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Evidence of Nest Site Fidelity in Four-Toed Salamanders Using Photo Identification and a 15-Year Nesting Dataset

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Abstract.—**Site fidelity refers to the tendency of an animal to return to the same location each year to breed, nest, or forage. The method for tracking returning individuals depends on the species, size of the animal, and the distance it travels. Some tracking methods are more invasive than others, which is why non-invasive techniques, such as pattern identification software, are used to recognize individuals based on their unique markings. Our objective was to evaluate whether breeding female Four-toed Salamanders (*Hemidactylium scutatum*) could be tracked year-to-year based on their ventral spot pattern measured via photographs and to determine whether individuals display moss clump nest site fidelity. To achieve this, we assessed a long-term dataset (15 y) of the ventral images of nesting females and analyzed them using HotSpotter photo identification software. We also used a subsample of our data to analyze site fidelity by measuring distances between nests of known matches of the same female and nests assigned a random match. HotSpotter successfully photo-matched nesting females using ventral images, and distances between known matches were significantly localized within nesting areas. Our findings confirm that females return to the same nesting areas each year and show strong fidelity to individual moss clumps, exceeding previous reports of pond-site fidelity. These results reinforce the importance of long-term datasets for amphibians with conservation needs.**

Key Words.—Appalachians; HotSpotter; plethodontid; spot-pattern; vernal pools; wetlands

Introduction

Wildlife biologists define site fidelity as the tendency of an animal to return to the same location it previously used for breeding, foraging, or hibernation (Switzer 1993). Site fidelity is a behavioral strategy with important ecological and evolutionary effects because it can influence dispersal, habitat specialization, and population connectivity. Species that repeatedly return to preferred breeding or nesting sites may be more dependent on the availability and quality of suitable habitat, which can limit dispersal, shape species distributions, and influence population genetic structure (Denoël et al. 2017). Site fidelity has been documented across a wide range of taxa, including Loggerhead Sea Turtles (*Caretta caretta*), which exhibit fidelity to seasonal migration routes, foraging areas, and natal nesting beaches (Evans et al. 2019), and amphibians, such as Marbled Salamanders (*Ambystoma opacum*) and Spotted Salamanders (*Ambystoma maculatum*), which return annually to their natal pool to breed (Gamble et al. 2007; Petranka 1998).

Understanding site fidelity requires the ability to identify and track individuals across multiple breeding seasons. Long-term monitoring of individuals provides insight into patterns of site reuse, reproductive longevity and movement within populations. Methods used to identify individuals vary among taxa. For migratory birds, researchers use leg bands with distinct identification numbers and various types of signal transmitters based on the size of the bird and the distance of the journey (Guilford et al. 2011). Researchers equip larger mammals (e.g., American Black Bears, *Ursus americanus*, and Jaguars, *Panthera onca*) with radio or GPS collars to observe daily activities and evaluate site revisitation within their home ranges (Bowersock et al. 2015; Pereira et al. 2022). For amphibians, common methods to identify individuals in sequent captures across seasons include implantation of either passive integrated transponders or visible implant elastomers, and toe clipping (Ferner 2007; Moon et al. 2022).

Individual identification through pattern recognition software has become increasingly common because it allows researchers to analyze large numbers of images and individuals efficiently (Nipko et al. 2020). These methods are also less invasive than traditional recapture techniques because individuals can be identified based on their natural markings rather

