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### ARTICLE

# Retention of Passive Integrated Transponder Tags in a Small-Bodied Catfish

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## Abstract

Members of the freshwater catfishes (order Siluriformes) are capable of transintestinal expulsion of foreign bodies, including internally implanted tags, which can bias movement and survival estimates. We evaluated long-term (120-week) retention rates of passive integrated transponder (PIT) tags in a laboratory setting to assess potential tag loss in Stonecat Noturus flavus. The PIT tags were surgically implanted into the peritoneal cavity of fish (n = 157) ranging from 71 to 213 mm TL. We demonstrated that Stonecats can successfully be tagged with 12- and 23-mm PIT tags with low levels of mortality (5.0%) and tag loss (9.6%). Based on individual encounter histories and covariates, we further evaluated our data set in a multistate framework using program MARK. Based on our findings, tag age has a negative effect on tag loss; if Stonecats lose tags, it is relatively soon after tagging. Additionally, Stonecat TL has a negative effect on tag loss, with tag loss decreasing with increasing fish TL.

Quantifying animal vital rates, movement, and habitat selection is essential to understanding population dynamics (McClintock et al. 2014) and making sound management decisions. Population parameters can be estimated using marking studies in which marked animals are actively recaptured (Williams et al. 2002) or passively monitored (Millspaugh and Marzluff 2001). Fisheries biologists use various marking techniques, including batch marks (e.g., fin clips or visual implant elastomer tags) or individual marks (e.g., radiotelemetry, coded wire tags, or

numbered floy tags), to monitor fish populations (Nielsen 1992; McKenzie et al. 2012). Each of these has limitations; batch marks typically do not provide data on individuals, radiotelemetry may be expensive and as such may limit sample size depending on budgets, and some individual marks (coded wire tags and numbered floy tags) typically require physical recapture. More recently, fisheries biologists have used passive integrated transponder (PIT) tags to study fish movement (Zydlewski and Casey 2003; Cucherousset et al. 2005), habitat selection (Roussel et al.

2000; Roussel et al. 2004), demography, population structure, and vital rates (Al-Chokhachy and Budy 2008).

The use of PIT tag technology has revolutionized our understanding of fish movement and habitat selection. Advantages of PIT tags include individual identification, long tag life span, easy application, and, when properly sized, minimal effects on growth, survival, and physiological performance (O'Donnell et al. 2010; Ficke et al. 2012). They are also relatively inexpensive compared with other individual-based tagging methods, such as radiotelemetry. Hence, PIT tags have allowed fisheries biologists to analyze movement and habitat preferences at much finer spatial and temporal scales than previously possible. However, like other tagging methods, it is important to explicitly address potential limitations of the technology that could influence parameter estimates. One critical factor that could affect PIT tag reliability is tag loss.

Tag loss can vary by tag type, species, TL, implantation location, and tagging procedures (Clugston 1996: Buzby and Deegan 1999) and should be evaluated carefully using tagging experiments to understand and address tag loss (Daugherty and Buckmeier 2009). Such studies should evaluate a variety of factors that are known to affect tag retention, and tag location and tagging procedure are particularly important (Jepsen et al. 2002). For example, PIT tags may be injected into muscular tissue or the body cavity using a hypodermic injector. Alternatively, intraperitoneal surgical implantation may be used and is regarded as a more appropriate method for long-term studies and small-bodied fishes (Bridger and Booth 2003; Swarr 2018). However, intraperitoneal implantation by any method may not be suitable for all species because of physiological processes that allow some species to remove foreign material from their body cavities (Moore et al. 1990).

Catfish species (order Siluriformes) from several taxonomic families have a demonstrated ability to expel internal tags that are implanted in their peritoneal cavity, and because of this there has been varied success in tagging catfish. Some examples of tag types that catfish have been known to expel include PIT tags (Moore 1992; Baras and Westerloppe 1999; Daugherty and Buckmeier 2009), ultrasonic tags (Sakaris et al. 2005), and Vemco V16 telemetry tags (Holbrook et al. 2012). Catfishes expel tags from the peritoneal cavity by first surrounding them in a fibrous capsule, migrating the fibrous capsule to the intestinal tract, creating a false intestinal wall around the fibrous capsule, absorbing the fibrous capsule into the intestine, and finally passing the capsule through the intestine. The process of transintestinal expulsion has been documented across fish in the order Siluriformes, including Channel Catfish Ictalurus punctatus (Summerfelt and Mosier, 1984; Marty and Summerfelt 1986), Brown Bullhead Ameiurus nebulosus (Sakaris et al. 2005), African Catfish Heterobranchus longifilis (Baras

