# The Influence of Weather on the Reproductive Behavior and Population Trends of Four-toed Salamanders (*Hemidactylium scutatum*)

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science

In

Fisheries and Wildlife Sciences

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March 20, 2026

Blacksburg, VA

Keywords: Plethodontid, Site Fidelity, Climate Change, Population Trends, Weather Patterns, Appalachians

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## ABSTRACT (ACADEMIC)

Environmental conditions influence amphibian reproduction, behavior, and development. Understanding these relationships is critical for developing effective management strategies to conserve populations and their habitats. However, knowledge gaps persist for many species, limiting our ability to predict responses to increasingly variable seasonal conditions driven by climate change. The first objective of this study was to further explain nest site fidelity in female four-toed salamanders (*Hemidactylium scutatum*) using photo-identification software (Hotspotter) to analyze individual ventral spot patterns. The second objective was to evaluate how seasonal weather patterns influenced the species' population abundance and trends over time at one site in Tennessee. I hypothesized that Hotspotter would accurately identify returning females and that females would exhibit nest site fidelity by returning to the same nesting area across multiple breeding seasons. To assess site fidelity, I used a Wilcoxon rank-sum test to compare re-nesting distances among individuals. To evaluate the effects of climate on reproduction, I examined the influence of daily maximum temperature, daily precipitation, and daily relative humidity on average clutch size and total annual nest abundance. Immediate and lagged climate effects were analyzed using a sliding window approach (1-48 months prior to nesting) within the R package *climwin*. I hypothesized that females would produce smaller clutches following unfavorable pre-nesting conditions and that the population would exhibit a recovery period of approximately two years. Results supported the site fidelity hypothesis, demonstrating that female-four-toed salamanders consistently rerun to the same or adjacent moss clump for oviposition. Climate analyses revealed that mid-fall to early-winter precipitation and early spring temperature in the year prior to nesting were the primary drivers of variation in clutch size, while only precipitation significantly influenced total annual nests. Our findings highlight the importance of long-term data for addressing knowledge gaps in nesting behavior and emphasize the need to protect critical habitat while continuing to collect data to better understand how four-toed salamanders may response to future environmental changes.

# The Influence of Weather on the Reproductive Behavior and Population Trends of Four-toed Salamanders (*Hemidactylium scutatum*)

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## ABSTRACT (GENERAL AUDIENCE)

Amphibians are especially sensitive to changes in their environment, making them important indicators of ecosystem health. Understanding basic aspects of their life history, such as how long they live, where they lay eggs, and how many offspring they produce is essential for effective conservation, yet there are several knowledge gaps about this information for many species. In this study, I examined the nesting behavior and reproduction in female four-toed salamanders (*Hemidactylium scutatum*) to better under how environmental conditions influence their populations. Using photographs of unique belly spot patterns, I tracked individual females with photo-identification software to determine whether they returned to the same nesting locations across multiple breeding seasons. I also examined how seasonal weather conditions, including temperature, rainfall, and humidity, affected the number of eggs females produced and the total number of nests each year. Results showed that female four-toed salamanders consistently returned to the same or adjacent moss clumps to lay their eggs, indicating a strong preference for the same nesting locations each year. The number of eggs laid by females depended on the amount of precipitation during the mid fall to early winter and temperature in early spring before nesting occurred. The total number of nests was influenced by only precipitation. These findings highlight the importance of the timing of seasonal weather conditions for four-toed salamander reproduction and emphasize that habitat management is critical for conserving their nesting habitat.

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# Acknowledgements

When I began my master's degree in 2023, I never anticipated the extent of the personal, academic, and professional growth I would experience, or the incredible people I would meet along the way. I am the person and student I am today because of the guidance, influence, and support of my outstanding mentors, friends, and family. Thank you for your unwavering support, patience, and encouragement over the past three years. Without these individuals, I would not have been able to accomplish my goals.

I would first like to thank my co-advisors for their endless support beginning with my time as an undergraduate student. My passion for four-toed salamanders and wetland conservation would not exist without Dr. Kevin Hamed, whose mentorship over the past eight years has been truly invaluable to my development as a researcher. I never would have imagined how that chilly, rainy night at the Weir Dam in 2019 would influence not only my academic path, but also the profound impact Kevin would have on my life. He has always been there when I needed him, whether helping me navigate classes in undergrad, finding housing for graduate school, offering guidance on career decisions, or providing thoughtful life advice throughout both my undergrad and graduate experiences. His constant encouragement, reassurance, and dedication as a researcher and as a person have meant more to me than words can express. I am deeply grateful for his unwavering belief in me, especially during moments of self-doubt.

Thank you, Dr. Holly Kindsvater, for taking a chance on me and welcoming me into your lab as a new transfer student in 2020. You taught me to believe in myself as a woman in natural resources and to advocate for myself with confidence, even when I doubted my own abilities. Your regular check-ins and goal setting through the "Three Buckets" framework kept me grounded and motivated, while pushing me to challenge myself, take initiative, and grow beyond what I thought I was capable of. Your mentorship has had a lasting impact on my confidence, independence, and sense of purpose, and I will carry those lessons with me as I continue my professional journey. I would also like to thank the rest of the Kindsvater lab for their feedback on manuscripts, conference posters, and presentations. Finally, I thank my committee member, Dr. Elizabeth Hunter, for her kindness and patience during our *climwin* meetings, as well as for the thoughtful questions that pushed me to think outside the box during my statistical analyses.

To the Mentorship & Academic Outreach Program (MAOP), your support, sense of community, and unwavering belief in me have shaped both my academic journey and personal growth. Thank you for welcoming me into the MAOP family and for funding my graduate assistantship; without your support, graduate school would not have been possible. Working with MAOP sparked an interest in me that I did not know I had and helped me clarify my interest in academic advising and student support. MAOP often says, "MAOP is family," and I have truly experienced that through the guidance and of Monica Hunter and Laila Kirkpatrick. They believed in me, my ability to support students, and in my potential to succeed as a professional in higher education.

I would also like to thank my friends (Austin Holloway, Olivia Madigan, Sarah Vollbrecht, Nathan Lucas, Tanner Humbert, and Ryan Adkins) for their constant positivity, laughter, and countless memories. Your presence made this journey more joyful and helped me stay focused during challenging times. I am grateful for your friendship and the encouragement you have given me over the years.

I must thank my family for without their encouragement and support I could not have completed this journey. I am grateful to my in-laws Darrell and Darlene Ferguson, for their ongoing support and encouragement. Your belief in me has meant so much, and your love and support throughout my education are greatly appreciated. I want to thank my mom, Wendy Davis, for always believing in me and reminding me that I could do it and tackle any challenge, no matter how big or small. Thank you for helping me grow as a person and for instilling in me the values of hard work, perseverance, and integrity in both life and my career. I am so grateful to call you my mom and will always carry your love and guidance with me.

Lastly, I must thank my husband of three years, Nathan, for being by my side through every long field day, stressful weeks, and every moment of self-doubt. I am endlessly grateful to have had you beside me through it all. Your love, support, and belief in me have made this achievement possible. Thank you for being my unpaid field technician in 90° weather, recording data on hundreds of salamanders, and for always showing up, even when you had didn’t have to. You celebrated every small victory, lifted me up when I felt discouraged, and reminded me to get out of my own head and trust myself when doubt set in. Thank you for making me laugh when I felt overwhelmed and for being my constant source of strength, comfort, and happiness throughout this journey.

## Introduction

Since the 1700s, an estimated 840 million acres of wetland habitat have been lost globally (Fluet-Chouinard et al. 2023). The United States has lost over half of its wetlands between the late 1700s and late 1980s (Asselen et al. 2013). This extensive loss is attributed to the high agricultural and forestry value of wetlands, as their soils are comprised of rich organic matter and nutrients, making them ideal for land conversion (Asselen et al. 2013; Fluet-Chouinard et al. 2023). Government programs initiated in the 1950s further accelerated wetland degradation, particularly in North America, China, and Europe, by promoting land conversion for agriculture, timber production, and urban development to accommodate growing populations (Asselen et al. 2013; Fluet-Chouinard et al. 2023). In recent decades, the importance of protecting wetlands has gained widespread recognition due to the services they provide (Xu etal. 2019). They enhance water quality by filtering out toxins and sediments, offer ecotourism opportunities for local communities, and play a vital role in flood control and storm protection (Jisha and Puthur 2022). Most importantly, wetlands are biodiversity hotspots and support a wide range of invertebrate and vertebrate species (Jisha and Puthur 2022). The Southern Appalachians in the United States are a prime example of how wetlands sustain biodiversity, including endemic and indicator species.

The Southern Appalachian Mountains are a biodiversity hotspot for many flora and fauna species. Many of its inhabitants, such as amphibians, are endemic and thrive in various microhabitat ecosystems created by elevational gradients and weather conditions (Walls 2014; Gade and Peterman 2019). Amphibians are often a good indicator of ecosystem health as they are found in both terrestrial and aquatic environments (Hopkins 2007). They serve as predators and prey to many species within an ecosystem and are essential in transferring energy throughout food webs (Sánchez-Hernández 2020). However, amphibians are at a greater risk of population declines than many vertebrates since many species breed and lay eggs in wetland systems containing ephemeral pools (Hopkins 2007). These pools are temporary and rely on seasonal rainfall for replenishment, yet they support more biodiversity than permanent ponds (Hopkins 2007; Walls et al. 2014).