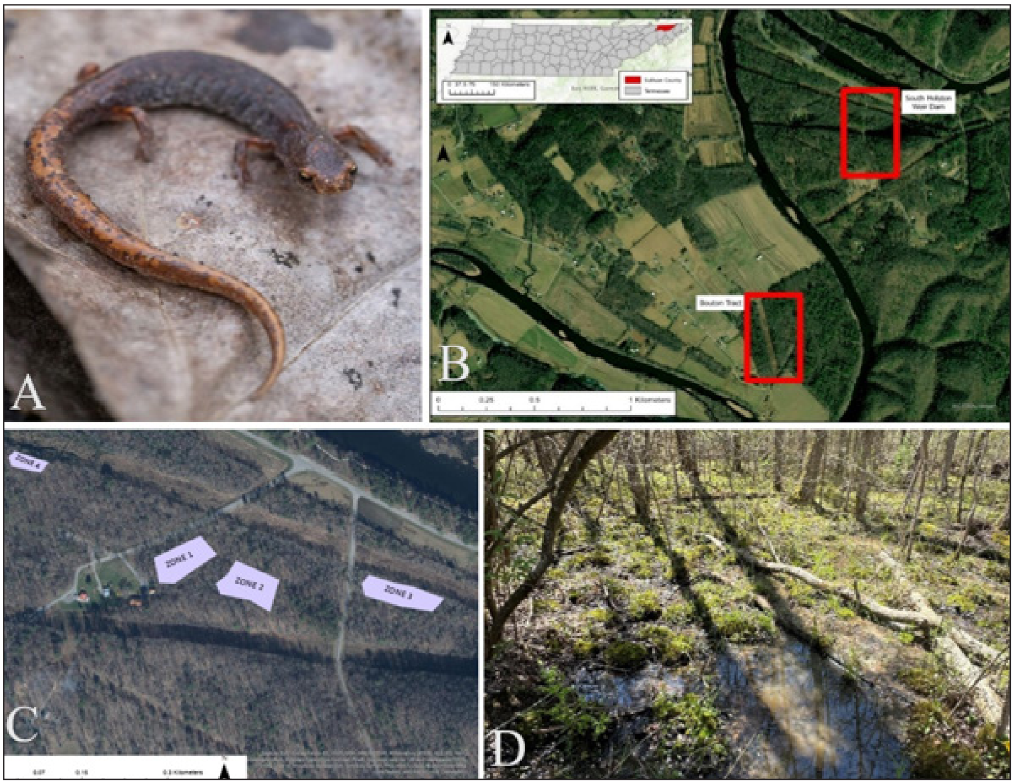

Figure 1. Study species, study sites, and nesting habitat of the Four-toed Salamander (*Hemidactylium scutatum*) in Sullivan County, Tennessee, USA. (A) Adult female from the Weir Dam study site in April 2022 (Photographed by Charlie Holguin). (B) Aerial imagery of the Weir Dam and Bouton Tract study sites, with the study sites indicated by red rectangles. (C) Aerial imagery of the Weir Dam study site, with nesting zones 1–4 highlighted with purple polygons to show size and shape. (D) Nesting habitat at the Weir Dam study site, showing shallow vernal pools surrounded by moss clumps. (Photographed by Kalin Ferguson).

than invasive marking methods (Suriyamongkol and Mali 2018). Researchers can import images taken manually on a camera or from a camera trap into the software to identify individuals automatically (Nipko et al. 2020; Dunbar et al. 2021). Among available platforms, HotSpotter has been successfully applied across taxa and has demonstrated high accuracy and efficiency in identifying individuals with unique markings (Suriyamongkol and Mali 2018; Nipko et al. 2020; Kakegawa and Hasumi 2024). Photo-identification software provides a non-invasive and effective tool for assessing site fidelity and improving understanding of species longevity and reproductive behavior.

Four-toed Salamanders (*Hemidactylium scutatum*; Fig. 1) are a distinctive member of the family Plethodontidae, representing the only species within its genus. The species has the largest geographic distribution of any plethodontid species (Herman and Bouzat 2016), with a broad, discontinuous range extending from southeastern Canada through the eastern U.S. to Florida, and westward to Oklahoma. Despite this large distribution, 17 of the 29 states within its U.S. range recognize *H. scutatum* as a species of conservation concern (Ferguson and Hamed 2024), reflecting its dependence on specialized forested wetland habitats and vulnerability to habitat loss and degradation. In Tennessee, Four-toed Salamanders are designated as a Species of Greatest Conservation Need (Ferguson and Hamed 2024), emphasizing the importance of understanding the ecological requirements that support population persistence within the state. Conservation concerns are closely tied to the specialized reproductive ecology of the species and its reliance on specific wetland habitat for successful nesting.

Compared to other plethodontids, Four-toed Salamanders have shorter larval periods and distinctive nesting preferences. Adults mate in the upland forest habitats during the fall or late winter and females subsequently migrate to wetland nesting sites in early spring (Petranka 1998). During this time, females lay their eggs within moss clumps (e.g., *Sphagnum* and *Climacium* spp.) located on steep edges above slow-moving or stagnant water within flooded forested vernal pools, bogs, and swamps (Gilbert 1941; Wood 1955; Petranka 1998; King and Richter 2022). Females exhibit pond fidelity, returning to the same breeding pond across years. For example, females repeatedly returned to two breeding ponds with circumferences of 37 and 45 m (Harris and Ludwig 2004). Fidelity to individual moss clumps, however, remains poorly understood. A solitary female may nest in a moss clump, or multiple females may nest in the same location (joint nesting) with the fittest female providing parental care throughout the nesting season (Blanchard 1934; Harris and Ludwig 2004).