and Westerloppe 1999), Blue Catfish *Ictalurus furcatus* (Holbrook et al. 2012), and Mekong Giant Catfish *Pangasianodon gigas* (Mitamura et al. 2006). Clearly, transintestinal expulsion could have negative consequences for PIT tagging studies that involve implanting tags into the peritoneal cavity (Arnason and Mills 1981; Seber and Felton 1981; Conn et al. 2004). Transintestinal expulsion has been documented in large and medium-sized catfishes but not in smaller catfishes such as madtoms *Notorus* spp. We were particularly interested in studying PIT tag retention in Stonecat *Noturus flavus*, a small catfish native to North America. Our interest in tag retention was motivated by a concurrent field study examining Stonecat habitat selection and vital rates.

The Stonecat is the largest species of madtom (maximum TL = 310 mm; Page and Burr 1991) and may share the ability to transintestinally expel PIT tags with larger species in the order Siluriformes. Our interest in tag loss was motivated by a concurrent field study examining Stonecat habitat selection and vital rates. Mark-recapture studies rely on a number of modeling assumptions, including no significant tag loss or mortality from tagging (Burnham et al. 1987). Unidentified tag loss on apparent survival estimates can negatively bias survival estimates by reducing the number of marked individuals available for recapture (Pollock et al. 1990; Bateman et al. 2009). Therefore, our specific objective was to conduct a laboratory experiment to assess rates of PIT tag loss and tag-related mortality in Stonecats prior to conducting our field study. We hypothesized that tag loss was affected by personnel experience, time since tagging, fish length, and tag type, as well as interactions between individual covariates.

## **METHODS**

Stonecats (n = 157; mean length = 152.8 mm TL, range = 71-213 mm TL; mean wet weight = 35.2 g, range = 3.60-86.15 g) collected via backpack and barge electrofishing in Horse Creek, Wyoming, were transported approximately 3 h in oxygen-saturated water to the Colorado State University Foothills Fisheries Laboratory, Fort Collins, Colorado, in a 113-L cooler on three occasions in May, August, and November 2015. During transport, fish condition, oxygen levels, and temperature were monitored approximately every 30 min. Upon arrival fish were transferred to four 340-L tanks receiving continuous flows (5 L/min) of air-saturated water matching the temperature of the capture location. After 1 week of acclimation to laboratory conditions, water temperature was increased to  $15 \pm 0.5$ °C at 1°C/d to allow sufficient time for physiological acclimation (Lyytikainen et al. 1997; MacNutt et al. 2004), and fish were held at this temperature. The laboratory was kept under a natural photoperiod for Fort Collins, Colorado (40.581°N, 105.138°W). Fish were

randomly assigned to one of four tanks. Initially, we held fish at densities of 17–20 fish per tank; however, after subsequent wild collections, we increased densities to 30–35 fish per tank due to constraints on the number of available holding tanks. Tanks included synthetic habitat elements constructed from PVC pipes cut in half lengthwise (approximately  $76 \, \text{mm} \times 300 \, \text{mm}$ ) as cover. Fish were fed to satiation daily on a mixed diet of frozen chironomid larvae and a commercially available trout feed (Bio-Oregon 2-mm pellets).

Fish underwent surgical tagging procedures after the laboratory acclimation period. Each collection occasion corresponded to an individual tagging event with similar tagging procedures and tagging personnel. Prior to surgery, fish were weighed (wet weight; g), measured (TL; mm), and anesthetized using neutrally buffered 40 mg/L of tricaine methanesulfonate (MS-222) to achieve complete immobilization. We defined complete immobilization as the point at which fish were unable to maintain equilibrium and no longer responded to stimuli before beginning the surgical procedure. Passive integrated transponder tags were surgically implanted into the peritoneal cavity of Stonecats (71–213 mm TL) through an incision in the peritoneal cavity. Incisions were made using a #12 curved scalpel blade. The incision was approximately the diameter of the tag and located approximately three-fourths up the length of the body cavity and slightly offset from the ventral midline. Incisions were offset from the ventral midline to minimize the probability of the wound becoming irritated while fish rested on the tank substrate. Tags were inserted anteriorly to avoid impacting the gut. Incisions were closed with a single suture using a Braunamid DS24 polyamide pseudo monofilament suture attached to a 24-mm curved suture needle (Swarr 2018). Fish between 71 and 150 mm TL (6.4 and 53.3 g) were tagged with Oregon RFID HDX 12-mm glass (n = 18) or polymer (n = 39)tags. Fish larger than 150 mm TL (26.3 to 86.2 g) were tagged with either Oregon RFID HDX 12-mm glass tags (n = 23), Oregon RFID HDX 12-mm polymer tags (n =32), or Oregon RFID HDX 23-mm glass tags (n = 45),

