# TECHNIQUES

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## Enhancing Ecological Investigations of Snakes with Passive Integrated Transponder (PIT) Tag Telemetry

The secretive nature of snakes presents challenges for researchers conducting ecological investigations in field situations (Fitch 1987; Parker and Plummer 1987; Dorcas and Willson 2009; Steen 2010). Many snake species exhibit cryptic coloration, limited activity patterns, and habitat use (e.g., use of subterranean, arboreal, or aquatic habitats) that preclude high rates of detection (Gibbons and Semlitsch 1987; Parker and Plummer 1987; Mazerolle et al. 2007). Indeed, studies have reported low detection rates for snakes, which may preclude the use of modern statistical methods (e.g., capture-mark-recapture [CMR] or occupancy analyses; Steen 2010; Willson et al. 2011). Thus, the development of field techniques directed towards enhancing snake detection would be quite valuable to our understanding of snake ecology.

Passive integrated transponder (PIT) tags have been established as an effective method of uniquely marking animals across a wide range of taxa (Elbin and Burger 1994; Gibbons and Andrews 2004). A recent advance in PIT technology, known as PIT tag telemetry, uses a portable reader and antenna that allow for PIT tag detection beyond tactile range, including detection of tags through a variety of media and materials. PIT tag telemetry has been successfully used to monitor fish (Zydlewski et al. 2001; Cucherousset et al. 2005) and salamander populations (Hamed et al. 2008; Connette and Semlitsch 2012, 2013; Ousterhout and Semlitsch 2014). This technique has not yet been applied to field studies of snakes. In this study, we examined the efficacy of PIT tag telemetry in studying a population of Queensnakes (*Regina septemvittata*). Our objectives were to assess the utility of PIT tag telemetry to: 1) enhance reencounter rates relative to hand-capture methods, 2) observe daily activity patterns in PIT tagged snakes, and 3) assess movements of PIT tagged snakes within a stream reach.

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### Methods

*Focal species and study site.*—Queensnakes are semi-aquatic snakes often found in shallow streams with rocky or sandy bottoms throughout the Midwest and Southeast USA (Conant 1960; Branson and Baker 1974). Individuals utilize rocks in and along streams as cover and bask in nearby vegetation (Gibbons and Dorcas 2004). We conducted a CMR study of Queensnakes along a 200-m reach of Little Hickman Creek in Jessamine County, Kentucky (USA). This second-order stream contained an abundance of limestone ledges and individual rocks, ranging from gravel to small boulders, within and adjacent to the streambed, which itself was largely bedrock. Numerous trees grew along the banks, yet canopy cover varied from no canopy cover along wider segments to 100% canopy cover along narrower portions of the stream.

*Snake PIT tagging and PIT tag telemetry surveys.*—Between May and July of 2013, we captured snakes within our study reach; most snakes were captured while basking or found while searching under cover objects within and adjacent to the stream. Captured snakes were measured (snout–vent length [SVL] and total body length [TBL]), sexed, aged (i.e., juvenile vs. adult) and weighed. Upon initial capture, we subcutaneously implanted 134.2 kHz, 12.5 mm PIT tags (Biomark HPT12) using the Biomark MK10 Implanter along the posterior third of the venter anterior to the cloaca of individuals. Individuals were identified as juveniles in our analysis of movement (see below) in accordance with the findings of Branson and Baker (1974) and Mitchell (1994). Females were categorized as juveniles with TBL < 344 mm and SVL < 318 mm. Males were categorized as juveniles with SVL < 305 mm. All snakes were released within 30 minutes after PIT tag implantation in the exact locations where they were captured.

Previous studies investigating the utility of PIT tags for marking adult and neonatal snakes have indicated minimal, if any, interference with normal physiology and behavior (Keck 1994; Jemison et al. 1995), but PIT tags have been expelled in some instances (Roark and Dorcas 2000). In order to ensure that detections were tagged snakes, rather than loose tags, we included only PIT-tagged individuals that were visually confirmed to have retained PIT tags during or after the study period in our analyses.