Four-toed Salamanders are the only salamanders in the U.S. that have a white belly punctuated with small black spots, which facilitates their identification (Herman 2013). Previously, researchers have used ventral spot patterns to distinguish individual females in the field while assessing site fidelity and nesting behavior (Harris and Gill 1980; Harris and Ludwig 2004; Harris 2008; Hamed 2014). Advances in artificial intelligence and computer recognition software have led to the development of several photo-identification softer programs that allow researchers to compare large numbers of individuals based on unique visual characteristics. Six commonly used photo identification software programs include APHIS, I3S, Wild-ID, Amphident, Hotspotter, and IBEIS (https://wildlife.ai/id-software-a-review/). We used the image-matching software HotSpotter (Rensselaer Polytechnic Institute, Troy, New York, USA; Crall et al. 2013) to evaluate its ability to reliably identify individual Four-toed Salamanders across nesting seasons and to determine if female Four-toed Salamanders exhibit fidelity to breeding sites by returning to the same or nearby moss clumps across nesting seasons.

Materials and Methods

Study sites.—We studied breeding activities of *H. scutatum* at two localities on Tennessee Valley Authority (TVA) property in northeast Tennessee, USA (Fig. 1). The forest canopy of the Weir Dam site consisted of Red Maple (*Acer rubrum*), Sweetgum (*Liquidambar styraciflua*), Sycamore (*Platanus occidentalis*), and Green Ash (*Fraxinus pennsylvanica*). The abundance of Green Ash trees within the study area declined since we started data collection in 2009 due to the Emerald Ash Borer (*Agrilus planipennis*). Most Green Ash trees died, resulting in significant gaps in the forest canopy. Moss species, primarily American Tree Moss (*Climacium americanum*), dominated the edge of small vernal pools within the study site, aided by partial shade conditions in the forest and the changes in hydrology from the vernal pool creation. This site has over 500 small, water-filled depressions and ditches that function as vernal pools, most of which measured < 2.5 m in circumference. Several amphibian species, such as Marbled Salamanders, Spotted Salamanders, and Spring Peepers (*Pseudacris crucifer*), use these pools during the breeding and nesting season (Hamed 2006). Four-toed Salamanders were first documented at the Weir Dam site in 2002 (Hamed and Gentry 2003).

The second site was on the southwestern side of the South Fork Holston River in a powerline right-of-way along the Bouton Tract Loop. This site lacked an overhead canopy and, therefore, differed from the Weir Dam site. *Sphagnum* spp. moss grew predominantly in field-like conditions among grasses and early successional vegetation. Because there was no forest canopy, the site may have had greater exposure to fluctuations in air temperature and moss moisture. TVA maintains the open canopy of the right-of-way with a mowing cycle every 3–5 y. We first documented Four-toed Salamanders at this site in 2022.

Surveys for adults.—We initiated annual surveys for nests of Four-toed Salamander at the Weir Dam site in 2008 and at the Bouton Tract site in 2022. We surveyed over a one-month from the last week in March to the last week in April from 2009–2025 at the Weir Dam site and from 2022–2025 at the Bouton Tract site. We did not conduct surveys in 2013 and 2014 at the Weir Dam site. Due to the COVID-19 pandemic, our nest surveys at the Weir Dam site in 2020 were incomplete; we surveyed for nests in zones 1 and 2 but not zones 3 and 4 (Fig. 1). Surveys at each site were completed systematically in attempted to identify all nesting females each year at each site. We searched sections of moss tussocks, beginning with moss in steep edges closest to the water, to check for the presence of females and their eggs. Females were typically found beneath the eggs or on the edge of the moss clump above the water line, fleeing from nest disturbance. Because females can nest jointly, all sides of the moss clumps were thoroughly examined to ensure all individuals were found and captured. We placed each female in an individual clear plastic petri dish and temporarily

