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Evaluation of p-Chip microtransponder tags on small-bodied salamanders (*Eurycea* spp.)

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Abstract.—Reliable approaches for tracking individual organisms are needed for research purposes and to inform the conservation and management of aquatic organisms. However, safe and dependable tagging methods are difficult to implement for small-bodied organisms. The objective of this study was to examine survival, tag retention, and growth in three aquatic salamander species of different sizes (Barton Springs Salamander, *Eurycea sosorum*; Comal Springs Salamander, *Eurycea pterophila*; Texas Blind Salamander, *Eurycea rathbuni*) injected with p-Chip tags in a captive setting. The ability of novice scanners to read p-Chips over the duration of the study was also assessed. Post-tagging survival was high across all treatments for all species (97–100%). Tag retention among species was similar (97–100%), and growth appeared unaffected by tagging. No relationship between success of tag readability and time since tagging was found, and all novice scanners were able to read the tags implanted in 100% of Comal Springs and Texas Blind Salamanders. However, variability was found with novice scanners reading tags in Barton Springs Salamanders, although all tags were successfully read by an experienced scanner. P-Chips provided an improved readability rate, reduced human error, and allowed for more individual identification codes than the visible implant elastomer tags commonly used for these species. This study shows that p-Chips are suitable tags for small-bodied aquatic salamanders.

Keywords. *Eurycea sosorum*, *Eurycea pterophila*, *Eurycea rathbuni*, retention, survival, tracking

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Introduction

Wildlife tagging provides a reliable method for the identification and differentiation of individuals that would otherwise be challenging to distinguish, and it allows for tracking a variety of life history parameters (Ricker 1956). In the field, mark-recapture efforts can provide information about growth, survival, habitat range, migration, age, condition, and other key parameters (Ricker 1956; Silvy et al. 2005; Osbourn et al. 2011; Moon et al. 2022) and marked organisms can be monitored over time in individuals, cohorts, and subsets of populations and communities. In captive settings, tagging eliminates the need to separate organisms or cohorts into different tanks or enclosures for identification, allowing enclosures to be stocked to capacity, thereby conserving space. It also facilitates the tracking of rare occurrences in captive individuals, such as reproductive events or illness. However, tags that are not compatible with a species can cause injury, mortality, or behavioral changes in the organism due to stress and difficulty in functioning normally (Musselman et al. 2017; Moon et al. 2022). In these cases, the results of any tagging program would be negatively affected by the tagging method.

A long and growing list of wildlife marking methods exists, allowing researchers to tailor methods to their study needs while accounting for the organism's biology and life history (Silvy et al. 2005). Tags should be selected to maximize various factors such as tag retention and longevity, cost efficiency, and ease of use while minimizing stress, handling time, and other factors that negatively affect growth, behavior, and survival (Osbourn et al. 2011). Failure to mitigate these negative effects can violate mark-recapture assumptions (Ricker 1956) and tagging ethics (Cooke et al. 2013), preventing the extrapolation of meaningful conclusions from data (Murray and Fuller 2000). General amphibian

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tagging practices include digit clipping (Phillott et al. 2007; Waddle et al. 2008), branding (Donnelly et al. 1994; Measey et al. 2001), affixing or implanting radio transmitters (Weick et al. 2005), staining (Carlson and Langkilde 2013; Fischer et al. 2020), pattern mapping and software assisted photo identification (Andreone 1986; Gamble et al. 2008; Bendik et al. 2021), injecting elastomers, and attaching transponders (Sinsch 1997; Donnelly et al. 1994; Moon et al. 2022).

Central Texas is populated by several species of endemic, paedomorphic salamanders of state and federal conservation concern with narrow distributions and poorly understood demographies. Among these are the state and federally endangered Texas Blind Salamander (*Eurycea rathbuni*) and Barton Springs Salamander (*Eurycea sosorum*), and a population of the non-listed Fern Bank Salamander from Comal Springs (reclassified from *Eurycea neotenes* to *Eurycea pterophila* by Devitt et al. 2019). The Barton Springs Salamander is protected under the Barton Springs Habitat Conservation Plan (Barton Springs/Edwards Aquifer Conservation District 2018), and the Texas Blind Salamander and Comal Springs Salamander are protected under the Edwards Aquifer Habitat Conservation Plan (Edwards Aquifer Authority 2012). Tagging these animals requires accommodating their small size and permeable skin (Heemeyer et al. 2007). This is achieved by selecting small tags with a high tag retention rate associated with that species. Additionally, it is important to minimize mortality when working with threatened and endangered species, so stress and handling time should be considered.

