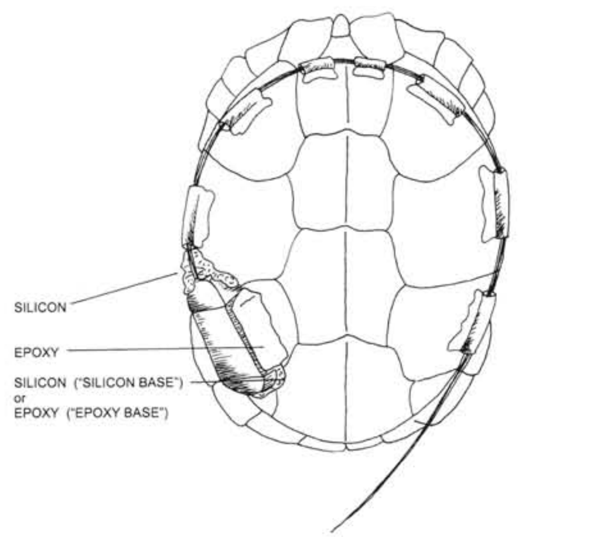

Figure 1. Dorsal view of a box turtle carapace showing position of radiotransmitter, antenna, and attachment materials.

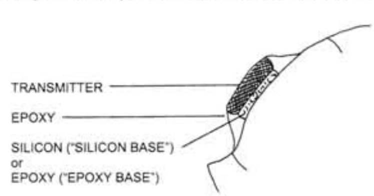

Figure 2. Cross sectional view of a box turtle carapace showing position of radiotransmitter and attachment materials.

ing. We prepared two strips of putty, each approximately 40 mm long by 10 mm wide, and placed a strip lengthwise along each side of the radiotransmitter. This epoxy putty was either added to the putty under the radiotransmitter (epoxy base), or formed brackets that held the radiotransmitter to the carapace (silicon base). We pressed the putty onto the radiotransmitter and scute and removed excess putty with a pocketknife. We removed the masking tape and any putty from the sutures.

We attached sections of aquarium mini-tubing to the first and second pleural scutes on the side with the radiotransmitter, first vertebral scute, and the first through third pleural scutes on the side opposite the radiotransmitter. A single 10 mm long piece of tubing encased in a small amount of putty was positioned on the bottom, center of each pleural scute and pressed against the carapace. Two 5 mm sections of tubing, one to either side of the keel, were attached to the vertebral scute. We threaded the antenna through the tubing as we attached each piece, and removed any putty that blocked the openings in the tubing or covered a suture. We then checked the antenna to ensure that it moved freely and would pull out of the tubing if it became entangled in vegetation. We removed excess putty from the sides of the tubing by cutting it away.

To prevent entanglement in vegetation or debris we filled and contoured the anterior and posterior ends of the radiotransmitter with silicon sealant. We filled the space between the anterior end of the radiotransmitter, antenna, and first piece of tubing (Fig. 1), and also filled the space between the posterior end of the radiotransmitter and the carapace with silicon.

Once the putty had set (ca. 10 min) we used non-toxic paint pens and permanent markers to camouflage the radiotransmitter and putty with colors and patterns similar to those of the turtle's carapace. We then measured the combined mass of the turtle and radiotransmitter. We wrapped masking tape over the radiotransmitter to cover the silicon until it was dry, and used non-toxic paint pens and markers to camouflage the tape.

We released the turtle and checked the signal. We relocated the turtle within two days of radiotransmitter attachment and removed any remaining masking tape. We located each turtle 1-3 times per week with a Telonics Model TR-2000 receiver and an "H" antenna from the day of capture through mid-November 1998 and sporadically thereafter through July 1999. We attempted to obtain visual confirmation of each turtle's exact location and to inspect radiotransmitter condition whenever we monitored our study animals.

Results. — We attached radiotransmitters to 19 adult eastern box turtles using the putty base, and to two turtles with the silicon base method. Handling time for both methods, including radiotransmitter attachment and data collection, was 38–70 min (mean = 49 min). Handling time generally decreased as personnel became more proficient in radiotransmitter attachment. Radiotransmitters and attachments (i.e., tubing and adhesives) added 2.5–5.0% (mean = 3.9%, $n = 19$, excluding two individuals that urinated during handling) or 14–26 g (mean = 19.71 g) to an animal's mass.

Radiotransmitters functioned properly and remained firmly attached to our study animals for as long as 407 days; in some cases, however, radiotransmitters apparently failed after only a few weeks. Despite extensive searches, we were only able to locate three individuals through days 16, 71, and 75 after radiotransmitter attachment; we believe that these radiotransmitters failed. One other individual left the study area and we were unable to track its exact location, although we were able to confirm that the radiotransmitter was working 165 days after it was attached. Two individuals died during the study; one of unknown causes 23–32 days after radiotransmitter attachment, and the second was crushed by land-clearing equipment off our study area 5 days after radiotransmitter attachment. Between May and July 1999, nine radiotransmitters were still functioning 347–407 days after attachment. Seven were removed and replaced and two were left "as is" on the turtles (M. Ciaranca, Camp Edwards Environmental Protection Office, *pers. comm.*).

