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Detection And Microhabitat Use Of The Cryptic Patch-nosed Salamander (Urspelerpes Brucei) In Three Occupied Streams

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DETECTION AND MICROHABITAT USE OF THE CRYPTIC PATCH-NOSED SALAMANDER (*URSPELERPES BRUCEI*) IN THREE OCCUPIED STREAMS

A Thesis
Presented to
the Graduate School of
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In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Wildlife and Fisheries Biology

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by
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ABSTRACT

Patch-nosed Salamanders (*Urspelerpes brucei*) are tiny (25.76 ± 0.17 mm [SE] snout-vent length) plethodontids endemic to headwater streams in a small (29 km^2 at the time of our study) geographic region of northeast Georgia (GA) and northwest South Carolina (SC). Due to its secretive nature and recent discovery (2007), little is known about *U. brucei* habitat, life history, or potential threats. Though environmental DNA (eDNA), aquatic leaf litter bags, and opportunistic active searches are successful detection techniques for *U. brucei*, and other factors influencing detection are unknown. Additionally, while occupied *U. brucei* streams have been characterized as shallow and steep-walled, previous work has not identified factors that influence *U. brucei* microhabitat use among patches within a single occupied stream. In Chapter 1, we applied six survey methods in three streams where *U. brucei* occur using a randomized complete block design and replicated each survey technique six times between August 2021 and June 2022. We used occupancy models to simultaneously investigate factors influencing *U. brucei* occupancy within a 5-m stream segment while estimating detection probability for each survey method and across a range of other survey-specific conditions. We found aquatic and terrestrial area-unconstrained surveys were at least 3.25 times better at detecting *U. brucei* than aquatic area-constrained surveys, while cloudless skies, recent rainfall amounts (> 5 cm in the previous week), and higher soil temperatures (≥ 20 °C) were positively associated with detection. Chapter 2 describes our finer scale analysis where we used occupancy models to investigate factors influencing *U. brucei* microhabitat use at the scale of a 31 x 31 cm patch. Notably, we used a subset of field data collected in Chapter 1 for analysis in Chapter 2. We found leaf litter accumulation represented the primary driver of microhabitat use for both *U. brucei* larvae and adults. Specifically, for larvae, shallow stream depths (≤ 4 mm) and deep leaf litter (≥ 70 mm) was the best predictor for microhabitat use, whereas adults had the highest probability of using microhabitat consisting of leaf litter depths ≥ 111 mm. Collectively, our findings emphasize the success of area-unconstrained surveys and leaf litter bag surveys under ideal weather conditions when detecting both larval and adult *U. brucei.* Additionally, we highlight how *U. brucei* distribution within streams can be highly variable, presumably because of variation in stream depth and leaf litter depth. We also found *U. brucei* seem to be rarer in SC relative to GA for reasons we do not fully understand. Moreover, our findings advance our understanding of effective *U. brucei* survey protocols, identifying microhabitat use, and baseline occupancy estimates that can be used to inform future research aiming to ascertain population demographics, further investigate distribution within occupied streams, and prioritize essential habitat to protect.

DEDICATION

To the overlooked slimy, scaly creatures scattered throughout stream bottoms and forest floors - you matter too.

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Chapter 1: Estimating Detection Probabilities of the Elusive Patch-nosed Salamander (*Urspelerpes brucei*) Using Six Survey Methods

ABSTRACT

Patch-nosed Salamanders (*Urspelerpes brucei*), discovered in 2007, are small (25.76 ± 0.17 mm [SE] snout-vent length) amphibians endemic to 28 first- and second-order streams in northeast Georgia (GA) and northwest South Carolina (SC; as of 2022). Due to its secretive nature and recent discovery, little is known about *U. brucei* habitat, occurrence, or potential threats. Though environmental DNA, aquatic leaf litter bags, and opportunistic active searches are successful detection techniques for *U. brucei*, other factors influencing detection are unknown. We applied six survey methods in three streams where *U. brucei* occur using a randomized complete block design and replicated each survey technique five to six times between August 2021 and June 2022. We used occupancy models to simultaneously investigate factors influencing *U. brucei* occupancy in 5-m stream segments while estimating detection probability for each survey method and across a range of other survey-specific conditions. We found aquatic and terrestrial area-unconstrained surveys and aquatic leaf litter bag surveys, were the most successful at detecting *U. brucei*, while cloudless skies, rainfall in the week prior (> 5 cm), and higher soil temperatures (≥ 20 °C) were positively associated with detection. Stream identity was the best predictor of occupancy with probability of *U. brucei* occurrence relatively similar between two streams and at least 4.38 times higher than occupancy in the SC stream most recently found to harbor *U. brucei*. Our findings emphasize the success of area-unconstrained surveys and leaf litter bag surveys under ideal weather

conditions for detecting *U. brucei* and highlight how variable distribution can be in streams.

INTRODUCTION

Amphibians are facing multiple threats, namely habitat degradation (Peterman et al. 2011), global climate change (Bernardo and Spotila 2006), and the spread of fungal pathogens (Bletz et al. 2013; Carter et al. 2020), which are associated with a sixth mass extinction event (Masters et al. 2006). Amphibians with small or restricted geographic ranges are even more susceptible to decline from direct (i.e., habitat loss) and indirect (i.e., global climate change) drivers of ecological disturbance (Sodhi et al. 2008).

A fundamental step in conserving any amphibian is to develop reliable detection methods and protocols. Gaining a better understanding of factors that influence our ability to detect rare amphibians can play an integral role in both discovering new populations and developing reliable practices for monitoring species at sites known to be occupied. For example, knowing that a species is more easily detected when exhibiting breeding behavior (e.g., vocalization of male frogs) can inform when or under what conditions to conduct surveys. Additionally, learning how likely we are to detect the target species on a single survey can help us determine the ideal number of surveys to conduct. Finally, maximizing effectiveness of survey efforts can drastically improve our understanding of species distributions.

The Patch-nosed Salamander (*Urspelerpes brucei*) is one example of a highly cryptic amphibian whose habitat preferences are largely unknown. *Urspelerpes brucei* was discovered in 2007 in the Appalachian region of northeast Georgia (GA) and is the first plethodontid to require the description of a new genus in 60 years (Camp et al. 2009). *Urspelerpes brucei* are tiny and slender with adult snout-vent lengths (SVL) averaging 25.76 ± 0.17 mm [SE] (Figure 1.1A, 1.1B) and larvae SVL averaging 18.01 ± 0.41 mm (Figure 1.1C; Camp et al. 2009). Due to its recent discovery and rarity, little is known about its life history, occurrence, habitat requirements, or possible threats. The GA State Wildlife Action Plan (SWAP; 2015) considers *U. brucei* to have a global and state rank of one, meaning it is critically imperiled due to rarity and is especially vulnerable to extirpation and/or extinction. The South Carolina (SC) SWAP (2015) does not have a state rank for *U. brucei* but lists it as a species of highest priority.

Urspelerpes brucei appears to have a patchy occurrence across its currently known range with identified populations occurring in a 29 km^2 region of northeast GA and northwest SC (Figure 1.2). *Urspelerpes brucei* have been detected in 28 headwater streams (25 in GA and 3 in SC; as of 2022), all of which are first- and second-order streams in steep-walled ravines (Camp et al. 2009), have closed canopies, and are within mature deciduous forests (Camp et al. 2018). *Urspelerpes brucei* research has yet to identify preferred habitat on a macro (i.e., stream-specific) or micro (i.e., in-stream) level for either *U. brucei* larvae or adults. Additionally, we do not know how common *U. brucei* are within occupied streams. Investigating *U. brucei* microhabitat preferences and distribution in streams will identify factors potentially influencing occupancy at broader scales and highlight critical habitat needs for this cryptic species.

To date, most *U. brucei* detections have resulted from opportunistic active searches (e.g., searching streams nearby known localities), aquatic leaf litter bags (Camp et al. 2009; Camp et al. 2018), and more recently, environmental DNA (eDNA; Pierson et al. 2016). Detections of adults, in comparison to larvae, are rare (Camp et al. 2009; Camp et al. 2018). For example, Camp et al. (2018) reported capture of 82 *U. brucei* individuals from 2009–2011, only 13 of which were adults. All observed adult *U. brucei* have been found near streams edge in loose leaf litter or, in a few instances, under rocks, suggesting a potentially significant refugia association with leaf litter (Camp et al. 2009; Camp et al. 2018). Conversely, *U. brucei* larvae have been successfully captured by active searches and aquatic leaf litter bags (Camp et al. 2018). Notably, Camp et al. (2018) found stream flow, depth, and width as factors that can potentially influence the capture rate of larvae in leaf litter bags. Thus, the highest rates of larvae capture have occurred near spring heads, where water depth is at its shallowest (Camp et al. 2018). Comparing multiple survey methods can identify the usefulness of each method, while directly informing survey protocols related to research goals.

The objectives of our study were to 1) investigate factors influencing *U. brucei* detection in occupied streams and 2) describe fine-scale (5-m stream segment) patterns of *U. brucei* occupancy in occupied streams. Specifically, we were interested in how weather condition, available refugia, survey method, and breeding activity potentially influence *U. brucei* detection probability and investigating how frequency of species occurrence throughout a stream might vary.

METHODS AND MATERIALS

Study sites

Our study was restricted to three streams known to be occupied by *U. brucei*, located in the Tugaloo Mosaic region where Habersham and Stephens counties (GA) and Oconee County (SC) meet. This region is located along the Brevard fault, where the Blue Ridge and Piedmont geologic zones converge (Camp et al. 2018). The Brevard fault is characterized by distinct soils, typical montane vegetation of the Blue Ridge, and elevations akin to the Piedmont (Menzel et al. 2016; Camp et al. 2018). Our sampling locations were tributaries of the Tugaloo River in the upper Savannah River basin. Each identified stream was representative of exactly 65 meters of flowing water at the start of our study (August 2021). Our study sites were first-order streams with depths ranging from 8–37 mm, average wetted widths ranging from 1.6–3.2 m, and average canopy closure ≥ 96% (Table 1.1). However, our northernmost SC stream had very few seepage areas, a steeper gradient, and multiple sections of cascading water, indicating a faster stream flow. Two streams were in SC on public land managed by the SC Department of Natural Resources (labeled as SC1 and SC2) and one stream was in GA (labeled as GA2) on private property. Due to species status and concerns of poaching, we are withholding exact stream locations. Two of our streams (GA2 and SC2) shared similar habitat characteristics in that they both contained multiple spring heads and seepage areas with few areas of fast-flowing water.

We chose our streams based on confirmed *U. brucei* presence and accessibility. Currently, *U. brucei* is only known from three locations in the state of SC. We excluded one SC stream because it did not meet stream length requirements for our study and seemed to potentially have an ephemeral water source. We chose to include one GA stream because preliminary findings suggest *U. brucei* is far more common in GA than in SC, and we wanted to determine if such differences were mirrored at a finer scale.

Study design

We used an occupancy approach to investigate factors influencing *U. brucei* detection and occupancy within occupied streams (MacKenzie et al. 2006). We defined occupancy as the probability that at least one *U. brucei* individual was present in a selected 5-m stream segment throughout our entire sampling period (August 2021–June 2022). We defined detection probability as the probability of detecting *U. brucei* in a truly used 5-m segment during a single replicate survey.

Before sampling, we subdivided each 65-m stream reach into 13 5-m linear segments and used segments as our sampling units for understanding occupancy (Figure 1.3). We used a randomized complete block design to compare *U. brucei* detectability of multiple survey methods and considered each 5-m stream segment as a ‘block.’ In each segment, we applied six survey methods: 1) aquatic area-constrained surveys, 2) terrestrial area-constrained surveys, 3) aquatic leaf litter bag surveys, 4) terrestrial leaf litter bag surveys, 5) aquatic area-unconstrained surveys, and 6) terrestrial area-unconstrained surveys.

Each of our survey methods was conducted in a predefined area that varied in size depending on the method employed (Figure 1.4). We implemented an aquatic and terrestrial version of each method to specifically target larvae (aquatic) and adults (terrestrial). Our aquatic surveys occurred within the wetted width of the stream. Because all prior observations of adult *U. brucei* have occurred within one meter of streams edge, we restricted terrestrial surveys to the 1-m riparian area on either side of a stream segment.

