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A novel indicator to assess PIT tag retention in an aquatic amphibian: impact of body size

Jacob M. Hutton[1*], Adrian D. Macedo[2], Jason L. Brown[1] and Robin W. Warne[1,2]

Abstract

The comprehensive study of organisms is often hindered by the difficulty of consistently capturing, detecting, and tracking all life stages and age classes. This challenge is particularly pronounced for aquatic amphibians such as *Siren intermedia* (lesser siren), which can aestivate underground in dry burrows during extended droughts. In addition, obtaining year-round data on the habitat use, occupancy, and movement ecology of *S. intermedia* is notably difficult due to their cryptic nature, mobility, aestivation behaviors, trap-shy habits, and the impracticality of outfitting hatchlings and small juveniles with telemetry devices like subcutaneous Passive Integrated Transponder (PIT) tags. Moreover, there is the lack of a non-physical method to distinguish retained tags from those that have been dropped. In this study, we developed a novel indicator to assess the field retention status of PIT tags inserted into the tail tissue of *S. intermedia*. This was achieved by analyzing individual spatial redetection patterns of 8- and 12-mm PIT tags inserted into juveniles and adults, respectively, over 2 years in a remnant Cypress–Tupelo swamp wetland complex in southern Illinois, using systematic dipnetting, trapping, and PIT scanning telemetry surveys. Tags were considered dropped if the average distance between subsequent scanned PIT detections after the first redetection was ≤ 5 m and if the distance between the second detection and final redetection location was also ≤ 5 m. We then examined PIT tag retention in relation to initial body size and marking parameters. Ultimately, 29% of the 8-mm PIT tags initially injected into juveniles with tail lengths (Tail) 46–84 mm were redetected at least once. Using our spatial–temporal PIT telemetry indicator, we found that 45% of the redetected PIT tags had been dropped by juveniles. In contrast, all three of the 12-mm tagged adults were redetected at least once, with movement patterns indicative of tag retention. Our findings suggest that 8-mm PIT tags are likely to be expelled from juvenile *S. intermedia* with a mean ± standard deviation (SD) Tail of 50.2 ± 2.8 mm. This study underscores the importance of determining appropriate size requirements and cutoffs for effective telemetry device application across different age classes of a species.

Keywords *Siren intermedia*, Passive integrated transponders (PIT), PIT telemetry, PIT tag retention, Movement ecology, Wetlands, Amphibian ecology

Background

Researchers have long endeavored to understand and quantify the spatial and temporal habitat, population, and movement dynamics of organisms on a landscape. This research carries far-reaching implications, spanning broader evolutionary and conservation impacts of population and metapopulation demographics to fundamental ecological mechanics such as ecosystem-level energy and nutrient fluxes and individual-level factors like fitness, resource acquisition, and dispersal [1–3]. Despite this extensive body of research, our understanding of long-term population-specific movement behaviors and population ecologies has predominantly focused on larger, more widely distributed, and/or visually conspicuous species and age classes [4, 5]. Consequently, a significant

*Correspondence:
Jacob M. Hutton
jacob.hutton@siu.edu
[1] Southern Illinois University, Carbondale, USA
[2] Prairie Research Institute, University of Illinois Urbana-Champaign, Champaign, USA

gap remains in our understanding of the movement behaviors and population ecologies of younger, smaller-bodied age classes, species inhabiting complex environments, and/or those with diverse and cryptic natural histories.

Siren intermedia (lesser siren) is a paedomorphic aquatic salamander that retains both fully developed gills and lungs throughout its life and can grow up to 500 mm in total length. They inhabit swamps and ponds across the eastern and central United States that are characterized by variable hydroperiods, which often support abundant populations [6–8]. Unlike most other aquatic amphibians*,* *S. intermedia* can aestivate during periods of drought or localized pond drying. Individuals will burrow into the substrate or utilize preexisting crayfish burrows, then envelope themselves in a mucous-like cocoon, which allows them to enter a hibernation-like state primarily associated with water conservation [9, 10]. When precipitation re-inundates the aestivation site, individuals can become active within 1 day [10]. Depending on local precipitation patterns and pond drying, individuals may undergo multiple cycles of aestivation within a year, with cycles lasting from just a few weeks to over a year [8, 10]. In southern Illinois, individuals have been observed aestivating at depths up to 1 m below dried pond basins, whereas in Indiana, they have been found at depths as shallow as 8 cm [9, 11]. However, despite their extensive range, there is considerable uncertainty about their average aestivation depths and histories or general movement patterns, particularly for juveniles. For example, some studies report the species as sedentary, with movement distances under 12 m and home ranges of 12 m^2 or less, while others report distances exceeding 30 m and home ranges larger than 95 m^2 [7, 9, 10]. These discrepancies are likely due to their extensive range and the challenges associated with their capture, marking, and recapture.

Previous studies of *S. intermedia* populations and their movements have often exclusively relied on passive funnel traps to capture individuals within inundated sites [7, 9, 12–16]. However, Davis et al. [1] and Thornton [12] raised concerns about high levels of trap avoidance or shyness in *S. intermedia*, while Gehlbach and Kennedy [7] noted that smaller individuals often escape funnel traps. Capturing population and movement data across all age classes is crucial, as juvenile age classes frequently drive dispersal, influencing processes like gene flow, adaptation, and speciation [4, 5, 17–19]. Yet, consistent data on juvenile *S. intermedia* remains elusive, with most studies focusing on sub-adults and adults.

Efforts to collect long-term data on *S. intermedia* have employed various capture–mark–recapture (CMR) techniques. Sawyer and Trauth [13] used uniquely-coded subcutaneous visible implant elastomer (VIE) tags, while Thornton [12] applied both VIE and visible implant alpha (VIA) tags. However, the dark pigmentation and thick skin tissues of sirenids hinder the observation and accurate identification of these tags [12–14]. Others have tried marking *S. intermedia* with uniquely numbered brands [7, 13, 14, 16]. Though the effectiveness of this method has been inconsistent. While Frese et al. [16] reported successful use of brands that remained visible during subsequent recaptures, Sorensen [14] found that the brands became illegible after just 2 months. Moreover, these methods rely on physically recapturing individuals, which poses unique challenges given their complex behaviors (e.g., aestivation), habitat, and trap avoidance/escape.