Among the Southern Appalachians' rich amphibian diversity, it is essential to highlight the salamander family, Plethodontidae, which accounts for the majority of the salamander biodiversity in the region (Kozak 2017). The family Plethodontidae is the largest and most diverse among the nine families of salamanders, comprising approximately 70% of the world's salamander species, with eight genera endemic to the eastern United States (Herman and Bouzat 2016; Kozak 2017). Plethodontids are lungless salamanders that prefer moist and cool environments to carry out cutaneous respiration, making them particularly sensitive to changes in precipitation and temperature (Milanovich et al. 2010; Kozak 2017). Plethodontids are excellent model group to study to reveal how environmental changes impact populations over time, as their phylogeny reveals their ability to withstand major interglacial periods with conditions similar to today (Kozak 2017). As climate change continues to reshape wetland ecosystems, understanding a species' life history is critical for predicting their response and susceptibility.

Life history traits offer insight into how a species optimizes its fitness and influences population growth despite environmental pressures (Stearns 2000). These traits include age and size at sexual maturity, clutch size, lifespan, and adult body size (Stearns 2000). Accounting for life history is vital in establishing management practices for species vulnerable to extinction. The four-toed salamander (*Hemidactylium scutatum*) is an ideal study species for examining life history traits, as there are still notable knowledge gaps in their nesting behavior and population dynamics. To address these gaps, my study focused on nest site fidelity and the effects of weather patterns on population trends, using a long-term dataset. Examining these factors offers valuable insight into the phenological windows that shape biological activities (e.g., egg-laying and feeding) of four-toed salamanders and provide key information about how wetlands play a key role in their persistence in the Southern Appalachian Mountains.

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# Chapter 1: Evidence of Site Fidelity in Four-toed Salamanders Using Photo Identification and Nesting Data

## Abstract

Site fidelity refers to an animal's tendency to return to the same location to breed, nest, or forage. Understanding how site fidelity varies with landscape features or weather conditions can inform management decisions aimed at protecting the ecosystem and its inhabitants. The method for tracking returning individuals depends on the species and size of the animal, as well as the distance it travels. Some tracking methods are more invasive than others, which is why new non-invasive techniques, such as pattern identification software, are used to recognize individuals based on their unique markings. The objective of the study 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 site fidelity. To achieve this, we assessed a long-term dataset (15-year dataset) comprised of the ventral images of nesting females and analyzed them using Hotspotter photo identification software. We also used a subsample of our long-term data to analyze site fidelity by measuring the distance of nests of known matches of the same female between years and nests assigned a random match using a nonparametric Wilcoxon rank sum test. Hotspotter successfully photo-matched nesting females using their spot pattern, and the distances between known matches were significantly localized within their nesting areas. Our findings confirm that females return to the same nesting areas year after year. These results reinforce the importance of gathering data to establish long-term datasets on the desired nesting habitat for amphibians with conservation needs.

# Introduction

Site fidelity is defined when an animal returns to the same place it previously utilized for foraging, mating, or hibernation, a behavior observed across several taxa (Switzer 1993). Migratory birds exhibit site fidelity as individuals return to the same seasonal breeding grounds (Greenwood 1980). American black bears (*Ursus americanus*) establish home ranges and revisit various locations within their home range based on food availability and breeding opportunities with limited competition (Rogers 1987). Reptiles (e.g., loggerhead sea turtles [*Caretta caretta*]) exhibit site fidelity as they often use the same seasonal migration route to reach the best foraging grounds and their breeding territory. Nesting females also return to their natal areas to lay eggs (Evans et al. 2019). This behavior also applies to amphibians (e.g., marbled salamanders [*Ambystoma opcacum*]), which return annually to their natal pool to breed (Gamble et al. 2007).

Areas where individuals present stronger site fidelity usually provide the highest quality of life to the individual and their offspring (Switzer 1993). However, environmental conditions can negatively affect the chance an individual returns to the same site by decreasing the habitat's overall quality or the number of surviving offspring. Tracking individuals within a population provides insight into how they respond to environmental changes and if they migrate to a more suitable habitat. The methods to track individuals vary by animal. For migratory birds, leg bands with distinct ID numbers and various types of signal transmitters are used in the field based on the size of the bird and the distance of the journey (Guilford et al. 2011). Larger mammals (e.g., American black bears and jaguars (*Panthera onca*) are equipped with radio or GPS collars to observe daily activities and the reason they return to precise locations within their home ranges (Bowersock et al. 2015; Pereira et al. 2022). For amphibians, common mark-recapture methods include PIT tagging, toe clipping, and nail clipping (Ferner 2007). These methods also work for reptilian species, in addition to the following: pattern mapping, painting, shell notching, and scale clipping (Ferner 2007). Drift fences and pitfall traps are also mechanisms used to catch individuals as they travel to and from their seasonal breeding grounds to evaluate if any individuals have been tagged or marked (Gamble et al. 2007).

Individual identification through pattern recognition software has become prevalent due to the feasibility of analyzing more images and individuals in a shorter time frame (Nipko et al. 2020). Using software to recognize individuals based on their unique markings is also less invasive than traditional recapture methods (Suriyamongkol and Mali 2018). In this method, 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). Six commonly used photo identification software programs include APHIS, I3S, Wild-ID, Amphident, Hotspotter, and IBEIS (Wildlife AI 2020). Many studies have been successful in using these programs to identify individuals. Suriyamongkol and Mali (2018) used Wild-ID, APHIS, and I3S Pattern+ to identify individual Rio Grande Cooters (*Pseudemys gorzugi*). They found Wild-ID to be the most effective in distinguishing plastron patterns and size. Dunbar et al. (2021) used Hotspotter to recapture hawksbill sea turtles (*Eretmochelys imbricata*) based on their dorsal head pattern. They found that Hotspotter had higher match accuracy in variable conditions than other studies using I3S for hawksbill turtles. Nipko et al. (2020) compared Hotspotter and Wild-ID to track jaguars and ocelots (*Leopardus pardalis*) in Belize. The authors found Hotspotter to be the most efficient and effective for both species. Kakegawa and Hasumi (2024) investigated using WildID to observe Hida salamanders (*Hynobius kimurae*) over a 16-year period based on their dorsal pattern. They found that WildID could easily distinguish individuals, even when females were gravid. The success of photo identification across diverse species demonstrates its effectiveness in recognizing individuals from images. Four-toed salamanders

(*Hemidactylium scutatum*) have distinguishing spot patterns on their underbelly (Harris and Gill 1980; Harris and Ludwig 2004). Tracking individuals with photo identification software is a unique opportunity to add to knowledge of their longevity and reproductive behavior.

Four-toed salamanders (Figure 1) are a distinctive member of the family Plethodontidae, representing the only species within its genus. The species has a broad, discontinuous range extending from southeastern Canada through the eastern United States to Florida, and westward to Oklahoma – the greatest geographic range of any plethodontid species (Herman and Bouzat 2016). Despite this wide distribution, the species has a recognized conservation status in 17 of the 29 states within its U.S. range (Ferguson and Hamed 2024). Compared to other plethodontids, four-toed salamanders have shorter larval periods and distinctive nesting preferences. Females migrate from upland forests to breeding sites in the fall and move to their nesting grounds in early spring (Petranka 1998). During this time, females will lay their eggs on the steep edges of moss clumps (e.g., *Sphagnum* and *Climacium spp.*) above slow-moving or stagnant water within forested vernal pools, bogs, and swamps (Gilbert 1941; Wood 1955; Petranka 1998; King and Richter 2022). A single female may nest in a moss clump, or multiple females may nest jointly in the same location with the fittest female providing parental care throughout the nesting season (Blanchard 1934).

Four-toed salamanders can easily be identified by the rusty coloration on their dorsal and solid white with small black spots on their ventral side. They are the only salamander in the United States with this ventral coloring (Herman 2013). In previous studies, individual females have been recognized in the field based on their ventral pattern to assess site fidelity and nesting behavior (Harris and Gill 1980; Harris and Ludwig 2004; Harris 2008; Hamed 2014). Given the success of Hotspotter in other studies, we chose Hotspotter for our analysis to evaluate nest site fidelity of nesting female four-toed salamanders over a 15-year period. We hypothesized that Hotspotter would correctly identify returning females with sufficient reliability and that females would display nest site fidelity by returning to the same or nearby moss clump across nesting seasons.

## Methods

### *Surveys for Adults*

Annual surveys of four-toed salamander nests were initiated by MKH at the Weir Dam site in 2008, and KJF and MKH began similar surveys at the Bouton Tract site in 2022. Nest surveys took place over a one-month period from the last week in March to the last week in April from 2009 to 2025 at the Weir Dam site, and from 2022 and 2025 at the Bouton Tract site. Surveys were not conducted in 2013 and 2014 at the Weir Dam site. Additionally, due to COVID-19 pandemic, Weir Dam surveys were incomplete in 2020. Surveys at each site were exhaustive, in that we tried to identify all nesting females at each year at each site. We systematically 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. Since females often nest jointly, all sides of the moss clumps were thoroughly examined to ensure all possible individuals were captured. Each female was placed in an individual clear plastic petri dish and temporarily set aside. If females were dropped or escaped during capture, we returned to the nest later in the day to allow time for their return.

To process a nest with a female present, we placed flags in the moss clump behind each cluster of eggs and labeled the nest with the date and nest number using a permanent marker. Next, we recorded the habitat type (e.g., forest, powerline, or edge), moss type, and the ID of nearest nests from past years. 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. Next, we photographed the nest flag, followed by a photo of the female salamander's ventral pattern, to organize which female belonged to the correct nest. We processed nests that did not have a female present (but did have eggs) the same way, excluding the morphometric measurements and ventral pattern.