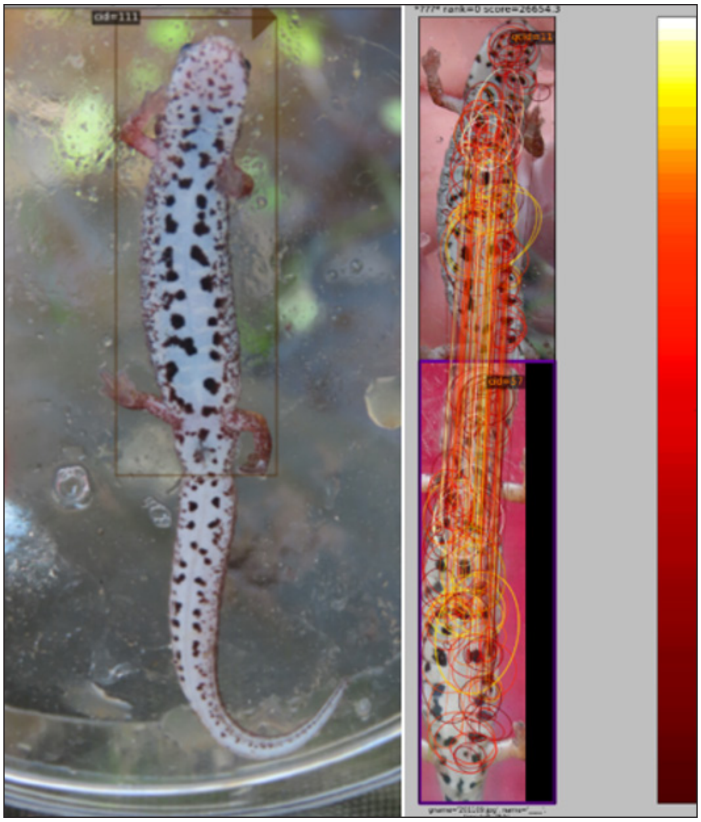

Figure 2. Example of HotSpotter image matching used to identify individual female Four-toed Salamanders (*Hemidactylium scutatum*) based on their ventral pattern at the Weir Dam site in Sullivan County, Tennessee, USA. The left panel shows the query image from an individual in April 2020 (Photographed by Kalin Ferguson), and the right panel shows a positive match from April 2009 and 2011 through the illumination of the circles matching specific spots. (Photographed by Kevin Hamed).

set them aside. If females were dropped or escaped during capture, we returned to the nest later in the day to allow time for their return; all females were observed to return.

To process a nest with a female present, we a placed a flag in the moss clump behind each cluster of eggs and labeled the flag with the date and nest number using a permanent marker. Next, we recorded the habitat type (e.g., forest, powerline, or edge), moss species, and the ID of nearest nests from past years (< 2 m away). We then removed the female(s) from the petri dish and placed them between the base and lid of the petri dish and positioned vertically to expose the ventral pattern. We then photographed the nest flag, and the ventral pattern of the female to document each female with her nest. If a nest did not have a female present (but did have eggs), we processed the same way, excluding the morphometric measurements and ventral pattern.

Ventral spot matching.—We captured all ventral pattern images during the nesting season, using a Nikon Coolpix 8700 (Nikon Corporation, Tokyo, Japan; 2008–2012) and two Canon cameras: the PowerShot SX50 (Canon Inc., Tokyo, Japan; beginning in 2014) and PowerShot SX70 PowerShot HS (Canon Inc.; beginning in 2019). Although the imaging process was not fully standardized, we used nest flagging and waders to reduce distracting backgrounds. We organized the images by year and assigned a specific identification number based on the year and est number (e.g., 201574; nest number 74 in 2015). We manually identified 105 factorial combinations of potential matches of ventral color patterns at the Weir Dam site from 2009–2025 and six factorial combinations at the Bouton Tract site from 2022–2025. To maintain organization, we sorted images into folders corresponding to each potential match and imported them into the software one at a time for analysis.

We cropped each image using two reference points in a process known as chipping. We chose a point at the top left and bottom right corner of the image, and the software automatically drew a box based on the bounds of the two points selected (Fig. 2). HotSpotter analyzed only the contents within each box. Each comparison of a chipped image against the dataset created a query. For each query, HotSpotter suggested six potential matches, highlighted corresponding points between images (Fig. 2), and assigned a and assigned a score to reflect the strength of the match. We manually reviewed all six candidate matches and confirmed positive matches based on similarities in ventral spots patterns (Crall et al. 2013). Confirmed matches had a similarity score ranging from 690.1–144,780.5. During verification, we evaluated potential false-positive matches, which most resulted from busy background images (e.g., water droplets). Based on previous studies demonstrating high identification success using photo-matching software (Dunbar et al. 2021), we believed that the correct photo match would appear within the first three suggested matches generated by HotSpotter (starting at image zero).