We defined *area-constrained surveys* as visual searches of static (31 x 31 cm) quadrats (*n* = 1 aquatic and 1 terrestrial per stream segment), that we assigned to a random (aquatic or terrestrial, depending on method) location in a 5-m stream segment. We used pin flags to permanently mark the top left and bottom left corners of all static quadrats for the entirety of the study. If pin flags were not applicable markers, we flagged rocks and/or vegetation to mark corners. We defined *leaf litter bag surveys* as a manual examination of contents from a statically placed leaf litter bag (31 x 31 cm; *n* = 1 aquatic and 1 terrestrial per segment) that was randomly assigned a location (aquatic or terrestrial, depending on method) in a 5-m stream segment. Each of our leaf litter bags was filled with leaf litter found in the assigned quadrat, and if aquatic, then completely submerged before securing. We secured our aquatic leaf litter bags with rocks or paracord to prevent displacement during rain events. To our knowledge, terrestrial leaf litter bags are a novel survey technique that we suspected might be useful in detecting *U. brucei* adults, given most encounters have been along the streambank in leaf litter (Camp et al. 2009). We defined *area-unconstrained surveys* (*n* = 1 aquatic and 1 terrestrial per segment) as visual, exhaustive searches of the entire aquatic and terrestrial portion of each 5-m stream segment. We used pin flags and/or flagging to permanently mark the beginning and end each 5-m stream segment for the entirety of the study.

Field surveys

We replicated all survey methods (*n* = 6) in each stream segment (*n* = 13 per stream) five to six times (i.e., Summer2021, Fall2021, Winter2021, Spring2022-A, Spring2022-B, Spring2022-C) between August 2021 and June 2022. However, our area-unconstrained surveys were not implemented until the Fall2021 seasonal window and were absent from the Summer2021 seasonal window. Additionally, we were unable to conduct surveys at one stream (SC1) during the Winter2021 seasonal window due to construction activity.

Area-constrained surveys---We conducted area-constrained surveys by exhaustively searching refugia that could potentially conceal a salamander in the predefined area. This included hand picking through leaf litter, pumping leaf litter and sediment into an aquarium net, and flipping potential refugia to uncover hidden salamanders.

Leaf litter bag surveys---To examine leaf litter bags, we set up two processing areas on dry ground, one for aquatic leaf litter bags and one for terrestrial leaf litter bags. For the aquatic station, we filled a 10-gallon tub with approximately two gallons of stream water using a manual pump. We then removed the aquatic leaf litter bag from the stream, placed it in a net to prevent salamander escapes, and then transferred it to the tub. We agitated the bag for 15–30 seconds, set it aside, poured tub contents through an aquarium net, and examined net contents for salamanders. To redeploy the aquatic leaf litter bag, we replaced a quarter of the leaf litter contents with fresh leaf litter, wetted it in the stream, and returned the bag to its original placement. To check terrestrial leaf litter bags, we emptied all contents into a tub and thoroughly searched for salamanders. We replaced a quarter of the leaf litter contents with fresh leaf litter and returned the bag to its original placement.

Area-unconstrained surveys---We conducted aquatic and terrestrial area-unconstrained surveys by exhaustively searching all aquatic and terrestrial refugia (i.e., leaf litter packs, rocks, logs), except for the area in static quadrats, that occurred in each 5-m stream segment. This included hand picking through leaf litter, pumping leaf litter and sediment into an aquarium net (e.g., where leaf packs are positioned in flowing water), and flipping potential refugia (e.g., rocks and logs). To minimize disturbance, we attempted to return any displaced natural cover to its original condition.

Due to logistics associated with processing the relatively large number of salamanders encountered during area-unconstrained surveys, we only recorded observations of *U. brucei*. During the other four survey methods, we captured and processed all salamanders encountered. We placed individuals in a 16 x 15 cm plastic bag with approximately 60 mL of stream water, recorded capture method, and identified to species when possible. We then processed individuals by recording stage (larvae, metamorph, or adult) and measuring SVL and total length (TL) to the nearest mm. Additionally, for *U. brucei* only, we recorded mass to the nearest 0.1 g, sex, and noted any sexual characteristics, such as the presence of eggs or cirri (Figure 1.5). If we were unable to distinguish species in the field, we took a dorsal and ventral photograph next to a metric ruler to later identify to the lowest taxonomic level possible. After individuals were processed, they were returned to the initial capture location.

Predictors

During each survey, we measured 14 covariates that we hypothesized might influence detection or occupancy of *U. brucei* (Table 1.2). We recorded cloud cover and weekly precipitation amounts by accessing historical weather data through Visual Crossing Corporation's Weather Query Builder from three local weather stations (KTOC, F6979, and 72525800382). We measured leaf litter and stream depth in each area-constrained quadrat (*n* = 4 per segment) using a metric ruler to serve as a proxy of survey-specific conditions in each segment for every seasonal window. We recorded soil temperature once per stream in the same location at the start of each survey using a digital probe thermometer. We averaged leaf litter depths collected in each segment across our entire sampling period (2021–2022) to serve as a segment-specific covariate that we hypothesized would influence *U. brucei* occupancy.

Data analysis

We used single-species, single-season occupancy models to estimate the probability of *U. brucei* occupying a 5-m stream segment over the course of the sampling period while accounting for imperfect detection (MacKenzie et al. 2006). Notably, single-season occupancy models assume the state of occupancy remains the same during the sampling period. While our study occurred over a relatively long period of time, we were comfortable assuming occupancy status remained the same in our 5-m stream segments. Specifically, because *U. brucei* are small and exhibit what seems to be a limited dispersal ability, likely indicating a small home range (≤ 5 m) which is consistent with other stream associated salamanders (i.e., Black-bellied Salamander [*Desmognathus amphileucus*]; Petermen et al. 2007).

Prior to analysis, we created detection histories for each 5-m segment (*n* = 13 segments/stream x 3 streams = 39 segments total) in our study. We defined replicate surveys as each time a survey method was used during a seasonal window (*n* = [4 survey methods x 6 seasonal windows] + [2 survey methods x 5 seasonal windows] = 34 replicate surveys). We converted *U. brucei* observations from each survey into a binomial variable (0 = no *U. brucei* detected and 1 = at least one *U. brucei* detected) and used the 5-m segment detection histories as the response data in our analysis. We evaluated all predictor variables for potential collinearity, and considered variables correlated when $|r|$ was ≥ 0.7. If variables were correlated, we disregarded one variable by selecting the variable we considered to have the most ecological relevance to our hypothesis.

Model development

We considered four ecologically relevant *a priori* hypotheses regarding *U. brucei* detection probability (Table 1.3). To represent our hypothesis that detection was driven primarily by weather condition, we used additive forms of cloud cover, weekly precipitation totals, and soil temperature in an additive model. We predicted that increased cloud cover, increased precipitation amounts, and higher soil temperatures would negatively impact *U. brucei* detection. We believed cloud cover would reduce visibility by limiting available sunlight in dense canopy habitats and result in reflections off the water surface. We also thought increased rainfall would lead to murky stream conditions, and higher soil temperatures associated with decreased salamander surface activity would negatively influence detectability. Our second hypothesis examined available refugia in additive forms of leaf litter depth and stream depth as drivers of detection. We hypothesized *U. brucei* (larvae and adults) were using leaf litter as their primary refugia based on previous observations and studies of the species; therefore, we predicted a positive relationship between detection probability and average leaf litter depth of a segment (Camp et al. 2018). Third, we hypothesized surface activity would peak during the *U. brucei* breeding season, which is proposed to be comparable to *Eurycea* species (spring and fall; Petranka 1998). We tested this hypothesis using a quadratic expression of sampling date ($x + x^2$), representing our prediction that detection probability would increase in the spring and fall relative to summer. Lastly, we examined survey method (aquatic area-constrained surveys, terrestrial area-constrained surveys, aquatic leaf litter bag surveys, terrestrial leaf litter bag surveys, aquatic area-unconstrained surveys, and terrestrial area-unconstrained surveys) as a categorical variable driving detection probability. We predicted that leaf litter bag surveys would be the most effective method due to increased refugia availability (leaf litter) and area-unconstrained surveys due to the exhaustive search effort of each segment. We also included a null model to represent the hypothesis that detection remained constant irrespective of all covariates considered.

When developing the occupancy component of our model, we considered two *a priori* hypotheses (Table 1.4). To represent our hypothesis that leaf litter accumulation was the primary driver of *U. brucei* occupancy at a 5-m segment scale, we included a single additive form of leaf litter depth. We predicted that average leaf litter depth of each 5-m segment would be positively associated with occupancy, because deeper leaf litter packs retain more moisture, provide refuge from predators, and increase foraging opportunities. To represent our hypothesis that *U. brucei* populations in SC exhibit local rarity compared to the GA population, we considered a model that included stream (GA2, SC1, and SC2) as a categorical variable and was the primary driver of occupancy. We predicted occupancy would differ among streams; specifically, GA2 would have the highest occupancy estimates based on preliminary observations. We also included a null model to represent our hypothesis that *U. brucei* occupancy would be similar among our study streams.

Model fitting and ranking

We used a two-step occupancy modeling approach (MacKenzie et al. 2006) to examine factors associated with detection and occupancy. In the first step, we evaluated support for factors influencing detection by examining Akaike's Information Criterion model weights corrected for small sample sizes (AIC_c; Akaike 1974) while holding occupancy constant. In the second step, we used the supported covariates (carrying the upper 90% of AIC_c model weight) from step one to model detection while varying the component of our model describing occupancy. We considered all models carrying any portion of the upper 90% of the AIC_c model weight to be well supported and warrant further examination. Though AIC_c is used for model selection in a confidence set, it does not assess model fit to observed data. Therefore, we used a Pearson chi-squared test with 5,000 bootstrap replications to determine if our global model adequately described our observed data (MacKenzie and Bailey 2004). We then examined our global model's fit by estimating our $\hat{c}$ value (values close to one indicate adequate fit; MacKenzie and Bailey 2004). We fit all occupancy models using the unmarked package (Fiske and Chandler 2011) in Program R (v 2023.03.0+386; R Core Team 2021).

RESULTS

We detected 92 *U. brucei* across our three streams between August 2021 and June 2022 (Table 1.5). Notably, 40 of the individuals we captured were adults (19 females and 21 males), 50 were larvae, and two were recently metamorphosed individuals (pooled with adults for analysis). Our captured *U. brucei* adults averaged 24.52 ± 2.03 [SD] mm SVL, while larvae averaged 15.86 ± 2.90 mm SVL. All our captured *U. brucei* adults displayed sexually dimorphic traits (Figure 1.5); 18 of the 19 females captured were gravid with 3–15 ova visible through a translucent venter, and all captured males exhibited prominent nasal cirri. We detected 45 *U. brucei* individuals using area-unconstrained surveys (aquatic = 24 larvae + 1 metamorph and terrestrial = 20 adults). Our aquatic leaf litter bags facilitated *U. brucei* detection of 21 larvae, three adults, and one metamorph, while the terrestrial leaf litter bags yielded capture of 14 adults. Our area-constrained surveys resulted in only eight *U. brucei* captures (aquatic = 1 adult + 5 larvae and terrestrial = 2 adults).

For larval *U. brucei* capture points, the average stream depth was 14 mm and average leaf litter depth was 35 mm (Table 1.6). Only one adult *U. brucei* was captured in an area where stream depth was > 1 mm. For adult *U. brucei* capture points, the average leaf litter depth was 84 mm. Our detections differed across streams with GA2 (n = 60 *U. brucei*) accounting for nearly twice the detections of SC1 (n = 3 *U. brucei*) and SC2 (n = 29 *U. brucei*) combined. We detected individuals in 9 of 13 (69%) GA2 segments, 2 of 13 (15%) SC1 segments, and 11 of 13 (85%) SC2 segments with most detections (77 of 92) occurring during the spring seasonal windows (14 March–13 June 2022).

Model ranking

In step one of our analysis, two detection models carried the upper 92% of the AIC_c model weight and were combined to inform detection for step two (Table 1.7). Our supported detection covariates included survey method and all those associated with weather condition (cloud cover, weekly rainfall totals, and soil temperatures). In step two, our top ranked model included stream as the only predictor of *U. brucei* occupancy, carrying 99% of the AIC_c model weight. Our global model adequately fit our data with an estimated $\hat{c}$ of 1.01.

Our top ranked model suggested survey method (Figure 1.6) and weather condition (Figure 1.7) play significant roles in detecting *U. brucei*, while stream is a potentially important predictor of *U. brucei* occupancy. We found the probability of detecting *U. brucei* in an occupied segment during an area-unconstrained survey was about three-times higher ($p \geq 0.13$ [0.08–0.21; 95% confidence intervals]) than during aquatic area-constrained surveys ($p = 0.04$ [0.02–0.08]). Additionally, detectability via our terrestrial leaf litter bag surveys was relatively low ($p = 0.07$ [0.04–0.14]) but still nearly twice that of either area-constrained survey ($p \leq 0.04$ [0.00–0.08]). Lastly, our terrestrial area-constrained surveys yielded the lowest detection probability (p = 0.01 [0.00–0.05]).

As we predicted, coefficients associated with weather variables indicated that detectability of *U. brucei* on a cloudless day (p = 0.24 [0.12–0.41]) was double that on a day with 68% cloud cover (p = 0.11 [0.07–0.19]; Figure 1.7A). However, in contrast to our predictions, increased weekly rainfall totals and higher soil temperatures were positively associated with detection. We found detection probability increased as weekly rainfall totals increased (Figure 1.7B) and was more than 2.33 times greater when soil temperatures were at 22.4 °C (p = 0.21 [0.12–0.36]) compared to 4.4 °C (p = 0.09 [0.05–0.17]; Figure 1.7C).