### *Ventral Spot Matching*

We captured all ventral pattern images during the nesting season from (mid-March to late April), using a Nikon Coolpix 8700 (2008–2012) and two Canon cameras: the Canon SX50 (beginning in 2014) and SX70 PowerShot HS (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 gave them a specific ID based on the year and nest number (e.g., 201574; nest number 74 in 2015). We identified 105 factorial combinations of potential photo matches at the Weir Dam site from 2009 to 2025 and six factorial combinations at the Bouton Tract site from 2022 to 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 a box would automatically be drawn based on the bounds of the two points selected (Figure 3). Only the contents within the box were analyzed in Hotspotter. Each comparison of a chipped image against the dataset was known as a query. For each query, Hotspotter suggested six potential matches and assigned a score to reflect the strength of the match. We reviewed all six suggested images manually to determine whether any represented a correct match.

### *Defining Site Fidelity*

To determine if female four-toed salamanders exhibit site fidelity not only to the same nesting site but also to the same or nearby moss clumps, we evaluated female presence at nests at the Weir Dam site from 2023 to 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 (Figure 4) based on distinct nesting areas within the study site and 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). In total, 26 random nests were selected and 26 known-match nests, resulting in 52 nests for analysis. 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 nests of individuals that re-nested within this period are closer than the average distance between a pair of random nests, it is evidence that re-nesting individuals exhibit moss clump fidelity (Nagle and Russell 2020). To further assess 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.

## Results

### *Ventral Spot Matching*

Over the 15 years 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 (Figure 5) and used Hotspotter to identify 156 females that returned over the nesting period (Figure 6). Each photo match analysis circled the spots that match each corresponding photo. When there was a positive correlation between the two images, the image was illuminated by the circles and given a score (Figure 7). A score between 690.1 to 144,780.5 indicated a match. We checked for false positive matches and determined that they occurred when images contained busy backgrounds (e.g., water droplets).

Of the 156 females that were observed returning at least once, 78 returned for two seasons and 45 for at least three (Figure 6). We discovered 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 (Figure 8). Several females exhibited gaps in detection with some nesting over a span of up to nine years, although they were not observed in every consecutive nesting season. The four-year dataset for the Bouton Tract site included 325 nests total nests with 213 present females. Two females were documented returning for two seasons and three were documented over three nonconsecutive nesting seasons between 2023 and 2025.

We hypothesized that the correct photo match would appear within the first three photo suggestions in Hotspotter (starting at image zero). However, there were two instances in which the correct match corresponded to the sixth suggestion. These results highlight the importance of manually reviewing all six image recommendations, as the top three are not always accurate. Nevertheless, the fact that most matches were in the top three suggestions indicates this is a reliable method of identifying females, even when spot patterns change slightly through time.

### *Site Fidelity*

We compared the distances between nests of known matches with the same female and nests with randomly assigned matches within four nesting zones at the Weir Dam site to evaluate whether female Four-toed Salamanders exhibited nest site fidelity by returning to the same moss clump for multiple years. The Shapiro-Wilk test indicated that the distance between matched and random nests did not follow a normal distribution ($P = 1.227 \times 10^{-6}$). The median distance between re-nesting locations was 1.55 ± 3.40 m (0.08–11.64 m) with 42% less than 1 m apart and 27% less than 25 cm a part. The median distance between random nests pairs was 12.10 ± 9.30 m (0.03–34.93 m). The Wilcoxon rank sum test showed that nests of the same individual re-nesting within the three-year period were closer together than pairs of random nests ($P = 4.662 \times 10^{-5}$). The one-sided Wilcoxon rank sum test identified zones at the Weir Dam site where nest distances were significantly shorter in specific zones, suggesting higher moss clump fidelity in those areas (Table 1). This comparison allowed us to identify how nest site fidelity varied among the four zones at the Weir Dam site. We found zones 2 ($P = 0.0039$) and 3 ($P = 0.0019$) to be statistically significant, while zones 1 and 4 did not reach significance (Fig. 3).

## Discussion

### *Ventral Spot Matching*

Non-invasive identification methods such as Hotspotter have shown 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 clear provides clear match outputs. However, its effectiveness for identifying individual salamanders has yet to be fully assessed. Our study was the first to apply Hotspotter to recognize nesting female four-toed salamanders based on their unique ventral pattern. The longest reproductive record we documented was a female 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 two years of age (Blanchard and Blanchard 1931), this individual is estimated to be at least 11 years old. To our knowledge, this 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. Some females in the field could have been overlooked, dropped, or escaped before students could find them, impacting the number of present females to photo match. To minimize missed detections, we revisited nests with eggs to recheck for any escaped females, and multiple searchers surveyed moss clumps where no nests were initially found. 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 to 2025, yet she was only documented in those two years. This individual may have contributed to joint nests 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).

### *Site Fidelity for Four-toed Salamanders*

The second goal of this 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 during the nesting season. 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.

However, for the females that survived to re-nest, our study provides evidence for significant nest site fidelity. Individuals consistently returned to the same nesting area each year, demonstrating strong site fidelity. Similar patterns have been reported in other pond-breeding salamanders, including ambystomatids such as spotted and marbled salamanders (Whiteford and Vinegar 1966; Gamble et al. 2007). In marbled salamanders, both first-time and experienced breeders exhibited high site fidelity to their natal breeding ponds, with return rates exceeding 90% (Gamble et al. 2007). Similarly, a mark recapture study, of spotted salamanders found that 86% of individuals returned for one breeding season, and nearly 77% returned across three breeding seasons (Whiteford and Vinegar 1966).

The habitat characteristics and spatial features of the Weir Dam likely contribute to nest site fidelity. The site is bordered by the South Holston River and upland habitat, both of which restrict dispersal. Rivers serve as a barrier to four-toed salamander movement (Herman and Bouzat 2016), while the surrounding upland areas, where adult females reside outside of the nesting period, may also limit movement, as four-toed salamanders are typically found within a few hundred meters from wetland habitat (Herman 2013; Vitale 2013). We systematically 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 survey as comprehensive and that few, if any, nesting females were overlooked.

In our examination of re-nesting location at finer scales, we found that females re-nest at moss clumps that are closer together that we would expect by chance in Zones 2 and 3. The four nesting zones at the Weir Dam site varied in habitat type and size. Nests in zones 1 and 2 were inside the forest canopy, while nests in Zones 3 and 4 were along the edge of the forest. Zone 4 was the smallest in size among the others and had limited nesting opportunities (King and

Ritcher 2022). Variation in the statistical support for fine-scale nest site fidelity among zones suggests that other factors besides habitat influence site fidelity among four-toed salamanders. The Weir Dam site is known to experience high levels of nest predation (Ferguson and Hamed 2024), which may influence finer-scale nest site fidelity, as moss clumps could become unsuitable for nesting due to damage from previous predation events. However, overall, our results support our hypothesis that female four-toed salamanders returning to the same wetland can and do return to the same or nearby moss clump to lay their eggs.

### *Management Implications*

Long-term datasets provide critical insight into species' preferred nesting grounds and 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. As a result, documented two females that were 10 and 11 years old returning 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. Females re-nested at distances 0.08–11.64 m apart (mean = 2.70 ± 3.35 m), with 27% returning within 25 cm indicating repeated use of the same moss tussock.

Our findings further underscore the critical importance of protecting nesting habitat for this species, given its conservation status in much of its geographical range. As climate change continues to influence seasonal precipitation, the protection of vernal pools (< 2 m in diameter) 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 management 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). Four-toed salamanders, which are semi-aquatic, rely on these ephemeral wetlands to complete their life cycle, nesting in the moss that surrounds the pools (King and Ritcher 2022). Given that wetlands with shorter hydroperiods have been shown to contribute equally to species richness (Vitale 2013), hydroperiod variability should be considered 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 each season. Evidence of site fidelity suggests that wetland protection is critical for population persistence, as females may not readily adapt to reproducing in new areas. 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, which helps regulate water and moss temperatures, both 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 is an effective way to reduce disturbances such as loggings and to support overall biodiversity. The size of the buffer depends on the movement patterns of the species and the proximity to nearby pools (Herrmann et al. 2005; Vitale 2013, Evans et al. 2017; Romano 2017). Evans et al. (2017) reported that most vernal pools within the Cumberland Plateau occur on private land. Implementing state incentive programs for private landowners could assist in promoting the conservation of critical wetland habitats, as those used by four-toed salamanders.

# Chapter 1 Tables

| | Zone | W | p_value |
|---|---|---|---|
| Zone1 | Zone1 | 17 | 0.065190365 |
| Zone2 | Zone2 | 0 | 0.003968254 |
| Zone3 | Zone3 | 13 | 0.001943103 |
| Zone4 | Zone4 | 4 | 0.500000000 |

Table 1: One-sided Wilcoxon rank sum test reveals zones 2 and 3 are significant when examining the distance of known matches nests is shorter than random nests. In the table, “Zone” represents the four different nesting zones at the Weir Dam site, “W” signifies the test statistic, and “p_value” indicates statistical measurement .

# Chapter 1 Figures

Figure 1: A female four-toed salamander (*Hemidactylium scutatum*) from the Weir Dam study site, Sullivan Co., TN. Photo credit: Charlie Holguin.

Figure 2: Aerial imagery of four-toed salamander (*Hemidactylium scutatum*) study sites in Sullivan County, Tennessee. The study sites are noted with red rectangles.

