Experimental Analysis of RFID Antennas for Use in Herpetological Studies Using PIT Tagged Salamanders (Ambystoma tigrinum)

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The study of life history, growth and population dynamics is critical in the development of comprehensive management strategies for amphibian populations. Typical methods include capturing and marking individuals and studies of an individual's growth and movement. Traditional amphibian marking has been some combination of toe clipping. Toe clipping is a good way to mark individual specimens but many species can regenerate toes within one season (Ott and Scott 1999). Additionally, toe clips can sometimes be confounded by individuals who have lost toes to accident or predation. Tracking movements has been accomplished by use of radio telemetry, but this technique is costly and time consuming (French et al. 1992; Oldham and Swan 1992; Madison and Ferrand 1998; Werner 1991). Currently, there are few studies focused on expanding the use of Radio Frequency Identification (RFID) antennas to track individuals buried in the soil. There is no single method that accomplishes individual marking and individual tracking with efficiency and economy (Ferner 1979).

One alternative is the use of Passive Integrated Transponder (PIT) tags and PIT tag antennas to mark and track individuals. Use of PIT tags and Radio Frequency Identification (RFID) antennas allow positive identification of individual animals over the life of the individual (Brown 1997). Many studies have used PIT tags for the assessment of movement, growth and population parameters (Eggert 2002; Matthews 2003; Richter and Seigel 2002). The use of PIT tags has several advantages in long term studies: PIT tags have high rates of retention if implanted properly; they have a low rate of mortality due to implantation; they can be functional for 10 to 20 years (greater than the lifespan for many salamanders); and they positively identify individuals (Kuhnz 2000; Matthews 2003; Pycke 2005).

Kuhnz (2000b) used PIT tags and RFID technology to track legless lizards in loose sandy soils at short distances. Several pertinent findings were presented including the following:

1) RFID antennas can detect PIT tags through sand, dust covered sand and wet sand to a distance of 12.0 cm.

2) RFID antennas can help to detect organisms in heavy vegetative cover.

3) Organisms can be located at short distances without disturbance using RFID antennas.

Kuhnz reported a minimum and a maximum detection distance for the unique circumstances involving legless lizards.

Ambystomatid salamanders spend much of their existence in the terrestrial environment in shallow tunnels either developed by
rodents or excavated on their own (Smith 2003). Because of this fossorial lifestyle, information on non-breeding habitat use and migration is incomplete. We evaluated the application of RFID antennas and PIT tags for tracking ambystomatid salamanders in their burrows. We tested the hypothesis that different antennas (described in methods) would have different detection distances (DD) and would perform differently when individuals were located at different depths within the soil. This experiment mimicked what we might expect to see in implementing RFID use in the field.

Our study uses the methodology of Kuhnz (2000b) but differs in the following ways:

1. We studied detection distances for two types of RFID antenna utilizing implanted salamanders in an open air treatment (control) and at different depths buried in soil in a 40 gallon Rubbermaid container.

2. We report maximal DD for both antenna types and describe their detection range and percent effectiveness in all experimental conditions.

3. We determined if the RFID tracking technique would be applicable in denser soils, which might block the antenna’s ability to pick up the tag more than loose sandy soil.

Methods and Materials.—PIT tags were implanted in seven, laboratory-housed Ambystoma tigrinum salamanders according to protocols outlined by Ott and Scott (1999). These individuals are also validation specimens for an ongoing drift-fence study where salamanders are tagged in the field in a similar fashion.

Methods were modified from Kuhnz (2000b). Antennas tested in our study were the Destron 2001 F-ISO Circular antenna and the Biomark Triangular Field antenna using attenuation box antenna. Antennas were supplied on loan for the experiment from Biomark, Inc. Both antennas tested are slightly different in design and application from the Avid Power Tracker II used by Kuhnz (2000). The Power Tracker II was designed as a handheld device with limited DD. The Biomark circular antenna is designed as a handheld but was designed with a greater DD. Additionally, the triangular antenna is reported to have a greater DD range and is designed for use with a handle at different distances. Our study was designed to determine the maximum DD in typical soil found in North Dakota. Soil used in the study was obtained from a local source and was classified as clay loam (NRCS, 2002).

To develop a comparable measure of antenna performance we use the term detection distance (DD), which is defined as the distance between the antenna and the PIT tag at which a read response (detection) is initiated. In this study DD was determined by progressively lowering the antennas from a height of 30 cm in a leveled, straight line above the specimen until the tag was identified or until the surface of the soil was reached. Two parameters were recorded: tag detection (positive or negative) and DD (in the case of a positive detection). For many of the trails the tag was detected well in advance of the antenna reaching the soil surface. Thus a soil depth of 13.2 cm might have a DD greater than 13.2.

