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Using PIT Tags to Evaluate Non-Individual-Specific Marks Under Field Conditions: A Case Study with Greater Siren (*Siren lacertina*)

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Mark-recapture models used in estimating population size require the capture, marking, and recapturing of marked animals (Donnelly and Guyer 1994). Although several methods are available for marking amphibians (see Ferner 1979), sirenids and Greater Siren (*Siren lacertina*), in particular, present several problems for marking schemes. Sirenids have fewer total toes (6 or 8) than most salamanders and this limits the applicability of toe clipping schemes. Additionally, the dark skin of Greater Sirens prevents marks made by tattooing and injectable dyes from being easily read (Sorensen 2003). The only known test of multiple marking techniques on *S. lacertina* was conducted on two captive animals (Sorensen 2003). The marking techniques used on the two captive animals included cyano-acrylic, tail-notching, heat-branding and Passive Integrated Transponders (PIT tags). Of these, only PIT tags were successful in creating a lasting mark and were later used in field studies. While it was not deemed applicable for Greater Siren, previous studies on Lesser Siren (*Siren intermedia*) used heat branding to create marks that lasted for up to 96 months (Frese 2000; Gehlbach and Kennedy 1978; Raymond 1991).

The required level of identity (e.g., individual, cohort) and persistence (e.g., permanent, month, day) for a mark is dependent on the specific goals of a mark-recapture study. I tested two types of non-individual-specific marks on *S. lacertina* in an isolated herbaceous bay wetland to determine their permanence and readability. Passive integrated transponder (PIT) tags are effective at providing a permanent individual mark in *S. lacertina* (Crabill 2007; Sorensen 2003). Their proven persistence as a mark for *S. lacertina* allowed me to use them as a redundant mark to test other marking techniques used in this study.

There are several individual marking schemes for toe clipping amphibians (see Donnelly et al. 1994). However the utility of toe clipping for individually marking *Siren* is fairly limited as they only have eight total toes (most toe-clipping schemes are designed for amphibians with 18 total toes). For this reason, toe-clipping in this study was considered to be a cohort mark (i.e., different toe-clip combinations can be used in order to separate animals into smaller groups by a pre-defined criterion such as period of capture). Tail notching has been successfully used as a marking technique for larval anurans (Turner 1960). Sirens often have minor damage to their tailfins that can resemble a tail notch (personal observation). To avoid confusion with naturally occurring tailfin damage, I used an elongate arc or "tail scoop" (see Luhring 2008) as a tailfin mark on each marked animal. Because there is not an effective way to vary the appearance of a tail scoop, this method was considered to be a non-specific capture mark.

Methods and Materials.—All animals were captured from September 2006 to September 2007 as part of an on-going study on greater siren and two-toed amphiuma at Dry Bay, a 5-ha fishless Carolina bay located on the Department of Energy's Savannah River Site in Aiken County, South Carolina, USA (Luhring 2008). A sampling period occurred each month for ten consecutive days (for a total of 130 nights of trapping over 13 months) with a fyke net, and multiple arrays of hoop nets, trashcan traps (Luhring and Jennison 2008; Luhring, *in press*), and plastic and steel minnow traps (see Luhring 2008 for details of trapping design). Upon return to the laboratory, animals were weighed to the nearest 0.1 g on a Mettler PC 440 electronic scale (Mettler Instrument Corporation, Hightstown, New Jersey), measured on a meter stick for snout-vent length (SVL) and total length to the nearest 1.0 mm, and were then marked. Animals were photographed with a Nikon D70 (model# 25218) or Nikon D200 (model# 25235) camera with a Nikon 18–70 mm f/3.5-4.5G ED IF AF-S DX Nikkor Zoom Lens (model#2149) mounted on a Bogen TC-2 copy stand (Bogen Imaging Incorporated, Ramsey, New Jersey) to document mark regeneration and for later use in morphometric measurements. Animals were restrained for marking by placing them on a wet cloth. The cloth was folded over the animal's head and then the side of the cloth was folded over the animal. The animal and cloth were then rolled together to the opposite end of the cloth (see Luhring 2008). This technique of restraining the siren permitted access to the area immediately posterior to the vent for injecting a PIT tag (AVID Marketing, Incorporated, Norco, California) and administering a tail scoop while restraining the siren. Sirens did not need to be restrained for toe-clipping as they typically did not react to this type of mark. Larger sirens (>300 mm SVL) also typically did not react to receiving a PIT tag, however, all animals were restrained in the cloth for PIT tagging and tail scooping.