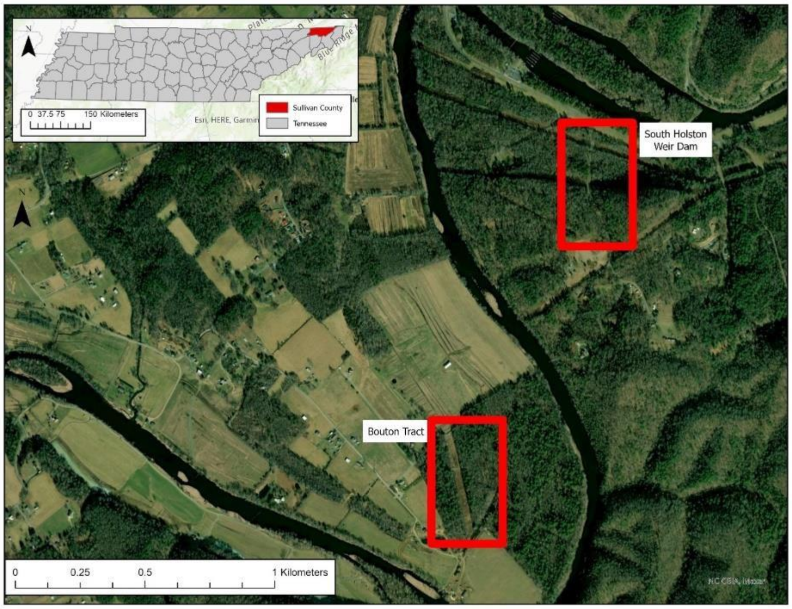


Figure 3: Photo of female four-toed salamander (*Hemidactylium scutatum*) with ventral spot pattern inside a red box created from chipping the image.

Figure 4: Aerial imagery of four-toed salamander (*Hemidactylium scutatum*) study site at the Weir Dam, Sullivan County, Tennessee. Zones 1–4 are highlighted with semi-transparent blue polygons to show the size and shape of each area.

Figure 5: Total number of nests with female four-toed salamanders present at the Weir Dam site from 2009 to 2025, excluding 2013 and 2014. The highest number of recorded females was in 2021 and 2022, with 148 nests with individuals, and 2010 had the lowest number, with 35 nests with individuals.

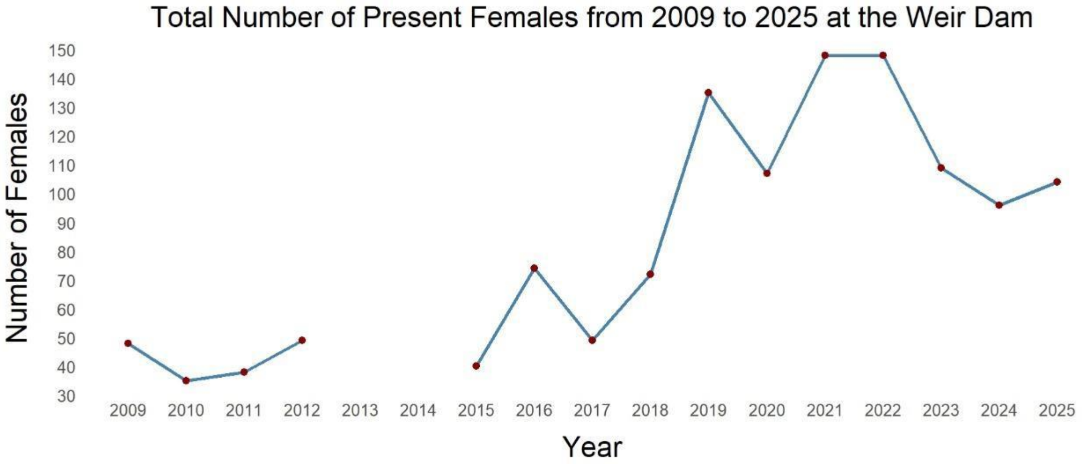


Figure 6: The number of female salamanders that we identified for multiple nesting seasons at the Weir Dam site. Most (73) returning females were identified for only two nesting seasons. One returning female was identified for eight seasons (2018– 2025) and another female was identified for nine nesting seasons (2017–2025). The female over eight seasons was detected only in 2018 and 2025, whereas the female observed in nine nesting seasons was present every year except 2020 and 2021.

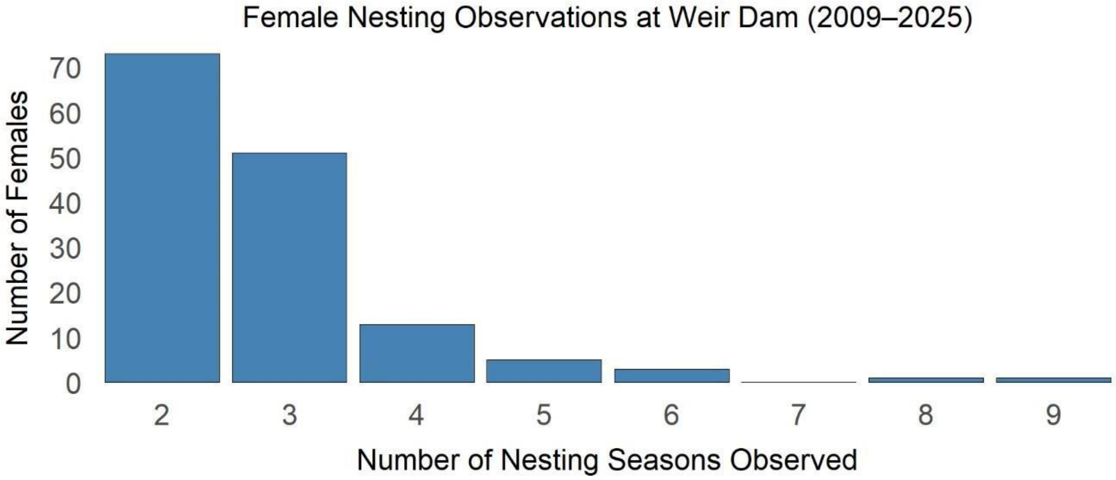


Figure 7: Photo of the same female four-toed salamander from April 2009 and 2011 at the Weir Dam site. This shows a positive match through the illumination of the circles matching specific spots.

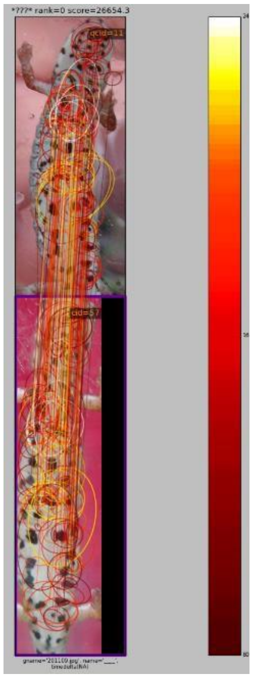
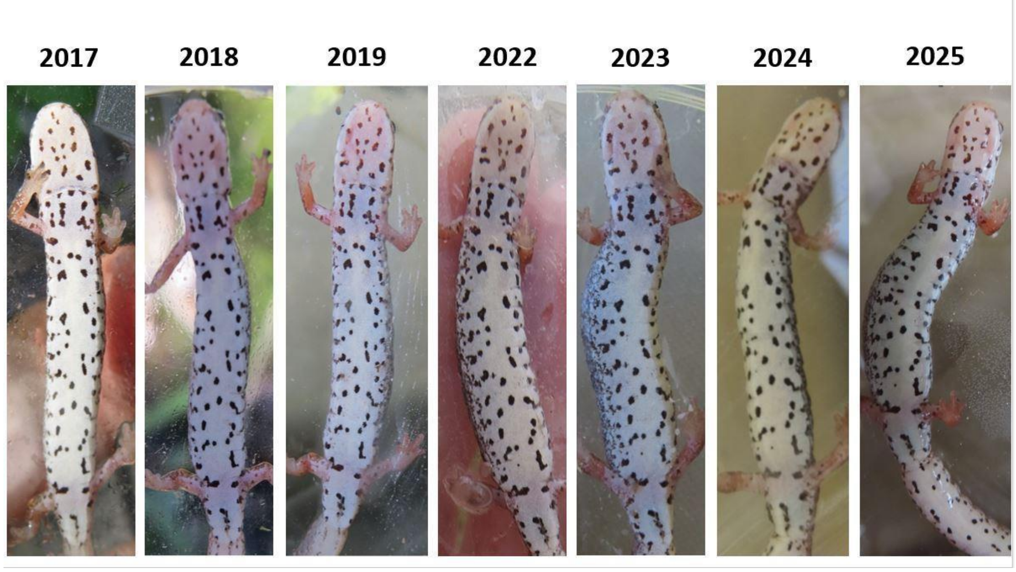


Figure 8: Photo of the same female four-toed salamander from April 2009 and 2011 at the Weir Dam site. This shows a positive match through the illumination of the circles matching specific spots.