Our only supported predictor of occupancy was stream. Though we predicted the highest occupancy estimates would be associated with the GA stream, our results suggested similar occupancy estimates between GA2 (Ψ = 0.70 [0.41–0.89]) and SC2 (Ψ = 0.94 [0.22–1.00]), which were at least 4.38 times higher than the estimate at SC1 (Ψ = 0.16 [0.04–0.47]; Figure 1.8). However, confidence intervals associated with the stream effects were large, indicating some uncertainty about differences in occupancy among streams (Table 1.8).

DISCUSSION

To our knowledge, ours is the first study to quantify occupancy of *U. brucei*. Our findings suggest *U. brucei* can be distributed relatively homogenously in some streams but patchily distributed in others. We suspect that *U. brucei* occupancy among 5-m stream segments is influenced by microhabitat preferences, though we failed to identify what those potential preferences are. Our results show aquatic leaf litter bag surveys, aquatic area-unconstrained surveys, and terrestrial area-unconstrained surveys were the most successful of the six survey methods. Though, detectability with any of these methods was still relatively low ($p \leq 0.14$), which highlights the cryptic nature of *U. brucei*. However, despite low detection probabilities across all our survey methods considered, the aquatic area-unconstrained survey method detection probability ($p = 0.14$ [0.09–0.22]) was close to the recommended detection probability threshold ($p = 0.15$) for reliably estimating occupancy (Bailey et al. 2004). Additionally, the conditions under which we surveyed influenced *U. brucei* detection success, suggesting time of year surveys are conducted may be a vital consideration. Our highest detectability estimates ($p \geq 0.19$ [0.11–0.31]) corresponded to weather conditions that align with spring, and 84% (77 of 92) of captures occurred in our spring seasonal window. Our findings provide baseline occupancy estimates for three *U. brucei* streams and highlight how sampling methods and survey conditions can be strategically combined to maximize detection, develop survey protocols, and more effectively monitor this species.

While our findings suggest *U. brucei* is cryptic, they also provide insight on how to successfully design sampling frameworks to maximize *U. brucei* detections and captures. Though we did not analyze detectability of larvae and adults separately, terrestrial methods singularly detected adults and the aquatic methods mostly detected larvae (Table 1.5). Our aquatic area-unconstrained and aquatic leaf litter bag surveys were the best methods for *U. brucei* larval detectability and are consistent with other studies assessing these survey methods' effectiveness at capturing aquatic salamanders (Barr and Babbit 2001; Waldron et al. 2003; Talley and Crisman 2007; Camp et al. 2009; Wilson and Gibbons 2009; Edwards et al. 2016; Camp et al. 2018). Interestingly, though *U. brucei* larvae have historically been detected far more frequently than adults, we found that terrestrial area-unconstrained surveys were similarly effective at detecting *U. brucei* individuals as aquatic area-unconstrained and aquatic leaf litter bag surveys. One possible explanation for our success with detecting adult *U. brucei* is that, unlike prior surveys, our study focused on the riparian area adjacent to occupied streams allowing us to survey an additional habitat that seems significant to adults. Even though our terrestrial leaf litter bag surveys were associated with a relatively low adult detection probability, which might be explained by cryptic habits, this novel survey method and terrestrial area-unconstrained surveys still accounted for 90% (38 of 42) of adult detections.

The performance of our survey methods associated with *U. brucei* detectability has several implications. Notably, our area-constrained surveys performed poorly ($p \leq$ 0.04 [0.00–0.08), likely due to a combination of random placement (i.e., poor microhabitat) and the small quadrat size (31 x 31 cm), which drastically limited the extent of refugia available to search. Though our study suggests detectability was often maximized with area-unconstrained surveys, such advantages should be considered alongside the potential negative impacts of active searches on habitat quality, especially in headwater stream systems where species exhibit specialized microhabitat requirements. For these reasons, Pauley and Little (1998) suggested leaf litter bags as an alternative to active searches because they are less disturbing to the habitat (i.e., displacing refugia and shifting substrate) and are equally, if not more, effective at capturing aquatic salamanders. However, while less labor intensive and destructive than area-unconstrained surveys, leaf litter bag surveys do require multiple stream visits via initial placement in the field, followed by a monitoring window.

Our results suggest weather conditions can impact *U. brucei* detectability and should be considered when conducting surveys. As predicted, we found percent cloud cover influenced the observer's ability to detect *U. brucei*, which may be a result of low light conditions and/or reflection off the water's surface during cloudy days. Although we predicted increased weekly precipitation totals to have a negative impact on detectability of *U. brucei* individuals, we found the opposite was supported. One explanation for the positive relationship between precipitation totals and detectability is that am increase in stream flows and higher moisture levels can activate salamander movement in microhabitat (i.e., leaf litter packs). Typical of plethodontid species, rainfall is documented to trigger immediate surface activity of adult salamanders (Petranka 1998) due to in an influx of available moisture in microhabitats (i.e., leaf litter packs adjacent to streams edge; Spotila 1972; Grover 1998; Farallo et al. 2018). In a previous study conducted by Johnson and Goldberg (1965), Two-lined Salamander (*Eurycea bislineata*) larvae were documented to limit activity during high stream flows after a precipitation event but experience peak activity following a return to normal flow conditions. Though a direct link between salamander activity and detectability has not definitively been supported, we speculate that encounter rates of *U. brucei* increase with higher weekly rainfall amounts. Specifically, the introduction of additional moisture in microhabitats may facilitate movement of adults above ground but within leaf packs, especially during breeding season. We also speculate that increased activity of benthic larvae, in this case foraging activity, results in higher encounter rates post rainfall. We predicted high soil temperatures would have a negative impact on detectability, but our results suggest otherwise. One explanation is that *U. brucei* experience low temperatures as a limiting factor instead of high temperatures, especially when considering breeding may occur in seasons when temperatures are known to peak. For example, leaf litter pack microclimates in a closed canopy system may provide an effective temperature buffer (Farallo et al. 2018) that allows *U. brucei* adults to remain aboveground during higher soil temperatures instead of moving underground. Lastly, ideal conditions for *U. brucei* detection may be influenced by seasonality. Our spring seasonal window accounted for 84% (77 of 92) of our *U. brucei* captures and were similar between life stages (*n* = 37 larvae and 40 adults). Though conditions varied, our spring surveys were typically characterized by minimal cloud cover, some rainfall in the week prior to surveying, and a trend of warming soil temperatures. Therefore, we speculate spring surveys may be optimal for *U. brucei* detections.

We demonstrated *U. brucei* occupancy at the 5-m segment scale can be highly variable among streams, though drivers of these differences remain somewhat unclear. *Urspelerpes brucei*, in SC2 and GA2, seem to be homogenously distributed throughout the stream, as evidenced by occupancy estimates and detections across multiple segments in the stream. However, in the SC1 population, *U. brucei* were comparatively less common and detections were concentrated in only two segments in the stream. The similar occupancy estimates and detections across segments at the SC2 and GA2 streams, potentially suggest a shared stream level characteristics influencing occupancy that we failed to quantify. Anecdotally, we noted lower leaf litter amounts, increased disturbance from feral hog (*Sus scrofa*) activity, and more frequent high stream flow events at the SC1 stream. Additionally, the SC1 stream was uniquely characterized by a relatively faster stream flow. Furthermore, unlike the SC2 and GA2 streams emerging from a springhead, our SC1 stream reach started at the base of a ~5-m cascade. Ultimately, more work is needed to identify stream-specific characteristics that influence *U. brucei* occupancy.

MANAGEMENT IMPLICATIONS

Our study highlights differences in the efficacy of multiple survey methods at detecting and capturing *U. brucei* while identifying optimal conditions for sampling. Ultimately, the utility of each survey method will vary in relation to the goal of sampling. For example, if the goal is to detect *U. brucei* presence or capturing individual *U. brucei* larvae, then we recommend using aquatic leaf litter bag surveys or aquatic area-unconstrained surveys. Practically, a simple power analysis based on our estimates of method-specific values of p suggests that confirming *U. brucei* occupancy within a randomly selected 5-m stream segment with 90% confidence would require 15 temporally replicated aquatic area-unconstrained surveys (cumulative $p = 1 – (1\text{-}0.14)^{15} = 0.90$). Alternatively, by increasing the density of aquatic leaf litter bags (i.e., spatial replicates) from one-per-segment to five-per-segment, one could increase p during a single survey to 0.50 (i.e., $p = 1 – (1\text{-}0.13)^5 = 0.50$), and thereby determine status of the patch with 90% confidence after only four replicate visits (cumulative $p = 1 – (1\text{-}0.50)^4 = 0.94$). Given the effort and habitat disturbance associated with exhaustive survey methods (Pauley and Little 1998; Barr and Babbit 2001), sampling eDNA collected from stream water may prove more appropriate for detecting *U. brucei* presence at a stream scale (Pierson et al. 2016; Moss et al. 2022; C. Jachowski, unpublished data). Though eDNA applications are evolving rapidly, current sampling approaches cannot accurately show demographic differences between populations (Yates et al. 2019; Spear et al. 2021; Yates et al. 2021), whereas traditional survey methods can. If the goal is to capture adult *U. brucei*, then we recommend terrestrial area-unconstrained surveys or modified terrestrial leaf litter bag surveys. In our study, we randomly deployed only 13 terrestrial leaf litter bags at each stream. Therefore, increasing the number of bags deployed and modifying these conditions may improve success. For example, we suspect a terrestrial leaf litter bag with slightly larger capacity for leaf litter, placed in moist, deep (> 200 mm) leaf litter, and located near a spring head or seep (Figure 1.9) might increase detectability of *U. brucei* adults. In considering weather conditions, we recommend conducting surveys when cloud cover is minimal (< 50 %), it has rained in the week prior to the survey (> 3 cm), and soil temperatures are greater than 15 °C, which generally aligns with spring conditions.

Our study of the two most permanent streams that harbor *U. brucei* in SC underlines the cryptic nature and likely rare status of *U. brucei* in SC. Based on low detection probabilities, we can confirm the cryptic nature of *U. brucei.* More importantly, occupancy of *U. brucei* drastically differs between the two SC streams. The rarity of *U. brucei* in SC1 ($\Psi = 0.16$ [0.04–0.47]) specifically raises significant conservation concern. We suggest investigating the roles available refugia (i.e., leaf litter), canopy composition, and stream flow variation (i.e., spring heads, seeps) play in characterizing ideal *U. brucei* habitat, which can be used to inform management decisions. We emphasize the importance of future research in describing population demographics and quantifying local abundance to better understand the overall health and status of *U. brucei* in SC. Though, successfully monitoring populations requires a reliable marking technique, which considering the tiny size of *U. brucei*, precludes traditional techniques like pit-tagging. However, identifying adult *U. brucei* individuals was possible via unique dorsal patterns (Figure 1.10). Due to dorsal melanophores and a snout patch on the larvae, we suspect they may also be individually distinguishable (Figure 1.1C). Therefore, we suggest considering photo identification as a unique identification technique for *U. brucei*, which is less invasive than other marking techniques (i.e., toe-clipping, elastomer marking) and has been successful in differentiating similarly sized and dorsally patterned salamander individuals (i.e., Blue Ridge Two-lined Salamander [*Eurycea wilderae*]; Bailey 2004).

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TABLES

Table 1.1. Stream characteristic averages and standard deviations based on stream segment (*n* = 13 per stream) measurements of three Patch-nosed Salamander (*Urspelerpes brucei*) occupied streams in Georgia and South Carolina, USA, 2021–2022.

Stream	**Stream depth (mm)**	**Wetted stream width (m)**	**Canopy closure (%)**
GA2	8.3 ± 3.0	1.6 ± 0.7	98.3 ± 2.4
SC1	37.2 ± 12.9	3.2 ± 1.1	97.7 ± 4.2
SC2	12.0 ± 5.7	1.8 ± 0.7	96.8 ± 8.4

Table 1.2. A description of stream-specific and survey-specific variables measured at three Patch-nosed Salamander (*Urspelerpes brucei*) occupied streams in Georgia and South Carolina, USA, 2021–2022.