Recently, advances such as as radio- and GPS-telemetry have addressed some of the difficulties in tracking smaller, more elusive, and cryptic species [3, 20–22]. In particular, radiotelemetry has proven effective in gathering spatial–temporal movement data for medium and large-bodied animals. However, the equipment cost, characteristics of materials (e.g., transmitter size, antenna length, limited transmitter battery life), and recurrent technical problems (e.g., often invasive attachment procedures, tag retention, limited function underwater) render them unsuitable for fossorial, smaller age classes, and/or species and groups with aquatic natural histories [3, 23–26]. Passive integrated transponder (PIT) telemetry offers a promising alternative to radiotelemetry through the utilization of miniature, subcutaneously implanted microchips encased in biocompatible glass [27]. The PIT tags themselves require no power source, are small ($\geq$ 8.4 mm and $\geq$ 0.03 g), and relatively inexpensive ($4–5 USD). The tags can be individually identified by a transceiver when activated by an electromagnetic field produced through a portable antenna that can penetrate and detect 8-mm PIT tags up to 21 cm away and 12-mm tags up to 34 cm away through water, organic debris, and bedrock [28].

Active PIT tag telemetry offers a powerful tool for long-term monitoring of individual detection and movement behaviors in smaller, cryptic, and/or aquatic organisms. However, PIT tag detection rates can be influenced by several factors, including tag size and orientation, tag distance from receiver antenna, environmental noise, and interference from metal or electromagnetic sources [28–32]. Previous studies on *S. intermedia* have exclusively employed larger 12-mm PIT tags, focusing primarily on sub-adults and adults [12–14, 33–36]. To date, no studies have examined the use of smaller 8-mm PIT tags or explored how the size of individuals at tagging affects PIT tag retention. Furthermore, past research has largely focused on identifying tagged individuals after physical recapture rather than active landscape-level telemetry redetections [37]. Although a recent study by Davis et al. [33] pioneered the use of active PIT telemetry surveys for environmental detection, without the individuals' physical recapture, there are significant challenges in determining whether the redetected individual is alive, aestivating, deceased, or if it ejected (i.e., dropped) its PIT tag.

In freshwater systems, PIT tags lost due to ejection or animal mortality are often undetected, as they can become buried too deep in the substrate to be detected or are removed from the study area by predators or flooding events [37, 38]. Historically, this issue was less significant in studies of non-fossorial species, but as PIT telemetry research has expanded to species with fossorial natural histories, there is an increasing need for reliable indicators of PIT tag retention. Allan et al. [38] addressed this issue in a tangential study of small freshwater fish by conducting repeated telemetry scans to distinguish between retained and dropped tags. However, this approach, which involved physical habitat disturbance to confirm dropped tags, is impractical for studies involving fossorial species or complex habitats, where such disruption could harm individuals or their burrows during vulnerable activities like aestivation or reproduction [8–11]. Thus, a non-invasive method to distinguish between retained and dropped tags in aquatic and fossorial species, such as *S. intermedia*, is urgently needed. In addition, researchers require reliable guidelines for selecting appropriate PIT tag sizes based on individual-specific parameters such as body size.

To address these challenges, we aimed to develop a robust indicator for determining the retention status of 8-mm and 12-mm PIT tags initially injected into juvenile and adult *S. intermedia* through the analysis of CMR spatial detection patterns combining dipnetting, trapping (funnel and trashcan), and active PIT scanning telemetry across 2 years. In addition, we tested if initial body size (e.g., tail length and mass) and marking conditions (e.g., survey, tag order, and water temperature) significantly affected the probability of PIT tag retention, with smaller individuals predicted to be more prone to tag loss due to less available tissue for secure attachment. We then also explored the relationships between movement/detection metrics and body size/marking parameters, with the goals of validating our tag retention indicator, identifying the most informative movement metrics, and refining our individual size-based PIT tag recommendations.

Methods

Study area

From January 2022 to December 2023, we conducted systematic dipnet and trapping surveys approximately every 15 days in Buttonland Swamp, a 1.8 km^2 (450-acre) cypress–tupelo wetland in southern Illinois. Buttonland Swamp is part of the Cache River State Natural Area managed by the Illinois Department of Natural Resources (IDNR), encompassing diverse habitats such as low-gradient rivers, shallow lentic areas, forested and emergent wetlands, and ephemeral ponds [39, 40]. It is the northernmost cypress–tupelo wetland in the United States, a Ramsar-recognized wetland [41], a dedicated Illinois Land and Water Reserve, and a focal area of the Illinois Wildlife Action Plan Streams Campaign. Our primary survey site was a large, semi-permanent, and fishless pond within Buttonland Swamp named Tupelo Pond.

Sampling and tagging procedure

Two researchers conducted dipnet surveys using long-handled dipnets (0.419 × 0.419 m, 794 µm mesh; Perfect Dipnet Model 7P, Jonah's Aquarium, Columbus, OH). Given the large size of Tupelo Pond (mean wetted perimeter ± SD: 294.9 ± 177.5 m, range: 0–659 m; area: 7137.9 ± 6073.2 m^2, 0–22798 m^2), its relatively flat contour, and consistent water depth from approximately 5 m from the shoreline to its center, we developed a modified sampling design to ensure thorough coverage of all potential microhabitats while avoiding locations least likely to be occupied. Adapting the dipnetting methods of Denton and Richter [42] and Hamer et al. [43], we conducted sweeps every 10 m along the pond's circumference. Our preliminary dipnet surveys indicated that *S. intermedia* were typically captured within 4.2 ± 3.2 m from the pond edge and never beyond 10 m, likely due to the absence of dense leaf litter and shaded areas in the open-canopied pond center, which are key daytime microhabitats for this species [6–8]. While individuals could theoretically move beyond this zone, particularly while foraging, the evidence from our earlier dipnet captures suggests that sampling up to 10 m from the shoreline effectively captures their primary daytime habitat, minimizing the risk of missed detections [44]. At each sampling location, we conducted three vertical sweeps from the shore, spaced approximately 2 m apart, where each sweep covered approximately 0.6 m in length. This incremental approach was designed to minimize capture biases (e.g., size or sex), while maximizing detection within the first 10 m from the shoreline. In addition, this design allowed for randomized sampling of key microhabitats (e.g., cattails, emergent grass, shallow and deep leaf litter) believed most likely to be occupied by siren within the 10 m zone. Based on previous studies, no dipnet detections have been observed beyond this distance, supporting the adequacy of this method in covering all significant microhabitats while minimizing the risk of missed detections and aligns with the assumptions of equal distribution and detection probability for the species [9, 13, 44].