All PIT tags were injected towards the distal end into the ventral side of the tail 1–3 cm posterior to the vent. This is the same area used by Sorensen (2003); however, I injected the PIT tag ventrally as the ventral aspect at this point was wider and doing so negated having to avoid the spinal column. Tail scoops were made with dissection scissors on the dorsal side of the tail fin. The scoop usually started at the widest part of the tail fin and was cut ~5–7mm deep in larger animals (>400mm SVL) and 2–5mm deep in smaller animals (<400mm SVL). The general rule of thumb in deciding tail scoop depth was that no cut should be deeper than a quarter of the tail depth (i.e., halfway to the middle of the tail). Tail tissue was easier

to cut if making an initial cut at a 60–90° angle and then cutting towards the distal end of the tail in a shallow arc for 25–35mm (variable with body size of animal). Tissue from tail scoops was saved for genetic analysis and thus scissors were cleaned with a 10% bleach solution (to degrade any remnant DNA), run under tap water (to wash off any bleach), and then submerged in 70% isopropyl alcohol between the marking of each animal. Syringe needles were stored in 70% isopropyl alcohol prior to being used on PIT tag applicators. All PIT tag applicators and needles were wiped with a paper towel (to remove tissue residue) and 70% isopropyl alcohol between animals to minimize cross-contamination.

Markings on recaptured animals were given a four-stage rating based on a combination of photographic records and notes taken during laboratory measurements. Toe clips and tail scoops were given a 1 if they were freshly clipped and did not show any evidence of regrowth. They were given a 2 if there was only minor regrowth. A toe clip was given a 3 if it was partially re-grown (more than half the original size but less than ¾ the size of a full grown toe). Tail scoops were given a 3 if the tissue had healed and the site of the mark was obviously discolored. Toes and tails that were fully regenerated were given a rating of 4. If a mark was considered to be borderline between categories, it was given the higher numerical rating to provide a conservative estimate of mark retention (for photos of marks at different stages, see Luhring 2008).

Toe clips were taken from the siren's second innermost toe (the longest toe) on the right foot by using a pair of sharp scissors to cut the toe at the base where it meets the hand. A few animals had deformities (not associated with toe-clipping) on their designated hand, in which case a toe was taken from the left hand. While toe clipping was administered from the beginning of the experiment, tail scooping was initiated in January of 2007 at which time all animals received a toe-clip, a tail scoop and a PIT tag. Animals recaptured within the same ten-day trapping period were recorded and released at the site of capture without taking additional measurements.

Mark ratings were tested for significant correlations to the age of the mark, and changes in SVL, total length, and mass since the original mark. Regressions were tested with an analysis of variance (ANOVA) with a lack-of-fit test. Statistical analyses were run in Statgraphics (Centurion XV Version 15.2.06.).

Results.—A total of 102 recaptures of 72 toe clipped animals and 94 recaptures of 58 tail scooped animals were analyzed for mark durability and readability. These data were grouped into 30-day intervals to represent monthly sampling efforts (Table 1). Days between captures ranged from 20 to 332 days for toe clips (100.2 ± 65.5 SD) and tail scoops (92.1 ± 57.3 SD). Most marks began showing signs of regeneration at around 30 days (Figs. 1, 2). Toe clips remained fresh or had only minor regeneration three times longer than tail scoops (61–90 days versus 20–30 days). Marks were readable (i.e., classified as a 1, 2, or 3) 180 days after they were given, however this is at the uppermost bound of tail scoop readability. The first observed loss of mark for a toe clip occurred 127 days after being administered. The first observed loss of mark for a tail scoop occurred 61 days after being administered. While the majority of toe clips were still readable within the 332 day maximum between captures (both recorded marks over 300 days were ranked as 3's) and one mark was a 1 at 216 days, most tail scoops were unreadable beyond 180 days.