Variable	Description
Stream-specific	
Canopy closure (%)	Continuous variable describing percent of shade at the top of each segment
Leaf litter depth (mm)	Continuous variable describing average depth of leaf litter for each segment
Leaf litter moisture (%)	Continuous variable describing average percent of leaf litter saturated for each segment
Stream depth (mm)	Continuous variable describing average depth of water column for each segment
Wetted stream width (m)	Continuous variable describing wetted width of stream channel for each segment
Distance to flowing water (m)	Continuous variable describing segment distance from segments characterized as flowing water
Survey-specific	
Survey method	Categorical variable describing each survey methods employed to detect *U. brucei* during a seasonal window
Cloud cover (%)	Continuous variable describing percent of cloud cover at the start of each survey
Soil temperature (°C)	Continuous variable describing soil temperature at the start of each survey
Total rainfall in past week (cm)	Continuous variable describing total local rainfall amounts for the week prior to each survey
Leaf litter depth (mm)	Continuous variable describing depth of leaf litter for each segment
Leaf litter moisture (%)	Continuous variable describing percent of leaf litter saturated for each segment
Soil moisture (%)	Continuous variable describing percent of soil moisture saturated for each segment
Stream depth (mm)	Continuous variable describing depth of water column for each segment

Table 1.3. *A priori* models for hypotheses regarding covariates associated with Patch-nosed Salamander (*Urspelerpes brucei*) detection at three occupied streams in Georgia and South Carolina, USA, 2021–2022.

Hypothesis	**Model structure**	**Predictions**
Detection is constant (null model)	*p*(.)	Covariates have no effect on detection
Detection is influenced by weather condition	*p* = β1(cloud cover) + β2(rainfall totals) + β3(soil temperature)	Cloud cover (-), rainfall totals (-), and soil temperature (-)
Detection is influenced by available refugia	*p* = β1(leaf litter depth + stream depth)	Leaf litter depth (+), stream depth (-)
Detection is influenced by breeding activity	*p* = β1(day of year survey conducted) + β2(day of year survey conducted^2)	Day of year survey conducted (+ with spring and fall)
Detection is influenced by survey method	*p* = β1(aquatic constrained) + β2(terrestrial constrained) + β3(aquatic leaf litter bag) + β4(terrestrial leaf litter bags) + β5(aquatic unconstrained) + β6(terrestrial unconstrained)	Aquatic constrained (-), terrestrial constrained (-), aquatic leaf litter bag (+), terrestrial leaf litter bag (+), aquatic unconstrained (+), and terrestrial unconstrained (+)

Table 1.4. *A priori* models for hypotheses regarding covariates associated with Patch-nosed Salamander (*Urspelerpes brucei*) occupancy at three occupied streams in Georgia and South Carolina, USA, 2021–2022.

Hypothesis	**Model structure**	**Predictions**
Occupancy is constant (null model)	Ψ(.)	Covariates have no effect on occupancy
Occupancy is influenced by leaf litter accumulation	Ψ = β1(leaf litter depth)	Leaf litter depth (+)
Occupancy is influenced by stream	Ψ = β1(site)	Site (occupancy varies by site)

Table 1.5. Summary of Patch-nosed Salamander (*Urspelerpes brucei*) captures for each survey method (ACS: aquatic area-constrained survey, ALLB: aquatic leaf litter bag survey, AUS: aquatic area-unconstrained survey, TCS: terrestrial area-constrained survey, TLLB: terrestrial leaf litter bag survey, and TUS: terrestrial area-unconstrained survey) across all seasonal windows at three occupied streams in Georgia and South Carolina, USA, 2021–2022. Values reported indicate the number of larvae detected / number of adults detected for each survey.

		Seasonal window						
Stream	**Survey method**	Summer2021	Fall2021	Winter2021	Spring2022-A	Spring2022-B	Spring2022-C	**Grand total**
GA2	ACS	**2/-**	-	-	-	-	**1/-**	**3/-**
	ALLB	**2/-**	-	**1/-**	**1/-**	**-/2**	**8/1**	**12/3**
	AUS	n/a	**1/-**	-	**5/-**	**2/-**	**9/-**	**17/-**
	TCS	-	-	-	-	**-/2**	-	**-/2**
	TLLB	-	-	-	**-/3**	**-/3**	-	**-/6**
	TUS	n/a	**-/1**	-	**-/3**	**-/9**	**-/4**	**-/17**
SC1	ACS	-	-	n/a	-	-	-	-
	ALLB	-	**2/-**	n/a	-	-	-	**2/-**
	AUS	n/a	-	n/a	**1/-**	-	-	**1/-**
	TCS	-	-	n/a	-	-	-	-
	TLLB	-	-	n/a	-	-	-	-
	TUS	n/a	-	n/a	-	-	-	-
SC2	ACS	**1/-**	-	**1/-**	-	**-/1**	-	**2/1**
	ALLB	**1/1***	**1/-**	**1/-**	**3/-**	-	**1/-**	**7/1***
	AUS	n/a	-	-	**1/-**	**2/1***	**3/-**	**6/1***
	TCS	-	-	-	-	-	-	-
	TLLB	-	-	-	**-/2**	**-/4**	**-/2**	**-/8**
	TUS	n/a	-	-	**-/1**	**-/2**	-	**-/3**
	Grand total	**6/1***	**4/1**	**3/-**	**11/9**	**4/24***	**22/7**	**50/42** = 92**

n/a Survey method not conducted.

*One metamorph included in adult count.

Table 1.6. Microhabitat characteristic averages and ranges based on Patch-nosed Salamander (*Urspelerpes brucei*) capture points at three occupied streams in Georgia and South Carolina, USA, 2021–2022.

Microhabitat characteristics	**Life stage**	
	Larvae	Adults
Leaf litter depth (mm)	35.3 [0–140]	83.8 [22–320]
Stream depth (mm)	14.2 [0–52]	1.2 [0–32]
Distance to stream edge (mm)*	-114.9 [-535–0]	211.6 [0–675]

*Values represent distance from the stream edge, where positive values indicate distance into the riparian area and negative values indicate distance toward the center of the wetted stream channel.

Table 1.7. Ranking of confidence set of models from most supported to least supported that are hypothesized to influence detection (step one) and occupancy (step two) of Patch-nosed Salamanders (*Urspelerpes brucei*) at three occupied streams in Georgia and South Carolina, USA, 2021–2022.

Model	**Model structure**	K^b	**AICc**	**ΔAICc**	**Wt**
Detection (step one) confidence set					
Weather condition	$\Psi(.)$ p(cloud + rainfall + soil temperature)	5	496.55	0.00	0.56
Survey method	$\Psi(.)$ p(method)	7	497.61	1.06	0.34
Available refugia	$\Psi(.)$ p(leaf litter depth + stream depth)	4	501.24	4.69	0.05
Breeding activity	$\Psi(.)$ p(day of year survey conducted + day of year survey conducted^2)	4	501.38	4.83	0.05
Null	$\Psi(.)$ $p(.)$	2	513.11	16.56	0.00
Occupancy (step two) confidence set					
Stream	Ψ(stream) p(method + cloud + rainfall + soil temperature)	12	472.52	0.00	0.96
Null occupancy	$\Psi(.)$ p(method + cloud + rainfall + soil temperature)	10	479.71	7.18	0.03
Leaf litter accumulation	Ψ(leaf litter depth) p(method + cloud + rainfall + soil temperature)	11	481.78	9.26	0.01
Null	$\Psi(.)$ $p(.)$	2	513.11	40.59	0.00

[b] Number of parameters in the model.

Table 1.8. Top ranked occupancy model parameters, estimates, standard errors (SE), and 95% confidence intervals (CI) for factors hypothesized to influence detection and occupancy of Patch-nosed Salamanders (*Urspelerpes brucei*) at three occupied streams in Georgia and South Carolina, USA, 2021–2022. Detection and occupancy parameter estimates are scaled.

Parameter	**Estimate**	**SE**	**Lower 95% CI**	**Upper 95% CI**
Detection (*p*)				
Aquatic area-constrained (Intercept)	-3.32	0.43	-4.17	-2.46
Terrestrial area-constrained	-1.14	0.83	-2.77	0.48
Aquatic leaf litter bag	1.40	0.50	0.43	2.37
Terrestrial leaf litter bag	0.85	0.52	-0.18	1.87
Aquatic area-unconstrained	1.50	0.51	0.50	2.50
Terrestrial area-unconstrained	1.43	0.51	0.42	2.43
Cloud cover	-0.39	0.20	-0.77	-0.01
Total weekly rainfall	0.36	0.13	0.10	0.61
Soil temperature	0.32	0.15	0.03	0.61
Occupancy (Ψ)				
GA2 (Intercept)	0.86	0.62	-0.36	2.08
SC1	-2.49	1.00	-4.44	-0.53
SC2	1.86	2.11	-2.28	6.00

FIGURES

Figure 1.1. Photos of Patch-nosed Salamander (*Urspelerpes brucei*) individuals (A: typical adult male with prominent nasal cirri, B: typical adult female, C: typical larva) captured at one stream in Georgia, USA, 2022.

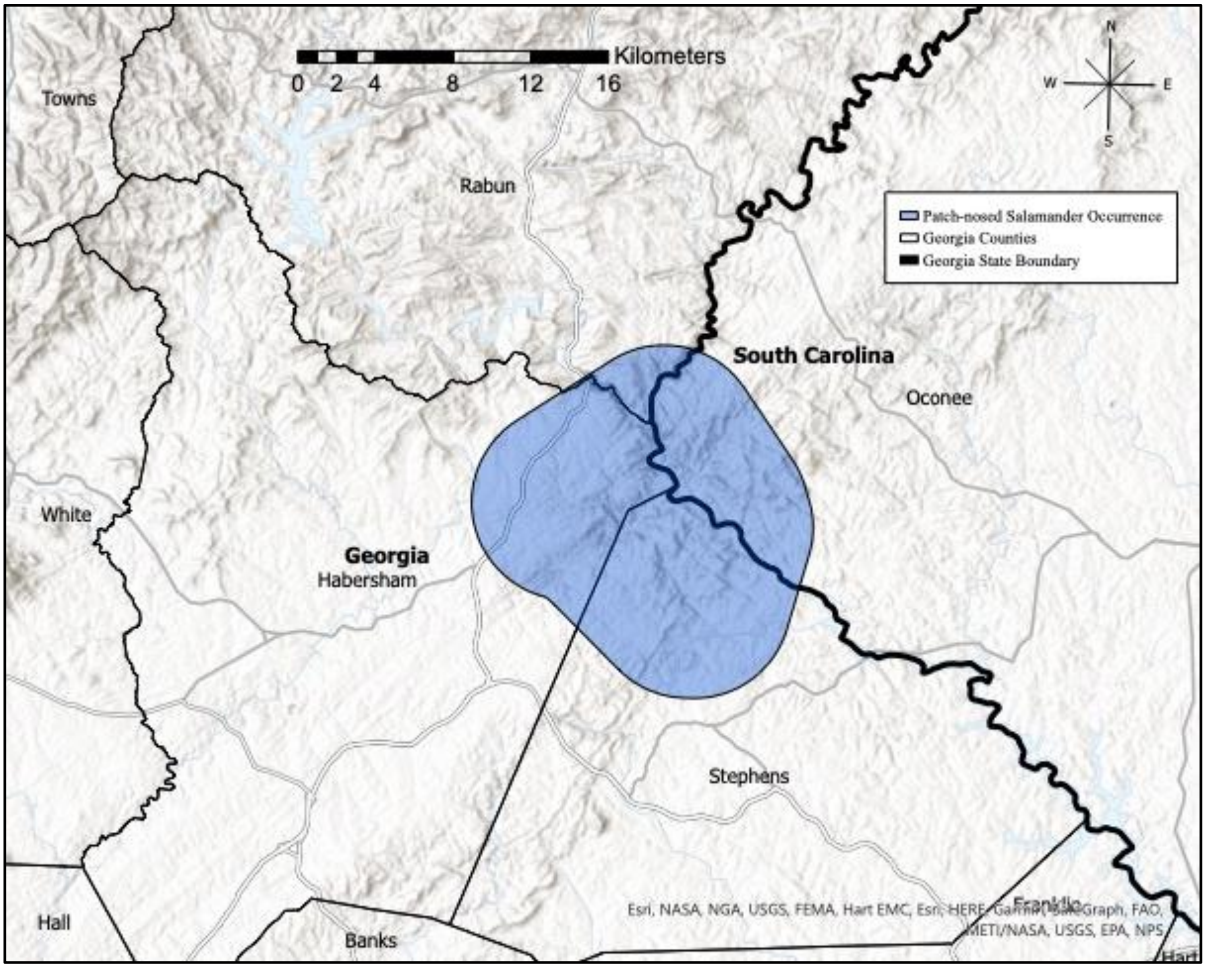

Figure 1.2. Map of Patch-nosed Salamander (*Urspelerpes brucei*) distribution in Georgia and South Carolina, USA, 2021–2022.

