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Evaluation of microtransponder tags on the threatened small-bodied San Marcos Salamander (*Eurycea nana*)

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***Abstract.*—Organism tagging can be used to examine and track important life history data that informs conservation efforts, but mortality and tag loss can mislead management decisions if not properly understood. To conduct mark-recapture studies that benefit conservation of the San Marcos Salamander (*Eurycea nana*), a tagging method that provides individual identification is needed. The goal of this study was to examine survival and tag retention of San Marcos Salamanders injected with microtransponder tags in captivity to determine if this is an appropriate tagging method. We tagged 23 San Marcos Salamanders with microtransponders at the base of the tail on the left side and monitored the survival daily and tag retention weekly for six months. We compared tagged salamander survival to that of 16 control salamanders. San Marcos Salamander survival (87%) was not affected by tagging. Additionally, no tag loss occurred in this study. Microtransponder tags provided high survival and retention and allowed for individual identification. This is a suitable tagging method for San Marcos Salamanders in captivity and could be considered for field mark-recapture studies to examine demographics through individual tracking.**

Keywords. *Eurycea nana*, retention, survival, tagging, microtransponders

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Introduction

Organism tagging can be used to examine and track important life history data that informs conservation efforts, but tag loss and latent mortality associated with tagging can mislead management decisions. Animal marking is used to estimate parameters like abundance, survival, movement, and habitat use (e.g., Holcomb et al. 2020; Miller et al. 2019; Moore and Brewer 2025). However, using tagging methods that are associated with low survival and tag retention could violate the assumptions of the studies and result in incorrect estimates (Malcolm-White et al. 2020; Sackett and Catalano 2017). It is imperative to determine the effects of a proposed tagging method on the species before using it to reduce harm to the organisms and ensure results are not negatively affected by the tagging method.

There are many tagging methods available for use under a variety of conditions and in different types of organisms. Choosing an appropriate tagging method for the target species and research needs is important to comply with study assumptions and ethical tagging (Cooke et al. 2013: Ricker 1956). Amphibian tagging methods available include injectable elastomers, externally attached transmitters and transponders, injected transponders, toe clipping, branding, and photo identification (Bendik et al. 2013; Measey et al. 2001; Moon et al. 2022: Silvy et al. 2005; Waddle et al. 2008). A more recent tagging method is p-Chips (PharmaSeq, Princeton, New Jersey), an injectable microtransponder (hereafter microtransponder tags). These are small (500 µm x 500 µm x 100 µm) computer chips with photocells that are read by a handheld laser reader to obtain a unique 9-digit code. Due to their small size, these microtransponders are associated with high survival and retention in small-bodied aquatic organisms such as fish (Chen et al. 2013; Faggion et al. 2020; Moore and Brewer 2021) and salamanders (Moore et al. 2024).

The federally threatened San Marcos Salamander (*Eurycea nana*; ESA 1973, as amended) is a small paedomorpic salamander endemic to Central Texas with research and conservation needs that could benefit

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from effective tagging methods (Chippindale et al. 1998; Diaz et al. 2015). This salamander is federally protected and managed under the Edwards Aquifer Habitat Conservation Plan (Edwards Aquifer Authority 2012), but little is known about the efficacy of conservation methods currently or future needs of the species. Due to this salamander's small range, reliance on Edwards Aquifer water, and unknown life history information, mark-recapture studies would greatly benefit conservation efforts (Chippindale et al. 1998; Diaz et al. 2015). However, few tagging methods have been examined in San Marcos Salamanders (Moon et al. 2022). The size and biological limitations (e.g., soft, permeable skin) of these salamanders limits the possible tagging methods available (Heemeyer et al. 2007). Although three Visible Implant Elastomer (VIE) tags (Northwest Marine Technology Inc. 2019) at the base of the tail had no effect on San Marcos Salamander growth or survival (Moon et el. 2022), VIE tags are limited in their ability to provide individual identification without employing multiple subcutaneous injections. The methods by Moon et al. (2022) provide 216 unique tag codes. Because our goal was to ultimately obtain individual identification for over 400 salamanders, we sought another method, such as microtransponder tags. Microtransponder tags have been successfully used in similar salamander species (Moore et al. 2024), but San Marcos Salamanders are known to be a particularly sensitive species (e.g., lower survival rate; Bockrath et al. 2023), especially females (Moore et al. 2023). Therefore, it is important to validate microtransponder tags as a viable tagging method before using it broadly.