which were randomly assigned (Table 1). We based tag size on findings from Winter (1996) that determined tag weight should be 1-2% of fish body weight (12-mm glass tag = 0.1 g, 12-mm polymer tag = 0.1 g, 23-mm glass tag = 0.6 g).

We monitored short-term tagging mortality related to surgical procedures with "control" and "sham" treatments using fish collected in May 2015. Our control treatment consisted of 12 fish that received no surgical procedure; our sham group consisted of 18 fish that received the full surgical procedure (anesthetization, incision, and suture) except a tag was not implanted. Control and sham fish were distributed equally among the four holding tanks to account for tank-related mortality. We attempted to identify control and sham fish based on scarring from incisions or sutures. In August 2015, we tagged control and sham fish because these fish were needed for a concurrent swimming performance study.

Tanks were monitored daily for mortality and tag loss. Whereas tags were easily seen on the tank bottom, we also used a magnetic wand to scan for expelled tags. Full counts and tag scans using an Oregon RFID HDX Proximity Reader of all fish in the tanks were conducted every 3 months to validate daily monitoring and determine whether fish lost tags that were not detected during checks. We attributed mortality within the first week to complications from surgical procedures, and mortality after the first week was attributed to causes not directly related to surgical procedures (Caputo et al. 2009). Due to space constraints, on July 20, 2016, one tank was removed from the study and the 47 Stonecats within it were euthanized (250 mg/L MS-222). Fish in the remaining three tanks (n = 87) were monitored for tag retention until the experiment was terminated on September 9, 2017.

We analyzed the laboratory PIT tag data set using a multistate mark–recapture framework (Brownie et al. 1993; Nichols and Kendall 1995; Lebreton and Cefe 2002; Fetherman et al. 2015) in program MARK version 6.1 (White et al. 2006) with two states: "TAG" and "NO\_TAG." Fish could stay tagged ( $\Psi_{TT}$ ) or transition to

TABLE 1. Tagging event date and associated number of tags implanted in Stonecats by tag type for the laboratory study on PIT tag loss. Tags lost for each tag type are given in parentheses. Mortalities by tag type in the last column are reported in the following order: for 12-mm glass tags, for 23-mm glass tags, for 12-mm polymer tags, and the total for each event (in bold italics). Mortality from euthanasia (n = 47) is not included in the mortality component of this table.

Tagging event and total		Tag type		Mortalities	
	12-mm glass	23-mm glass	12-mm polymer	Total tags lost	(by tag type)
May 2015	41 (6)	11 (1)	0	7	4, 2, 0, <b>6</b>
August 2015	0	0	26 (7)	7	0, 0, 1, <i>1</i>
November 2015	0	34 (1)	45 (0)	1	0, 1, 0, <i>1</i>
Total	41 (6)	45 (2)	71 (8)	15	8

NO\_TAG ( $\Psi_{TN}$ ). Fish could not transition from NO\_TAG to TAG ( $\Psi_{NT}=0$ ). If fish transitioned from TAG to NO\_TAG, we removed them from the data set as fish were not retagged ( $\Psi_{NN}=0$ ). Our parameter of interest was  $\Psi_{TN}$ : tag loss, conditional on survival. Fish were housed in enclosed tanks, and we assumed that all mortalities and lost tags were observed. Therefore, we set detection probability for both states equal and fixed at 1 ( $P_{TAG}=P_{NO\_TAG}=1$ ; i.e., we detect all lost tags). We also set survival probability for both states (TAG and NO\_TAG) equal and fixed at 1 ( $S_{TAG}=S_{NO\_TAG}=1$ ) because we observed few mortalities during the experiment. As such, we did not explicitly model mortality or detection probability.

