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Ex-Situ PIT-tag Retention Study in Two *Desmognathus* Species

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The use of capture-mark-recapture (CMR) models to study salamanders is a crucial component to understanding survivorship, demographics, habitat preferences, and conservation in the wild. The various CMR models operate under several basic assumptions, but all CMR models assume animals retain their marks. This assumption is often ignored or very difficult to quantify unless individuals are double-marked, but nonetheless tag or mark loss can violate model assumptions, strongly bias model estimates, and lead to invalid conclusions about a population (Arnason and Mills 1981; Grant 2008). However, poor estimates can be corrected within models with knowledge of tag loss rates (Arnason and Mills 1981). Often, field studies do not have the power to detect errors such as mark loss or misidentification, which can bias estimates of demographic parameters (Grant 2008). Mark misidentification, mark loss, or mark migration cannot always be controlled in a CMR study; therefore these errors should be incorporated into the modeling of the capture histories (Grant 2008).

There are various methods (e.g., photography, toe clips, radiotelemetry, elastomer tags, and implanted reflector tags) to uniquely mark or identify individual salamanders (Hamed et al. 2008), but passive integrated transponder (PIT) tags are becoming more commonly used in salamander research, especially of larger species (e.g., Gibbons and Andrews 2004, Table 1). One major advantage of using PIT tags over other marking methods is that researchers can repetitively identify individuals without disturbing the habitat or the individual; thus minimizing handling. However, PIT tags have their limitations, including size and cost limitations. For example, in small species and juveniles of larger salamander species, PIT tag size becomes a limiting factor of their usage. The smallest tags available to researchers is 8.4 x 1.4 mm in size (Biomark, Idaho), which are not feasible for injection into smaller individuals. Compared to toe clips and other marking methods, PIT tags are more expensive (< US $5.00 each) and PIT tag readers can cost US $300–600.

Although the use of PIT tags is commonplace in salamander research today, relatively few studies have assessed tag retention rates in salamanders (see Unger et al. 2012). To our knowledge, tag retention studies using salamanders are limited to nine species representing four families (Ambystomatidae, Cryptobranchidae, Plethodontidae, Salamandridae) and despite the increase in CMR studies with plethodontids, few studies have quantified their long-term retention of marks (Table 1). In this study, we quantified the retention rate of PIT tags in two *Desmognathus* species, *D. monticola* (Seal Salamander) and *D. quadramaculatus* (Black-bellied Salamander). The first is a widely distributed species occurring from Pennsylvania to the panhandle of Florida, but the species' distribution mostly coincides with the Appalachian Mountains (Petranka 1998). The second species, *D. quadramaculatus*, has a more restricted distribution relative to *D. monticola* and is mostly confined to the Southern Appalachians (Petranka 1998). Both are among the larger *Desmognathus* species, but adult *D. quadramaculatus* (9–21 cm TL) are larger than *D. monticola* (7.5–15 cm TL; Petranka 1998, Bruce 2011). Using these two species of salamanders, our goal was to determine the reliability of PIT tags as a permanent "mark" for CMR studies in *Desmognathus*.

Methods

Twenty-four *D. monticola* and eight *D. quadramaculatus* were collected from Mill Creek in Murray County, Georgia, USA (34.87835N, 84.70915W; WGS84). All salamanders were transported to Sewanee: The University of the South (Sewanee), where they were PIT tagged with Biomark HPT8 tags (8.4 mm length x 1.4 mm diameter). Salamanders were anesthetized using Orajel per protocols described in Cecala et al. (2007), and tags were injected anterior to the right rear leg into the body cavity. We did not use any sealant (e.g., VetBond) to close the injection site in this study. Snout–vent length (SVL, mm) and mass (g) were recorded while salamanders were anesthetized. Snout–vent length was measured to the posterior end of the cloaca. Mean length and mass of *D. monticola* was 56.83 mm (standard error [SE] = 1.20 mm) and 3.90 g (SE = 12.26 g), while mean length and mass of *D. quadramaculatus* was 59.0 mm (SE = 2.15 mm) and 4.65 g (SE = 3.71 g). The minimum length and weight marked successfully for *D. monticola* was 45 mm SVL and 2.24 g and the minimum length and weight marked successfully for *D. quadramaculatus* was 50 mm SVL and 2.73 g. Recovery was monitored for 1 h following the procedure, and salamanders were checked after 24 h to ensure normal behavior (alertness and movement). Salamanders were transported to the Tennessee Aquarium Conservation Institute, where they were held at 4°C in individual containers with dechlorinated water and a small piece of paper towel for 3 days before the start of the experiment. This retention study coincided with another study focusing on interactions between *D. monticola* and *D. quadramaculatus*, which ran for 37 days. In the interaction experiment, salamanders were paired by size and pairs were randomly placed into one of sixteen fiberglass tanks (0.53 x 0.60 x 1.20 m). Each tank had terrestrial (e.g., sand and gravel) and aquatic habitat with each habitat having one cover object (e.g., cobble). Each tank system was a closed recirculating system and for details about their design refer to Ennen et al. (2016). After the interaction experiment was concluded, 10 *D. monticola* were transported to Sewanee and held for an additional 223 days in 35 x 22 x 8 cm plastic containers at 12°C. We used a Biomark HPR Plus Reader to record retention of the PIT tags after the conclusion of the interaction experiment and after 260 days. Salamanders were fed small (6.35mm) crickets weekly throughout the length of this experiment.