PIT implanted salamanders were placed in a cloth bag to limit movement and then covered with differing levels of soil. The DD was recorded for each trial with every salamander tested five times for each of the different antennas within each experimental condition. This allowed for a total of 175 tests of detection and DD per antenna (350 detection attempts).

Antennas were analyzed for detection and DD using differing levels of soil coverage as experimental conditions. The following amounts of soil were used as mimics of soil depth in field conditions: 0 cm, 2.2 cm, 6.6 cm, 13.2 cm, and 15.4 cm of loose soil. Data were log-transformed for DD, arc sine transformed for % effectiveness, and analyzed with separate two-factor ANOVAs. Factors were antenna type and depth of soil covering salamander. Additionally, to avoid pseudoreplication only the mean values of each salamander were used for running the ANOVAs. In all hypothesis tests, α was set at 0.05. Where appropriate, significant hypothesis tests were followed by post hoc analysis with the least significant difference (LSD) test.

Results.—No significant differences in DD were observed between antenna types, or among soil depth profiles (Table 1). However, significant differences were observed when the % effectiveness was analyzed (Table 1). The circular antenna read at a greater distance in all treatments except the treatment without soil (Table 2).

There was a progressive loss of effectiveness in both antennas at increases in soil depth beyond 6.6 cm, at 13.2 cm and 15.4 cm the circular antenna showed less of a loss in effectiveness than the triangular antenna (Table 2).

Both antennas recorded at least one detection in each of the experimental conditions. There was a maximal DD for each of the antennas in all treatments (Table 2). The loss in effectiveness for the triangular antenna at 15.4 cm was 10 times greater than the loss for the circular antenna (Table 2).

Discussion.—Our study results support the use of PIT tags and RFID antenna to track and locate ambystomatid salamanders in fossorial burrows and in the terrestrial and aquatic environment. Both antennas tested could locate pit tagged salamanders in the loosely packed soil used during the tests. Additionally, both antennas could detect tags that were implanted in salamanders in soil of at least 15.4 cm; this condition approximates real field conditions. Of importance to researchers is the fact that this range of

**Table 1. ANOVA table of mean detection distances and arcsine transformed % effectiveness of experimental RFID antennas.**

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
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<td>1.438E-02</td>
<td>1.322</td>
<td>.277</td>
</tr>
<tr>
<td>Antenna</td>
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<td>6.928E-03</td>
<td>.637</td>
<td>.429</td>
</tr>
<tr>
<td>Depth X Antenna</td>
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<td>1.962E-02</td>
<td>1.804</td>
<td>.145</td>
</tr>
<tr>
<td>Error</td>
<td>44</td>
<td>1.087E-02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
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<td>29.354</td>
<td>&lt;.001</td>
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<tr>
<td>Antenna</td>
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<tr>
<td>Depth X Antenna</td>
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<td>1.726</td>
<td>.156</td>
</tr>
<tr>
<td>Error</td>
<td>60</td>
<td>540.251</td>
<td></td>
<td></td>
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</tbody>
</table>

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The DD for an antenna is influenced by many factors including but not limited to: position of the tag in the individual, chemical component of the medium between the tag and the antenna (air, water, and soil), additional obstacles between tag and antenna (i.e., rocks, metal, landfill debris, other field equipment, or somebody's leg) and the angular relationship between the antenna field and the tag. It was not within the practical scope of this study to test these additional variables yet these are all factors that have a great deal of influence on antenna performance.

The tests that were performed supported the hypothesis that RFID antennas can locate herpetological study specimens at some distance beneath the soil. It follows that this method may also be utilized to track paedomorphic ambystomatids in aquatic habitat if they are within the DD for the antenna.

Both of the antennas tested showed a considerable loss in tag reading performance when the individual was buried in 15 cm of soil (Table 2). Each antenna showed an optimal range of performance (between 8 and 18 cm) and a discernable limit to the DD in open air and in soil covered treatments (Table 2). Beyond 6.6 cm the circular antenna was more effective at reading tags (Table 2).

Overall, both antennas showed the ability to read pit tags implanted in salamanders in typical loose packed North Dakota soil at least 15.4 cm deep. From our experiments we have determined the effective detection range for these antennas in our experimental conditions to be anywhere from 8 to 22 cm. Both antennas detected pit tags right at the limit of their reported detection range (Biomark reports a 25.4 cm limit for these antennas).

The valuable discovery for the field herpetologist is that the circular antenna was much more effective compared to the triangular antenna. The current cost of the triangular antenna is three times the cost of the circular antenna (please consult with Biomark for current prices and additional considerations). This type of cost differential is a real consideration for the researcher juggling limited budgets.