Few studies have used tagging techniques specifically on either Barton Springs, Texas Blind, or Comal Springs Salamanders. Bendik et al. (2021) successfully employed photographic identification software to track Barton Springs Salamanders without placing a tag. However, when using photo identification in Jollyville Plateau Salamanders (*Eurycea tonkawae*), another paedomorphic salamander species found in Central Texas, misidentification rates slowly increased over time in adults and rapidly increased (within 2 months) in juveniles due to growth-associated pigment changes (Bendik et al. 2013). Passive Integrated Transponder (PIT) tags, bio-compatible glass-encased microchips typically ranging in size from 8–14 mm (Gibbons et al. 2004), yielded a low retention rate in adult Texas Blind Salamanders (Moon et al. 2022). PIT tags are best suited for larger individuals and species over 50 mm in standard length (Musselman et al. 2017), rendering them unsuitable for juveniles and smaller species. Visible Implant Alphanumeric (VIA) tags, small (1.2 mm x 2.7 mm to 2.0 x 5.0 mm) fluorescent plastic tags printed with visible 3-digit alphanumeric codes (Northwest Marine Technology Inc. 2019), were rejected by Comal Springs Salamanders by tearing through the skin and falling out through the resulting wound (Moon et al. 2022). Even when salamanders successfully retained VIA tags (e.g., Texas Blind Salamanders; Moon et al. 2022), reading was inhibited when the tag was injected too deeply beneath an insufficiently translucent tissue (Osbourn et al. 2011), or at an angle to the epidermal surface (Moon et al. 2022). Visible Implant Elastomer (VIE) tags, injectable colored liquid tags that cure into flexible, fluorescent, bio-compatible solids (Northwest Marine Technology Inc. 2019), exhibited great ease of use and retention in Texas Blind and Comal Springs Salamanders (Moon et al. 2022) and had no observable effects on survival or growth in San Marcos salamanders (*Eurycea nana*) (Phillips and Fries 2009). VIE tags are injected with a 29-gauge needle (Davis and Ovaska 2001), making them suitable for small salamanders. Tag reading success was generally high (Moon et al. 2022), but has been reported to be reduced in some cases by the propensity of the tags to break, degrade, and migrate (Heemeyer et al. 2007). Additionally, certain elastomer colors are difficult to discern even by a trained eye (DMM, TSF, pers. obs.; Northwest Marine Technology Inc. 2021), occasionally resulting in human error. As a result, the sparse color palette available limits the number of differentiable markings possible (≤ 10) unless using multiple color injections, which increases handling time (Davis and Ovaska 2001), requires additional wounds, and exacerbates stress.

As a relatively new technology, p-Chips are injectable microtransponders with a unique set of characteristics that present an alternative option for tagging small-bodied species, including neotenic salamanders. Tag detection and reading is accomplished with a laser wand that transmits information in the form of a 9-digit serial number from the p-Chip photocells to a computer using specialized software (PharmaSeq, Princeton, New Jersey, USA), which expedites tag detections and readings and eliminates the potential for human error. The small size of p-Chips (500 µm x 500 µm x 100 µm) renders them nearly invisible and should not provoke social (Fiske 1997; Frommen et al. 2015; Fischer et al. 2020) or predatory (Catalano et al. 2001; Carlson and Langkilde 2013) behavioral responses from surrounding animals, which are sometimes associated with colored tags. P-Chips have shown high tag retention (Chen et al. 2013; Moore and Brewer 2021) and subject survival rates (Faggion et al. 2020; Moore and Brewer 2021) in studies performed on small-bodied fishes. Thus, p-Chips could be effective for other small-bodied aquatic species, including salamanders.

To our knowledge, p-Chip microtransponder tags have not yet been tested in salamanders or any other amphibians. The purpose of this study was to examine the efficacy of p-Chips in small-bodied aquatic salamanders by measuring survival and tag retention in three salamander species of different sizes (*Eurycea sosorum, Eurycea pterophila*, and *Eurycea rathbuni*) injected with p-Chip tags in a captive setting.