Fig. 1. Passive Integrated Transponder (PIT) tag telemetry was used to detect Queensnakes (*Regina septemvittata*) in Jessamine County, Kentucky (USA). Telemetry involved the use of the Biomark HPR Plus portable reader (orange) and BP Portable Antenna.

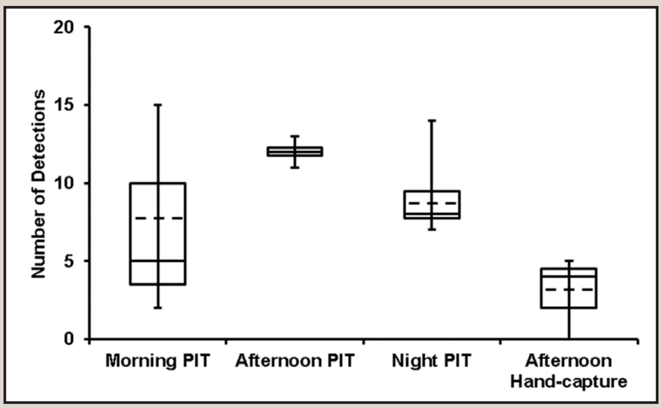


Fig. 2. Box plot showing medians (horizontal lines), means (dashed lines), and 25th and 75th percentiles of numbers of Queensnakes detected using morning, afternoon, and night PIT tag telemetry surveys and afternoon hand-capture surveys. Note the median value for afternoon PIT telemetry surveys is equal to the mean value (i.e., 12 detections).

Subsequently, PIT tag detections from individuals that we did not obtain a visual observation were excluded from our analyses.

*Encounter rate, activity, and movement.*—To examine differences in encounter rates, we compared numbers of detections of PIT-tagged snakes using hand-capture surveys with data generated from PIT tag telemetry surveys. Hand-capture surveys consisted of two observers visually searching for Queensnakes along the 200-m stream reach. Specifically, our searches consisted of examining basking sites and lifting rocks and other potential cover objects in and along the stream banks up to the high water mark. All three hand-capture surveys occurred during afternoon hours (1200–1600 h); each survey generally lasted 120 minutes although environmental conditions, such as water level, influenced the amount of time necessary to search the entire transect thoroughly. PIT tag telemetry surveys consisted of walking along the same 200-m stream reach as hand-capture surveys, using the Biomark HPR Plus portable reader and BP Portable Antenna to detect individuals (Fig. 1). The Biomark BP Portable Antenna has a maximum reading distance ranging from 30.5 cm to 43.2 cm (for 134.2 kHz Biomark 12.5 mm PIT tags), depending on tag orientation and electromagnetic interference. We swept the antenna over and around rocks, roots, and any other objects in the survey reach. Surveys were conducted with teams of two people operating the antenna and portable reader; one person operated the antenna and the other monitored the portable reader for encounter events. Upon detection of a tagged snake, the Biomark HPR Plus portable reader recorded the unique identification number associated with the PIT tag, as well as time and geographic coordinates at the moment of detection. Once detected with the reader, we visually confirmed the presence of the PIT-tagged individual. We conducted three morning PIT tag telemetry surveys (0800–1100 h), four afternoon PIT tag telemetry surveys (1200–1600 h), and four night (2000–2300 h) PIT tag telemetry surveys. A minimum period of three days separated each survey. Although generally lasting 90 minutes, PIT tag telemetry surveys were not explicitly timed as environmental conditions dictated the amount of time necessary to scan the 200-m reach. Because air temperature influences snake behavior and activity, air temperatures were recorded at the beginning of each hand-capture and PIT tag telemetry survey. All surveys were conducted in August and early September 2013.