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# Chapter 2: How Weather Patterns Influence Clutch Size and the Total Number of Nests in Four-toed Salamanders

## Abstract

Climate change can alter weather patterns and increase the frequency of extreme weather conditions, including droughts and shifts in seasonal precipitation, which may in turn influence animal population dynamics. Salamanders may be particularly sensitive to environmental changes because they rely on wet conditions for survival and reproduction. We evaluated the effects of moisture and temperature on four-toed salamander (*Hemidactylium scutatum*) population abundance and reproductive output in northeastern Tennessee using a 15-year dataset of clutch size, snout-vent-length (SVL), and total annual nest counts. Seasonal climatic influences on reproduction were assessed using models incorporating daily maximum temperature, daily precipitation, and daily relative humidity across 1-month sliding windows, examining immediate effects within 12 months on clutch size and lagged effects up to 48 months the total number of annual nests prior to April nesting. Larger females produced significantly larger clutches, clutch size increased with precipitation four to six months prior to April nesting and decreased with maximum temperature one to two months prior, indicating that conditions during October-December and February-March of the previous year were influential for egg production. Lag analyses showed that precipitation, but not temperature or humidity, influences annual nest counts, suggesting that climatic effects impact multiple life stages. Overall, these results highlight the importance of seasonal precipitation and temperature in shaping salamander reproductive output; and emphasize the need for more long-term datasets to understand how climate change may effect this species.

## 1. Introduction

The loss of species diversity driven by climate change is expected to be the most severe in regions designated as biodiversity hotspots, such as the Southern Appalachians, where species may experience significant shifts in geographical ranges and ecological niches as early as 2040 (Milanovich et al.2010; Zhu et al. 2021). This rapid decline in biodiversity through climate change and habitat looks has been described as a global “biodiversity crisis” (Blaustein et al. 2010). These changes are projected to have substantial effects on native and endemic species worldwide, particularly those associated with aquatic systems, which are highly sensitive to environmental fluctuations (Blaustein et al. 2010;Walls et al. 2013; Evans et al. 2017).

Among vertebrates, amphibians are considered the most susceptible to extinction due to their reliance of water availability not only for survival and reproduction, but also because their limited dispersal capacity restricts their geographical range (Blaustein et al. 2010). This vulnerability arises because many amphibians function as wetland specialists, depending on consistent moisture throughout their life cycle, and making them particularly vulnerable to variation in temperature and precipitation (Blaustein et al. 2010; Walls et al. 2013; Evans et al. 2017). Temperature and precipitation are the primary drivers of amphibian behavior, survival, and recruitment (Walls et al. 2013). Species that breed and hatch in vernal pools are especially at risk, as shifts in temperature and precipitation can shorten hydroperiods and negatively affect larval development by reducing oxygen availability (Blaustein et al. 2010). Drought-like conditions can further disrupt both short-and-long-term weather patterns, altering population dynamics over time (Blaustein et al. 2010). Plethodontid salamanders, which occupy aquatic and terrestrial habitats, rely on cutaneous respiration and require sufficient moisture in terrestrial environments to facilitate oxygen exchange across their skin (Blaustein et al. 2010).

Understanding the climatic factors that influence reproduction, growth, and adaptive capacity is crucial for predicting how species will respond to climate change. If conditions are poor prior to nesting (i.e. limited rainfall and warmer temperatures), female salamanders may be smaller and produce fewer eggs, and individuals may skip breeding years in search of more favorable conditions (Semlitsch 1981; Walls et al. 2013). Long-term datasets are valuable, as they enable monitoring of populations under varying environmental conditions and help identify which demographic responses to environmental drivers most strongly influence population trends. These insights are critical for addressing knowledge gaps and informing effective conservation and management strategies.

Plethodontid salamanders, including four-toed salamanders (*Hemidactylium scutatum*), are an excellent model species for studying environmental responses, as they exhibit distinct nesting preferences and rely on specific microhabitats for reproduction, growth, and survival (Chalmers and Loftin 2006). Female four-toed salamanders nest in moss clumps which require consistent rainfall to maintain moisture during egg incubation and keep the female's body moist during parental care, allowing for gas exchange (Blanchard 1923). Temperature is also a crucial factor as it affects larvae development and the duration of metamorphosis; warmer temperatures can accelerate water loss within the moss, influencing larval survival and maturation while shortening the hydroperiod of vernal pools that larvae use to continue their life cycle after hatching (Chalmers 2004).

We studied nesting female four-toed salamanders at one site in Tennessee, USA, over a 15-year period to examine how weather patterns alter population abundance and fluctuation over time in relation to three climate covariates: maximum daily temperature, daily precipitation, and daily relative humidity. We hypothesized that unfavorable conditions prior to nesting (e.g., warmer temperatures or limited rainfall) would reduce clutch size per female. We also considered female body size as an important predictor of reproductive output, as larger females generally produce more eggs and larger clutches than smaller females (Semlitsch 1981; Hamed 2014). Using our extensive dataset, we also aimed to understand the relationship between total nest abundance and each climate variable to better understand variation among the four different nesting zones. We hypothesized that climatic conditions during the nesting and early larval development period would influence recruitment, such that unfavorable conditions during this time would reduce survival of early life stages. Because individuals require multiple years to reach sexual maturity, reduced recruitment in a given year may cause lower nest abundance after a lag of approximately two to three years.

## 2. Materials and Methods

### *2.1 Study Site*

The South Holston Weir Dam, located on Tennessee Valley Authority (TVA) property in Sullivan County, Tennessee (Figure ), supports a diverse community of local herpetofauna and plant species. The site (11,656 m$^2$) contains one of the most productive vernal pools in Northeast Tennessee (Hamed 2006), four-toed salamanders were first documented nesting in 2002 (Hamed and Gentry 2003). The surrounding forest canopy is dominated by red maple (*Acer rubrum*), sweetgum (*Liquidambar styraciflua*), sycamore (*Platanus occidentalis*), and green ash (*Fraxinus pennsylvanica*). American tree moss (*Climacium americanum*) is the most abundant moss species along vernal pool margins.

Following mating in late summer and fall, female four-toed salamanders migrate from the upland forests to vernal pools and ditches in the early spring to lay eggs within moss clumps (e.g., *Sphagnum* and *Climacium spp.*). Oviposition timing varies by location, beginning as early as February and concluding by mid-May (Petranka 1998). At our study sites, oviposition was observed from mid-March through the first week in April. Females remain with their clutches throughout development, providing parental care for up to 60 days (Herman 2013). Communal nesting is common, with kinship and body condition influencing which female remains with the eggs and provides care by secreting a substance that inhibits fungal growth (Harris et al. 2003; Harris 2008; Banning et al. 2008). Females abandon the nests shortly before hatching and migrate back to the upland forest within a few hundred meters away (Herman 2013). Upon hatching, larvae drop into the water and begin their larval period of approximately 20–40 days, the most rapid development of any plethodontid salamander (Herman 2013). Juveniles disperse back into the upland forest and reach sexual maturity in approximately two to three years (Petranka 1998; Herman 2013).

### *2.2 Field Data Collection*

We surveyed four-toed salamander nests at the Weir Dam site from 2009 to 2025, beginning at the end of March until late April. Nest surveys were done systematically by separating the study site into four zones, each ranging from 1,058 to 3,780 m$^2$ (Figure 1), defined by the location of distinct nesting clumps across the study site. Surveys in 2020 were partially completed due to the COVID-19 pandemic, with sampling conducted only in zones 1 and 2. Upon locating a nest, we placed flagging behind the eggs and recorded the date and nest number on each flag. Using the nest number and year, we created unique identifications for each female (e.g., 202537) to track individuals across years (Chapter 1). When a female was present at the nest, we temporarily placed her inside a clear plastic petri dish while counting the number of viable eggs in each clutch. If multiple females were present, each female was placed in a separate petri dish. Embryos were noticeable by their white appearance, surrounded by a clear, jellylike substance, and measured as approximately 3.0 mm in diameter (Petranka 1998; Figure 2). We deemed embryos with white coloration as viable and embryos yellow in color with a cloudy appearance as non-viable (Banning et al. 2008). We only included viable eggs in our dataset. After counting the eggs, we measured each female's snout-vent-length (SVL), before returning her to the nest.

### *2.3 Fecundity-body size relationship*

Given the influence of female body size on clutch production (Chapter 1), we included SVL and as a predictor of clutch size in a linear model using data from 2009–2025 in R 4.3.2 (R Core Team 2025), with α = 0.05. Joint nest data were excluded from all biological data analyses to ensure that each nest represented only a single female. Clutches containing more than 49 eggs were removed, as they likely reflected contributions from multiple females (Harris and Gill 1980). Females missing SVL measurements or egg counts (n=280) were excluded, resulting in a final dataset of 918 individuals. Additionally, we calculated the average clutch size for each year.

### *2.4 Climate Variables*

We obtained climate data from the National Oceanic and Atmospheric Administration (NOAA). Our focal climate variables included daily humidity (%), maximum daily temperature (°F), and daily precipitation (inches), which we used to assess influence on clutch size and annual nest totals. The climate datasets ranged from 1 January 2005 to 25 August 2025 (including four years of data before the beginning of our biological data). Humidity and temperature data were retrieved from the Bristol Airport station in Sullivan County, Tennessee, while precipitation data were obtained from the South Holston Dam rain gauge maintained by TVA, also located in Sullivan County. Maximum daily temperature showed no correlation with daily precipitation (r = -0.008) and a weak positive correlation with relative humidity (r = 0.078), while daily precipitation and relative humidity were weakly positively correlated (r = 0. 294).

