

Visible Implant Fluorescent Elastomer: A Reliable Marking Alternative for Snakes

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It is important to note that gloves have not been found to negatively affect juvenile or adult amphibians. The use of gloves to handle amphibians is widespread in the field and lab. Changing gloves between amphibians remains an important hygiene measure to prevent transmission of infectious agents such as *B. dendrobatidis* and ranaviruses between individual amphibians and aquaria. However, given our tadpole results, it would be useful to formally investigate potential non-lethal effects of gloves on adult and juvenile amphibians to ensure that gloves really are entirely non-injurious.

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Studies in population ecology require use of reliable marking techniques to estimate various parameters (e.g., population size, density, demographics, movement, or behavior; Penney et al. 2001; Perret and Joly 2002; Walsh and Winkelman 2004; Woods and Martin-Smith 2004). However, it is imperative that marking techniques meet standard assumptions: 1) marks must remain visible for the duration of the experiment, 2) marks are correctly recorded, 3) marks do not affect the survival of the animal, and 4) marks do not affect the recapture probability of animals (Goldsmith et al. 2003; Otis et al. 1978).

Visible implant fluorescent elastomer (VIE; Northwest Marine Technology, Inc., Shaw Is., Washington, USA) was initially developed for batch marking migratory fish, but has recently been used to mark amphibians and lizards (Bailey 2004; Losos et al. 2004; Nauwelaerts et al. 2000; Nishikawa and Service 1988; Penney et al. 2001). Visible implant fluorescent elastomer consists of a liquid polymer added to a curing agent to create a flexible plastic mark. Color kits are available, capable of marking 15,000 individuals depending on the number of colors used and marking design. Our objective was to determine if VIE was an appropriate marking technique for snake research based on the marking assumptions of Otis et al. (1978) and Goldsmith et al. (2003). We hypothesized that VIE would be a reliable marking technique for snakes. To our knowledge, our study is the first to apply VIE to snakes.

We conducted this empirical study in a laboratory setting at North Carolina State University, Raleigh, North Carolina, USA. We marked Red Cornsnakes (*Pantherophis guttatus*; N = 18) between 19 and 29 April 2006. Each snake received three doses (1, 2, and 3 μ l) of yellow VIE randomized to the general area of three locations (neck, midbody, and pre-caudal). We injected marks subcutaneously and dorsolaterally on left sides using a graduated 1cc Luer-lok syringe with a 25-gauge needle (Becton-Dickinson, Franklin Lakes, New Jersey, USA). We used 1cc syringes to better approximate volumes, which required the 25-gauge needle for a secure fit. We injected additional *P. guttatus* (N = 4) and Common Kingsnakes (*Lampropeltis getula*; N = 6) with

blue and red to examine VIE color, ground color, and species effects, but did not quantify results from these 10 snakes.

All snakes were captive-raised and housed individually at a constant 26.6°C with food, water, and substrate provided regularly. We checked snakes for marks every two weeks using a UV-B light. We collected shed skins to record shedding frequency and expulsion of marks. Our study concluded on 4 May 2007 after 370 days.

We calculated retention time as the number of days we detected a mark until the median date between when a mark was last detected and the following examination. We derived mean retention times for each mark volume (1, 2, and 3 μ l). To discern the effects of mark volume or individual variation on retention time, we analyzed retention time as a dependent variable with mark volume and individuals as independent variables in an additive, 2-factor analysis of variance (ANOVA). Similarly, we employed two 1-factor ANOVAs with retention time as the dependent variable and shedding frequency (i.e., number of sheds/individual) or mark location (i.e., neck, midbody, and pre-caudal) as independent variables. We performed analyses using PROC GLM (SAS 9.1, Cary, North Carolina, USA). We calculated the percentage of marks retained to demonstrate mark performance by volume.