To complement dipnet surveys and reduce potential detection/capture method-specific biases (i.e., dipnet only) toward different size classes, sex, or diel activities, we used unbaited funnel traps (i.e., minnow) and modified aquatic trashcan traps deployed for 24 h before each dipnet survey [45, 46]. We used vinyl-dipped steel mesh funnel traps (41.9 × 22.9 cm) and created modified trashcan traps by attaching four funnel halves to the bottom sides of 120-L (56.9 × 53.3 × 84.9 cm) outdoor, heavy duty, polypropylene trashcans [46]. If the water was deep enough to cover the funnel trap entrances, one funnel trap per 25 m of pond circumference was randomly placed in shallower areas, with the trap top resting above the water surface to prevent animal drowning [15]. Trashcan traps were placed in deeper areas where the attached funnels were fully submerged but the trashcan lid remained above the water surface. We used one trashcan trap per 75 m, with a maximum of four traps due to material constraints. When the pond was too shallow to deploy traps, only dipnetting was performed.

We conducted all dipnet and trap checks in the morning. Captured siren were placed in individual containers and the exact location was flagged and given a unique temporary identifier. As previously utilized for *S. intermedia* in Western Kentucky by Davis et al. [33], we too anesthetized our siren in a solution of maximum, double-medicated Church and Dwight© Orajel® (Active ingredients: 20% benzocaine and 0.26% menthol; Ewing Township, NJ) for processing (see Davis et al. [33] and Cecala et al. [47] for concentrations). Once individuals failed to respond to tapping and could not self-right, we immediately removed them from the solution and measured their snout-vent length (SVL), total length (TL), tail length (Tail), and mass (Mass). Individuals > 245 mm TL (≥ 165 mm SVL) were considered adults [7, 48, 49].

Each individual was then scanned with a Biomark© BP portable antenna attached to an HPR Plus portable PIT tag reader (Biomark, Boise, Idaho, USA). If untagged, we injected the individual with a new, sterilized PIT tag. As PIT tags weigh much less than traditional radio-and GPS-telemetry transmitters [50], our tag sizes were selected based on PIT tag recommendations by Vollset et al. [51] to not exceed 17.5% of the tagged body length. Consequently, individuals < 145 mm TL and 45 mm Tail were not PIT-tagged due to concerns for animal safety. New individuals 145–245 mm TL and 46–69 mm Tail (i.e., juveniles) received 8-mm PIT tags (Biomark MiniHPT8; 134.2 kHz, 8.4 × 1.4 mm, and 0.033 g) via injection with a Biomark MK165 Implanter and individuals > 245 mm TL and 69 mm Tail (i.e., adults) received 12-mm PIT tags (Biomark HPT12; 134.2 kHz, 12.5 × 2.0 mm, and 1.06 g) via injection with a Biomark MK10 Implanter (Biomark, Boise, Idaho, USA). PIT tags were injected towards the distal end into the ventral side of the tail, approximately 1–3 mm posterior to the cloaca [12, 13, 52]. Similarly to all previous *Siren* PIT tag studies, we did not use any sealants at the injection site [12–14, 33–36, 52]. Syringe needles and PIT tags were sterilized with 70% isopropyl alcohol before use to minimize cross-contamination and infection [52].

To avoid potential researcher measuring and tagging bias, the same researcher processed all sirens [53]. To assess whether the researcher's confidence and/or the specific order in which a siren was tagged each survey influenced the probability of PIT tag retention, we recorded both the Julian Date (JD) and the relative order in which individuals were tagged (Tagorder). We also measured the recovery container water temperature using a digital thermometer (Water). After processing and full recovery, indicated by continuous, rapid, and upright responses to tapping, the sirens were promptly returned to their exact capture locations and scanned with the portable antenna to record their initial capture coordinates and date. All individuals were processed in the field and returned to their exact capture point within 1 h, and no mortalities occurred following anesthesia and PIT-tagging.

Assessment of PIT tag retention

To monitor movements and assess tag retention, we conducted PIT scanning telemetry surveys [28, 33, 38]. Due to personnel limitations and concurrent research activities in our study area, we conducted telemetry surveys approximately once a month, where each survey was limited to 45 min. To minimize potential surveyor bias, the same researcher conducted all telemetry surveys during daylight hours [53]. During each telemetry survey, the pond was randomly scanned across its known maximum wet perimeter, including both submerged and dried basin areas. To do so, the pond was divided into four sections, aligned by their cardinal direction and extending equally from the center of the pond to each sections' maximum perimeter. Equal time was allocated to randomly scanning both wet and dry areas within each section. When a tag was detected by the receiver, the surveyor physically flagged the location and recorded the inundation state. In cases where the tag was detected in a submerged area, the distance to the shoreline was recorded, whereas if the location was dry, the distance to the wetted pond section edge was recorded instead.

Prior to statistical analyses, individual PIT tag telemetry detection histories were downloaded from the tag reader. We then calculated Euclidean distances and the number of days between each subsequent detection.

Biomark reports a read error rate of less than 1 in 106 and an HPR Plus GPS accuracy of within 3 m of the reader's physical location [28]. However, we observed an accuracy closer to 5 m in our study area when using 8-mm PIT tags. Specifically, the average distance between consecutive telemetry detections for tags that did not physically move (regardless of pond inundation state) was 3.3 ± 1.2 m. Following this accuracy, each PIT tag was assigned to a retention category: either retained (1) or dropped (0). PIT tags with two or more telemetry detections were classified as dropped if they met both of the following criteria: (1) the average distance between subsequent detections after the first redetection (RdavgDist) was ≤ 5 m and (2) the distance between the second detection (i.e., first redetection) and the final detection (SecondLast) was also ≤ 5 m. For PIT tags with only one scanned telemetry redetection, we determined if the distance from the initial capture to the first and only redetection (FirstDist) was ≤ 5 m and if the location had been both dry and wet after detection. Individuals scanned once and never detected again and those captured in nets and also never telemetry-detected again were assumed to have retained their PIT tags.

The meta-analysis by Vollset et al. [51] found that post-tagging mortality increased in juvenile salmonids when the PIT tag size relative to body length exceeded 17.5%, with a predicted mortality rate of 2%. In our study, none of the redetected PIT tags came from individuals with an inital tag-to-tail length (Tagtail) greater than 17.4% (14.2 ± 2.3%; 9.5–17.4%). Moreover, the fish considered by Vollset et al. [51] were PIT tagged in the body cavity, which can interfere with organ function, blood circulation, immune responses, and buoyancy control [51, 54]. In contrast, we injected PIT tags into the tail tissues of sirens, a region rich in fat reserves, which reduces the risk of organ damage [36, 52]. Previous studies on sirens have not reported any mortality associated with post-cloacal PIT tagging, likely due to the consistent use of sterilized equipment [17, 33–36, 52]. Since all sirens were fully responsive and mobile after recovery and observed swimming away upon release, we assumed that tag loss was not related to mortality. Instead, tags were likely dropped during recovery, immediately after release, or shortly thereafter, as in the cases where the initial PIT tag redetection occurred > 5 m from the sirens' release location and all subsequent redetections (RdavgDist and SecondLast) were < 5 m.