In pursuit of an effective tagging method providing individual identification for future field applications, it is important to first determine the efficacy of tagging under controlled conditions. Therefore, the objectives of this study were to 1) determine if tagging San Marcos Salamanders with microtransponder tags affects survival and 2) examine tag retention associated with microtransponder tags in captive salamanders.

Materials and Methods

All San Marcos Salamanders used in this study were part of a captive-assurance population (i.e., animals held in captivity for potential reintroduction purposes) located at the United States Fish and Wildlife Service San Marcos Aquatic Resources Center in San Marcos, Texas. Our research was conducted January to June 2023 and used 39 captive-bred individuals to minimize potential harm to the wild-collected population. Salamanders were held in a 265-L (259 cm x 20.3 cm x 55.8 cm) fiberglass tank with a water depth of 23 cm. The tank was divided into five equal sections (3 tagged, 2 control) by water-permeable barriers to maintain controlled environmental conditions while keeping track of treatment groups. Salamanders received flow-through water from an Edwards Aquifer well at 20-23 °C. All salamanders were provided similar habitat structure (rocks, aquarium plants, etc.) and were fed live blackworms and live *Daphnia* on Fridays and live *Daphnia* and frozen *Mysis* on Tuesdays. All feeds were provided at a portion of 0.25 mL/salamander. Tanks were cleaned weekly.

We anesthetized salamanders before randomly placing them into treatment groups and measurements were taken. We immersed salamanders in tricaine methanesulfonate (MS-222, 0.5 g/L) buffered with sodium bicarbonate using established protocols (Wright, 2001). Then we randomly assigned them to treatment groups (tagged and control) using a random number generator. The sample size was larger for the tagged group (N = 23) than the control group (N = 16) to ensure the ability to examine tag retention. Salamanders were placed in a clear plastic bag for easier handling (Heemeyer et al., 2007). We measured each salamander's snout-vent length (SVL, mm; Petranka, 1998) and sexed them using the candling method when possible (Gillette and Peterson, 2001). Additionally, any unique characteristics were recorded (e.g., the presence of eggs or regurgitation).

There were two treatment groups (tagged and control) which allowed us to determine the effects of p-Chips on San Marcos Salamander survival. Following manufacturer guidelines (Pharmaseq Inc., 2020), a 0.8 mm hypodermic needle was used to inject a 500 µm x 500 µm x 100 µm microtransponder tag subcutaneously at the base of the tail just dorsal and posterior to the left hindlimb of each salamander in the tagged group (Moore et al. 2024). After tagging, the tags were scanned with the tag reader to record the 9-digit tag number. Control salamanders were handled the same as tagged salamanders but were not tagged or pierced with a needle. After handling, all salamanders were held in a recovery tank until recovery was evident (i.e., they were able to right themselves and swim normally). For consistency, one researcher (Moore) performed all tagging. Salamander survival and tag retention was monitored for six months. We monitored survival daily as a part of normal salamander care. Each week, an experienced tag scanner (Moore) scanned all tagged salamanders to record retention. Tags were considered lost if the scanner could not detect that tag for the rest of the trial.