Materials and Methods

All salamanders used in this study, namely Barton Springs Salamanders (*Eurycea sosorum*, n = 95), Comal Springs Salamanders (*Eurycea pterophila*, n = 111), and Texas Blind Salamanders (*Eurycea rathbuni*, n = 78), were part of the captive-assurance populations (i.e., Critically Endangered and threatened animals in captivity for reintroduction purposes) located at the United States Fish and Wildlife Service San Marcos Aquatic Resources Center in San Marcos, Texas, USA. When possible, we prioritized using captive-bred salamanders to minimize potential harm to the wild stock population. However, due to the limited availability of captive-bred individuals, 51 captive-held wild stock Texas Blind Salamanders were included. All salamanders in this study were adults except for 19 juvenile captive-bred Texas Blind Salamanders. Adult salamanders were held in seven tanks, each with a volume of approximately 265 L (70 gal), maintained at a depth of 23 cm. Each tank of adult salamanders contained one species of salamander and was divided into equal sections with water-permeable barriers to separate the treatment groups while maintaining controlled environmental conditions across the groups. Juvenile salamanders were held in three 38 L (10 gal) aquaria with an 18 cm water depth. Each aquarium contained one treatment group, but all aquaria received water from the same source to keep the water quality consistent. Tanks were supplied with flow-through well water at a temperature of 20–23 °C. Each tank section and aquarium had a similar assortment of habitat structures (rocks, aquarium plants, etc.). Adult salamanders were fed live blackworms and live *Daphnia* once weekly, and live *Daphnia* and frozen *Mysis* once weekly. Additionally, because of their larger size, adult Texas Blind Salamanders were fed live red worms (*Eisenia fetida, Eisenia hortensis,* and *Perionyx excavatus*) cut into small pieces each week. Juvenile Texas Blind Salamanders were fed live *Artemia* and *Daphnia* twice weekly. All feeds except for the red worms were supplied at a portion of 0.25 mL/salamander. Red worms were supplied at a portion of 1.6 cm/salamander. Tanks were cleaned weekly.

Prior to launching the full study, we ran a pilot study with five salamanders of each species tagged using the methods described below and monitored for one month to assess potential mortality in the federally listed species. All salamanders survived and retained their tags. Pilot study salamanders were not used in any analyses.

Salamanders were anesthetized before being placed into treatment groups, and measurements were taken. Salamanders were anesthetized via immersion in tricaine methanesulfonate (MS-222, 0.5 g/L) buffered with sodium bicarbonate using previously established protocols (Wright 2001). The salamanders were randomly assigned into treatment groups (tagged, sham, and control) using a random number generator. Sample size varied among treatments due to the limited availability of salamanders (Table 1). The tagged groups were the most numerous for each species to ensure the validity of statistical analyses examining tag retention. Salamanders were placed in clear re-sealable, sliding channel, polyethylene storage bags for easier handling (Heemeyer et al. 2007). Each salamander was measured for weight (g) and snout-vent length (SVL, mm; Petranka 1998; Table 1), sexed using the candling method (Gillette and Peterson 2001), and any distinguishing features or behaviors were recorded (e.g., the presence of eggs or regurgitation). Technical difficulties prohibited the weighing of 40 of the 95 Barton Springs Salamanders (Table 1).

Fig. 1. The left side of a gravid female salamander. The black arrow points to the p-Chip tag.

Three treatment groups were used to examine the effects of p-Chips on salamander survival. Following the manufacturer's guidelines (Pharmaseq Inc. 2020), a 0.8 mm diameter hypodermic needle was used to inject a 500 µm x 500 µm x 100 µm p-Chip subcutaneously at the base of the tail just dorsal and posterior to the left hindlimb of each salamander in the tagged groups (Fig. 1). After tagging, the p-Chips were scanned with the laser reader to record the unique 9-digit tag number. Sham salamanders were treated the same as tagged salamanders (e.g., handled and punctured with the needle) except no tag was placed. Control salamanders were handled the same as the tagged and sham salamanders but were not tagged or pierced with a needle. Using both sham and control groups allowed us to distinguish between the effects of the handling process and the effects of the tag itself (Jepsen et al. 2015; Moore and Brewer 2021). After handling, the salamanders were placed in a small recovery tank until they were able to right themselves and swim normally. Salamanders were then moved to the appropriate section of their holding tank. For consistency, one researcher (DM) performed all the tagging.