Defining nest site fidelity.—To determine if female Four-toed Salamanders exhibit nest site fidelity not only to the same nesting site (small pool or ditch) but also to the same or adjacent moss clumps, we evaluated female presence at nests at the Weir Dam site from 2023–2025. We used the date and nest number written on all the flags corresponding to the previously assigned ID of each female. We divided the entire nesting area into four zones ranging from 1,058 to 3,780 m^2 (Fig. 1) based on distinct nesting clumps within the study site, and we measured the distance from the base of each flag to nests of known matches corresponding to the same female. We selected random nests from each zone that did not have a known match using a table of random numbers (Hayek 1994). We selected 26 random nests and 26 known-match nests, resulting in 52 nests for analysis in R version 4.3.2 (R Development Core Team 2023). We ran a Shapiro-Wilk Normality Test to assess the spread of the data. We used a nonparametric Wilcoxon Rank Sum Test to evaluate if the distance between nests of the same female differed significantly from nests assigned a random match. If re-nesting individuals nested closer together than random nests, this would indicate moss clump fidelity (Nagle and Russell 2020). To further assess nest site fidelity in specific nesting areas, we conducted a one-sided Wilcoxon Rank Sum Test for each of the four nesting zones, comparing distances between re-nesting females and random matches. We used $\alpha = 0.05$ for all statistical tests.

Results

Ventral spot matching.—Over the 15 y of data for the Weir Dam site, we recorded 1,450 nests of female Four-toed Salamanders. From this, we documented 1,252 nests (including both single and joint nests) with females present (Fig. 3) and used HotSpotter to identify 156 females that returned at least once (Fig. 3). For most photo matches, the correct match was ranked within the first three HotSpotter suggestions; however, two matches occurred at the sixth suggested match.

Of the 156 females that we observed returning at least once, 78 returned for two seasons and 45 for at least three (Fig. 3). We recorded 16 females over four nesting seasons and 11 over five nesting seasons. We identified four females returning for six nesting seasons. We successfully photo-matched one female over eight nesting seasons and another over nine seasons (Fig. 4). Several females exhibited gaps in detection with some nesting over a span of up to 9 y, although we did not observe them in every consecutive nesting season. The four-year dataset for the Bouton Tract site included 325 nests with 213 nests in which females were present. We documented five instances of females returning across nesting seasons (two females returning for two seasons and three females over three nonconsecutive nesting seasons between 2022–2025), representing a substantially lower rate of site fidelity compared to the Weir Dam site.

Nest site fidelity.—The distance between matched and random nests did not follow a normal distribution ($W = 0.81$, $P < 0.001$). Across all four nesting zones, re-nesting locations were significantly closer together than randomly paired nests (Fig. 5). The median distance between re-nesting locations was 1.55 ± (standard deviation) 3.40 m (range of values, 0.08–11.64 m) with seven nests (27%) located < 25 cm apart, whereas the median distance between random nests pairs was 12.10 ± 9.30 m (range of values, 0.03–34.93 m). Nests of the same individual re-nesting within the three-year period were significantly closer together than pairs of random nests ($W = 124.0$, $P < 0.001$). There were zones at the Weir Dam site where nest distances were significantly shorter in specific zones, although sample sizes varied among zones (Table 1). This comparison allowed us to identify how nest site fidelity varied among the four zones at the Weir Dam site. Distances between known re-nesting locations and randomly paired nests differed significantly in zones 2 and 3 but not in zones 1 and 4

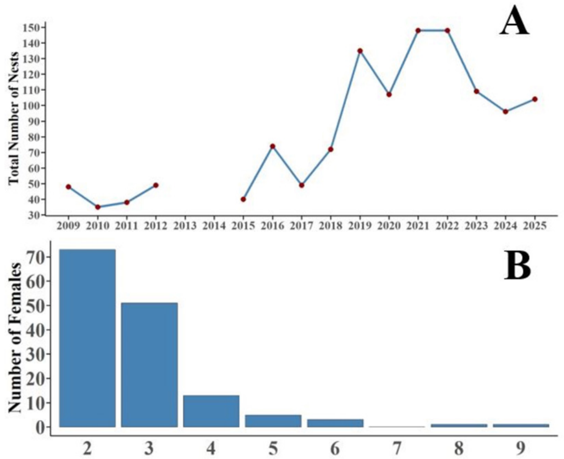

Figure 3. Long-term nesting patterns of female Four-toed Salamanders (*Hemidactylium scutatum*) at the Weir Dam site in Sullivan County, Tennessee, USA. (A) Number of female Four-toed Salamanders identified across multiple nesting seasons. Most females (73) were identified during only two nesting seasons. (B) Total number of nests with female Four-toed Salamanders present from 2009 to 2025, excluding 2013 and 2014.

(Table 1).