To determine if PIT tag telemetry increased the number of encounters, we used a one-way analysis of variance (ANOVA) to compare mean numbers of tagged snakes detected using afternoon hand-capture surveys with mean number of tagged snakes detected during afternoon PIT tag telemetry surveys. To assess activity periods of Queensnakes at our study location, we used a one-way ANOVA to compare mean numbers of tagged individuals detected during PIT tag telemetry surveys conducted during morning, afternoon, and night, assuming that periods of increased detections were a result of enhanced activity (e.g., basking and foraging; Robertson and Weatherhead 1992). We also used a one-way ANOVA to compare mean air temperatures among surveys. For all analyses, we used a Shapiro-Wilk test to examine normality of residuals and a Tukey's HSD test was used to determine pairwise differences, if applicable. ANOVA and other tests were conducted in R (version 3.2.3) with alpha level set at 0.05. Dispersion around means is indicated by +/- one standard error unless otherwise indicated.

Finally, the portable receiver and antenna used during PIT tag telemetry surveys provided geographic coordinates for each encounter event (accuracy +/- 3m). We used ArcGIS (version 10.1) to map encounters of tagged individuals using these coordinates and determined maximum distances moved by individuals between encounters.

### Results

We tagged a total of 33 Queensnakes within our 200-m stream reach from May to July 2013. Tagged Queensnakes at initial capture ranged from 2.4 g and 175 mm SVL to 105.6 g and 570 mm SVL. We limited our statistical analyses to 22 individuals (9 adults [4 males and 5 females] and 13 juveniles) that we visually confirmed during or after PIT tag telemetry surveys. During afternoon hand-capture surveys, we recorded nine encounters of eight PIT-tagged Queensnakes. Conversely, PIT tag telemetry surveys conducted in the afternoon resulted in 48 total encounters of 18 PIT-tagged Queensnakes. Mean encounters during afternoon PIT tag telemetry surveys were significantly greater than afternoon hand-capture surveys ($F$ = 43.39, df = 1, P = 0.001; Shapiro-Wilk test, P = 0.57; Fig. 2). Air temperatures for afternoon PIT tag telemetry surveys averaged 29.3 ± 0.55ºC and these mean air temperatures were not significantly different from those during afternoon hand-capture surveys (29.1 ± 0.74ºC, $F$ = 0.04, df = 1, P = 0.85; Shapiro-Wilk test, P = 0.21).

Mean number of Queensnake encounters during morning, afternoon and night PIT tag telemetry surveys were 7.33 ± 3.93, 12 ± 0.41, and 9.25 ± 1.60, respectively. Despite the variable number of encounters, mean encounters were not significantly different among PIT tag telemetry surveys ($F$ = 1.24, df = 2, P = 0.34; Shapiro-Wilk test, P = 0.19; Fig. 2). However, air temperatures were significantly different among PIT tag telemetry survey types ($F$ = 7.19, df = 2, P = 0.02; Shapiro-Wilk test, P = 0.51); morning surveys had lower mean temperatures than afternoon and night surveys (Tukey's HSD test, P = 0.02). Overall, mean encounters per individual using PIT tag telemetry were 4.9 ± 0.56, with a range of 1 to 11.

During PIT tag telemetry surveys, we detected and recorded geographic coordinates for 19 individual Queensnakes (7 adults [4 females and 3 males] and 12 juveniles) two or more times. Maximum linear distances that adults were detected from initial locations ranged from 1.0 m to 164.1 m, with a mean of 37.3 ± 21.44 m (Fig. 3). Maximum linear distances that juveniles were detected from initial capture locations ranged from 11.2 m to 155.1 m, with a mean of 46.3 ± 11.67 m (Fig. 3).

### Discussion

PIT tag telemetry surveys detected significantly more tagged Queensnakes than hand-capture surveys, demonstrating the advantage of this technique in relocating previously tagged individuals. Previous studies have found recapture rates of Queensnakes to be low; Branson and Baker (1974) recaptured only 13 of 70 (18.6%) marked Queensnakes in Kentucky. Our surveys using hand-capture searching methods revealed similarly low encounter numbers (i.e., eight of 33 tagged individuals; 24.24% of our tagged population). However, our PIT tag telemetry surveys were more successful; we encountered and visually confirmed 22 of 33 (66.66% of our tagged population) PIT-tagged individuals during these surveys. If we included detections of snakes that we did not visually confirm during surveys, our encounter rate increased to 96.97% (i.e., 32 of 33 tagged snakes had at least one detection).