Salamander survival, tag retention, and tag readability were monitored for eight months, and growth was examined at the conclusion of the study. Survival was monitored daily as part of the normal husbandry care. Each week, a researcher with experience in scanning p-Chips (DM) scanned all tagged salamanders to check

Table 1. Mean snout-vent lengths (SVL; ±SD), weights (±SD), ranges, and sex (M = male, F = female, U = unknown) for each treatment at the start of the study. Only a subset of the Barton Springs Salamanders were weighed due to technical difficulties, and the number weighed is indicated in parentheses after the sample size (*n*). The final SVL was obtained at the conclusion of the study.

Treatment	*n*	Mean SVL (mm) ± SD	SVL (mm) range	Mean weight (g) ± SD	Weight (g) range	Final mean SVL (mm) ± SD	Final SVL (mm) range	M	F	U
Barton Springs Salamander (*Eurycea sosorum*)										
Total	95 (55)	41.5 ± 4.1	32–52	1.8 ± 0.6	0.8–3.7	41.6 ± 4.4	32–52	33	53	9
Control	32 (18)	40.8 ± 3.5	35–47	1.5 ± 0.4	0.8–2.4	40.9 ± 3.8	33–47	14	14	4
Sham	31 (18)	41.1 ± 4.6	32–48	1.8 ± 0.6	1.1–3.6	41.0 ± 4.8	32–51	17	11	3
P-Chip	32 (19)	42.4 ± 4.0	36–52	2.0 ± 0.7	1.1–3.7	42.7 ± 4.4	35–52	22	8	2
Comal Springs Salamander (*Eurycea pterophila*)										
Total	111	32.9 ± 2.2	29–39	0.7 ± 0.1	0.4–1.1	32.4 ± 1.8	28–38	67	44	0
Control	34	32.6 ± 2.2	29–37	0.6 ± 0.1	0.4–0.9	31.7 ± 1.9	28–36	18	16	0
Sham	34	33.0 ± 2.1	30–39	0.7 ± 0.2	0.4–1.1	33.2 ± 1.8	30–38	21	13	0
P-Chip	43	33.0 ± 2.4	29–39	0.7 ± 0.1	0.4–1.1	32.3 ± 1.7	28–36	28	15	0
Texas Blind Salamander (*Eurycea rathbuni*)										
Total	78	49.4 ± 11.0	26–67	3.3 ± 1.8	0.35–8.1	50.5 ± 10.2	28–68	33	18	27
Control	20	48.6 ± 12.1	28–64	3.2 ± 1.9	0.5–6.3	49.7 ± 10.6	32–66	13	2	5
Sham	20	47.9 ± 11.8	26–64	3.1 ± 1.9	0.4–6.9	49.6 ± 10.4	31–64	5	5	10
P-Chip	38	50.6 ± 10.0	26–67	3.4 ± 1.7	0.35–8.1	51.3 ± 9.4	28–68	15	11	12

retention. Tags were considered lost if the scanner could not detect a tag for the remainder of the trial. Tag readability, i.e., the ability to obtain the identification code of an implanted p-Chip tag, was assessed over time by novice scanners monthly. A novice scanner, i.e., someone who never scanned or had experience with p-Chips before participating in this study, scanned a subset of at least 20% of the salamanders each month to assess any tag readability differences between the experienced and novice scanners. The subset was selected by randomly selecting a tank tagged group and requiring the novice scanner to scan all individuals in that group. A new novice scanner was used each month to examine readability across many individuals and avoid bias due to any experience gained over the duration of the study. Readability was quantified as the percentage of salamanders successfully scanned by the novice scanner. Salamanders were not anesthetized during scanning events to reduce unnecessary stress. There were not enough participants to have a novice scanner for each species each month; so, the order in which salamander species were scanned varied across novice scanners to reduce bias due to any experience gained during the scanning process. SVLs were recorded at the conclusion of the study to determine if growth was affected by tagging, although final weights were not recorded to reduce unnecessary salamander stress.