Discussion

Ventral spot matching.—Non-invasive identification methods such as HotSpotter have demonstrated high success in tracking a variety of amphibian species (Patel and Das 2020; Lukanov et al. 2024). The software is free, easy to use, and provides clear match outputs; however, researchers have not yet fully assessed its effectiveness for identifying individual salamanders. Our results demonstrate that HotSpotter is a reliable method for identifying female Four-toed Salamanders, even when spot patterns change slightly through time (Harris and Gill 1980; Harris and Ludwig 2004; Harris 2008). Because individuals were not independently validated with a permanent marking technique, however, we cannot completely exclude the possibility of misidentification, although we believe this likelihood is very low given the distinctiveness and long-term consistency of ventral spot patterns. Some correct matches were not ranked among the top suggestions, though, so we recommend manually reviewing all images. These findings highlight that photo-matching accuracy may vary among species and that identification outputs should be carefully evaluated.

Long term ecological datasets are rare because they require sustained sampling effort over many years, yet they are invaluable for understanding life-history traits, demographic processes, and behavioral patterns that cannot be detected through short-term studies (Lindenmayer et al. 2012; Cusser et al. 2021). The datasets used in this study represents a 15-y continuous monitoring effort at the Weir dam site and 4 y of monitoring at the Bouton Tract site, allowing for repeated identification of individual nesting females across multiple breeding seasons. Compared to the Weir Dam site, repeated observations of individual females at Bouton Tract were uncommon, with only a small proportion of the 213 documented females detected across multiple nesting seasons. Specifically, we identified two females returning for two nesting seasons and three females observed across three nonconsecutive nesting seasons from 2022–2025. This lower frequency of recapture

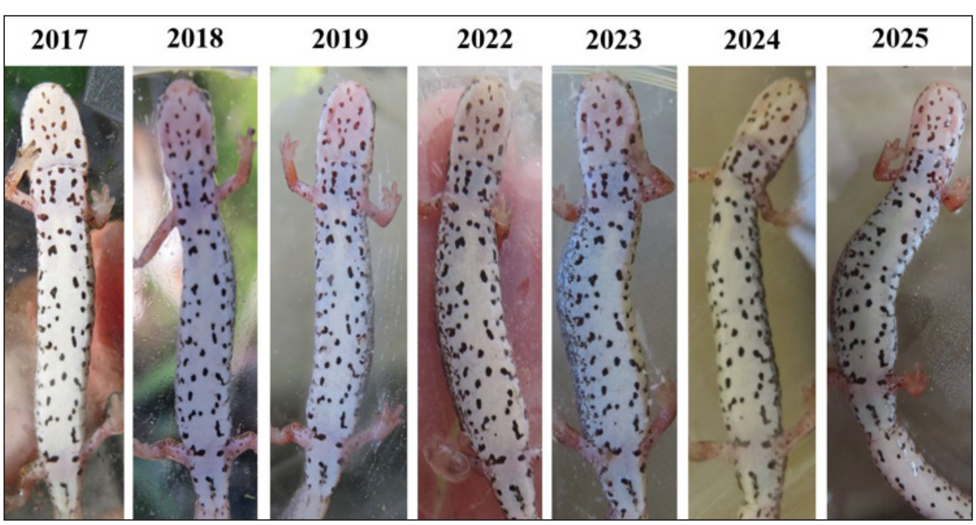

Figure 4. Photographs of the same female Four-toed Salamander (*Hemidactylium scutatum*) over seven nesting and 9 y from 2017 to 2025 at the Weir Dam site in Sullivan County, Tennessee, USA. (Photographed by Kalin Ferguson: 2022; 2023; 2024; 2025 and Kevin Hamed: 2017; 2018; 2019).

Table 1. Sample sizes (n; for renesting), random-pair distances in meters (MRPD), and median re-nesting in meters (MRD) of the Four-toed Salamander (*Hemidactylium scutatum*) within each of the four nesting zones at the Weir Dam site in Sullivan County, Tennessee, USA, and results of a one-sided Wilcoxon Rank Sum Test (*W*) and associated *P*-values.

Zone	n	MRD (m)	MRPD (m)	*W*	*P*-value
1	15	5.1	17	17	0.065
2	10	2.7	0	0	0.004
3	20	5.7	13	13	0.002
4	6	2.0	4	4	0.500

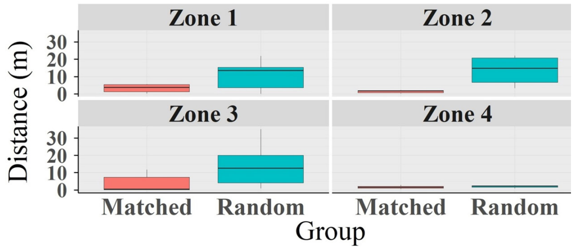

Figure 5. Median distances by group and zone between known re-nesting locations of individual female Four-toed Salamanders (*Hemidactylium scutatum*) and randomly paired nests within each of the four nesting zones at the Weir Dam site in Sullivan County, Tennessee, USA. Center line represents medians, whiskers extend to 1.5 x the interquartile range, and points beyond the whiskers represent outliers. Orange boxes are matched sites and blue boxes are random sites.

highlights the importance of long-term monitoring and individual identification methods, as repeated breeding records may represent only a small fraction of the population. By applying HotSpotter to this dataset, we conducted the first long-term assessment of individual female Four-toed Salamanders using their unique ventral pattern.