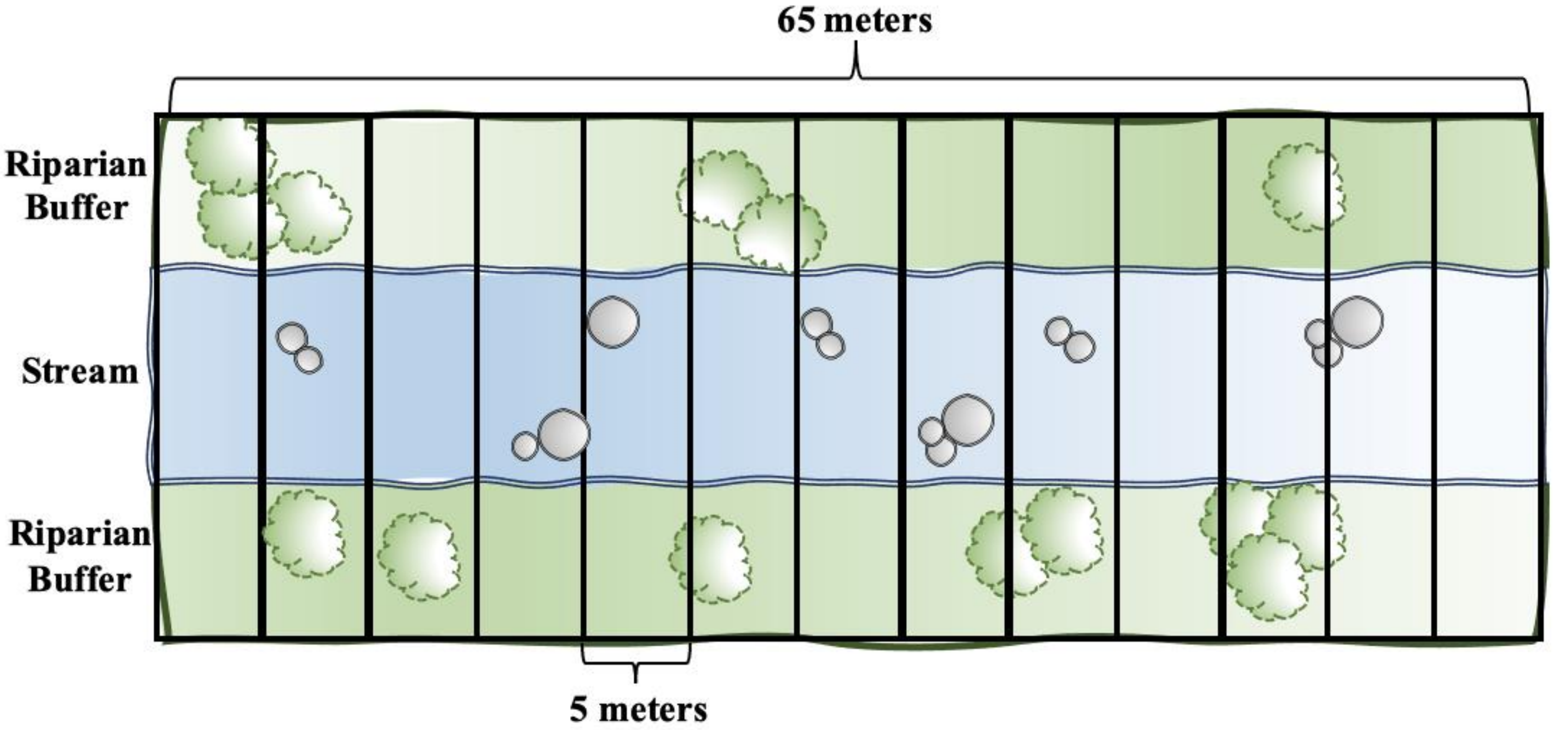

Figure 1.3. Representation of experimental design for evaluating Patch-nosed Salamander (*Urspelerpes brucei*) occupancy. We subdivided each 65-stream reach into 13 five-meter-long stream segments that included an aquatic (represented by stream) and terrestrial (represented by riparian buffer) portion, which we then used as our sampling units for understanding occupancy.

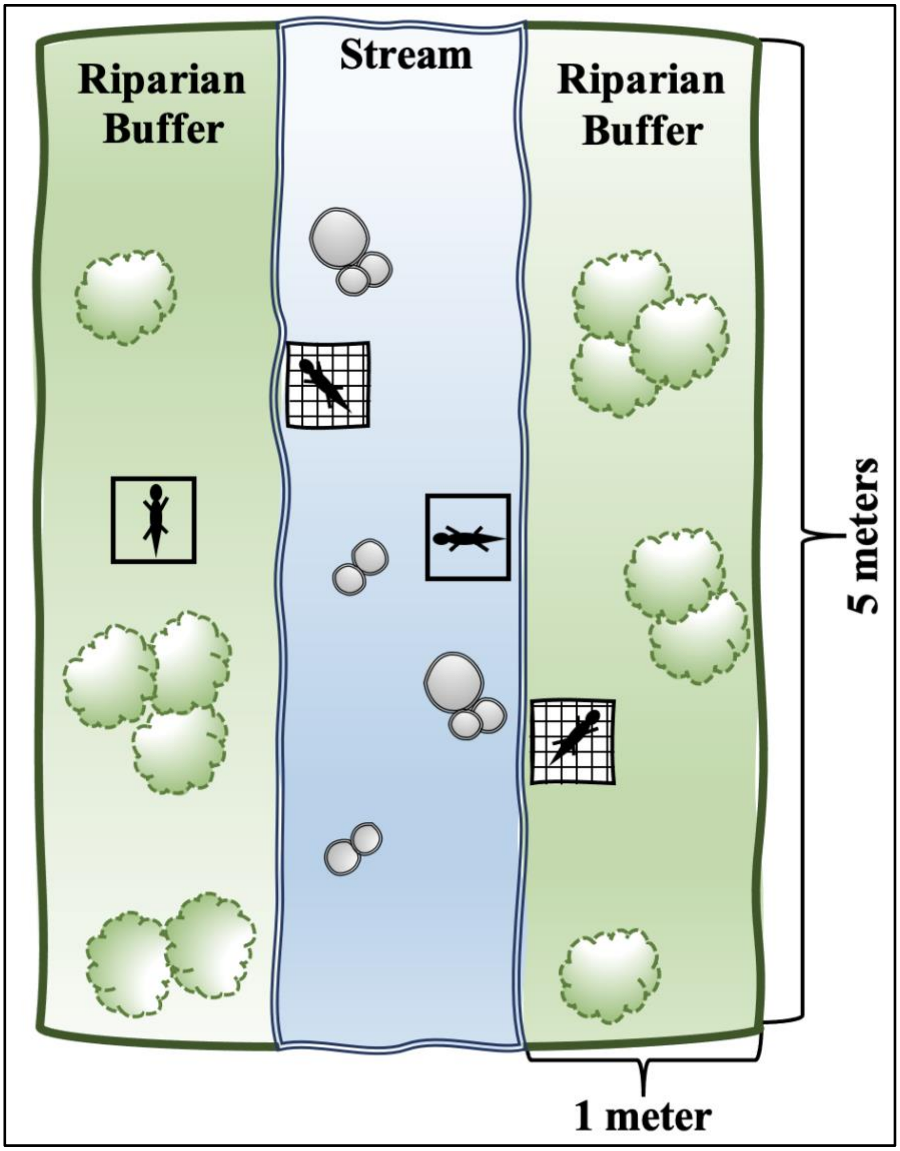

Figure 1.4. Experimental design for evaluating Patch-nosed Salamander (*Urspelerpes brucei*) detectability using six survey methods. Five-meter-long stream segments were used as an experimental blocking factor. Gridded squares represent leaf litter bags (one aquatic and one terrestrial), and open squares represent constrained survey quadrats (one aquatic and one terrestrial). Area-unconstrained survey areas (aquatic and terrestrial) are represented by the stream or riparian buffer of each segment.

Figure 1.5. Photos of Patch-nosed Salamander (*Urspelerpes brucei*) adults exhibiting sexual characteristics (A: gravid female (ventral view), B: male with prominent nasal cirri) captured at one stream in Georgia, USA, 2022.

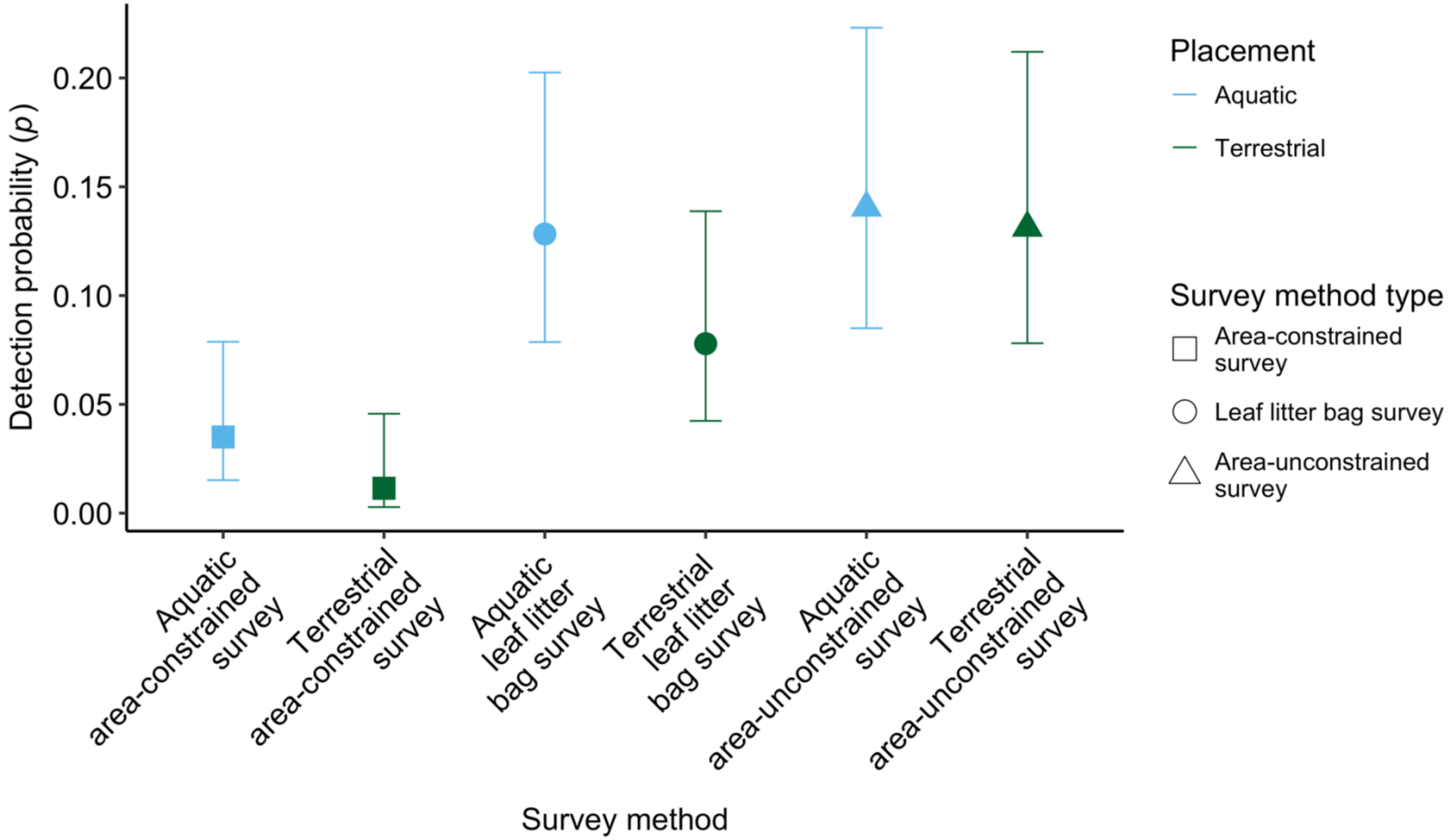

Figure 1.6. The effect of survey method on Patch-nosed Salamander (*Urspelerpes brucei*) detection probability at three occupied streams in Georgia and South Carolina, USA, 2021–2022. The solid point represents predicted detection probability, and the bars represent 95% confidence intervals.