Our ability to detect a signal varied with the location and behavior of the turtle. When turtles were underground in burrows (generally ≤ 0.3 m below the surface), the distance at which we could detect the signal was highly variable. In most cases, signals from burrows could be detected several hundred meters from the burrow. However, in a few instances antennas appeared to have grounded out against the walls of the burrow and signal strength was greatly reduced. In one such case we were able to detect a signal from a turtle in a burrow only from approximately 10 m away. This turtle's signal was later discernable 100–200 m from the burrow (although the turtle remained inside). When turtles were active or in forms above ground, however, we could monitor their signals from as far as 963 m away through flat woodland habitat. Signal range also increased when part of the antenna pulled out of the plastic tubing and trailed freely. In all cases, we were able to identify an individual's exact location whenever a signal was detected and the turtle accessible.

Field observations suggest that our radiotransmitter attachment design did not impede reproduction. On two separate occasions we observed females with radiotransmitters copulating with unmarked males.

Discussion. — We attached radiotransmitters to the first 19 adult turtles that we captured during our study by bonding the entire bottom surface of the radiotransmitter to the carapace (epoxy base) with the same brand of putty adhesive used by Boarman et al. (1998). Although the manufacturer's stock number of the epoxy putty that we used was different from that used by Boarman et al. (1998), the manufacturer assured us that the two products were identical with the exception of package size. We therefore expected similar results with radiotransmitter removal. When one of the turtles in our study died 15–21 days after radiotransmitter attachment, however, we tried to remove the radiotransmitter from the frozen specimen with a pocketknife (as recommended by Boarman et al., 1998) and found that we were unable to do so. Our attempts to cut away the putty failed and eventually a large section of the scute broke away from the carapace. Had the turtle been alive, it may have been seriously injured. We had similar results when trying to remove a radiotransmitter from the remains of a second individual that was killed by land-clearing equipment. These observations reinforce the caution made by Belzer and Reese (1995), who noted that prying off radiotransmitters bonded to turtles with epoxy can tear scutes. However, in May 1999, approximately 12 months after the start of our study, radiotransmitters that had been attached to turtles for 347–366 days were easily removed without damaging scutes (M. Ciaranca, *pers. comm.*). Whether the putty degraded over time or whether natural sloughing of scute material, or both, caused the radiotransmitters to loosen over time is unknown. Regardless of cause, it appears the putty base method of attachment is acceptable if radiotransmitters are expected to remain attached to turtles for extended periods of time, such as the 11–12 months that elapsed between attachment and removal in this study. However, we are hesitant to recommend this method in cases where radiotransmitter life is shorter, incidence of radiotransmitter failure is high, or recovery of radiotransmitters after only brief periods of attachment is otherwise anticipated.

Because attempts to remove radiotransmitters from two dead turtles resulted in damage to scutes, we further modified the method used by Boarman et al. (1998) in order to minimize the amount of putty in contact with the carapace. Our silicon base method created a space between the radiotransmitter and carapace that provided working room for tools that would later be used to remove the radiotransmitter. We anticipated that a carefully used cordless rotary hobby tool (e.g., Dremel) with a cutting disk could readily cut through the strips of epoxy putty along the sides of the radiotransmitter. Because the radiotransmitter was not in direct contact with the carapace (due to the silicon pad) the cutting disk would not contact the scute and therefore would not damage the turtle. After the old radiotransmitter was removed, a new radiotransmitter could be attached to the putty that remained bonded to the carapace.

We believe the silicon base method appropriate for both long and short-term attachment of radiotransmitters. One turtle with a silicon base mounted radiotransmitter was relocated 357 days after attachment, indicating that the longevity of this attachment method is comparable to that of the putty base method. The main benefit of the silicon base method is that it permits the safe removal of radiotransmitters at any time without requiring either the epoxy or scute surface to degrade. The ability to remove radiotransmitters shortly after attachment may be especially important in the event of premature radiotransmitter failure. We recommend this approach for all applications because it minimizes the likelihood of turtles being injured during the radiotransmitter removal process.

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Rediscovery of the Critically Endangered River Terrapin, *Batagur baska*, in Cambodia, with Notes on Occurrence, Reproduction, and Conservation Status

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The river terrapin, *Batagur baska* (Gray, 1831), inhabits coastal rivers, estuaries, and mangrove swamps from eastern India and Bangladesh, to Myanmar, southern Thailand, Cambodia, Cochinchine (southern Vietnam), peninsu-