Analyses. Kaplan-Meier time-at-event curves (Goel et al. 2010) were built to examine survival and retention over time. These curves estimated the probability of an event (survival or retention) occurring at each time interval. Days post tagging and weeks post tagging were used as the time increments for survival and retention, respectively. This approach could reveal any differences across time that may be missed with other methods. For example, high mortality immediately following tagging might be an indication of harm from tagging even if survival rates are somewhat similar across groups. The two null hypotheses tested using log-rank tests were that survival curves did not differ among treatments or by sex for each species and that retention curves did not differ among species. Only salamanders that could be sexed were included in tests comparing survival between sexes (Table 1). Data for juvenile and adult Texas Blind Salamanders were pooled due to the similarity in results. Differences were considered significant at $\alpha \leq 0.05$. Kaplan-Meier curves and log-rank tests were performed in the "survival" package (Therneau 2020) in the program R version 4.2.2 (R Core Team 2022).

Tag readability over time and the effects of tagging on growth were examined. Tag readability was assessed using Pearson's pairwise correlation coefficient to determine the correlation between the percentage of scanned tags to the time since tagging in months. A correlation was considered to be strong at $|r| \geq 0.50$. One-way ANOVAs were performed to confirm that there were no differences in initial SVLs among treatments for each species. To determine the effects of tagging on growth, two-sample t-tests were conducted to compare the growth of tagged salamanders to control salamanders for each species. Growth was calculated by subtracting the initial SVL from the final SVL of each salamander, and differences were considered significant at $\alpha \leq 0.05$. All assumptions for analyses (normality, homoscedasticity, independence, and no outliers) were met by the data in this study. The base package in the program R version 4.2.2 (R Core Team 2022) was used to calculate the correlation coefficients and conduct the t-tests.

Results

Tagging salamanders with p-Chips had no effect on their survival, and no difference in survival was evident between male and female salamanders (Figs. 2–4). Survival did not differ among treatment groups for Barton Springs Salamander ($\chi^2 = 0.5$, $p = 0.8$), Comal Springs Salamander ($\chi^2 = 2.3$, $p = 0.3$), or Texas Blind Salamander ($\chi^2 = 1.1$, $p = 0.6$). Additionally, survival did not differ between males and females among the treatment groups for Barton Springs Salamander ($\chi^2 = 4.5$, $p = 0.5$), Comal Springs Salamander ($\chi^2 = 5.2$, $p = 0.4$), or Texas Blind Salamander ($\chi^2 = 2.4$, $p = 0.8$). Three Barton Springs Salamander mortalities (two tagged and one control) occurred on day 138 of the study, and an additional mortality in the sham group occurred on day 177 (Fig. 2). One Comal Springs Salamander mortality occurred in the control group on day 150 (Fig. 3), and one adult Texas Blind Salamander mortality occurred in the tagged group on day 191 (Fig. 4). Tag retention was relatively high and did not differ among species ($\chi^2 = 1.1$, $p = 0.06$; Table 2). One Barton Springs Salamander tag was lost in week 1 of the study, and one tag in a Comal Springs Salamander was either lost or shifted to the point that it could not be read in week 6 of the study (Fig. 5).

Tag readability for Barton Springs Salamanders varied across novice scanners, but all Comal Springs Salamander and Texas Blind Salamander tags were readable by every novice (Table 3). There was not a strong correlation between readability and time since tagging for Barton Springs Salamanders over the eight months of this study ($r = 0.31$). Only the experienced scanner was able to read one of the Barton Springs Salamander tags. This salamander was randomly selected for reading by novice scanners in months 1–4 and 6. Novice scanners in months 3 and 4 were unable to read other salamander p-Chip tags, but those tags were successfully read by novice scanners thereafter (Table 3). The novice scanners in months 3 and 4 both attempted to read the Barton Springs Salamanders first and then read the other species' tags afterward. All novice tag scanners were able to accurately read all Comal Springs Salamander and Texas Blind Salamander tags throughout the study.

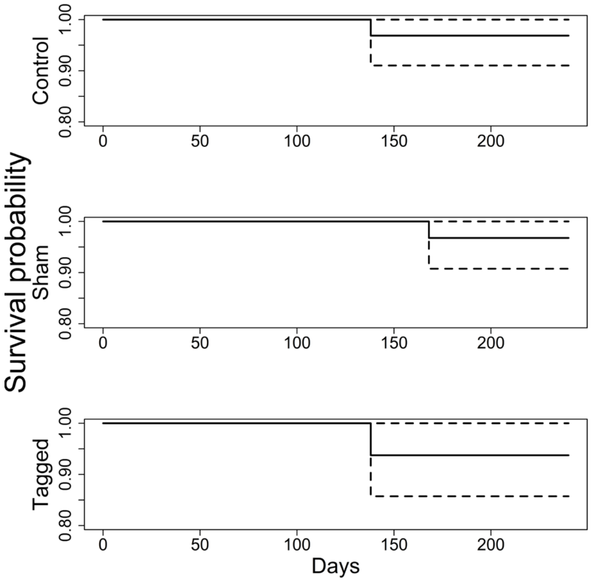

Fig. 2. Kaplan-Meier survival curves for Barton Springs Salamanders in the control, sham, and tagged groups. The probability of survival is shown with 95% confidence intervals (dashed lines) over time in days since tagging.