To further validate the categorization of PIT tags as retained or dropped, we examined the recorded inundation state (wet or dry) at each detection location during and between telemetry scans. Based on our retention criteria and the only reported aestivation behaviors [9–11], we assumed that dropped PIT tags would be redetected multiple times at the same location, regardless of inundation state, across extended wet and dry periods. Considering the accuracy of the HPR Plus GPS reader, we expected that dropped tags would be redetected within ~ 5 m. Flags were placed at each detection location, irrespective of inundation status, and the area was thoroughly scanned during the subsequent telemetry survey to determine if the individual had moved. We also recorded each tags total number of Redetections, as repeated detections at the same location in both wet and dry conditions were likely indicative of dropped tags. Supporting this assumption, we recovered three 8-mm PIT tags in 2022 from the dried pond surface at depths too shallow for aestivation (i.e., 1–3 cm). In contrast, all three of our redetected 12-mm PIT tags inserted in adult sirens (> 245 mm TL) showed movement histories consistent with tag retention (i.e., all individual movements were significantly > 5 m).

Statistical analyses

Given the limited number of adult sirens (i.e., 3), we developed two sets of models to assess the factors influencing 8-mm PIT tag retention among juvenile sirens and the various movement/detection metrics of their dropped and retained tags. Our primary goal was to use a multi-model framework to test a priori hypotheses regarding the role of body size and tagging-related variables in predicting tag retention and post-tagging movement/detection. Before building the models, we centered and scaled each continuous variable [55] and assessed the correlations among the predictor variables (body size and marking parameters) using Spearman's correlation coefficients (r; Table S1). Variables with a high correlation ($r > 0.7$) were not included together in the same model to avoid multicollinearity [56].

Model set 1: PIT tag retention

We used generalized linear models (GLMs) with a logistic link function and binomial error distribution to evaluate the relationships between PIT tag retention (binary response: retained = 1, dropped = 0) and several initial size and tagging-related variables (SVL, TL, Tail, Tagtail, Mass, JD, Tagorder, and Water; Table 1). We hypothesized that larger body size would predict higher tag retention due to greater tissue availability for securing the PIT tag. We included JD as a proxy for researcher experience, hypothesizing that tagging precision would improve over time, reducing the likelihood of tagging errors. Tagorder was considered as an indicator of researcher confidence, with the expectation that later-implanted tags in a survey would have a higher retention probability. Lastly, Water was included as an exploratory variable since the effect of water temperature on PIT tag-specific wound healing

Table 1 Individual *Siren intermedia* PIT tag retention status, size, marking, movement, and detection parameters, their descriptions, and associated model sets

Parameter	Description	Model Set/Type
Tag retention	Dropped (0) or Retained (1)	Size/Marking Response Variable
SVL	Snout-vent length (mm) at first capture	Size/Marking and Movement/Detection Predictor Variable
TL	Total length (mm) at first capture	Size/Marking and Movement/Detection Predictor Variable
Tail	Length of tail (mm) at first capture	Size/Marking and Movement/Detection Predictor Variable
Tagtail	PIT Tag length relative to tail length (%)	Size/Marking and Movement/Detection Predictor Variable
Mass	Mass (g) at first capture	Size/Marking and Movement/Detection Predictor Variable
JD	Julian Date of first capture from 1/1/2022	Size/Marking and Movement/Detection Predictor Variable
Tagorder	Daily order in which an individual was tagged	Size/Marking and Movement/Detection Predictor Variable
Water	Post-tagging recovery water temperature (℃)	Size/Marking and Movement/Detection Predictor Variable
FirstDist	Distance (m) between the 1st and 2nd detection	Movement/Detection Response Variable
FirstLast	Distance (m) between the 1st and last detection	Movement/Detection Response Variable
SecondLast	Distance (m) between the 2nd and last detection	Movement/Detection Response Variable
MaxDist	Maximum movement distance (m)	Movement/Detection Response Variable
AvgDist	Average movement distance (m)	Movement/Detection Response Variable
RdavgDist	Average subsequent movement distance (m)	Movement/Detection Response Variable
Redetections	Total number of tag/siren redetections	Movement/Detection Response Variable

Only PIT tags with ≥ 2 telemetry redetections were included for the parameters SecondLast and RdavgDist

and retention is unknown. We also tested for interaction effects between our body size variables and JD, Tagorder, and Water, provided there were no significant correlations between them (Table S1).

Model set 2: movement and detection metrics

The second set of models explored the relationships between initial size/marking parameters and various movement/detection metrics (e.g., FirstDist, FirstLast, SecondLast, MaxDist, AvgDist, RdavgDist, and Redetections; Table 1) to differentiate between retained and dropped tags. Our goal was to understand how initial siren size and tagging conditions influenced post-tagging movement behaviors and detection histories in relation to tag retention. For metrics with repeated observations (AvgDist and RdavgDist), we initially used linear mixed-effects models with individual ID as a random effect. However, as the variance for the random effect was near zero, we instead used individual-averaged values for these metrics in our subsequent linear regression models.

Thus we built linear models (lm) to examine the relationships between body size variables, JD, Tagorder, Water, and each tag's first redetection distance (FirstDist), hypothesizing that smaller juveniles would drop their tags more quickly (i.e., closer to the release location). We also analyzed the distance between the first capture and final detection location (FirstLast), expecting that more mobile and/or slightly larger individuals would drop their tags after moving > 5 m from the release location. To further distinguish dropped and retained tags that moved > 5 m, we separately analyzed the distance between the second detection and the final detection (SecondLast), assuming tags with movements > 5 m between these points were retained. We then considered each tags' single maximum movement distance (MaxDist), hypothesizing that although some individuals dropped their tags > 5 m from their release location, they likely still did not move as far as individuals with retained tags. We also analyzed each tags' average movement distance, including the first (AvgDist), to distinguish dropped tags with larger first movement distances from retained tags with more than one movement > 5 m. Furthermore, we considered the average distance of all movements after the first (RdavgDist), hypothesizing that all dropped tags would have subsequent movements ~ 5 m as a result of our equipment's GPS accuracy. Lastly, we considered each tags' total number of Redetections, where we predicted that repeated detections of a PIT tag at the same location in both inundated and dry basin conditions would most likely represent dropped tags.