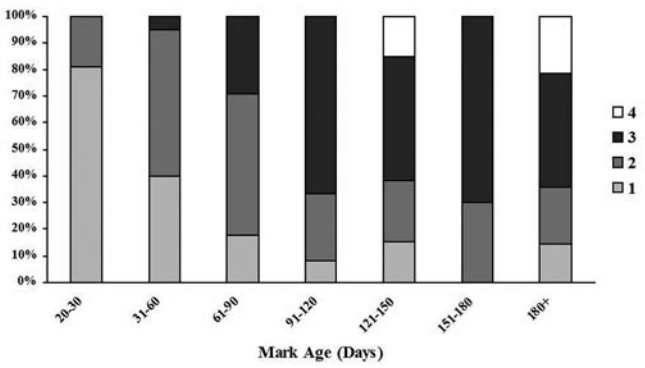

FIG. 1. Proportion of toe clip ratings by mark age of recaptured animals. 1 = fresh, 2 = minor regeneration, 3 = partial regeneration, 4 = loss of mark.

Toe clip and tail scoop mark readability were correlated to mark age, and change in SVL, TL, and mass ($p<0.05$). While tail scoop regeneration was best explained by mark age ($F_{1,87} = 144.59$, $p<0.0001$, $r^2 = 0.79$), toe clip regeneration was explained slightly better by change in SVL ($F_{1,98} = 96.15$, $p<0.0001$, $r^2 = 0.70$) than by mark age ($F_{1,98} = 89.65$, $p<0.0001$, $r^2 = 0.69$; see Luhring 2008 for complete ANOVA tables). A Fisher's least significant difference (LSD) test was used to compare the mean mark age (days) to mark rating (1–4) within toe clips and tail scoops and between toe clips and tail scoops (Table 2). Two outliers were removed from the toe clip statistical analyses to create a better fit for the model without affecting significance. The outliers belonged to the same animal that did not regenerate any toe tissue after 194 and 216 days. One outlier was removed from the tail scoop analyses for similar reasons (no tail tissue regeneration in 63 days) and did not affect significance. All three outliers are included in non-statistical figures and tables (Figs. 1, 2; Tables 1, 2).

Discussion.—Toe clips lasted longer than tail scoops and can also be used to create a finer level of distinction between groups

TABLE 1. Breakdown of toe clip (c) and tail scoop (s) mark readability (1–4) by mark age (days) for recaptured animals.

Readability	Mark Age 20-30 C	S	31-60 C	S	61-90 C	S	91-120 C	S	121-150 C	S	151-180 C	S	180+ C	S
1	13	4	8	0	3	1	1	0	2	0	0	0	2	0
2	3	11	11	5	9	2	3	0	3	0	3	0	3	0
3	0	1	1	13	5	10	8	10	6	7	7	5	6	1
4	0	0	0	0	0	3	0	2	2	3	0	4	3	9
Total	16	16	20	18	17	16	12	12	13	10	10	9	14	10

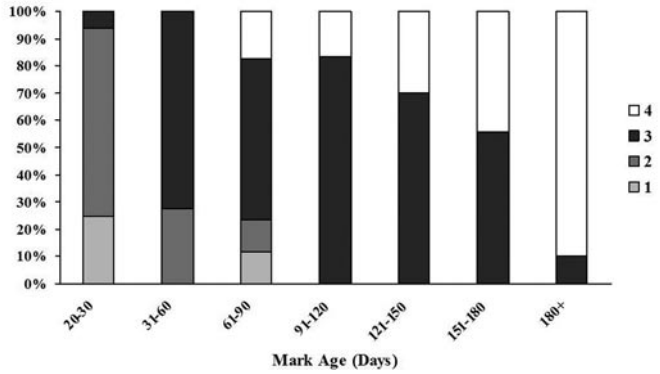

FIG. 2. Proportion of tail scoop ratings by mark age of recaptured animals. 1 = fresh, 2 = minor regeneration, 3 = partial regeneration, 4 = loss of mark.

of animals (cohorts). Most toe clips were still readable through the end of the study and one animal that was caught outside of the study period on 31 January 2008 had a readable toe clip that was 353 days old. Although there were five "lost marks" (a rating of 4) of toe clips, most of these were actually still distinguishable marks that surpassed the ¾ of original length threshold between a rating of 3 and 4. However, they were not easy to distinguish and may have only been distinguishable to someone familiar with what that specific toe should look like.