Analyses

We used t-tests to determine if salamander lengths differed between treatments or sexes. We created Kaplan-Meier time-at-event curves (Goel et al. 2010) to visualize survival over time. These curves are used to estimate the probability of an event (e.g., survival) occurring at each given time interval. The time interval for survival was days since tagging because survival was determined daily. This method reveals differences across time that might not be exposed with other methods. All assumptions for Kaplan-Meier time-at-event curves were met by our dataset. We used a log-rank test to determine if the survival curves differed between treatments or by sex. Salamanders of unknown sex were not included in tests comparing survival between sexes. We intended to visualize tag retention in the same way, but no tag loss occurred during the study. We used $\alpha \leq 0.05$ to determine statistical significance. These analyses were performed in the program R version 4.4.2 (R Core Team 2024). The Kaplan-Meier curves and log-rank test were performed using the "survival" package (Therneau 2024).

Results

San Marcos Salamander lengths did not differ between treatments or sexes. Salamander SVL was similar between tagged (30.30 ± 1.79 STDEV) and control salamanders (30.25 ± 1.53 ; $t(35.39) = 0.10$, $p = 0.92$). We were able to sex 38 of the salamanders (97.4%), so only one individual was excluded from analyses regarding salamander sex. Of those 38 salamanders, 11 were female and 27 were male. Salamander SVL was also similar between male (30.41 ± 1.72) and female salamanders (30.36 ± 1.03; $t(30.50) = 0.10$, $p = 0.92$).

Tagging San Marcos Salamanders with microtransponder tags had no effect on survival, survival was not different between male and female salamanders, and tag retention was high. Survival was not statistically different between tagged (87.0%) and control (87.5%) salamanders ($\chi2 = 0.0$, $p = 1.0$; Figure 1). Two tagged and two control salamanders perished on day 14 presumably due to an unrelated technical malfunction at the facility.

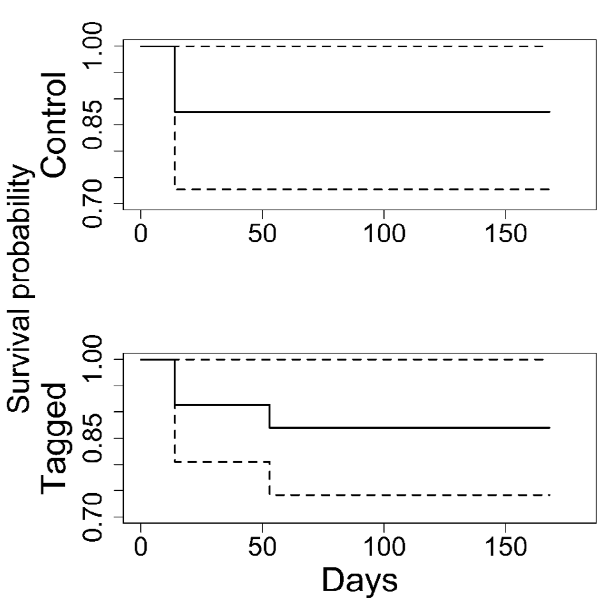

Fig. 1. Kaplan-Meier survival curves created for San Marcos Salamanders (*Eurycea nana*) in the control and tagged groups over six months. Salamanders in the tagged group were injected with microtransponder tags at the base of the tail on the left side. The probability of survival is shown with 95% confidence intervals (dashed lines) over time in days since tagging.

Although these were not considered mortalities due to the study, we did include them in analyses because it could not be definitively determined that tagging did not contribute to mortality in some way. Additionally, tagged salamander mortality was recorded on day 53 of the study. Survival did not differ between males and females ($\chi2 = 0.4$, $p = 0.6$). No tag loss occurred during this study; therefore, we did not perform any analyses of these data.

Discussion

Determining an appropriate tagging method for research is necessary, particularly for at-risk and sensitive species. We found high survival (87%) and tag retention (100%) associated with microtransponder tags used in San Marcos Salamanders, indicating the tags are suitable and there is potential to use these tags in field studies. Additionally, this tagging method provided individual identification codes allowing for tracking of individual information. To our knowledge, this is the first study examining this tagging method in San Marcos Salamanders.