Model selection

We selected and compared models within each set using the Bayesian Information Criterion (BIC), which balances model accuracy with complexity (i.e., number of parameters), applying a stronger penalty for complexity than Akaike's Information Criterion (AIC [57]). BIC is particularly sensitive to non-significant interactions, thus offering a more conservative model selection process

[58]. Lower BIC values indicated a better fit. Models with ΔBIC > 6 were considered top models, providing strong support. Among the models with ΔBIC < 6 that represented similar biological information (e.g., SVL, TL, Tail, Tagtail, and Mass), we typically selected Tail as the representative and most informative body size variable as it is most relative to the tagging location, unless another variable demonstrated stronger predictive power or better model fit [59]. In our analyses, BIC rankings differed from those based on AIC, with interaction terms being less supported by BIC due to its stricter penalization of complexity. Given these differences, we report BIC values and weights as the primary model ranking criterion. All analyses were conducted in R (Version 4.4.1; [60]) using the 'sf' package (Version 1.0–12; [61]), 'adehabitatLT' package (Version 0.3.27; [62]), 'lme4' package for mixed models (Version 1.1–33; [63]), and base R 'stats' for cor, glm, and lm functions. Plots were generated using 'ggplot2' (Version 3.5.1; [64]) and all data were back-transformed to their original scale for Table 3 and all figures.

Results

Captures and PIT tag redetections

We recorded 75 physical captures of 73 *S. intermedia*, with 64 individuals (88%) initially captured in dipnets, 6 (8%) in funnel traps, and 3 (4%) in trashcan traps. Among these, 68 (93%) were juveniles and 5 (7%) were adults. Specifically, 62 juveniles (91%) were captured in dipnets, while 6 (9%) were caught in funnel traps. For the adults, 2 (40%) were captured in dipnets and 3 (60%) were caught in trashcan traps. Unfortunately, two of the adults captured in trashcan traps died, likely due to thermal stress, as they were captured during the warmest summer surveys. Out of the 71 PIT tags (68, 8 mm and 3, 12 mm), 23 (32%) were redetected at least once. Specifically, we redetected 20 (29%) of the 8-mm tags initially injected into juveniles and all 3 of the 12-mm tags from adults. Among the 8-mm tag redetections, 2 (10%) were from juveniles physically recaptured in dipnets, while the remaining 18 (90%) were redetected during PIT telemetry surveys. In contrast, all of the 12-mm tags were redetected via telemetry.

PIT tag retention

Utilizing our PIT tag retention indicator, we determined that all 3 of the 12-mm tags were retained by the adults. The average movement distance between adult telemetry detections was 43.5 ± 15.2 m (13.8–91.5), with 14–427 days between them. For the 8-mm tags, we estimated that 9 (45%) were dropped from juveniles 162.9 ± 8.0 mm TL (148.0–174.9) and 50.2 ± 2.8 mm Tail (46.1–53.5). Specifically, the dropped tags were first redetected 8.8 ± 7.2 m (2.0–25.3) away from and 9–382 days after their initial siren capture. Conversely, the retained 8-mm PIT tags were from juveniles initially 199.3 ± 26.9 mm TL (163.3–245.0) and 64.6 ± 11.9 mm Tail (50.4–84.0) and were first redetected via either dipnet or telemetry 31.5 ± 26.3 m (9.8–89.1) away from and 18–545 days after their first capture. The average distance between subsequent dropped 8-mm tag redetections (RdavgDist) and the distance between the second and last redetection (SecondLast) were 3.3 ± 1.2 m (0.6–5.0) and 2.4 ± 0.8 m (1.4–3.9), respectively. Whereas for the retained tags, these distances were 11.1 ± 2.6 m (8.2–13.2) for both metrics.

The dropped 8-mm tag with the greatest initial distance had a 48.6 mm Tail length and was first scanned in an inundated pond area 25.3 m away and 130 days after its initial capture. Over the following ~ 400 days, it was redetected every 54 ± 32 days across eight telemetry surveys, under both wet and dry basin conditions. The average distance between these redetections was 2.7 ± 1.0 m (1.2–4.3). In contrast, the dropped tag with the shortest FirstDist was first redetected 2.0 m away, just 9 days after its initial capture, and from a juvenile with a Tail length of 46.1 mm. Over the next 120 days, it was redetected across four telemetry surveys every 28 ± 11 days, again in both wet and dry conditions with an average distance of 2.9 ± 1.5 m (0.8–4.4) between redetections. We ultimately found the tag buried just a few centimeters in the ground. Whereas a retained tag from a juvenile with a Tail length of 55.0 mm was redetected 18 days after its first capture and found to have moved 13.9 m. On their first redetection, all dropped PIT tags were located either immediately after release (i.e., within 5 m: 3.6 ± 1.2 m, 2.0–4.8) or shortly thereafter, as in the cases with tags that were first redetected > 5 m (13.1 ± 6.5 m, 6.7–25.3). In contrast to the dropped tags, the retained 8-mm and 12-mm PIT tags were not redetected within 5 m of their initial capture location during either their second or last redetection (i.e., redetections occurred 9.8–77.8 m away).

Among the telemetry-redetected and PIT tag-retained juveniles, 3 (27%) were redetected twice, while the rest were redetected once. Whereas 2 (67%) of the adults were telemetry-redetected twice, with the remaining individual being redetected only once. Notably, 7 (78%) of the telemetry-redetected and tag-retained juveniles and 2 (67%) of the adults, were scanned within inundated pond areas after their first aquatic capture. Overall, 4 (44%) of the redetected juveniles and all 3 of the adults with retained PIT tags exhibited telemetry detection patterns suggestive of aestivation behaviors and successful subsequent emergence and were never detected again after their last flagged locations became re-inundated.

PIT tag retention and movement/detection model results

For our size/marking PIT tag retention model set, model analysis indicated that Tail, Tagtail, and SVL were significant predictors of 8-mm PIT tag retention (Table 2). Nonetheless, none of the ΔBIC values for the five size-specific (SVL, TL, Tail, Tagtail, Mass) models indicated a single best fit (i.e., ΔBIC were < 6; Table 2). However, as Tail had a lower BIC value, smaller estimate errors, and the most relative interpretability, we report Tail length as the best predictor of 8-mm PIT tag retention (Tables 2 and 3). As such, our modeling showed that juvenile *S. intermedia* Tail length was positively associated with the likelihood of 8-mm PIT tag retention (Fig. 1). In our second model set, focused on movement/detection metrics in relation to the size/marking parameters, Tail length best predicted all of the movement/detection metrics and out-performed the other four size parameters, except among Redetections, where instead, Tagtail was the strongest predictor (Table 2). Overall, RdavgDist performed better than all other movement/detection models, followed closest by SecondLast. Our top model showed a strong positive relationship between Tail length at PIT tagging and the average distance between subsequent redetections ($R^2 = 0.94$, $P < 0.005$; Fig. 2a; Table 3).