All 18 *P. guttatus* used in the experiment were of similar length (mean = 990.39 $\pm$ 79.41 mm snout–vent length) and weight (mean = 370.33 $\pm$ 81.93 g). After 370 days, 94, 83, and 100% of low (mean = 354 days), medium (mean = 333 days), and high (mean = 370 days) mark volumes were retained. A 2-factor ANOVA revealed no differences in retention time between mark volumes ($F_{2,34} = 1.27$, $P = 0.2940$) or individuals ($F_{17,34} = 0.88$, $P = 0.6045$). Shedding frequency (mean = 5.05 $\pm$ 1.21 sheds/snake) did not have a significant effect on mark retention time at low ($F_{1,16} = 0.79$, $P = 0.3860$), medium ($F_{1,16} = 0.00$, $P = 0.9501$), or high mark volumes (100% retention). Analysis of mark location revealed a 13% lower mean retention time for marks located pre-caudally (mean = 321 days) compared to neck (mean = 366 days) and midbody (mean = 370 days) mark locations. However, we did not detect a significant difference among locations ($F_{2,51} = 3.00$, $P = 0.0588$).

Our results indicated that VIE was a reliable marking technique for snakes, with 94, 83, and 100% retention for low, medium, and high volumes after 370 days and no mortalities recorded. Elastomer marks were easy to identify and record due to fluorescent colors. We observed a significantly lower retention time for pre-caudal marks. In fact, 3 of the 4 marks lost (1-low and 2-medium volume) were located in the pre-caudal region and were lost through expulsion within the first few examinations. If we removed these early losses from our analyses, retention times would be 100, 94, and 100% for low, medium, and high volumes, respectively.

Our results demonstrated that VIE marks last at least 370 days and satisfy the marking assumptions proposed by Otis et al. (1978) and Goldsmith et al. (2003). Branding and scale clipping have been reported to last $\geq$ 3 years (Brown and Parker 1976; Winne et al. 2006) and elastomer marks have been reported lasting well over a year in amphibians (Davis and Ovaska 2001) and are capable of permanence (Kinkead et al. 2006). We acknowledge our short study duration (370 days), but believe VIE satisfies assumptions

for correct recording, and survival and recapture effects (Davis and Ovaska 2001; Kinkead et al. 2006) and is a reliable marking technique for snakes. Equipment costs were initially higher for VIE (US \$465) compared to scale clipping (i.e., scissors) or branding (i.e., cautery units ~US \$20–25; Winne et al. [2006]), but marking costs per snake were small (~\$0.10–\$0.29 for marks of 1–3 μ l). Further, our retention of all mark volumes suggested the usefulness of VIE in snakes of any size; small marks can be applied to small-bodied species and individuals ($\leq$ 26 cm), which may be too small for PIT tags or scale clipping (Spellerberg 1977). However, problems were encountered with the technique. Pre-caudal marks had a 13% lower retention time and accounted for 75% of marks lost. Mark losses occurred from 23 days to 310 days, and were likely due to expulsion from the site of injection. Similarly, fragmentation of marks into several pieces could cause detection problems. The application of a liquid bandage product would likely deter expulsion and pathogen introduction.

We recommend future studies evaluate the use and efficacy of VIE in snakes, both in lab and field settings. Future studies should evaluate using VIE in different species of snakes with various ground colors and at various mark locations. Anecdotally, we can report that yellow, blue, and red VIE colors were detectable in Red Cornsnakes at all volumes, but blue VIE was difficult to detect in Common Kingsnakes due to the dark ground color of this species. Mark volume should be studied in the field to better understand mark retention under natural conditions and field and laboratory research should focus on survival and recapture rates for VIE over longer periods. Future research should evaluate the effects of growth on mark detectability and compare stress levels incurred by traditional and VIE marking techniques.