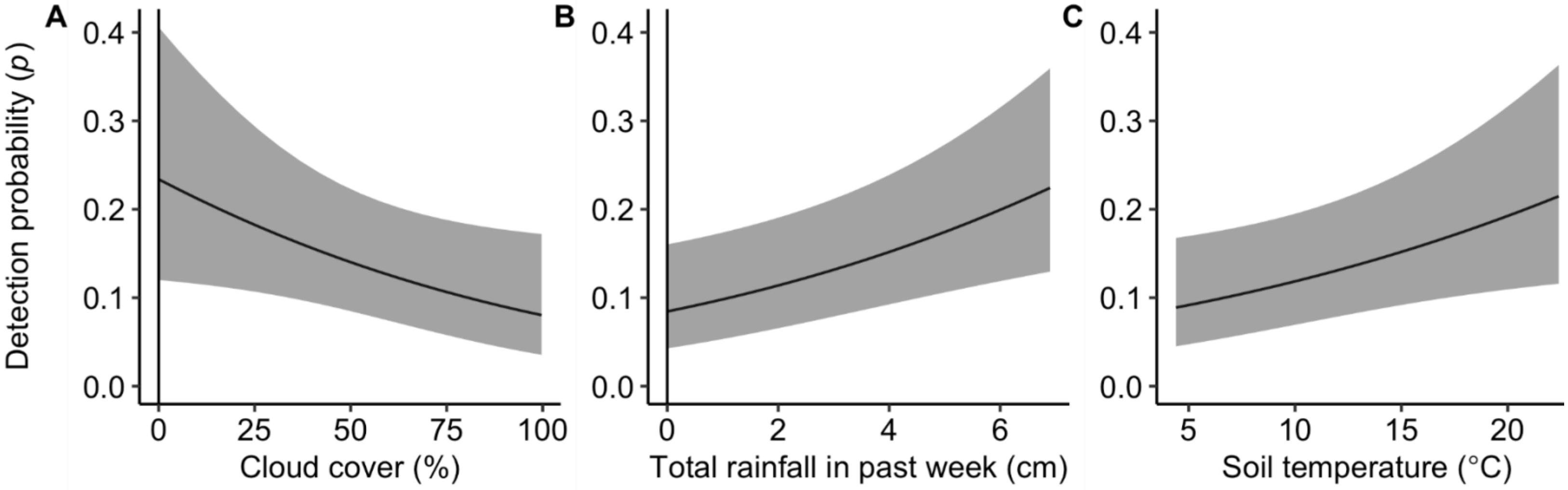

Figure 1.7. The effect of weather condition (A: cloud cover (%), B: total rainfall in past week (cm), C: soil temperature (°C)) on Patch-nosed Salamander (*Urspelerpes brucei*) detection probability at three occupied streams in Georgia and South Carolina, USA, 2021–2022. The solid line represents the predicted detection probability, and the shaded areas represent 95% confidence intervals. Estimates shown here were generated assuming the aquatic area-unconstrained survey was the method being used.

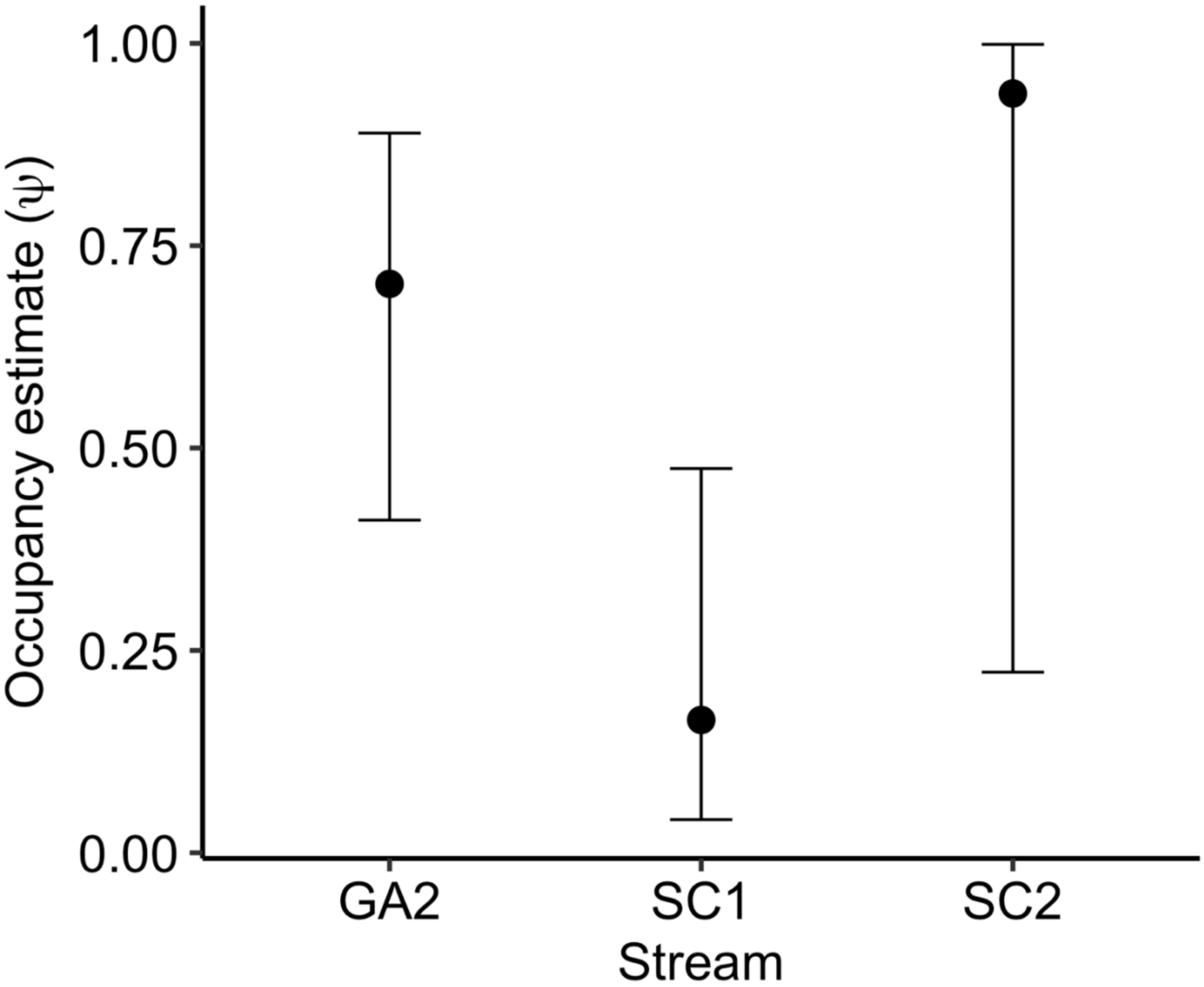

Figure 1.8. The effect of stream identity on Patch-nosed Salamander (*Urspelerpes brucei*) occupancy estimates at three occupied streams in Georgia and South Carolina, USA, 2021–2022. The solid point represents the predicted occupancy estimate, and the bars represent 95% confidence intervals.

Figure 1.9. Photo of ideal Patch-nosed Salamander (*Urspelerpes brucei*) microhabitat located in Georgia, USA, 2022.

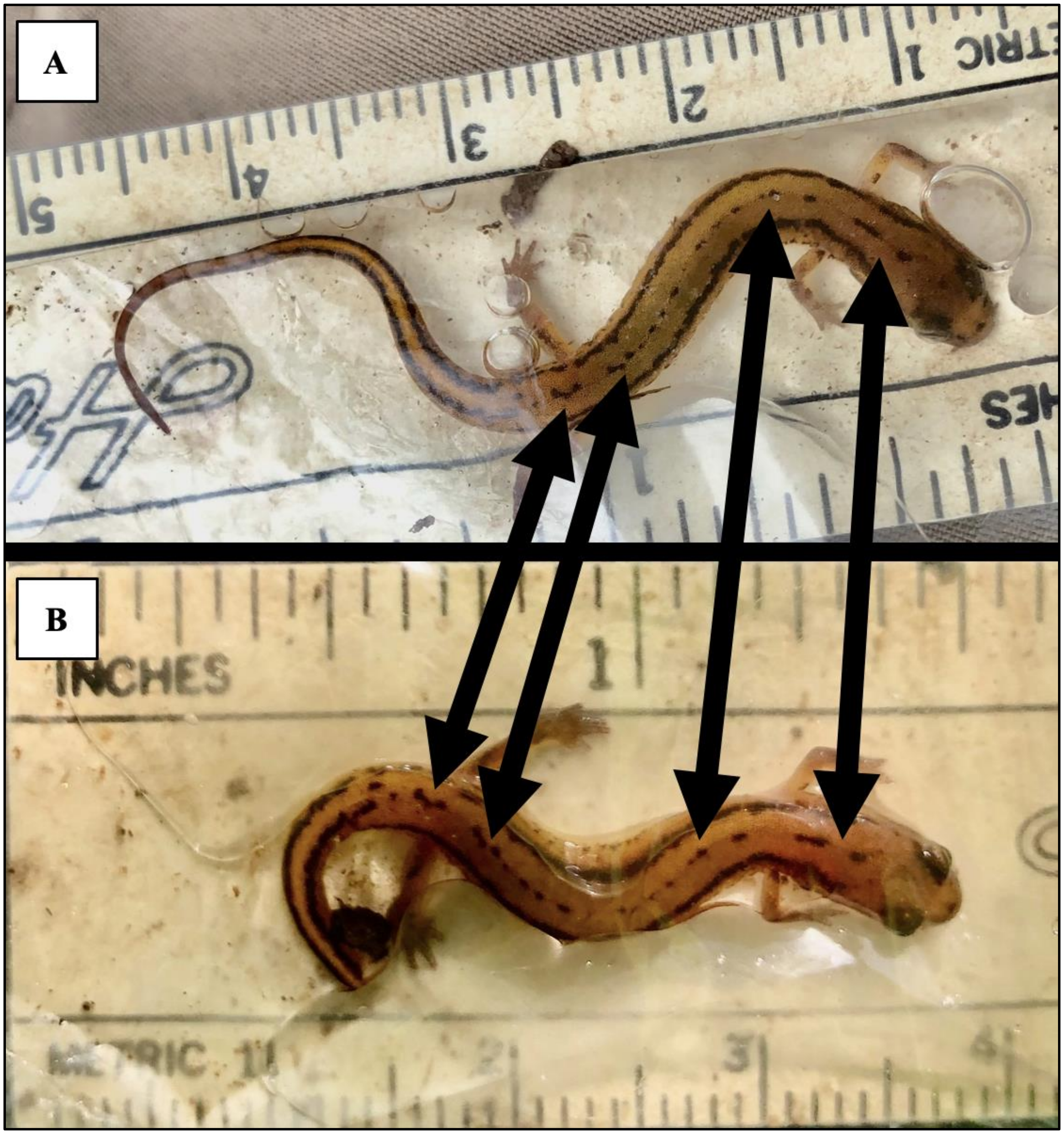

Figure 1.10. Photos (A: taken on 12 April 2022, B: taken on 23 April 2022) showing unique dorsal pattern of a male Patch-nosed Salamander (*Urspelerpes brucei*) found in Georgia, USA that could be used as a form of individual identification.

Chapter 2: Comparing Microhabitat Use of Larval and Adult Patch-nosed Salamanders (*Urspelerpes brucei*) in Three Occupied Streams

ABSTRACT

Patch-nosed Salamanders (*Urspelerpes brucei*) are small, enigmatic plethodontids discovered in 2007, with a patchy distribution across a small geographic range (29 km^2; at the onset of our study), and low encounter rates, especially for adults. Though some *U. brucei* stream level characteristics have been described, factors associated with microhabitat use are poorly understood but are essential components of effective conservation and management. We used an occupancy approach to investigate microhabitat use for *U. brucei* larvae and adults separately. In the larval *U. brucei* analysis, our best supported model describing microhabitat use supported leaf litter accumulation as the primary predictor (when stream depth was $\leq$ 4 mm and leaf litter depth was $\geq$ 70 mm). Similarly, in our adult *U. brucei* analysis, our best supported model describing microhabitat use also supported leaf litter accumulation as the principal driver (when leaf litter depth was $\geq$ 111 mm). Collectively, our findings indicate microhabitat use (i.e., leaf litter) may explain patterns of *U. brucei* occurrence at broader scales when considering distribution among occupied streams and possibly inform occurrence from stream to stream throughout *U. brucei*'s geographic range.

INTODUCTION

Biodiversity loss is proceeding at an alarming degree with the rate of extinction exponentially increasing (Masters et al. 2006; Johnson et al. 2017; Sage 2020). Amphibians, 40% of which are classified as threatened (Johnson et al. 2017), face multiple conservation risks, especially global climate change (Stuart et al. 2004; Bernardo and Spotila 2006), habitat degradation (Vié et al. 2009; Peterman et al. 2011), and the spread of fungal pathogens (Rohr and Raffel 2010; Bletz et al. 2013; Carter et al. 2019). Amphibian species thought to be particularly vulnerable to climate change express sensitivity attributes like environmental thresholds (i.e., species already living at the limit of tolerable conditions; Sage 2020), rarity (i.e., small population sizes, small geographic range), high exposure to other pressures (i.e., disease outbreaks, habitat degradation), and have specialized microhabitat requirements (i.e., require multiple habitats associated with life stages; Vié et al. 2009; Foden 2018).