Since only three of the retained 8-mm tags had two or more telemetry redetections, we also considered the top movement/detection metric model for all tags with at least one redetection (FirstDist, FirstLast, MaxDist, AvgDist). Here, Tail length again best predicted each, with AvgDist outperforming the other movement/detection models (Table 2). The model indicated a moderate positive relationship between Tail length and the average distance between all redetections ($R^2 = 0.39$, $P < 0.005$; Fig. 2b; Table 3). Notably, neither JD, Tagorder, Water,

Table 2 Model parameters, number of coefficients (K), Bayesian information criterion (BIC), difference in BIC from top model (ΔBIC), and model weight (w_i) for all binomial logistic regression models used to estimate the individual-specific size/marking factors that best predict *Siren intermedia* PIT-tag retention probability and the linear regression models for the size/marking parameters that most influence each movement/detection factor

Model Set/Analysis	Parameters	K	BIC	ΔBIC	w_i
Size/Marking					
Tag Retention	Tail	2	18.58		0.35
–	Tagtail	2	21.66	3.08	0.22
–	SVL	2	22.92	4.34	0.15
–	TL	2	23.19	4.61	0.14
–	Mass	2	23.42	4.84	0.14
–	1 (null)	1	30.52	11.94	8E-4
–	JD	2	30.81	12.23	7E-4
–	Tagorder	2	32.20	13.62	3E-4
–	Water	2	33.38	14.80	2E-4
Movement/Detection					
RdavgDist	Tail	2	0.85		0.99
SecondLast	Tail	2	15.05	14.20	8E-4
AvgDist	Tail	2	49.87	49.02	2E-11
Redetections	Tagtail	2	51.01	50.16	1E-11
FirstLast	Tail	2	51.15	50.30	1E-11
FirstDist	Tail	2	51.52	50.67	9E-12
MaxDist	Tail	2	54.71	53.86	2E-12

Each model is listed in order of overall rank

Table 3 Top overall ranked model parameters, their estimates, standard errors, and significance values for PIT tag retention and movement/detection metrics as functions of initial body size and marking parameters

Model	Parameters	Estimate ± SE	*p-value*
Tag Retention ~ Tail	(Intercept)	−27.14 ± 13.01	0.033
	Tail	0.21 ± 0.09	0.031
Tag Retention ~ Tagtail	(Intercept)	22.36 ± 9.01	0.035
	Tagtail	−1.49 ± 0.81	0.033
Tag Retention ~ SVL	(Intercept)	−27.27 ± 13.03	0.036
	SVL	0.23 ± 0.11	0.038
Tag Retention ~ TL	(Intercept)	−32.13 ± 17.16	0.040
	TL	0.19 ± 0.10	0.064
Tag Retention ~ Mass	(Intercept)	−10.49 ± 5.52	0.052
	Mass	1.00 ± 0.55	0.055
Tag Retention ~ 1 (null)	(Intercept)	0.20 ± 0.45	0.665
Tag Retention ~ JD	(Intercept)	−0.98 ± 0.87	0.148
	JD	0.01 ± 0.01	0.140
Tag Retention ~ Tagorder	(Intercept)	−1.05 ± 1.21	0.427
	Tagorder	1.93 ± 1.73	0.496
Tag Retention ~ Water	(Intercept)	0.78 ± 1.67	0.650
	Water	−0.03 ± 0.08	0.682
RdavgDist ~ Tail	(Intercept)	−10.53 ± 1.27	< 0.005
	Tail	0.28 ± 0.02	< 0.005
SecondLast ~ Tail	(Intercept)	−12.59 ± 1.75	< 0.005
	Tail	0.30 ± 0.03	< 0.005
AvgDist ~ Tail	(Intercept)	−38.69 ± 16.38	< 0.005
	Tail	0.94 ± 0.28	< 0.005
Redetections ~ Tagtail	(Intercept)	−7.21 ± 3.76	0.007
	Tagtail	0.77 ± 0.27	0.023
FirstLast ~ Tail	(Intercept)	−53.62 ± 18.65	0.010
	Tail	1.29 ± 0.31	< 0.005
FirstDist ~ Tail	(Intercept)	−59.69 ± 19.87	0.007
	Tail	1.39 ± 0.34	< 0.005
MaxDist ~ Tail	(Intercept)	−58.95 ± 19.74	0.008
	Tail	1.38 ± 0.33	< 0.005

Estimates and their errors were back-transformed to the original scale of the data

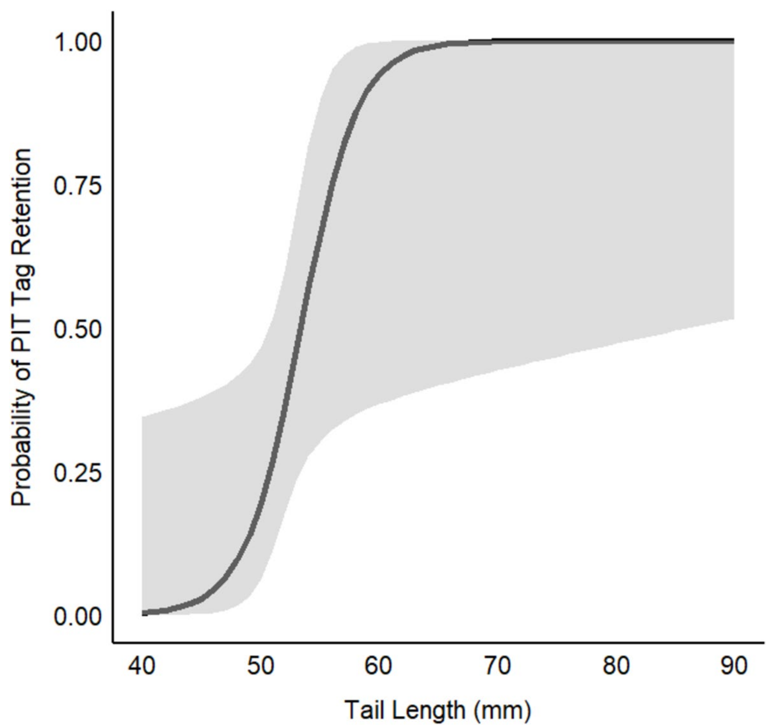

Fig. 1 Probability (± 95% confidence intervals, grey buffer) of 8-mm PIT tag retention as a function of tail length in juvenile *Siren intermedia*