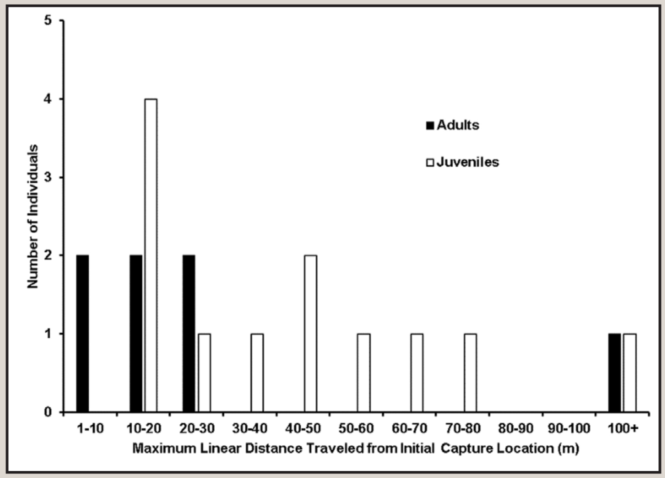


Fig. 3. Maximum linear distances (m) between first and second capture locations for adult (N = 7) and juvenile (N = 12) Queensnakes.

We observed relatively uniform daily activity patterns, as indicated by encounter numbers during PIT tag telemetry surveys conducted at different times of day. Our observations confirm prior research that suggests Queensnakes are active during both night and day during the active season (Branson and Baker 1974; Mount 1975; Ernst and Ernst 2003). Furthermore, our results indicate that PIT tag telemetry is a useful tool to examine daily activity patterns. As an extension, this technique may also be useful if applied across annual active periods to provide further insight into patterns associated with seasonality. In addition, we were able to quantify movements of individuals within the study reach using the geographic coordinates recorded by the portable reader. Our multiple encounters of individual Queensnakes indicate somewhat limited movement and site fidelity, although we did find movements greater than 100 m for two individuals. During a previous CMR study of Queensnakes in Kentucky, the majority of recaptured marked individuals were found within 25 m of release sites, although one was observed to move 45 m and another was captured 135 m from the original capture location (Branson and Baker 1974).

PIT tag telemetry represents a minimally invasive method of obtaining data from animals that have in the past been difficult to study, either because target species are too small to successfully employ radiotransmitters, or because hand-capture sampling is extremely time-consuming, intensive or results in the destruction of habitat (Ousterhout and Semlitsch 2014). Our results suggest that PIT tag telemetry offers much promise for improving reencounter rates of snakes, which is crucial when estimating population parameters, such as survivorship. However, not all habitats may be suitable for PIT tag telemetry; specifically some habitat characteristics (i.e., dense vegetation) may impede researchers in conducting surveys with portable antennas (Cucherousset et al. 2008; Fig.1). The natural history of species to be used in PIT tag telemetry studies may also be an important consideration. We focused on a species that is generally restricted to a linear, aquatic habitat. Species with high site fidelity or specific habitat preferences may be excellent candidates for PIT tag telemetry investigations. Conversely, species that have large home ranges may not be as suitable for PIT tag telemetry, given the limitation of detection distance. Finally, as PIT tag size dictates detection ranges, selection of appropriate tags with regard to animal body size and desired detection distance is important to consider (Ousterhout and Semlitsch 2014). Biomark produces a range of tag sizes (8, 9, 10, 12.5, and 23 mm) and detection range decreases as tag size is reduced. We found that the manufacturer's estimated detection distance of up to 43.2 cm for 12. 5 mm, 134.2 kHz tags appeared to be relatively accurate. Many Queensnakes were often detected underneath rocks and rock ledges that were equal to or slightly exceeded 25 cm in depth whereas others were detected 30 cm under water and/or within crayfish burrows. Thus, our study demonstrates the utility of using PIT tag telemetry in snake detection and that this technique represents an important tool to add to the list of standard practices for investigating snake ecology.

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