As a result of our research, we will be incorporating several aspects of tracking and locating salamanders in the field while utilizing RFID antennas. We will be working on a protocol for randomized and transect oriented surveys for our tagged salamanders in the terrestrial and aquatic environment and we will continue to test some of the other variables affecting antenna effectiveness and DD. Multipurpose use of PIT tags and antennas will allow the herpetologist to optimize the value of the cost of using pit tags in research. Continued research into alternative uses for pit tags will assure that manufacturers incorporate added value in the future development of PIT tag hardware.

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**Literature Cited**


Use of Anesthesia Increases Precision of Snake Length Measurements

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Researchers typically use body length (snout–vent length; SVL) as the principal measure of snake size. Several techniques for determining SVL have been reported (e.g., Blouin-Demers 2003; Fitch 1987; Quinn and Jones 1974). However, numerous researchers have also commented on the imprecision of common methods of measuring snake length (e.g., Blouin-Demers et al. 2002; Fitch 1987; Madsen and Shine 2001; Measey et al. 2003; Taylor et al. 2005). In at least one publication, SVL measurements were considered so imprecise that head length was considered a more consistent measure of snake size than SVL (Houston and Shine 1994). Despite recognition of this problem, until recently, little effort has been made to report the precision of different measurement methods, or to assess sources of variation. Recently, several researchers have quantified measurement error in SVL measurements. However, these studies stopped short of testing factors which influence measurement error (e.g., Bertram and Larsen 2004; Blouin-Demers 2003).

Variation between SVL measurements may be partitioned into three source categories: variation between measurements made by a single measurer using a single technique (e.g., the “intrinsic limitations” of a measuring method; Blouin-Demers 2003), variation between measurements made by different measurers using the same technique, and variation between measurements made using different techniques.

It is likely that the greatest source of variation in SVL measurement results from differences between measurements of stretched and unstretched snakes (Fitch 1987). I consider measurement of unstretched snakes preferable for numerous reasons. 1) snakes spend the majority of their lives in relaxed postures, thus measurement of an unstretched snake is a truer measure of snake length; 2) stretched measurements are positively biased in comparison to measurements of preserved snakes (Fitch 1987; Reed 2001) and indirect measurements (e.g., squeeze box measurements; Bertram and Larsen 2004); 3) large snakes may be difficult to completely stretch, resulting in decreased precision (Fitch 1987; but see Blouin-Demers 2003); 4) snakes may vary in elasticity as well as in behavioral resistance to stretching, biasing interspecies comparisons; 5) stretching venomous snakes poses a danger to measurers; and 6) stretching can injure snakes (Fitch 1949, 1987). For these reasons, I chose to only evaluate methods for measuring unstretched snakes.

Here, I present the results of two separate evaluations of SVL measurement precision. In the first part of this study, I evaluated sources of variation in repeated SVL measurements of a diverse group of snakes. My objective was to quantify the effect of each source of error in SVL measurement, as well as to identify the most precise measurement technique. In the second portion of this study, I quantified the precision, under research conditions, of the most precise of the previously evaluated techniques.

Materials and Methods.—In the first portion of this study, I evaluated repeated SVL measurements of 24 snakes using three snake measurement techniques: squeeze box (Quinn and Jones 1974), conscious-straightened, and anesthetized. To increase the breadth of the statistical population, snakes from seven species (Charina bottae, Crotalus horridus, C. lepidus, Elaphe emoryi, Lampropeltis pyromelana, L. triangulum, and Heterodon nasicus), four clades (Colubrinae, Crotalinae, Erycinae, and Xenodontinae), and comprising a range of sizes (210–957 mm SVL) were selected. Measurements were recorded based on the following design: two measurers measured each snake three times per technique, resulting in 18 measurements of each snake. All measurements were recorded during a single session. Most measurements were performed independently, and each measurer was unaware of values recorded by the other measurer. However, measurers helped one another restrain venomous snakes during conscious-straightened measurements, and thus may have been aware of the values of these measurements.

In the second portion of this study, I quantified the precision of measurements of 24 anesthetized adult Crotalus polystictus recorded under more typical research conditions (i.e., taken by different measurers over a longer time frame while measurers were primarily focused on tasks other than snake measurement). Each snake was measured once by three measurers, totaling three measurements per snake. Snakes were measured as they were captured over a nine-day period. Measurers were instructed on measurement technique the first day, but were not provided subsequent instruction. All measurements were taken independently. However, some measurers were aware of some previously recorded values, possibly influencing subsequent measurements.

During both portions of the study, measurers attempted to measure from the snout to the posterior tip of the anal plate of each snake. For measurements made with a squeeze box, the position of each snake’s anal plate was marked on the snake’s dorsum using a temporary marker prior to measurement. To measure snakes with a squeeze-box, snakes were placed within a wooden squeeze box (61 cm × 61 cm) outfitted with a plexiglass lid and lined with a 5 cm thick foam rubber pad. Snakes were pressed against the pad with enough force to temporarily retard movement while measurers traced the dorsal mid line of the snake from snout to the anal plate mark using a temporary...