### *2.5 Pre-Nesting Climate Analysis on Clutch Size*

We used sliding window analyses, following Hunter et al. (2021), to identify which climate variables most strongly influenced reproductive output using the R package ‘climwin’ (Bailey & van de Pol 2020). For each climate variable, we calculated the mean value within each climate window to determine the optimal window for predicting clutch size per female and the total number of nesting females each year. For the clutch size analysis, we assessed all possible climate windows from 1 to 12 months in the 12 months preceding the 1 April nesting season (the date by which most females had completed laying eggs), in 1 month increments, resulting in 91 candidate models based on climate data from 2007–2025. Climate variables were averaged across each candidate window prior to analysis. The baseline model included a linear regression of clutch size and female body size (SVL), with the three climate variables being incorporated as additional linear effects. All climate variables were standardized before running climate windows.

All candidate models were compared using Akaike’s information criterion adjusted for small sample size (AICc). For each model we recorded the model window, ΔAICc, ß coefficient, standard error, and model weight. To reduce the risk of Type 1 error, we performed a built-in randomization test in ‘climwin’, repeating the climate window analysis 100 times with randomized biological dates (Van de pol et al. 2016). We compared the top model (‘best’ climate window) to the ΔAICc distribution from the randomized datasets to estimate the likelihood that the observed ΔAICc resulted from random variation. We used PΔAICc < 0.05 to show support for our climate windows against the random variation.

### *2.6 48-Month Climate Lag Analysis on Total Nests*

To assess longer-term effects of seasonal climate variability on recruitment, we incorporated a 48-month lag and examined all possible climate windows ranging from 1 to 12 months in duration within the 48 months prior to 1 April nesting data. This resulted in 1,225 candidate models per climate variable using data from 2005–2025, beginning four years prior to the biological dataset. The four nesting zones were included as a random effect and treated as independent sites, supported by site fidelity results in Chapter 1 indicating independence among zones. A baseline model was fit using lmer(Total Nests ~ (1| Zone)), and candidate models incorporated standardized climate variables. We applied the same randomization process used in the pre-nesting climate analysis and used significance level of $P_{\Delta AICc} < 0.05$.

### *2.7 Assessment of Long-Term Climate Trends*

To quantify the change in the three climate variables in Northeastern Tennessee over the 18-year study period (1 January 2007–25 August 2025), we plotted the yearly mean values within the previously identified climate windows preceding the 1 April nesting period. We included linear trendlines with 95% confidence intervals to illustrate long-term patterns relative to the overall mean and to identify years in which values fell above or below average. We also estimated the average yearly change for each variable.

## **3. Results**

We documented 1,450 total nests of female four-toed salamanders from 2009 to 2025 (Figure 3). In this analysis we had a total of 1,070 remaining nests for analysis. Following the removal of joint contributions and missing SVL or clutch size values, the dataset included 918 females. The average body size for all females was 36.30 ± 3.85 mm with the largest female with a SVL of 49.50 mm.

### *3.1 Fecundity-body size relationship*

We conducted a linear regression model to examine the relationship between female body size (SVL) and clutch size over the 15-year period. This allowed us to assess if larger females produced more eggs and larger clutch sizes. We found this positive relationship to be statistically significant (*P* = 5.15 x $10^{-4}$), indicating that clutch size increased with female body size. We also calculated the average clutch size per year. The smallest average clutch size occurred in 2025, with 25 ± 9.22 eggs, while 2016 had the largest average clutch size, with 38 ± 5.34 eggs (Figure 4).

### *3.2 Effects on Pre-Nesting Climate on Clutch Size*

Daily precipitation from October–December in the year prior to the nesting season (6–4 months prior to 1 April) had a positive effect on clutch size, after accounting for female size (ß = 21.32; PΔAICc = 0.04; Figure 5). Daily maximum temperature from February–March (2–1 months prior to 1 April) had a negative effect on clutch size (ß = -15.50; PΔAICc = 0.04; Figure 6.) Daily relative humidity (6–1 months prior to 1 April) was not a significant predictor of clutch size (PΔAICc = 0.10), although the positive coefficient (ß = 6.71) suggests a potential biological association with increased clutch size.

### *3.3 Climate Lag Effects on Total Nest Abundance*

Daily precipitation 46–7 months prior to 1 April nesting had a positive effect on total nest abundance (ß = 24.50; PΔAICc < 0.001; Figure 7). Daily maximum temperature (48–1 months prior to 1 April; ß = 9.46; PΔAICc = 0.14) and daily relative humidity (38 months prior; ß = 1.30; PΔAICc = 0.11) were not significant predictors of total nest abundance, but both exhibited positive trends.

### *3.4 Long-Term Trends in Climate Variables*

The climate window for the mean daily precipitation effect spanned October-December (Figure 8). Over the years, monthly precipitation showed a slight decline (ß = -0.004 inches, *P* = 0.381), corresponding to an overall decrease of approximately 0.074 inches. Recent years, including 2021–2024 were below the long-term mean, indicating relatively drier conditions. The climate window for daily maximum temperature ranged from February–March (Figure 9). Monthly maximum temperature increased significantly overtime (ß = 0.0171 inches, $P$ = 0.027), suggesting a steady increase in temperature over time of approximately 0.293°F. Since 2016, temperatures have followed a more consistent warming pattern, whereas earlier years had greater variability. The climate window for daily relative humidity ranged from October-March (Figure 10). Relative humidity showed a minimal upward trend (ß = 0.004 %, $P$ = 0.653), increasing approximately 0.080%. Humidity remained fairly consistent across years, indicating that moist conditions during this period have been relatively stable.

## 4. Discussion

We examined how seasonal weather patterns across an 18-year period (2007–2025) influenced clutch size in female four-toed salamanders prior to oviposition, as well as total annual nest abundance at one salamander breeding site in northeastern Tennessee. Our climate analysis identified daily precipitation during mid-fall to early winter and maximum temperature in early spring as the most influential predictors of clutch size. These results suggest that moisture conditions several months prior to reproduction, along with temperatures immediately preceding oviposition, play important roles in shaping female reproductive output. Increased precipitation during the fall-winter period may enhance moss moisture and improve nesting habitat quality, while also supporting greater foraging conditions and energy storage prior to nesting. In contrast, higher spring temperatures may influence female body condition and reproduction, potentially affecting whether individuals exhibit lay-and-stay or lay-and-leave behaviors (Harris et al. 1995).

We found that higher temperatures were associated with smaller clutch sizes, whereas increased precipitation corresponded with larger clutches. Both patterns are crucial for plethodontid salamanders, which rely on moist conditions for cutaneous respiration (Blaustein et al. 2010; King and Ritcher 2022). Warmer and drier conditions may increase physiological stress, reduce foraging success, and limit the availability of suitable nesting microhabitats, therefore constraining the energy available for egg production and reducing nesting opportunities (Harris and Ludwig 2004). Overall, our findings are consistent with previous studies demonstrating that precipitation and temperature are important for salamander reproductive success (Harris and Ludwig 2004; Wahl et al. 2008).

Our 48-month lag analysis on total nest abundance did not identify a single critical window influencing annual nest counts, suggesting that precipitation across the preceding four years may affect multiple life stages of four-toed salamanders. This pattern indicates that climatic conditions influencing egg and larvae survival, juvenile growth, and adult body condition can all contribute to the variation in annual nest abundance. Additionally, our analysis of climate trends over the 18-year period revealed a statistically significant increase in temperature during February-March, which coincides with oviposition. Although not all the variables were statistically significant predictors, they can still influence conditions before, during and after nesting for both females and their eggs.

Because climate effects vary among individuals, it is critical to understand how body size influences reproductive outcomes. Smaller females are prone to water loss due to their higher surface-area-to-volume ratio (Semlitsch 1981), whereas larger females are able to tolerate moisture and temperature related stress (Hamed 2014). As a result, larger females can forage for longer periods under dry or warm pre-nesting conditions, experience more courtship opportunities, and remain with their eggs longer, providing parental care that can enhance embryo survival (Harris and Gill 1980; Semlitsch 1981; Feder 1983; Harris et al. 1995). We found that larger females produced larger clutches, indicating that body size is positively associated with reproductive output. Larger females may therefore represent the fittest individuals, as their greater size likely reflects better body condition before oviposition and a higher capacity for nest attendance during periods of elevated temperature or reduced moisture. Extended nest guarding by larger females can enhance offspring survival, and larger females are also less likely to skip nesting years compared to smaller individuals, particularly in years with warmer temperatures and lower precipitation (Harris and Ludwig 2004; Walls et al. 2013). However, larger clutch size does not guarantee recruitment success. Physiological and behavioral differences in four-toed salamanders, combined with their sensitivity to environmental conditions, highlight the importance of habitat characteristics in influencing nest success and population dynamics across their range.

Four-toed salamanders have the most extensive yet disjunct distributions among plethodontid salamanders, occurring in Canada and throughout both the eastern and western United States (Herman and Bouzat 2016). Because of this, populations experience a wide range of climatic conditions and may exhibit region-specific responses to environmental changes (Blaustein et al. 2010). Widmer et al. (2022) further found that four-toed salamanders are likely to experience both range limitations and expansions across much of their distribution as climate-driven habitat changes alter niche suitability. This species exhibits specialized habitat requirements for nesting and larval development and has a short larval stage, making its persistence on the landscape highly dependent on understanding how they utilize their preferred habitat in the nesting season (Blaustein et al. 2010; Sutton et al. 2015; King and Ritcher 2022).