Though mature, complex forest structures have been described as major climate buffers (i.e., lower forest floor temperatures; Frey et al. 2016; Davis et al. 2018), microhabitat (i.e., leaf litter, substrate, burrows, stream) can also act as a climate refuge for amphibians (Seebacher and Alford 2002; Shoo et al. 2011; González-del-Pliego et al. 2020; Tong et al. 2023). Therefore, conserving microhabitats that serve as a climate buffer for amphibians may be especially important when considering the likelihood of global temperature rise, changing precipitation patterns (Sage 2020), and the inability of some species to disperse over larger areas in search of more suitable habitat. Microhabitat is an integral component of amphibian survival because it can function as refugia (i.e., predator avoidance), facilitate ideal microclimates (i.e., desiccation avoidance), and provide foraging opportunities (Keen 1979; Feder 1983). Specifically, because microhabitat affects temperature and humidity levels, it can indirectly influence amphibian body temperature and water retention (Seebacher and Alford 2002; Shoo et al. 2011), which regulates amphibian activity, especially for plethodontid salamanders (Feder 1983). Broad scale disturbance can have trickle down effects on amphibian health by changing microhabitat quality. For example, harvesting timber alters the canopy structure resulting in less overall leaf litter (Likens et al. 1978; Pough et al. 1986), lower moisture gradients, and higher forest floor and stream temperatures (Frey et al. 2016; Davis et al. 2018); all of which have been linked to amphibian declines (Nowakowski et al. 2016).

One example of a species that may be particularly vulnerable to global climate change and other negative pressures (i.e., land use change) is the Patch–nosed Salamander (*Urspelerpes brucei*). *Urspelerpes brucei* is a small plethodontid salamander that occurs in first- and second-order streams across a small Appalachian region (29 km^2; as of 2022) of northeast Georgia (GA) and northwest South Carolina (SC; Camp et al. 2009). Though some *U. brucei* stream level characteristics have been described (Camp et al. 2009; Camp et al. 2018), factors associated with *U. brucei* microhabitat use are poorly understood. The objective of our study was to investigate *U. brucei* larvae and adult microhabitat use separately in three occupied streams to better understand this species of high conservation concern (GA State Wildlife Action Plan [SWAP] 2015; SC SWAP 2015).

METHODS AND MATERIALS

Study sites

Our study sites included three 65-m streams known to harbor *U. brucei* in northeast GA (*n* = 1) and northwest South Carolina (*n* = 2; Figure 1.2) that are tributaries of the Tugaloo River and located in the upper Savannah River basin. Located in the Tugaloo Mosaic physiographic region, this area is characterized by unique soils and flora similar the Blue Ridge (Menzel et al. 2016). All reaches occurred in first-order streams located on either SC Department of Natural Resource managed land (labeled as SC1 and SC2) or private property in GA (labeled as GA2). We are withholding stream locality due to conservation concerns.

Our streams were typically shallow (< 38 mm) with steep ravines, closed canopies, and contained at least one spring head and/or seep (Table 1.1, Figure 2.1). We chose streams based on known *U. brucei* occurrence and accessibility of each. Though *U. brucei* is known from three localities in SC, we excluded one because it did not meet stream length requirements (≥ 65 m) and was an isolated observation from a potentially ephemeral water source. We chose to include one GA stream to compare *U. brucei* probability of use between SC and GA since, before the onset of our study, anecdotal observations of *U. brucei* seem more common in the latter.

Study design

We used occupancy monitoring approach to investigate factors influencing *U. brucei* microhabitat use in occupied streams. We defined occupancy as the probability that at least one *U. brucei* used a selected quadrat (31 x 31 cm) throughout our sampling period (August 2021–June 2022). Due to our small quadrat size, we relaxed the closure assumption (i.e., that occupancy status remains static for each habitat patch throughout the sampling period) that is inherent to a more common occupancy analysis and, following advice of MacKenzie et al. (2006), interpret occupancy estimates as probability of use. This approach allowed us to account for imperfect detection by repeating surveys throughout our sampling period (MacKenzie et al. 2006). We defined detection probability as the probability of detecting at least one *U. brucei* individual within a truly used quadrat during a single replicate survey.

We used a random stratified design to investigate factors influencing *U. brucei* microhabitat use (Garton et al. 2012). We defined strata as the 1) terrestrial and 2) aquatic portion of our 65-m streams (Figure 2.2). We considered the entire wetted width of the stream to fall within our aquatic strata. Because all prior observations of adult *U. brucei* have occurred in one meter of streams edge, we defined our terrestrial strata to include a 1-m riparian area on either side of a stream segment (Camp et a. 2018; pers. comm., Todd Pierson). To ensure even sampling along our stream reaches, we sub-divided each stratum into 13 linear segments (5-m each) and randomly selected four static quadrats (2 aquatic and 2 terrestrial; 31 cm x 31 cm each) from each segment to use as our sampling units for monitoring microhabitat use (4 quadrats per segment x 13 segments/stream x 3 streams = 156 quadrats total; Figure 2.3). Because we were unsure about the most effective survey method for detecting *U. brucei* larvae and adults at the onset of our study we assigned each of the four static quadrats in a segment to a unique survey method. We randomly assigned aquatic quadrats to either 1) aquatic area-constrained surveys or 2) aquatic leaf litter bag surveys and randomly assigned terrestrial quadrats to either 3) terrestrial area-constrained surveys, or 4) terrestrial leaf litter bag surveys. Notably, we used a subset of field data collected in Chapter 1 for analysis in Chapter 2; specifically, we did not consider data associated with aquatic or terrestrial area-unconstrained surveys. We used pin flags to permanently mark the top left and bottom left corners of each static quadrat for the entirety of the study. If pin flags were not applicable markers, we flagged rocks and/or vegetation to mark corners. We set out leaf litter bags by first filling them with leaf litter found in the assigned quadrat, and if aquatic, then we completely submerged the bag before securing in the quadrat. We secured aquatic leaf litter bags with rocks or paracord to prevent displacement during rain events. To our knowledge, terrestrial leaf litter bags are a novel survey method that have never been used prior to this study. We suspected terrestrial leaf litter bag surveys might be useful at detecting *U. brucei* adults given most adult captures have been along the streambank in leaf litter (Camp et al. 2009).

Field surveys

We searched each quadrat five to six times (i.e., Summer_2021, Fall_2021, Winter_2021, Spring_2022-A, Spring_2022-B, Spring_2022-C) between August 2021 and June 2022 (Table 2.1). However, we were unable to conduct surveys at one stream (SC1) during the winter seasonal window due to construction work limiting access.

Area-constrained surveys---We defined area-constrained surveys as visual searches of static microhabitat quadrats (n = 1 aquatic and 1 terrestrial per stream segment), that we assigned to a random (aquatic or terrestrial, depending on method) location in a 5-m stream segment. We conducted area-constrained surveys by exhaustively searching refugia (i.e., hand picking through leaf litter, pumping leaf litter and sediment into an aquarium net, and flipping potential refugia) that could potentially conceal a salamander.

Leaf litter bag surveys--- To conduct leaf litter bag surveys, we set up two processing areas on dry ground, one for aquatic leaf litter bags and one for terrestrial leaf litter bags. For the aquatic station, we filled a 10-gallon tub with approximately two gallons of stream water using a manual pump. We then removed the aquatic leaf litter bag from the stream, placed it in a net to prevent salamander escapes, and then transferred it to the tub. We agitated the bag for 15–30 seconds, set it aside, poured tub contents through an aquarium net, and examined net content for salamanders. To redeploy the aquatic leaf litter bag, we replaced a quarter of the leaf litter contents with fresh leaf litter, wetted it in the stream, and returned the bag to its original placement. To check terrestrial leaf litter bags, we emptied all contents into a tub and thoroughly searched for salamanders. We replaced a quarter of the leaf litter with fresh leaf litter and returned the bag to its original placement.

Salamander processing---We captured and processed all salamanders that we encountered while examining each quadrat. We placed individuals in a 16 x 15 cm plastic bag with approximately 60 mL of stream water, recorded capture method, identified to species when possible, and subjected each to standard processing. We processed individuals by recording stage (larvae, metamorph, or adult) and measuring snout-vent length (SVL) and total length (TL) to the nearest mm. Additionally, we processed all *U. brucei* by recording mass to the nearest 0.1 g, sex, and noting sexual characteristics (i.e., gravid, cirri). If species were not identifiable in the field, we took a dorsal and ventral photograph next to a metric ruler to later identify to the lowest taxonomic level possible. After an individual was processed, we returned it to its initial capture location.

Predictors

Throughout our sampling period, we measured six survey-specific, four quadrat-specific, and two stream-specific covariates that we hypothesized might influence *U. brucei* detection and microhabitat use (Table 2.2). For each survey, we recorded cloud cover and preceding weekly precipitation amounts by accessing historical weather data through Visual Crossing Corporation's Weather Query Builder from three local weather stations (KTOC, F6979, and 72525800382). Additionally, we recorded soil temperature in the same location at the start of each survey using a digital probe thermometer. Also, during each survey, we measured leaf litter and stream depth using a metric ruler in the center of each quadrat to serve as a survey-specific covariate informing detection. We then separately averaged each survey-specific measurement of leaf litter and stream depth for each unique quadrat to serve as a quadrat-specific covariate that we hypothesized would influence *U. brucei* probability of use. We also measured quadrat distance (± 5 m) from nearest flowing water (i.e., portion of the wetted stream with visible flow) and distance to nearest spring head/seepage; Figure 2.4).

Data analysis

We used single-species, single-season occupancy models to estimate the probability of *U. brucei* using a microhabitat quadrat over the course of the sampling period while accounting for imperfect detection (MacKenzie et al. 2006). We modeled use separately for *U. brucei* larvae and adults.

We created two separate detection histories, one for *U. brucei* larvae and one for adults, based on observations of each in our quadrats. Because *U brucei* larvae are fully aquatic, we only considered aquatic microhabitat quadrats ($n = 78$) in our analysis of larvae microhabitat use. For this analysis, we coded detection histories for each quadrat such that ‘1’ represented an occasion when at least one larval *U. brucei* was observed in an aquatic quadrat, and ‘0’ indicated no larval *U. brucei* were detected. Because adult *U. brucei* can use aquatic and terrestrial microhabitats, we considered all quadrats ($n = 52$ per site) in our adult analysis. However, due to zero adult *U. brucei* captures at our SC1 stream over the course of our study, we removed all SC1 associated data for the adult analysis. We coded detection histories for each quadrat such that ‘1’ indicated detection of at least one adult *U. brucei* using a quadrat, and ‘0’ indicated that no adults were detected. Replicate surveys corresponded to each time a quadrat was examined throughout our study ($n = 5$ to 6 times per quadrat). We used the detection histories of larvae and adults as the response data in each occupancy analysis.

Model development

Prior to each analysis, we evaluated all predictor variables for potential collinearity, and considered variables correlated when $|r|$ was ≥ 0.7. If variables were correlated, we discarded one variable by selecting the variable we considered to have the most ecological relevance to our hypothesis.

Larval microhabitat use

We considered two *a priori* hypotheses thought to influence larval *U. brucei* microhabitat use (Table 2.3). We hypothesized leaf litter accumulation in the form of leaf litter depth and stream depth was the driver of larval microhabitat use. We predicted average leaf litter depth of each microhabitat quadrat would be positively associated with larval microhabitat use due to a greater moisture retention capacity of deeper leaf litter packs, which is vital for shallow streams. We also predicted shallower stream depths would be positively associated with larval microhabitat use because it indicates slower stream flows (i.e., seepages) and allows for more significant leaf litter buildup. We also considered the hypothesis that larval *U. brucei* may use seep and spring heads to avoid deeper water columns and faster flows (Camp et al. 2018). Therefore, we considered a model that included an additive effect of quadrat distance from flowing water. We predicted microhabitat use estimates would increase as distance from flowing water increased. We also included a null model to represent our hypothesis that larval *U. brucei* microhabitat use would be constant across our quadrats.

We considered five *a priori* hypotheses to evaluate detectability of larval *U. brucei* (Table 2.4). We hypothesized weather condition (cloud cover, weekly precipitation, and soil temperature) would negatively impact larval detection by reducing visibility during surveys (i.e., darker environment, muddy stream). We hypothesized *U. brucei* larvae were primarily using leaf litter in the stream as their refugia. However, based on previous *U. brucei* larvae encounters near spring heads (Camp et al. 2018), we hypothesized they were using leaf litter in shallowest portions of the stream. Therefore, we predicted a positive relationship between detection probability and leaf litter depth but a negative relationship between detection probability and stream depth. Though *U. brucei* breeding and larval emergence seasons are unknown, *Eurycea* are known to oviposit February through May, with emergence occurring 4–10 weeks later (Petranka 1998), and observations of captive *U. brucei* larvae suggest emergence at least occurs through July (Miller-Murthy et al. 2021). Therefore, we hypothesized larval detectability would increase in spring and potentially fall relative to summer. We represented this hypothesis by using a quadratic expression of survey date ($x + x^2$). We hypothesized that microhabitat use might be driven by competition and predator avoidance given the small size of *U. brucei* larvae (18.01 [$\bar{x}$] ± 0.41 mm [SE] SVL; Camp et al. 2018) and their assumed susceptibility to depredation by larger salamanders (Petranka 1998). To represent our predator avoidance hypothesis, we created a detection history of other salamander species for each static quadrat and predicted microhabitat use would increase in quadrats where other species were detected less often. Notably, we considered predator avoidance a detection covariate instead of a probability of use covariate due to the likely fluid movement of larger salamander species in and out of our small quadrats. Lastly, we evaluated survey method (aquatic area-constrained surveys, aquatic leaf litter bag surveys) as a categorical variable driving detection probability of *U. brucei* larvae. We predicted aquatic leaf litter bag surveys would be the most successful larval *U. brucei* survey type due to previous studies success utilizing this method (Camp et al. 2018). We also included a null model to represent the hypothesis that both detection and probability of use remained constant despite hypothesized detection covariates.