The high survival rate and perfect retention found in this study are similar to those found using these tags other aquatic organisms (Chen et al. 2013; Faggion et al. 2020, Moore and Brewer 2021) and other aquatic salamanders (Moore et al. 2024). Tagging and scanning these tags took very little time for an experienced tagger and provided individual identification for any number of salamanders, unlike the previously used VIE tags which can only provide individual identification for a limited sample size (Moon et al. 2022). Although this study did not examine the use of these tags in the wild where habitats are more variable and there are predators, this provides the foundation needed to begin exploring that possibility.

Microtransponder tags are suitable for use in San Marcos Salamanders, especially when individual identification is needed. However, other tags (e.g., VIE tags) may be preferable in projects with a limited budget, small sample sizes, or without need for individual identification. We did not find a limit to the duration of efficacy for these tags in San Marcos Salamander, but our study monitored them for only six months. Studies examining the long-term efficacy of the tags are needed to inform the use of these tags in long-term studies. At the end the year-long VIE tagging study in San Marcos Salamanders, the VIE had some signs of deterioration and breakage (Moon et al. 2022). However, it is unclear when that deterioration began. Additionally, we only examined tag use in adult salamanders. Research determining size limits of San Marcos Salamanders that can safely be tagged with microtransponder tags would be beneficial for studies examining recruitment, growth, and survival of juveniles. We did not examine the effects of tagging on growth and behavior, which would be advantageous to determine. Furthermore, examination of survival and retention of San Marcos Salamanders tagged with microtransponder tags in the wild could benefit studies aiming to use these tags in the wild population.

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Dr. Katie Bockrath earned a Ph.D. in Genetics from the University of Georgia (2015) and a bachelor's degree in biology at the University of West Georgia (2009). Katie's Ph.D. research focused on assessing aquatic biodiversity using genetics, population surveys, and habitat measurements. She joined the U.S. Fish and Wildlife Service in 2016 as a Geneticist with the Whitney Genetics Lab at the Midwest Fisheries Center in Onalaska, Wisconsin, where she and her team used environmental DNA (eDNA) to monitor invasive species , specifically invasive Carp. When she was not testing water samples, optimizing lab methods, or experimenting with new methods, Katie attended pragmatic and scientific meetings where she presented research and advancements in the eDNA field. Katie fostered collaborations with multiple state agencies and conservation offices, USGS labs across the Midwest, and with biologists across U.S. Fish and Wildlife Service programs. In addition to working on eDNA methods and applications, Katie built and managed the next-generation sequencing lab and used high-throughput sequencing methods for early detection of Aquatic Invasive Species and conservation of priority native species. In June of 2021, Katie joined team at the San Marcos Aquatic Resources Center as a Biologist and Research Lead where her and her team have made significant advancements in the understanding of biology and ecology of many understudied spring decent species. Katie continues collaborative work with universities and state agencies to carry out fundamental research of species dependent on the Edwards Aquifer. Katie enjoys spending time outdoors with her husband and dog, playing board/card games, and working on home improvement projects.

Desiree Moore is a research biologist with the U.S. Fish and Wildlife Service at the Ash Meadows Fish Conservation Facility (Amargosa Valley, NV, USA) working to conserve Devils Hole Pupfish but was previously a part of the Edwards Aquifer Refugia Program at the San Marcos Aquatic Resources Center (San Marcos, Texas, USA), conducting research to benefit federally listed aquifer species. Moore graduated with a Master of Science degree from Oklahoma State University (Stillwater, Oklahoma, USA) examining movement patterns and developing relationships between flow regime characteristics and occupancy probability of freshwater pelagic broadcast spawning minnows, with an emphasis on the federally threatened Arkansas River Shiner. Moore is continuing her research with federally listed aquatic organisms and specializes in tagging small-bodied aquatic animals.