Female four-toed salamanders typically select north facing edges of *Sphagnum, Climacium,* or *Thuidium* mosses to deposit their eggs just above the waterline of vernal pools (Blanchard 1923; Wahl et al. 2008). This positioning allows larvae to readily drop into the water to begin their short larval period of up to 40 days (Herman 2013). During hotter months, adults have been observed burrowing into the ground to maintain moisture (Herman 2013), and larvae may exhibit a similar behavior if vernal pools experience shortened hydroperiods due to climatic conditions. Females have been documented to show strong site fidelity, returning annually to the same nesting areas (Harris and Gill 1980; Harris and Ludwig 2004; Harris 2008; Hamed 2014) and, in some cases, even to the same or nearby moss clumps used in previous nesting years.

Wetlands in the Southern Appalachians have already experienced warming trends and are projected to warm up to 6.3° F over the next century (Schultheis et al. 2010). Warming can alter vegetation dynamics, allowing grasses and other plants to dominate moss clumps, competing for water and nutrients and causing the moss to dry out and become unproductive (Schultheis et al. 2010). At our study sites, we have observed that moss clumps once occupied by nesting females have become unsuitable over time, as they have turned brown and dry, emphasizing the vulnerability of these unique microhabitats. Across nearly 59% of their U.S. range, four-toed salamanders are recognized with a conservation status or are included in state wildlife action plans (Ferguson and Hamed 2024), highlighting their potential vulnerability. Long-term biological datasets across their range are essential for understanding how climate change influences populations differently and for determining which populations are most vulnerable. Four-toed salamanders return to the same nesting site each year, which may limit their ability to adapt if these habitats become unsuitable under changing conditions. Effective management of vernal pool systems is therefore critical, as their ecological importance is often underestimated.

## **Acknowledgements**

We thank Tennessee Valley Authority for funding and their ongoing support throughout this project. We also acknowledge the Tennessee Wildlife Resources for scientific collection permits (1986 and 1434), the Virginia Tech Institutional Animal Care and Use Committee (protocols 25–214, 22–223, and 19–242), and The University of Tennessee Institutional Animal Care and Use Committee (protocol 2130–0412). We are grateful to the undergraduate student volunteers from the Virginia Tech Department of Fish and Wildlife Conservation, Virginia Highlands Community College, and The University of Tennessee for their dedication and assistance in the field. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the use of the U.S. Government.

# Chapter 2 Figures

Figure 1: Aerial imagery of four-toed salamander (*Hemidactylium scutatum*) study site at the Weir Dam, Sullivan County, Tennessee. Nesting areas are designated with semi-transparent blue polygons labeled zones 1–4. Each polygon shows the size and shape of each zone.

Figure 2: Four-toed salamander (*Hemidactylium scutatum*) nest from the Weir Dam study site, Sullivan County, Tennessee. Viable embryos are while in color enclosed within a clear outer later, embedded in *Sphagnum* spp. moss.

Figure 3: Line graph showing the total number of nests per year at the Weir Dam Site in Sullivan County, Tennessee from 2009 to 2025, excluding 2013 and 2014. The highest number of nests was recorded in 2022 (128 nests), while the lowest occurred in 2010 (34 nests).

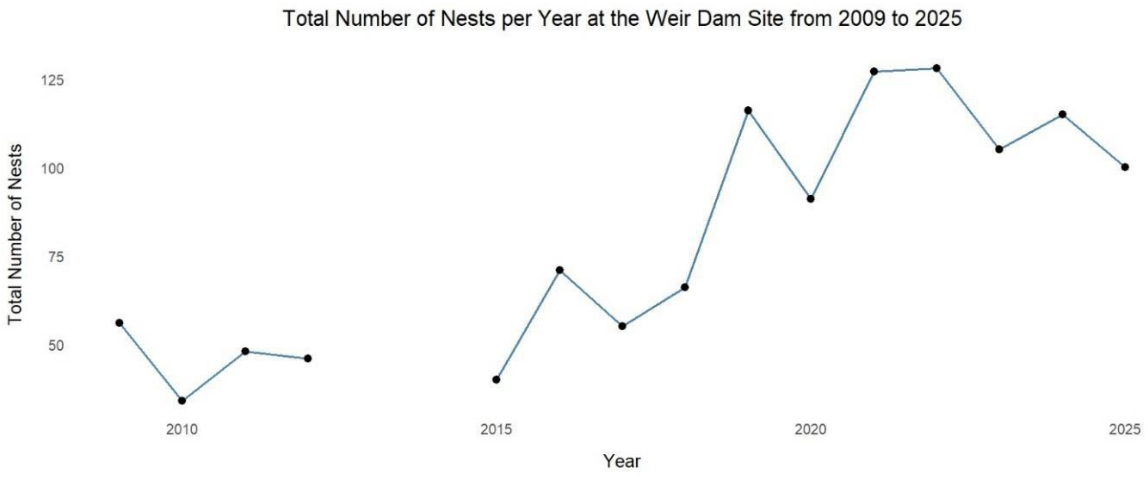


Figure 4: Line graph showing the average female clutch size per year at the Weir Dam Site in Sullivan County, Tennessee from 2009 to 2025, excluding 2013 and 2014. Mean clutch size was lowest in 2025 (25 eggs) and highest in 2016 (38 eggs).

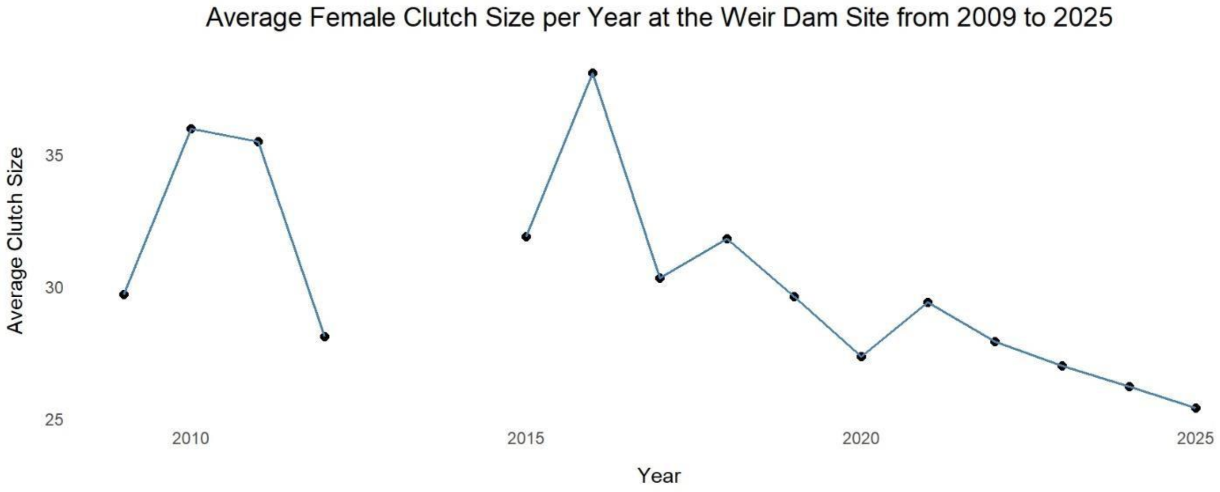


Figure 5: Output from *randwin* analysis examining the relationship between daily precipitation and clutch size. (Top left) Heat map of ΔAICc values for all fitted climate windows relative to the null model. (Top middle) All fitted climate windows, with those included in the 95% confidence set. (Bottom left) Histogram of ΔAICc values for all fitted climate windows compared to null model (dashed line indicates threshold).

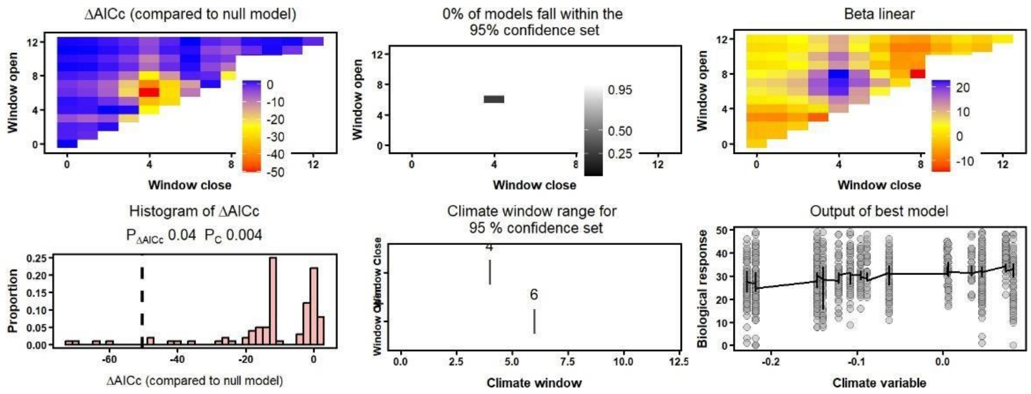


Figure 6: Output from *randwin* analysis examining the relationship between daily maximum temperature and clutch size. (Top left) Heat map of ΔAICc values for all fitted climate windows relative to the null model. (Top middle) All fitted climate windows, with those included in the 95% confidence set. (Bottom left) Histogram of ΔAICc values for all fitted climate windows compared to null model (dashed line indicates threshold).