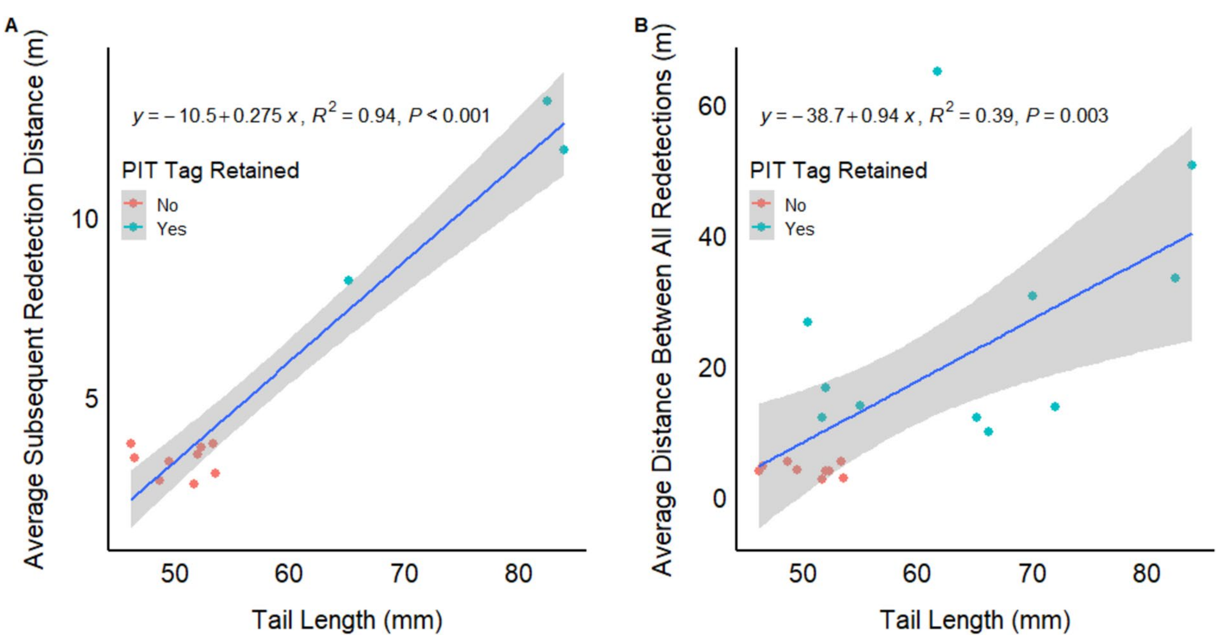

Fig. 2 Relationships between tail length and (**A**) the average distance between subsequent (i.e., only those following the first) telemetry redetections (RdavgDist) and (**B**) the average distance between all redetections (AvgDist) among juvenile *Siren intermedia*. The grey outlines represent the SE around the regression line estimates

nor their interactions with the size variables were significant or included in any of the top selected PIT tag retention and movement/detection metric models (Tables 2 and 3). Overall, model diagnostics revealed no evidence of outliers or problematic residual distributions, suggesting that the models fit the data well.

Discussion

In this study, we found that PIT tag retention in *S. intermedia* was influenced by body size, specifically Tail length. While the three adult salamanders retained their 12-mm tags, juveniles with 8-mm tags showed lower retention, with smaller individuals being more likely to lose their tags. Our models identified Tail length as the most reliable predictor of 8-mm tag retention and movement metrics, confirming that larger juveniles had a greater likelihood of retaining their tags. Furthermore, our retention indicator, based on PIT telemetry data, proved effective in distinguishing retained tags from dropped ones without invasive methods. These results emphasize the need to consider the size of the specific body area/part when selecting PIT tag sizes and highlight the utility of PIT telemetry for monitoring small-bodied, aquatic, and/or fossorial species. Ultimately, this approach can improve survey accuracy and inform better conservation practices for aquatic salamanders and similar taxa.

Assessment of PIT tag detection

Previous studies have identified several factors influencing PIT tag detection, including tag size, orientation of both the tag and antenna, and the tag injection site, which can affect telemetry detection, animal survival, and tag retention [3, 65–67]. Tag size, in particular, plays a crucial role in detection. For instance, 12-mm Biomark tags can be detected up to 34 cm away from the scanner, whereas 8-mm tags are only detectable up to 21 cm [28]. This difference likely explains the seemingly high detection rate for the 12-mm tagged adults in our study, compared to only 29% for the 8-mm tags. It is likely that many of the undetected 8-mm tags were either too far from the scanner or in individuals aestivating deeper than 21 cm. Although *S. intermedia* have been found aestivating at depths up to 1 m in southern Illinois and at depths as shallow as 8 cm in Indiana [9, 11], there is limited research on average aestivation depths, particularly for juveniles. In addition, substrate and basin characteristics vary regionally [68, 69], potentially affecting aestivation depths. Given the large size and complexity of the pond basin in our study, it is possible that some 8-mm tags were missed due to time constraints during our scanning surveys. Thus, utilizing multiple scanners and/or extending survey durations would likely improve PIT tag detection rates.

Assessment of PIT tag retention

PIT tag retention in aquatic species has shown mixed results, often depending on both tag size and the injection site. Mamer and Meyer [70] and Saboret et al. [37] reported that PIT tags implanted in the body cavity of female trout and salmon were expelled with their eggs during breeding. Johnson and Blackwell [35] injected *S. intermedia* with 12-mm PIT tags in their abdominal cavities and attributed the low recapture rates to mortality from organ damage. In contrast, most PIT tag studies on *S. intermedia*, including ours, inserted the tags dorsally, just posterior to the vent, in an area rich in fat reserves, minimizing the risk of organ damage [12, 33, 52]. Therefore, tag retention is likely influenced by the structure and thickness of the tissue at the tagging site. As such, Tail length was the strongest predictor for tag retention probability and each of our movement-specific metrics.

Unger et al. [71] reported high retention rates for tags injected post-cloacally into the large-bodied aquatic salamander *Cryptobranchus alleganiensis*, and those that were lost, were most likely ejected before the injection site had fully healed. In our study, two juveniles were redetected via telemetry just 9 and 18 day post-capture. The first redetected tag, from a juvenile with a shorter Tail length, was found only 2.0 m from its release site and was determined to have been dropped, while the second tag, from an individual with a Tail 17.6% longer, was redetected 13.9 m away and later determined to be retained. Thus, the smaller individual likely dropped its tag either in the recovery container or immediately during release. As such, 8-mm PIT tag injection wounds appear to heal in juvenile *S. intermedia* in at least 18 days. Interestingly, some of our dropped tags were first redetected more than 5 m away from their original location up to 382 days after initial capture, most likely as a result of our large site area and telemetry survey limitations.