Hotspotter allowed us to identify females across multiple nesting seasons, enabling us to document reproductive longevity and nest site fidelity. These basic natural-history parameters improve our understanding of population dynamics and provide valuable information for conservation assessments of Four-toed Salamanders. The longest reproductive record we documented was a female that we observed over nine nesting seasons at the Weir Dam site from 2017 to 2025 (excluding 2020 and 2021). Given that Four-toed Salamanders reach sexual maturity at approximately 2 y of age (Blanchard and Blanchard 1931), this individual was at least 11 y old. To our knowledge, this record represents the longest documented lifespan of Four-toed Salamanders in the wild (Harris 2005).

It is important to note that various student volunteers assisted with adult surveys for both sites. This meant that while we conducted exhaustive searches, detection likely varied among seasons and sites. A few female salamanders could have evaded capture, affecting the number of females available for photo matching. To minimize missed detections, we revisited nests with eggs each season to recheck for any escaped females, and multiple searchers surveyed moss clumps where no nests were initially found. Given the geography of the Weir Dam rivers system and surrounding topography restricting dispersal, we are confident that these efforts accounted for all present individuals (Herman 2013).

Female Four-toed Salamanders may skip nesting years when inadequate foraging limits the energy available for egg production and oviposition, or when environmental conditions suggest low egg survival (Harris and Ludwig 2004). At the Weir Dam site, we identified a female across eight potential nesting seasons from 2018–2025 yet documented her in only two of those years. This individual may have contributed to a joint nest in previous years and, if in poor condition, may have abandoned her eggs after oviposition, leaving them to be guarded by the fittest female (Harris 2008).

Nest site fidelity.—The second goal of our study was to determine the fidelity of Four-toed Salamanders to specific locations within the Weir Dam site by examining whether females return to the same moss clump within their nesting area to lay their eggs. Of the 1,252 females found at the Weir Dam site, we documented 11.7% in more than one nesting season. This small percentage of returning females is consistent with high adult mortality overall. Predation of nests and presumably the guarding female by mesopredators like racoons is common (Ferguson and Hamed 2024); however, for the females that survived to re-nest, our study provides evidence of significant nest site fidelity. Harris and Ludwig (2004) documented females returning to the same larger nesting ponds (37 and 45 m circumference) at their study sites but only investigated pond site fidelity rather than on a smaller scale. In their study, limited nesting options may have contributed to high annual return rates, as females had few preferred sites available. In contrast, the Weir Dam site contained more than 500 potential nesting pools (< 2.5 m circumference) and numerous moss clumps suitable for nesting, yet females consistently returned to the same or adjacent moss clump within the site, showcasing high nest site fidelity even when vast nesting options were present. Our study therefore expands the definition of nest site fidelity beyond large nesting pools and provides evidence of fine-scale microhabitat selection across nesting and breeding seasons.

The habitat characteristics and spatial features of the Weir Dam likely contribute to animals returning to the same zones. The site is bordered by the South Holston River and surrounding upland habitat, both of which can limit dispersal. Rivers have been shown to act as barriers to Four-toed Salamander movement (Herman and Bouzat 2016), and adult females typically remain within a few hundred meters of wetland habitat outside the nesting season (Herman 2013; Vitale 2013). Similar barriers to movement have been documented in other salamander species, where habitat alteration from timber harvesting and road construction reduces dispersal and population connectivity (Semlitsch et al. 2008, 2012). We searched all available ditches and pools within the site for potential nests. Given the natural boundaries of the river and uplands, we are confident that our surveys were comprehensive and that few nesting females were overlooked.

In our examination of re-nesting location at finer scales, we found that females re-nested at moss clumps that are closer together than expected by chance in zones 2 and 3. The distribution of distances between known female nest matches and randomly assigned nests illustrates this pattern, which matched nests generally occurring at shorter distances than random comparisons at the Weir Dam site; however, the strength of this relationship varied among nesting zones. The four nesting zones differed in habitat characteristics, available nesting habitat, and sample sizes, which likely influenced our ability to detect significant patterns of fine-scale site fidelity. Zones 1 (3,490 m^2) and 2 (3,780 m^2) occurred beneath forest canopy, whereas zones 3 (3,328 m^2) and 4 (1,058 m^2) were located along forest edge. Zone 4 was the smallest nesting area and contained fewer available moss clumps, resulting in fewer documented re-nesting females and reduced statistical power. Therefore, the lack of a significant relationship in some zones may reflect limited sample sizes rather than an absence of fine-scale nest site fidelity.