As mentioned above, each collection occasion corresponded to an individual tagging event with similar tagging procedures and personnel. Due to tag availability, not every tag type was available in every tagging event. Therefore, we analyzed how individual covariates influenced  $\Psi_{TN}$  within their respective tagging event. Covariates included time since tagging, fish TL, and tag type. Furthermore, tagging personnel were held constant throughout the experiment. Modeling  $\Psi_{TN}$  within each tagging event allowed us to indirectly assess tagging experience by examining if tag loss decreased with subsequent tagging events as the tagging crew became more experienced. The three tagging events resulted in a staggered time since tagging. Specifically, individuals tagged in May 2015 were at large for 120 weeks, individuals tagged in August 2015 were at large for 108 weeks, and individuals tagged in November 2015 were at large for 96 weeks.

Candidate models were developed starting with the simplest model in which  $\Psi_{TN}$  was not influenced by tagging event or covariates  $[\Psi_{TN}$  (.)]. We then parameterized the model by allowing  $\Psi_{TN}$  to vary by tagging event  $[\Psi_{TN}$  (event)], followed by the addition of covariates (time since tagging, tag type, and TL) within tagging event. We also included an interaction term between tag type and fish length because small fish (70–180 mm TL) received 12-mm

tags but large fish (>180 mm TL) were randomly assigned either a 23-mm or a 12-mm tag. All models were analyzed concurrently in program MARK, and results were ranked based on Akaike information criterion corrected for small sample sizes (AIC<sub>c</sub>) (Burnham and Anderson 2002).

### **RESULTS**

Raw estimates of Stonecat tag retention (>90%) and survival (95%) were high. After 120 weeks, 15 fish (9.6%) lost tags and there were eight mortalities (5.0%) (Table 1). Three mortalities occurred within 1 week posttagging and were attributed to surgical procedures and confirmed via necropsy. The other five mortalities occurred later, and the causes are unknown but did not appear to be related to the tagging procedure. The May 2015 tagging event had four mortalities among unmarked fish (control and sham groups) during the three months of evaluation (two in the first month, one each in the second and third), and we could not assign these to treatments as incisions had healed and scars were not evident. After the 47 fish had to be euthanized due to space constraints in July 2016, 87 individuals remained alive and were monitored for tags until the experiment was terminated in September 2017. Length was significantly correlated to weight  $(r^2 = 0.96)$ ; therefore, weight was not included as a covariate.

Based on the multistate mark-recapture model, two models are plausible and account for 79% of the model weight (Table 2). Both top models include the covariates of time since tagging and length. The top model contains the additional covariates of tag type and the length × tag type interaction (Table 2). Three parameters in the top model (time since tagging, fish length, and the interaction of length × tag type) have 95% confidence intervals for their respective beta estimates that do not overlap zero (Figure 1B). Time since tagging has a negative effect on the beta estimate for estimated probability of weekly tag loss, indicating that tag loss occurs early and that the longer a tag is in a fish the less likely it is to be lost. Fish

TABLE 2. Model selection results from the laboratory study on PIT tag loss in Stonecats.

Model	$\mathrm{AIC}_c$	$\Delta { m AIC}_c$	AIC <sub>c</sub> weights	Model likelihood	Number of parameters	Deviance
$\Psi_{TN}$ (event) + time since tagging + length + tag type + length × tag type	244.3	0	0.488	1	7	230.2
$\Psi_{TN}(\text{event})$ + time since tagging + length	245.2	0.934	0.306	0.627	5	235.2
$\Psi_{TN}$ (event) + time since tagging + length + tag type	246.9	2.663	0.129	0.264	6	234.9
$\Psi_{TN}(\text{event})$ + time since tagging	247.9	3.669	0.078	0.16	4	239.9
$\Psi_{TN}(event)$	304.7	60.43	0	0	3	298.7
$\Psi_{TN}(.)$	327.4	83.14	0	0	1	325.4

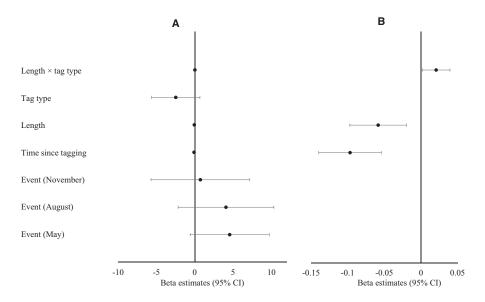


FIGURE 1. Panel (A) shows beta estimates (black dots; logit scale) and associated 95% confidence intervals (error bars) for all parameters in the top model. Panel (B) shows a close-up of several parameters in the top model, with beta estimates and 95% confidence intervals shown on a scale that clearly shows that the estimates do not overlap zero.