While sealants (e.g., super or surgical glue) are commonly used in terrestrial vertebrates to ensure wound closure after trauma from procedures such as PIT tag injection [72–74], their use in aquatic amphibians is debated. Sealants may actually delay healing, increase infection risk, contain toxic compounds, and be ineffective in water [75–78]. Although no previous studies on sirenid PIT tagging have used sealants, the further development of a safe, reliable product for aquatic amphibians is worth investigating. Although our modeling did not find a strong influence of water temperature on tag retention, temperature is known to affect physiological responses in aquatic ectotherms, particularly to stressors initiated by superficial wounds [79, 80]. It is, therefore, possible that interactions between an individual's size, tagging-specific tissue characteristics, tag size, and water temperature play an important role in tag retention not captured in our data. Lastly, we did not find any evidence that the researcher processing individuals inadvertently influenced tag retention either within or among surveys.

Although there are few studies on the use and retention of 8-mm PIT tags in small-bodied animals with fossorial natural histories, we report the first usage in aquatic post-metamorphic caudates. Recio et al. [66] reported 100% retention of 8-mm tags in a fossorial amphisbaenian lizard. While Ousterhout and Semlitsch [3] reported 100% retention in the post-metamorphic adults of the fossorial salamander *Ambystoma annulatum*. However, the researchers found higher detection and recapture rates in individuals injected with 12-mm tags relative to 8-mm tags, likely due to increased detection distances afforded to larger tags [3, 27]. Davis et al. [33] was the first to use active PIT telemetry to detect 12-mm PIT-tagged *S. intermedia* in the field, redetecting 78% of their individuals during two of three telemetry surveys, compared to only 22% physically recaptured over 11 funnel trap surveys. They speculated that additional telemetry surveys would have further increased their redetection rate. Notably, their redetected tags were not from individuals aestivating, dead, or that dropped them, as they were scanned along the wetted pond perimeter and were not redetected in subsequent telemetry surveys [33].

In contrast to the 12-mm tags in our study and Davis et al. [33], 45% of our 8-mm tags initially injected into juvenile *S. intermedia* had numerous telemetry detections at the same location—indicative of dropped tags. Given our telemetry equipment's' GPS accuracy, repeated detections within 5 m of each other, in both wet and dry pond conditions, most likely belonged to dropped tags. Conversely, 55% of our redetected juveniles and all three of the adults appeared to retain their tags, as indicated by telemetry patterns suggestive of multiple movements greater than 5 m and/or aestivation behaviors and successful emergence from aestivation, as they were not detected again after their previous detection location became re-inundated. These results underscore the importance of our PIT tag retention indicator, which suggests that tags with average distances less than 5 m between their subsequent telemetry redetections and their second and last detection most likely indicate dropped tags.

Body size, PIT tag size, and retention

Prior sirenid studies exclusively used 12-mm tags [12, 14, 15, 33–36, 52]. Based on Vollset et al.'s [51] 17.5% Tagtail threshold, 8-mm tags accounted for approximately 15% of the total post-cloacal Tail length in our juveniles, whereas 12-mm tags would have accounted for 22%. In comparison, Davis et al. [33] tagged larger *S. intermedia* with 12-mm tags comprising roughly 13% Tagtail. Interestingly, Raymond and Hardy [36] tagged 243 juveniles as small as 50 mm Tail with 12-mm tags and failed to recapture them. The smallest 12-mm tagged individual recaptured by Davis et al. [33] had an initial SVL of 142 mm (individual Tail length not reported). Unfortunately, all other prior studies did not report the sizes of their recaptured individuals at tagging. Though none of our 12-mm tagged adults were physically recaptured, the smallest redetected individual had an initial Tail length of 74 mm. Overall, this study is the first to report the successful retention of 8-mm PIT tags in recaptured juvenile *S. intermedia* as small as 163 TL and 52 mm Tail. For optimal safety and to maximize long-term PIT tag retention and detection, we recommend using 8-mm PIT tags in *S. intermedia* with Tail lengths over 50 mm and 12-mm tags in individuals with Tail lengths greater than 70 mm.

Conclusions and management implications

This study highlights that examining PIT telemetry detection patterns across different pond basin inundation conditions allows researchers to distinguish between aestivation behaviors and successful emergence in sirenids and other similar burrowing/fossorial organisms from dropped PIT tags. Depending on the specific telemetry equipment, dropped tags can be identified by their consistent detection within a 5 m radius across multiple scans, especially when detections occur over extended periods that exceed expected natural behavior. In addition, if all redetections occur within 5 m of each other despite transitions in the site's wet and dry conditions, this suggests a dropped tag rather than an actively moving individual. Our study provides a transferable method to assess PIT tag retention in the field by analyzing telemetry detection patterns across the landscape. This approach underscores the importance of establishing size guidelines and thresholds for the effective use of telemetry devices across different age and size classes within a species. Ultimately, ensuring appropriate tag size is critical for maximizing detection success and minimizing tag loss, thereby improving the reliability of PIT telemetry in ecological studies.

Abbreviations

PIT	Passive integrated transponder
Tail	Tail length (mm)
SD	Standard deviation
CMR	Capture–mark–recapture
VIE	Visible implant elastomer tags
VIA	Visible implant alpha tags
IDNR	Illinois department of natural resources
SVL	Snout-vent length
TL	Total length (mm)
Mass	Mass (g)

JD	Julian Date from 1/1/2022
Tagorder	Relative survey-specific PIT tagging order
Water	Post-tagging recovery water temperature (℃)
FirstDist	Distance (m) between the first and second detection
FirstLast	Distance (m) between the first and last detection
SecondLast	Distance (m) between the second and last detection
MaxDist	Each individuals' maximum movement distance (m)
AvgDist	Average of all of an individual's movement distances (m)
RdavgDist	Average of subsequent redetection movement distances (m)
Redetections	Total number of tag/siren redetections

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40317-024-00392-z.

Additional file 1 (Table S1. Spearman's correlations (r) between the explanatory variables tested in our model set 1 and 2 models. Highly correlated variables (r > 0.7) are indicated by an asterisk and were not included together in the same model to avoid multicollinearity.)

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Author contributions
All authors participated in writing and structuring the manuscript. Data Analysis was performed by JMH and figures were created by JMH. All authors read and approved the final manuscript.

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Availability of data and materials
The data that support this study will be shared upon reasonable request to the corresponding author.

Declarations

Ethics approval and consent to participate
All contributors to this research observed appropriate ethical and legal guidelines and regulations for the use of animals in this study. This study was approved by the Illinois Department of Natural Resources (IDNR) and by the Southern Illinois University Institutional Animal Care and Use Committee (Protocol 22-035). All animals were handled under IDNR Permit No. 14420.

Consent for publication
All authors have read and approved the submission of the manuscript.

Competing interests
The authors declare no competing interests.

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