TABLE 1. Comparison of retention rates of passive integrated transponder (PIT) tag studies in nine salamander species representing four families.

Family/Species	Retention rate	Duration	Tag size[a]	Implant method	Tag location in body	Reported size[b]	Citation
Ambystomatidae							
Ambystoma annulatum	100%	6 weeks	8.5 x 1.4	Surgical incision	Body cavity	42.86 ± 0.70 mm, 1.573 ± 0.163 g	Ousterhout & Semlitsch 2014
Ambystoma annulatum	100%	6 weeks	12.5 x 2.12	Surgical incision	Body cavity	43.48 ± 1.89 mm, 1.579 ± 0.138 g	Ousterhout & Semlitsch 2014
Ambystoma opacum	89%	24 weeks	not reported	Surgical incision, wound closed with New Skin® liquid bandage	Body cavity, anterior to hind limb	Range = 1.67–4.05 g	Ott & Scott 1999
Cryptobranchidae							
Cryptobranchus alleganiensis	100%	2 years	12.5 x 2.12	Injected with syringe	Tail musculature posterior to hind limb	not reported	Unger et al. 2012
Plethodontidae							
Desmognathus monticola	100%	260 days	8.4 x 1.4	Injected with syringe	Body cavity, anterior to right rear leg	*56.83 ± 1.20 mm, 3.90 ± 12.26 g	this study
Desmognathus quadramaculatus	100%	37 days	8.4 x 1.4	Injected with syringe	Body cavity, anterior to right rear leg	*59.0 ± 2.15 mm, 4.65 ± 3.71 g	this study
Plethodon shermani	100%	9 weeks	12.5 x 2.07	Surgical incision	Body cavity, anterior to left hind limb	52.52 ± 4.87 mm, 3.30 ± 0.76 g	Connette & Semlitsch 2012
Salamandridae							
Calotriton asper	84%	19 months	11.5 x 2.1	Injected with syringe	Ventral part of peritoneal cavity	*Males-71.5 mm ± 0.3, Females-71.3 mm ± 0.4	Ducherousset et al. 2008
Salamandra salamandra	100%	2 years	12 x 2.2	Surgical incision	Subcutaneously, dorsolateral trunk above costal grooves	> 12 cm	Schulte et al. 2007
Triturus dobrogicus	89.5%	11 years	not reported	Injected with syringe, wound closed with tissue glue VET SEAL	Abdominal body cavity	> 2 g	Jehle & Hoedl 1998

[a] Measured in mm

[b] Size of salamanders; represents standard deviation

* Represents standard error

Results and Discussion

Our *ex situ* experiment provides evidence that *Desmognathus* species do not reject PIT tags after injection and can retain PIT tags for extended periods of time. We observed no tag loss in 8 *D. quadramaculatus* and 24 *D. monticola* to 37 days or in the 10 *D. monticola* to 260 days. To our knowledge, this is the first study to investigate PIT tag retention for *Desmognathus* species.

In general, PIT tag retention rates in salamanders are high, ranging from 84–100% (Table 1). Our retention rates fall at the upper limits of that range. Comparison of retention rates among studies could potentially be confounded by several factors, including study duration, PIT tag size, anatomical placement of the PIT tag, and implantation method. For example, Dieterman and Hoxmeier (2009) found that anatomical placement influenced PIT tag retention rates in Brook Trout (*Salvelinus fontinalis*), where injection of PIT tags into dorsal muscular tissue had a higher retention than the body cavity. Likewise, in Corn Snakes (*Pantherophis guttatus*), anteriorly placed PIT tags were expelled in 53% of individuals, while no tags were expelled when placed in the midbody region (Roark and Dorcas 2000). However, it appears that the different methodologies have no effect on PIT tag retention rates in salamanders (see Table 1), but more studies should be conducted to statistically tested retention rates among the various methods.

Although tag retention studies reported high retention rates in salamanders, there are other studies, which did not calculate retention rates *per se*, that provide anecdotal evidence suggesting PIT tag retention is variable among ambystomatid salamanders (Table 1). For example, Ryan et al. (2014) reported that 45% of their detections of Blue-spotted Salamanders (*Ambystoma laterale*) were actually expelled PIT tags. In a study using metamorph *A. californiense*, only 1% of individuals with PIT tags were recaptured, while 5% of individuals marked by toe-clipping were recaptured (Trenham et al. 2000). The authors interpreted this discrepancy in recaptures between the marking techniques as evidence that juveniles expel and/or experience higher mortality from PIT tags than toe clipping.

Expanding the knowledge of PIT tag retention rates in salamanders can benefit CMR models by eliminating tag loss bias. By adding *Desmognathus* species to the small list of salamander PIT tag retention studies, future CMR studies using these species could potentially incorporate retention rates in the models. However, more retention studies are needed to understand how various factors (e.g., body size and anatomical location) influence PIT tag retention rates in salamander species.

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