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Monitoring Non-breeding Habitat Activity by Subterranean Detection of Ambystomatid Salamanders with Implanted Passive Integrated Transponder (PIT) Tags and a Radio Frequency Identification (RFID) Antenna System

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Pond breeding amphibians require terrestrial habitat during the non-breeding season in addition to aquatic reproductive habitat. Ambystomatid salamanders such as spotted (*Ambystoma maculatum*) and marbled salamanders (*Ambystoma opacum*) have been previously studied to determine migration movements and terrestrial habitat requirements. Several migration studies have estimated the “life” or buffer zones required for terrestrial non-breeding habitat. Estimates of habitat area that encompass 95% of the population have ranged from 159 m to 370 m (Faccio 2003; McDonough and Paton 2007; Rittenhouse and Semlitsch 2007; Semlitsch 1998; Semlitsch and Bodie 2003). Few studies have examined migration and non-breeding home range of *Ambystoma* species over multiple years because of the difficulties of long term tracking and monitoring of these animals. Recent studies

have shown that vertebrates with implanted passive integrated transponder (PIT) tags are detectable subterraneously (Cabarle et al. 2007; Kuhn 2000). The purpose of this study was to examine the utility of PIT tags and a multidirectional radio frequency identification (RFID) antenna system (FS 2001 Destron reader and Biomark triangle antenna) for tracking and monitoring ambystomatid salamanders during the non-breeding season.

Identifying and locating individual salamanders is important in determining terrestrial home range and migration. Several methods of identification have been used including: photographs of spot patterns (Stenhouse 1985), toe clips (Ott and Scott 1999), radiotelemetry (Faccio 2003; Madison 1997; McDonough and Paton 2007), PIT tags (Blackwell et al. 2004; Gibbons and Andrews 2004; Ott and Scott 1999), and subcutaneously implanted reflector tags (Moseley and Castleberry 2005). Drift fences have been frequently used to monitor migrants to and from breeding pools (Kleeberger and Werner 1983; Sexton et al. 1990). Concentric circles of drift fences spaced at regular intervals allow researchers to determine the approximate area in which salamanders establish a home range. However, drift fences only capture salamanders moving on or just below the surface. Radiotelemetry studies can track animals underground, but transmitters have a short (< 5 months) battery life and can have high cost (> US \$150 each). Radioisotopes were used to determine the home range and summer movements of *A. maculatum* (Kleeberger and Werner 1983), *A. talpoideum* (Semlitsch 1983), *Plethodon jordani* (Madison and Shoop 1970), and *Desmognathus fuscus* (Ashton 1975), but do not allow for individual specific identification.

Materials and Methods.—In this study we attempted to locate and track ambystomatid salamanders using RFID antenna systems with animals that had been marked during previous reproductive seasons with PIT tags. The study area was a constructed vernal pool on the Tennessee Valley Authority’s (TVA) South Holston Weir Dam property (36.5239°N, 82.1100°W) in Sullivan County, Tennessee, USA. The floodplain forest at this location supports populations of both *A. maculatum* and *A. opacum*, with the population of *A. opacum* being distinct and disjunct in eastern Tennessee (Hamed et al. 2007). A two lane paved road (Holston View Dam Road) bisects the property and salamanders living on the north side of the vernal pool must cross the road to reach the pool. The forested area (0.79 ha) north of the road consists mainly of Virginia Pine (*Pinus virginiana*), Boxelder (*Acer negundo*), and Sycamore (*Plantus occidentalis*). A mixed deciduous forest composed mainly of Sweetgum (*Liquidambar styraciflua*), Sycamore, White Oak (*Quercus alba*), and a 0.5 ha patch of non-native bamboo (*Phyllostachys aureosulcata*) border the south side of the vernal pool.

Previous studies have reported variable detection distances of PIT tagged vertebrates beneath the soil surface (12–22 cm) in both *in situ* (Kuhn 2000) and experimental (Cabarle et al. 2007) conditions. The observed variability was a result of antenna sensitivity and environmental conditions. We first used a preserved specimen of *A. maculatum* obtained from the East Tennessee State University teaching collection to determine the sensitivity of subterranean detection of PIT tags specific to our study site in a series of ten location accuracy trials. A TX-1411-SST PIT tag (Biomark, Idaho) was injected into the body cavity of the preserved specimen anterior to the rear limbs. To