length also has a negative effect on estimated probability of weekly tag loss, indicating that tag loss is higher in smaller fish (Figure 1B). Interaction between length and tag type has a positive effect on estimated probability of weekly tag loss (Figure 1B). We arcsin transformed the estimated weekly probability of tag loss and the confidence bounds postanalysis (Figure 2) to better visualize how the probability of tag loss decreases across three discrete time periods (1 week posttagging, 52 weeks posttagging, and 120 weeks posttagging) for three fish length categories (<75 mm, 76–120 mm, and 121–215 mm).

Estimated weekly probability of tag loss decreased with increasing fish size during tagging periods (1, 52, and 120 weeks) and with increasing length of time since fish were tagged (Figure 2).

## **DISCUSSION**

We demonstrated that Stonecats can successfully be tagged with 12- and 23-mm PIT tags with low levels of mortality (5.0%) and tag loss (9.6%), which means that PIT tags are a good choice for field studies on Stonecats.

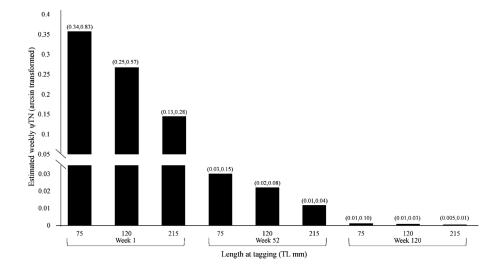


FIGURE 2. Arcsine-transformed estimated weekly probability of tag loss ( $\Psi_{TN}$ ) and associated 95% confidence intervals (numbers in parentheses above each bar) as a function of fish length at tagging (<75, 76–120, and 121–215 mm TL) for week 1, week 52, and week 120 after tagging.

Tag loss was influenced by time since tagging, fish length, tag type, and interaction of fish length by tag type.

One concern was mortality associated with surgically implanting PIT tags. However, mortality in this study was low and usually occurred within 1 week of tagging, indicating that mortality was due to surgery as suggested by Caputo et al. 2009. Musselman et al. (2017) provided a synthesis of various studies involving PIT tags and diminutive fishes and found that tag retention (90%) and survival (92%) were high in the majority (85%) of studies reviewed, which was also reflected in their own study involving PIT tags in small-bodied warmwater fishes. Burdick (2011) had similar results of low average mortality rates  $[9.8 \pm 3.4\% \text{ SD}]$ , with most mortality occurring within 48 h of tagging when injecting juvenile Lost River Sucker Deltistes luxatus with 12-mm PIT tags. Additionally, Bruyndoncx et al. (2002) tagged European Bullhead Cottus gobio, another small-bodied benthic fish, with 12mm PIT tags and found no tag loss or mortality during a 4-week laboratory experiment and only one tag loss in a 7-week field experiment. Similar to our study, they concluded that tagging small-bodied benthic fish is possible without high levels of tag loss or mortality. Based on the results of our study, we feel confident that Stonecats can be successfully PIT-tagged without biasing subsequent field-based survival estimates, especially with experienced tagging personnel.

In our study, fish lost tags in the first few weeks posttagging and time since tagging was inversely related to the estimated probability of weekly tag loss. Welch et al. (2007) concluded that loss of surgically implanted acoustic tags in steelhead Oncorhynchus mykiss was a function of time since tagging, with most tag loss occurring between 6 and 8 weeks after tagging. In a 5-year study, Feldheim et al. (2002) used dorsally injected PIT tags to identify individual Lemon Sharks Negaption brevirostris and found that about 12% lost their tags and 23.4% of tag loss occurred within the first week posttagging. Studies on Shovelnose Sturgeon Scaphirhynchus platorynchus showed a different trend than our study, with increasing injected PIT tag loss over time in both the operculum and dorsal musculature (Hamel et al. 2012). Whether tag loss is high initially or increases over time, it is necessary to account for the relationship between time since tagging and tag loss to avoid biasing field-based vital rate estimates. As such, time since tagging is an important covariate to consider in any mark-recapture study where tag loss is a concern.