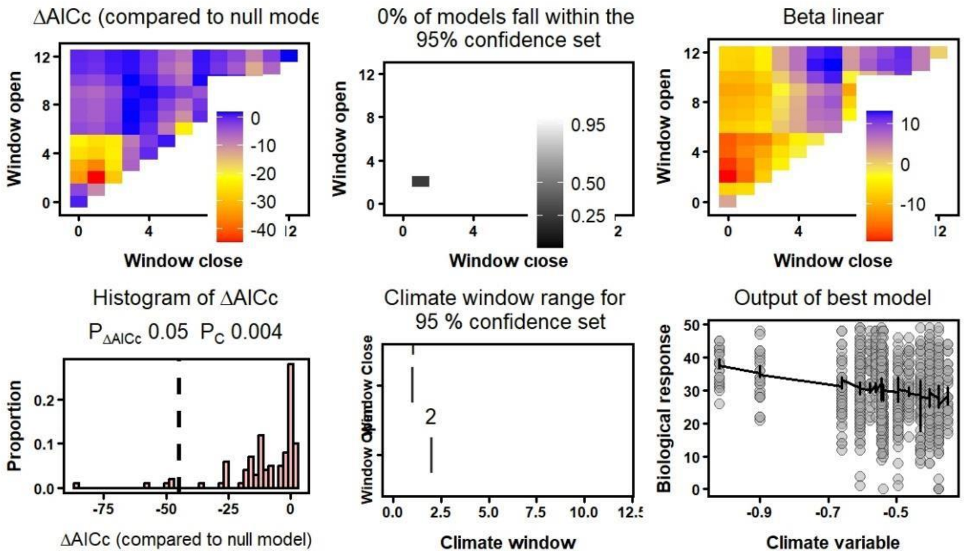


Figure 7: Output from *randwin* analysis examining the relationship between daily precipitation and total nests. (Top left) Heat map of ΔAICc values for all fitted climate windows relative to the null model. (Top middle) All fitted climate windows, with those included in the 95% confidence set. (Bottom left) Histogram of ΔAICc values for all fitted climate windows compared to null model (dashed line indicates threshold).

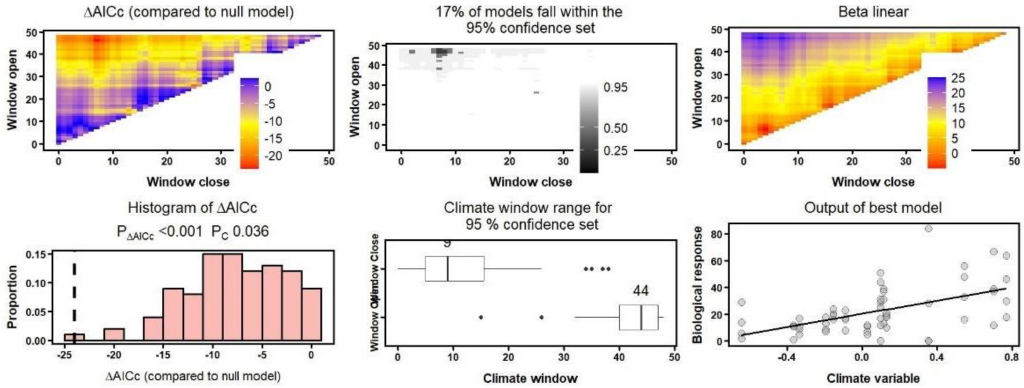


Figure 8: Mean daily precipitation (inches) during October–December from 2007 to 2025 at the Weir Dam site in Sullivan County, Tennessee. Blue points indicate annual deviations below the long-term mean, and red points indicate deviations above the mean, with lines connecting years to illustrate variability. The solid red line shows a weak negative trend overtime, and the shaded gray band represents the 95% confidence interval.

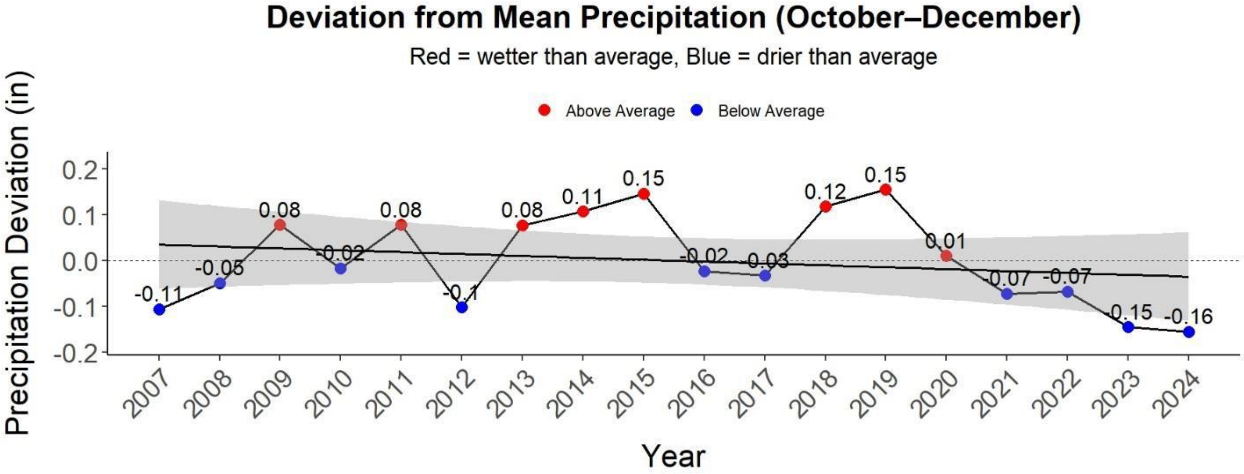


Figure 9: Mean daily maximum temperature (°F) during February–March from 2007 to 2025 at the Weir Dam site in Sullivan County, Tennessee. Blue points indicate annual deviations below the long-term mean, and red points indicate deviations above the mean, with lines connecting years to illustrate variability. The solid red line shows a weak negative trend overtime, and the shaded gray band represents the 95% confidence interval.

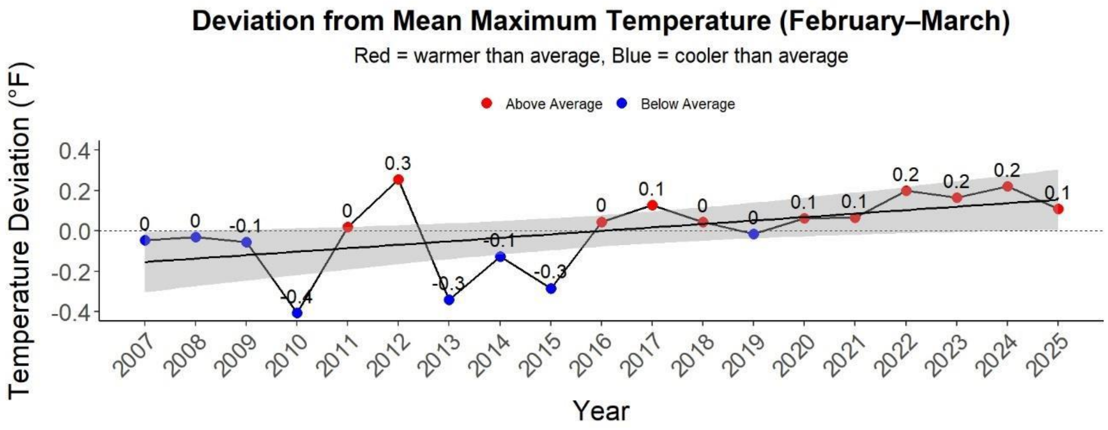
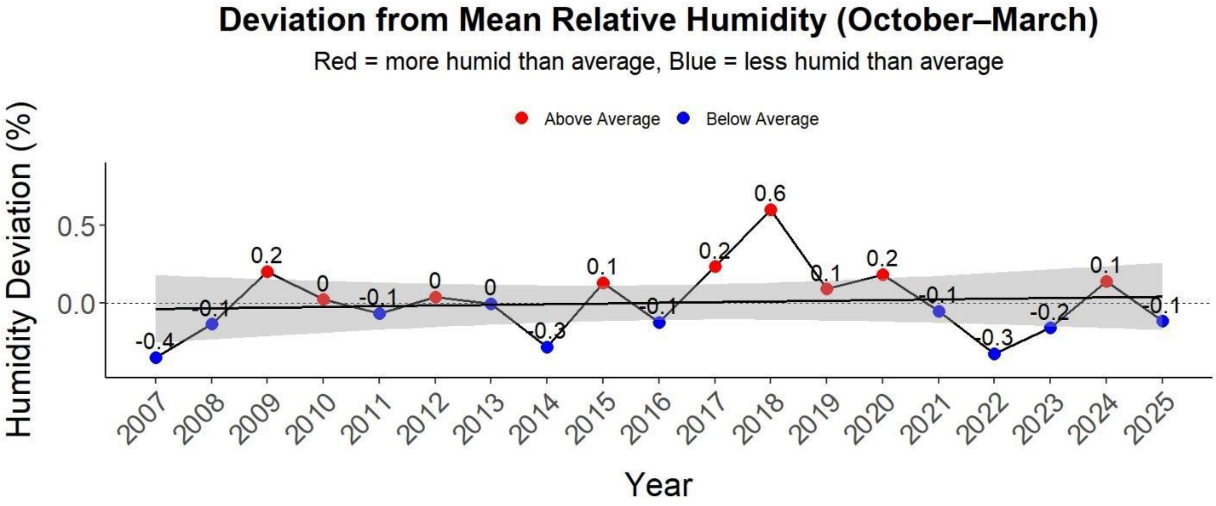


Figure 10: Mean daily relative humidity (%) during October–March from 2007 to 2025 at the Weir Dam site in Sullivan County, Tennessee. Blue points indicate annual deviations below the long-term mean, and red points indicate deviations above the mean, with lines connecting years to illustrate variability. The solid red line shows a weak negative trend overtime, and the shaded gray band represents the 95% confidence interval.



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