In addition to habitat structure and sample size, predation pressure may influence patterns of fine-scale nest site fidelity. The Weir Dam site experiences high levels of nest predation (Ferguson and Hamed 2024), particularly at moss growing in edge habitat, which may influence fine-scale nest site fidelity, as predation events can render moss unsuitable for future nesting. We expected that female Four-Toed Salamanders would demonstrate nest site fidelity at a finer scale than previously reported by returning to the same of nearby moss clump to lay their eggs. Our results support this expectation, showing that females can return not only to the same wetland but also to specific nesting areas within the wetland.

Management implications.—Long-term datasets provide critical insight into preferred nesting grounds for a species, and into the characteristics that make them suitable for raising their young. Our study produced one of the most extensive long-term datasets on Four-toed Salamanders. We document two females, estimated to be 10 and 11 y old, that returned to the same nesting area to lay their eggs. By examining a subsample of females re-nesting over a shorter period, we determined that nesting site fidelity was significant within the wetland.

Our findings further underscore the critical importance of protecting nesting habitat for this species, given its conservation status throughout much of its geographical range. As climate change continues to influence seasonal precipitation, the protection of small vernal pools and ditches has become increasingly urgent, as these changes can significantly affect population dynamics over time (Walls et al. 2013). Despite the ecological importance of small vernal pools and ditches, they are often excluded from federal and state protection plans due to their small size and limited connectivity (Evans et al. 2017). Our findings help clarify the strong nesting preferences and patchy distribution of Four-toed Salamanders, both of which raise conservation concerns (Vitale 2013).

Moss species, such as *C. americanum,* create preferred nesting habitat for female Four-toed Salamanders and provide moist conditions for their eggs until hatching. Females prefer wetlands with higher amounts of moss and moss presence is correlated with breeding success (Wahl et al. 2008; King and Richter 2022). Predation events by animals, such as raccoons, can reduce available nesting habitat by creating gaps in moss clumps, which may limit nesting opportunities in subsequent seasons (Ferguson and Hamed 2024). Illegal moss harvesting in the Appalachians is also a concern, as commercial demand exceeds regrowth rates (Muir et al. 2006). Unregulated moss collection has been speculated to serve as a threat to Four-toed Salamander populations (Mitchell and Gibbons 2010; Petranka 1998). Our work shows that even small illegal collection attempts could disrupt future nesting attempts for female salamanders that exhibit extreme site fidelity. Although *C. americanum* can be cultivated under controlled conditions, which makes it a promising candidate for habitat restoration (Hilt et al. 2025), moss regeneration requires substantial time in both the laboratory and in the field (Martin 2015). This regeneration period represents a critical window during which even small-scale habitat loss may influence nest site fidelity and could disrupt successful nesting.

Given that wetlands with shorter hydroperiods contribute equally to species richness (Vitale 2013), managers should consider hydroperiod variability in federal wetland regulations (Snodgrass et al. 2000). Our results aligned with previous studies (Harris and Gill 1980; Chalmers 2004; Harris and Ludwig 2004; Harris 2008; Hamed 2014) indicating that, like many amphibians, this species is habitual and often returns to the same nesting areas, even to the specific clump of moss, each season. Evidence of nest site fidelity suggests that wetland protection is critical for population persistence; however, the essential habitat for this species extends beyond the vernal pools themselves. The surrounding forests of wetlands also play a vital role in maintaining the health of vernal pools and supporting species that return to the same area year and year. Tree canopy provides shade that regulates water and moss temperatures, which are important for Four-toed Salamander egg and larval development (Blanchard 1923). After providing parental care to the eggs, females return to the upland area, emphasizing the need to protect aquatic and terrestrial habitat (Petranka 1998). Enhancing forested buffer zones around vernal pool systems effectively reduces disturbances, such as logging, and supports overall biodiversity. Buffer size depends on species movement patterns and the proximity to nearby pools (Herrmann et al. 2005; Vitale 2013, Evans et al. 2017; Romano 2017). Evans et al. (2017) report that most vernal pools within the Cumberland Plateau occur on private land. Implementing state incentive programs for private landowners could promote conservation of critical wetland habitats.

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