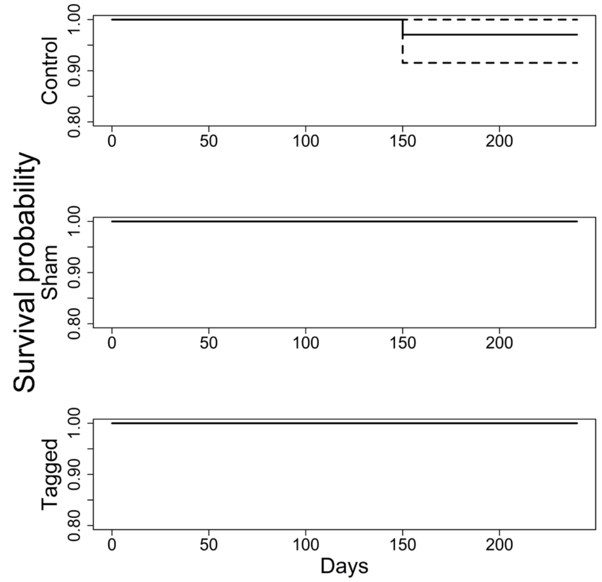

Fig. 3. Kaplan-Meier survival curves for Comal Springs Salamanders in the control, sham, and tagged groups. The probability of survival is shown with 95% confidence intervals (dashed lines) over time in days since tagging.

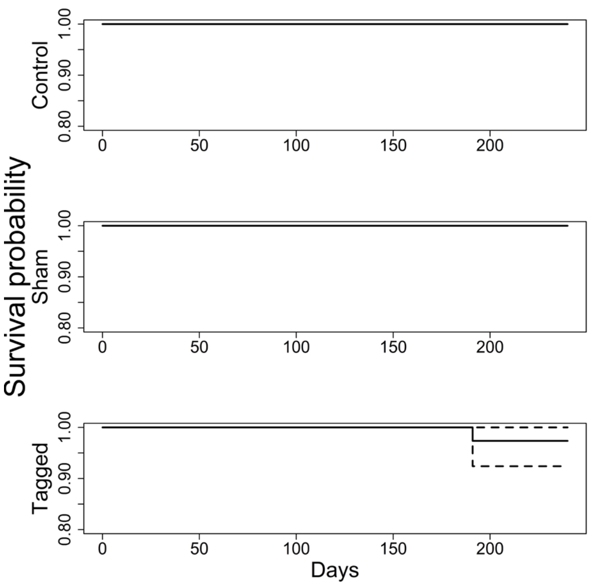

Fig. 4. Kaplan-Meier survival curves for Texas Blind Salamanders in the control, sham, and tagged groups. The probability of survival is shown with 95% confidence intervals (dashed lines) over time in days since tagging.

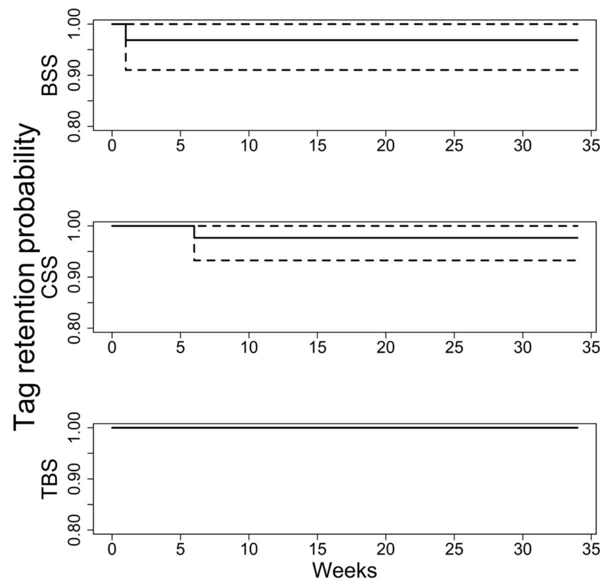

Fig. 5. Kaplan-Meier p-Chip retention curves for Barton Springs Salamanders (BSS), Comal Springs Salamanders (CSS), and Texas Blind Salamanders (TBS). The probability of p-Chip retention is shown with 95% confidence intervals (dashed lines) over time in weeks since tagging.