Stonecat size was an important predictor of tag loss in our study, with the likelihood of tag loss decreasing as fish TL increased. Similar to our study, PIT tag retention increased with fish length for Southern Redbelly Dace *Chrosomus erythrogaster* (Pennock et al. 2016), Brown Trout *Salmo trutta* (Richard et al. 2013), Humpback

Chub Gila cypha (Ward et al. 2015), and Spot Leiostomus xanthurus (Brewer et al. 2016). However, some studies report little to no relationship between fish length and tag loss, including studies on members of families Salmonidae (Dare 2003; Dieterman and Hoxmeier 2009), Cyprinidae (Archdeacon et al. 2009), Fundulidae (Clark 2016), and Sparidae (Navarro et al. 2006). Although conclusions vary on the relationship between tag loss and fish size, the relationship is important to consider in studies using PIT tags.

Tagging event (May 2015, August 2015, and November 2015) served as a surrogate for tagging personnel experience, and presumably increased experience could lead to higher tag retention. However, beta estimates and associated 95% confidence intervals for individual tagging events overlapped zero and each other. Although beta estimates and variation indicate caution in interpreting the tagging event variable, it was a factor in the top five of six models. The decreasing trend in beta estimates across the three tagging events suggests that tagging experience may be important. Dare (2003) concluded that there is an effect of tagging experience on tag loss in Chinook Salmon Oncorhynchus tshawytscha. Furthermore, Navarro et al. (2006) found that small fish survival and tag retention were higher when tagged by experienced tagging personnel compared with inexperienced personnel. Therefore, to minimize tag loss and maximize survival in a tagging study on smallbodied fish (such as a Stonecat), tagging personnel should be trained on the specific organism and methods.

Our modeling suggested that tag type was an important consideration; however, beta estimates overlapped zero, indicating that the relationship was weak. Additionally, due to limited availability of tags, we did not use each tag type on every tagging event, complicating inferences regarding tag type. Our analyses also indicated an interaction between tag size and fish length. However, we feel that the interaction is an artifact of tagging protocols because large fish (>180 mm TL) received either a 23-mm or a 12-mm tag, whereas small fish (70–180 mm TL) only received 12-mm tags. Additional species-specific tagging studies are needed to clarify relationships between tag type and tag loss.

Our experimental fish were used in an independent swimming performance study evaluating the effects of slope on Stonecat passage success in rock ramp fishways (Swarr 2018). Fish use in the passage structure was not treated as a factor in our analysis of tag retention because these data were being used for another study. However, fish used in the passage structure, were exposed to varying and turbulent flow, and were swimming over rock substrate. Therefore, we feel that the passage trials would likely increase tag loss and provide us with a conservative overall estimate when considering using PIT tags to determine habitat selection and movement of Stonecats in field studies. However, it is important to consider that

controlled laboratory estimates of tag loss may not accurately estimate tag loss in a harsher field environment.

The goal of our study was to estimate PIT tag loss in Stonecats and was motivated by the need for estimates to inform our concurrent field study. We were concerned about potential tag loss because of transintestinal expulsion observed in other large catfishes (Moore 1992; Baras and Westerloppe 1999; Mitamura et al. 2006; Daugherty and Buckmeier 2009), although we were not trying to identify the process underlying tag loss. However, anecdotally, we did not observe obvious external signs of transintestinal expulsion (i.e., irritation and/or inflammation of the anus) and our observed tag loss was low compared with other studies on larger catfishes (Summerfelt and Mosier 1984; Marty and Summerfelt 1986; Holbrook et al. 2012).

Our results indicate that PIT tags are a viable method for individually identifying and tracking Stonecats and that long-term tag loss is low. Time since tagging and fish length appear to be the best predictors of tag loss. The effects of unidentified tag loss on apparent survival estimates in field studies can be important (Pollock et al. 1990) as tag loss imposes a negative bias on survival estimates due to a reduction in marked individuals available for recapture (Bateman et al. 2009). Initial loss could be adjusted for directly by correcting overall tag loss with apparent survival (Bateman et al. 2009) or by allowing initial apparent survival to be modeled differently from subsequent apparent survival (Pradel et al. 1997). By understanding the probability of tag loss from our laboratory study, we can correct for potential bias in field studies of Stonecat populations and better estimate key demographic parameters, such as survival and movement.

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