Toe clips would be best suited for mark-recapture studies lasting for a field season (January to September) and provide an opportunity for a cohort marking scheme. Toe clipping may also be used in conjunction with studies on skeletochronology (Bruce and Castanet 2006; Halliday and Verrell 1988) or for any studies needing tissue samples. The number of toes on *S. lacertina* (8) limits toe-clipping schemes. Additionally, I caution against removing multiple toes on the same foot because *S. lacertina* use their feet as much, if not more than, their tail (Schalk and Luhring, unpubl. data) to pull themselves through the water, vegetation, and organic debris, and the extent to which the loss of multiple toes would impact their survival is not known.

Although one tail scoop was readable 332 days later, 11 out of the 12 tail scoop marks that were over 165 days old were completely regenerated and no longer visible. After 63 days, all tail scoops were healed and only discolored tissue (a rating of 3) in the area of the original mark distinguished these animals as having been given a tail scoop. Of all the techniques tested, tail scoops might have had the shortest-lived effects on sirens. Tail scoops would be ideal for marking efforts that last for a short period of time (<60 days) and do not require a specific mark. The tissue taken from tail scoops is sufficient for quality DNA extraction (personal observation) and the rapidity of tailfin regeneration suggests that there are not any likely long-term effects of tail scoops. While mark age, and change in SVL, TL, and mass were significantly correlated to the quality of marks, these correlations were not directly tested for causation of mark regeneration. Future studies that test mark regeneration as it relates to the growth rates of aquatic salamanders may show differences for regeneration rates of different types of tissue. Greater Sirens show a reduced growth rate upon reaching a length of 400 mm SVL (Luhring 2008), and toe regeneration may be retarded in these animals (pers. obs.).

TABLE 2. Results of a 95% Least Significant Difference (LSD) test on the mean ages of mark type readability. Letters in homogenous groups column denote significantly different groups.

Mark Type-Readability	No. Ind.	Mean Age	Homogeneous Groups
Tail Scoop-1	4	25.0	A
Tail Scoop-2	18	35.5	A
Toe Clip-1	27	47.0741	A
Toe Clip-2	35	87.1429	B
Tail Scoop-3	47	94.2553	B
Toe Clip-3	33	143.242	C
Tail Scoop-4	21	160.095	CD
Toe Clip-4	5	193.6	D

There are still several other types of marking techniques available for testing on sirens and other permanently aquatic salamanders (e.g., visible implant alpha-numeric tags). While PIT tags work well for permanent individual identification, they cannot be used in larva and small juveniles of *S. lacertina* because of the large gauge needle needed to insert the tag into the tail (Sorensen 2003; personal observation). The golden flecking on *S. lacertina* is highly variable in respect to the amount present, as well as the shapes and sizes of individual flecks. This variation is possibly unique and might be useable for individual identification with photo-identification programs (see Gamble et al. 2008).

Until the validity of other such techniques are tested on *S. lacertina* in the field, I suggest using PIT tags for long-term studies or those requiring individual identification and either toe clipping or tail scooping for short-term studies not requiring individual identification. The use of a PIT tag as a permanent individual-specific mark was essential for determining the applicability of non-individual-specific marking techniques in the field. Field-testing marking techniques would presumably be the best indicator of how well those techniques would work in the field on the species or groups of species of interest.

The initial investment of time and money required to establish the validity of cost-effective marking techniques enables long-term savings through cheaper marking schemes. Many population level studies on animals are cost-prohibitive. For example, only two published mark-recapture studies are available for *S. lacertina* and more information on population monitoring is unavailable at this time. Prior to this study, only PIT tags were proven to work as a marking scheme and the cost of PIT-tagging a population of sirens would prevent many investigators from being able to conduct even a simple population estimate. Now that there are two cheap, quick, simple, and field-proven marking techniques for sirens, population monitoring is cheaper and more realistically achievable.

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