Growth was not affected by tagging in any of the three species. Initial lengths and weights were closely correlated (Pearson's product moment coefficient |r| = 0.93), and so the lengths were used for growth analyses. The initial lengths did not differ among treatments for Barton Springs ($F_{2, 92} = 1.39$, $p = 0.25$), Comal Springs ($F_{2, 108} = 0.39$, $p = 0.68$), or Texas Blind ($F_{2, 75} = 0.47$, $p = 0.63$) salamanders. Although the final mean SVL was smaller than the initial mean SVL for some groups (Table 1), growth did not differ between the tagged and control groups for Barton Springs Salamander ($p = 0.84$), Comal Springs Salamander ($p = 0.53$), or Texas Blind Salamander ($p = 0.64$). Visible growth was noted in the juvenile Texas Blind Salamanders, but not in any other groups. Although not formally examined, we noted that the control and tagged groups of Comal Springs and Texas Blind Salamanders produced multiple clutches of viable eggs over the duration of the study.

Discussion

Improving tagging methods for small-bodied aquatic organisms is important for conservation and management, and progress is underway with new technological developments. P-Chips resulted in high survival (97–100%) and tag retention (97–100%) without inhibiting growth in aquatic salamanders, indicating the potential for using p-Chip tags in lab and field studies involving aquatic salamander species. Additionally, p-Chips provided an improved readability rate, reduced human error, and allowed for a greater number of individual identification codes than the VIE tags commonly used for these species. To our knowledge, this is the first study examining p-Chips in aquatic salamander species.

We found p-Chips to be appropriate and versatile tags for aquatic salamanders. P-Chips provided high survival and retention rates in Barton Springs, Comal Springs, and Texas Blind Salamanders. Our results are similar to those of studies examining p-Chips in other aquatic organisms (Chen et al. 2013; Faggion et al. 2020; Moore and Brewer 2021). Although photo identification has been used in Barton Springs Salamanders (Bendick et al. 2021), we are unaware of any other published studies examining the efficacy of photo identification in any of the species in our study. Photo identification is labor intensive as it requires time to take and process the photos. Tagging and scanning p-Chips requires only seconds for an experienced tagger. P-Chips would be preferred to photo identification when time is a concern and an experienced tagger is available. P-Chips were more versatile than the previously used VIE tags. Although survival and tag retention were similar in previous VIE tagging research (Phillips and Fries 2009; Moon et al. 2022), p-Chips provided individual identification with a single tag. To achieve the same resolution of individual information provided by a single p-Chip tag, especially in large sample sizes, VIE tag codes would require the injection of multiple tags per individual. Increasing the number of wounds might increase animal stress, the possibility of infection, and mortality over time. P-Chips also enabled individual identification in small (e.g., ≤ 35 mm SVL) salamanders that might not be able to survive the injection of several VIE tags. There were fewer opportunities for human error when using p-Chips because tag codes were recorded directly from the laser reader into a CSV file instead of being manually observed, interpreted, and written or typed. Additionally, novice scanners were more successful at reading p-Chips (100%) compared to novice scanners reading VIE tags in Comal Springs and Texas Blind salamanders (Moon et al. 2022). It is not currently known whether p-Chips would perform as well when applied to salamanders in a wild setting where habitats are more variable and predators might be present.

Although tag readability was optimal for Comal Springs and Texas Blind Salamanders, novice scanners made occasional errors when reading p-Chips in Barton Springs Salamanders. Unlike with VIE tags in *Eurycea* spp. (Moon et al. 2022), tag readability in Barton Springs Salamanders was not related to time since tagging. Instead, the difficulty reading tags seemed to be related to individual salamander tag placement and variations in scanner ability, and was unique to the Barton Springs Salamanders in this study. For example, one individual salamander was unreadable by all novice scanners who attempted to scan it. The experienced scanner noted that the tag in this salamander was at an angle, and the tag had to be read by pointing the laser upward from the underside of the salamander. The novice scanners did not have the experience to identify and troubleshoot this issue and were unable to read that tag. This instance indicates that the experience of the tagger might be important for overall readability, since a less experienced tagger might not be able to tag as many individuals with consistent placement regarding depth, angle, and location. Another possibility is that readability may have been increased for the tagger, as hypothesized with VIE tags (Moon et al. 2022). The wide range of readability scores (50–100%) indicated that individual scanner variation might affect novice readability in Barton Springs Salamanders. Possible reasons for this variation include variation in eyesight, patience, interest, and similar experiences. For example, individuals that have read other types of tags in the past might be more able to read p-Chips without direct experience.

It is notable that readability issues were only present for Barton Springs Salamanders, indicating there might be some anatomical or behavioral traits that reduce readability overall. Another possibility is that this issue was partially due to novice scanners having more trouble with the first species they read. However, this issue was not seen for novice scanners that read other species first, and some scanners that began with other species were not able to read all the Barton Springs Salamander tags. On several occasions, the experienced and novice scanners noted that the Barton Springs Salamanders seemed more physically active than the other species, so it was difficult to keep them positioned long enough to find the proper angle for reading. Anesthetizing salamanders during the scanning event might improve the ability of novice scanners to read these tags. However, repeated anesthetization in these species is not well studied and might have negative effects. Additionally, the Barton Springs Salamanders are more pigmented than Texas Blind Salamanders and seem to have thicker skin than Comal Springs Salamanders, a trend that might be related to size (i.e., larger salamanders tended to have thicker skin during tagging). Future work should examine the differences among species and ontogenetic stages that could be contributing to this readability issue, which could indicate those species that are most suitable for using p-Chip tags.

The data indicated no evidence that growth was affected by p-Chip tagging or contributed to the migration of the p-Chips. Like VIE tagging in San Marcos Salamanders (Phillips and Fries 2007), growth was unaffected by p-Chip tagging in Barton Springs, Comal Springs, and Texas Blind Salamanders in this study. However, we only examined growth at the conclusion of the study rather than at various points throughout the study, so we may have missed any variation in growth rates earlier in the study (e.g., Baras et al. 1999; Ruetz et al. 2006). Additionally, we did not examine any other metrics that might have been affected by tagging, such as behavior. Growth often affects the migration of subcutaneous tags in aquatic organisms (Linnane and Mercer 1998; Haddaway et al. 2011). For example, growth was shown to increase VIE breakage and deterioration in *Eurycea* spp. (Moon et al. 2022). However, we found no evidence of p-Chip migration with growth. Juvenile Texas Blind Salamanders had the highest growth rate but were also associated with 100% readability and tag retention. Growth might affect the migration of p-Chips used in individuals smaller than the ones we examined. More research is needed to evaluate the use of p-Chips in smaller (≤ 25 mm SVL) individuals of each of these species.

The results of this study show that P-Chips are suitable tags for small-bodied aquatic salamanders, especially for projects that require individual identification. However, other tags such as VIE tags might be more appropriate in projects where individual identification is not needed or short-term studies with few individuals, especially when costs must be reduced. The duration of effectiveness for subcutaneously injected p-Chips in aquatic organisms remains unknown. Longer-term monitoring is needed to determine the endpoint of p-Chip efficacy in long-lived aquatic organisms. Although no deterioration of p-Chip readability was observed over the eight months of this study, they may become more difficult to read with age. Additionally, more research is needed to determine the size limits of salamanders that can be tagged with p-Chips, particularly in the smaller salamander species in which we only tagged adults. Tagging juveniles could be beneficial for examining recruitment and growth rates, and for tracking individual metrics such as genetic, collection, and rearing information. Research comparing the efficacies of photo and p-Chip identification in these species is needed. Photo identification is an effective method of the identification for some species at a low cost and might be preferable to, or used in conjunction with, p-Chips for some projects. Although we found no effect on growth from tagging with p-Chips, additional studies examining the effects of p-Chip tagging on behaviors such as swimming, hunting, and reproduction are recommended. Because survival and retention are often different in captive and wild settings (e.g., Musselman et al. 2017), studies examining the effects of p-Chips on organisms in the wild are needed to confirm the utility of this tagging method under wild conditions.

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