Adult microhabitat use

We considered two *a priori* hypotheses concerning microhabitat use (Table 2.5). We hypothesized adults would use areas with greater leaf litter accumulation measured as leaf litter depth. Microhabitat is a principal mediating factor influencing moisture retention and hydration of lungless salamanders (Heatwole and Lim 1961). Therefore, we predicted higher average leaf litter depths would be positively associated with adult microhabitat use. Analogous to *U. brucei* larvae, we hypothesized adults were occupying microhabitats associated with seeps and spring heads based on previous observations (Camp et al. 2009; Camp et al. 2018), which we represented as an additive form of distance from flowing water. We predicted *U. brucei* adults would occupy riparian quadrats that were further away from riparian areas adjacent to flowing water. We also included a null model to represent our hypothesis that adult *U. brucei* microhabitat use would be constant across our quadrats.

We examined five relevant *a priori* hypotheses associated with adult *U. brucei* detection probability (Table 2.6). We hypothesized weather condition (cloud cover, weekly precipitation totals, and soil temperature) would influence *U. brucei* adult detections in a similar manner to larvae. Therefore, we predicted as cloud cover and weekly precipitation totals increased, adult detection probability would decrease. We predicted high soil temperatures would negatively impact adult detectability because plethodontids seem to remain underground in warmer months, especially during the day (Petranka 1998). We also hypothesized available refugia represented as leaf litter depth would influence *U. brucei* adult detections. We predicted a positive relationship between detection probability and leaf litter depth based on anecdotal descriptions of adult *U. brucei* observations (i.e., loose leaf litter ≤ 1 meter from stream edge in the riparian area; Camp et al. 2009; Camp et al. 2018). We also hypothesized adult surface activity would increase during *U. brucei* breeding season. Though exact seasonality of *U. brucei* breeding is unknown, eight adults captured between April and May in 2007 exhibited sexual characteristics associated with breeding conditions (i.e., gravid, obvious nasal cirri and mental glands; Camp et al. 2009), and a nest was observed in March 2020 (Miller-Murphy et al. 2021), indicating a breeding season associated with spring. Though, we hypothesized, analogous to *Eurycea* species found in the region, breeding may also occur in the fall. Therefore, we predicted adult detection would increase during the spring and fall seasonal windows, which we represented by a quadratic expression of survey date ($x + x^2$). Due to the small size of *U. brucei* adults (25.76 ± 0.17 mm SVL; Camp et al. 2018), we hypothesized adult detectability might also be influenced by competition and predator avoidance; similarly sized salamander species (e.g., Two-lined Salamanders [*Eurycea bislineata*]) have been documented prey of larger salamanders (Petranka 1998) known to co-occur with *U. brucei*. Thus, we predicted *U. brucei* adult detections would be lower in quadrats where we detected other salamander species present, which we analyzed by creating a separate detection history of other species for each unique quadrat. We considered predator avoidance a detection covariate instead of a probability of use covariate for the same reasons listed in the *Larval microhabitat use* section. We hypothesized survey method would influence *U. brucei* adult detectability and represented survey method as a categorical variable (aquatic area-constrained surveys, aquatic leaf litter bag surveys, terrestrial area-constrained surveys, terrestrial leaf litter bag surveys). We predicted terrestrial leaf litter bag surveys (described in Chapter 1) would be the most successful at detecting *U. brucei* adults due to the riparian placement within one meter of the stream edge and the semi-permanent refugia availability (i.e., leaf litter). We also included a null model to represent the hypothesis that both detection and probability of use remained constant despite included detection covariates.

Model fitting and ranking

For each analysis (larval and adult), we used a two-step occupancy modeling approach (MacKenzie et al. 2006). In the first step, we evaluated relative support for our *a priori* hypotheses regarding detection while holding probability of use constant using Akaike's Information Criterion corrected for small sample sizes (AIC_c; Akaike 1974). In the second step, we carried over all supported detection covariates from step one (terms in models carrying upper 90% of AIC_c model weight) to model detection while varying the component of our model describing probability of use. We based our final inference on models that fell in the confidence set (carrying the upper 90% of the AIC_c model weight) in step two. Though AIC_c is used for model selection in a confidence set, it does not assess model fit to observed data. Thus, we used a Pearson chi-squared test with 5,000 bootstrap replications to determine if our global model adequately described our observed data (MacKenzie and Bailey 2004). We determined adequate model fit using $\hat{c}$, where we assumed values close to one indicate adequate fit (MacKenzie and Bailey 2004). We fit all occupancy models using the unmarked package (Fiske and Chandler 2011) in Program R (v 2023.03.0+386; R Core Team 2021).

RESULTS

We detected 26 larvae, 20 adults, and one metamorph (adults and metamorph combined for adult analysis) using our quadrats throughout our sampling period (Table 2.7). Larvae were primarily detected using aquatic leaf litter bag surveys, which accounted for 21 of 26 captures, as opposed to aquatic area-constrained surveys (5 of 26). Of the captured adults, 70% were detected using terrestrial leaf litter bag surveys (14 of 20). The one detected metamorph was captured using an aquatic leaf litter bag. Most *U. brucei* larvae (22 of 26) were encountered in saturated leaf litter that averaged 59 mm (range = 17–91 mm) deep and stream depths that averaged 14 mm (0–32 mm) deep; though, four individuals were found resting openly on sediment in the stream, lacking natural cover. All *U. brucei* adults were captured in moist leaf litter with an average depth of 91 mm (range = 22–320 mm). Over half of the larval *U. brucei* detections were from GA2 (15 of 26) and only two larvae were captured at SC1. We detected larvae in eight of 26 (31%) GA2 quadrats, seven of 26 (27%) SC2 quadrats, and only one of 26 (4%) SC1 quadrats. Half (11 of 20) adult detections were from seven unique quadrats in GA2, while the remainder were captured from four unique SC2 quadrats (9 of 20 adults). We did not detect adults from SC1. Among adults observed, 80% (16 of 20) were detected in terrestrial quadrats. Notably, only one of the four aquatic quadrats where *U. brucei* adults were detected had a stream depth > 1 mm, and the adult found there was a gravid female. We included the single *U. brucei* metamorph detected in the adult analysis for simplicity. The sole metamorph was detected at SC2 in an aquatic quadrat that had a stream depth of 31 mm.

Model ranking

Larval analysis

In our larval analysis, assessment of our global model indicated adequate fit to our data ($\hat{c}$ = 0.47). In step one, we had three models in our 90% confidence set of models describing larval detectability. Our first ranked detection model (w_1 = 0.70) represented survey method, a categorical covariate (aquatic area-constrained survey, aquatic leaf litter bag survey; Table 2.8) and was carried over to inform detection in step two since the second ranked model was the null model. In step two, our best supported model (w_1 = 0.93) suggested survey method alone influenced larval *U. brucei* detection probability while leaf litter accumulation (leaf litter depth and stream depth) was the primary driver of microhabitat use. Terms in the occupancy component of our model indicated that larval microhabitat use was positively associated with leaf litter depth and negatively associated with stream depth. For example, our larval microhabitat probability of use estimates were $\geq$ 0.91 [0.25–1.00; 95% confidence intervals] once leaf litter depths reached 70 mm (Figure 2.5A). Probability of larval use was 0.84 [0.31–0.98] when stream depths were $\leq$ 4 mm and decreased by half (Ψ = 0.46 [0.17–0.78]) once stream depth increased to 16 mm (Figure 2.5B). Detectability of larvae (p = 0.14 [0.07–0.24]) was two times higher when using aquatic leaf litter bag surveys than aquatic area-constrained surveys (p = 0.07 [0.02–0.19]; Figure 2.6). Though, 95% confidence intervals associated with use predictors were relatively large, indicating uncertainty regarding strength of effect (Table 2.9).

Adult analysis

In our adult analysis, $\hat{c}$ based on our global model was 0.99, indicating adequate fit to our data. In step one, a single detection model carried the upper 90% of the AIC$_c$ model weight and included leaf litter depth as the only driver of detection; thus, leaf litter depth was the single detection covariate used in step two (Table 2.10). In step two, our best supported model carried 94% of the AIC$_c$ model weight and supported leaf litter depth as the primary driver of microhabitat use.

In our best supported model, terms in the occupancy component showed a positive relationship with leaf litter depth. Specifically, our adult microhabitat use estimates were six times greater in quadrats where leaf litter depth approached 111 mm (Ψ = 0.92 [0.35–1.00]) than in quadrats where leaf litter depth was less than 47 mm (Ψ = 0.15 [0.06–0.32]; Figure 2.7). Additionally, our best supported model indicated a positive effect of leaf litter depth on detection probability (Table 2.11). Specifically, adult detectability was almost five times greater (p = 0.23 [0.12–0.40]) when leaf litter depths approached 269 mm than when leaf litter was absent (p = 0.05 [0.00–0.19]; Figure 2.8).

DISCUSSION

To our knowledge, our study is the first to investigate factors influencing *U. brucei* microhabitat use. We found leaf litter was an important microhabitat characteristic for *U. brucei*, but larvae and adults microhabitat use differed. Salamanders with biphasic life cycles typically rely on multiple habitat types with both aquatic and terrestrial components (Petranka 1998). For example, *E. bislineata* and Ocoee Salamanders (*Desmognathus ocoee*) exhibit a fully aquatic larval stage occupying slow-moving portions of the stream, while juveniles and adults inhabit riparian areas along the stream and moist substrate in adjacent forests (Petranka 1998). Our findings suggest leaf litter accumulation in concert with desiccation avoidance, increased foraging opportunities, and shelter provision associated with *U. brucei* life stage may dictate microhabitat use. Specifically, larvae were primarily found in thick leaf litter packs (≥ 70 mm) in shallow water (≤ 4 mm) and adults were predominately found in riparian areas characterized by deep leaf litter (≥ 111 mm). Notably, one possible shortcoming of our study is that microhabitat use estimates were based on all quadrats, including those representing leaf litter bag surveys which may have influenced our conclusions regarding *U. brucei* reliance on leaf litter. For example, one possible outcome of using leaf litter bags to understand microhabitat use is that leaf litter bags may have introduced more favorable microhabitat (i.e., deep leaf litter) to patches that previously had little available leaf litter. Therefore, in patches where *U. brucei* were previously absent due to unfavorable microhabitat, introducing leaf litter bags may have played a role in attracting *U. brucei* to the patch. Although leaf litter depths varied patch-to-patch throughout the stream, the deployed leaf litter bags had leaf litter depths comparable to patches without leaf litter bags. Thus, microhabitat conditions associated with leaf litter bags were also documented to naturally occur in patches lacking bags. Regardless, the reliance of *U. brucei* on leaf litter in both aquatic and terrestrial habitats make them vulnerable to a myriad of threats that impact leaf litter accumulation and stream quality.

Consistent with our hypothesis, we found leaf litter accumulation most influenced *U. brucei* microhabitat use. Previous *U. brucei* larval findings suggest larvae use microhabitat associated with spring heads and seepages, which our study indirectly supports considering the microhabitat larvae were found to use (Camp et al. 2018). Additionally, we found larval *U. brucei* exhibit similar patterns of microhabitat use to *E. bislineata* larvae (Petranka 1998). For example, *U. brucei* larvae most often used shallow, slow-moving portions of the stream where leaf litter builds up and acts as an additional desiccation buffer. Furthermore, when stream depths are shallow by nature (i.e., first- and second-order streams) leaf litter acts as refugia and potentially provides more foraging opportunities (Petanka 1998; Crawford and Semlitsch 2007). The relationship we found between adult *U. brucei* probability of use and leaf litter is comparable with general patterns for stream associated salamanders using areas with deeper leaf litter (Petranka 1998, Crawford and Semlitsch 2007). Our findings are also consistent with other research assessing the importance of microhabitat in preventing dehydration of plethodontid salamanders. Principally, water loss is a limiting factor for lungless salamanders, which is influenced by temperature, humidity, weather conditions, and ultimately microclimate to name a few (Pough 1986; Jorgensen 1997; Petranka 1998). Therefore, plethodontid salamanders, especially those with life stages inhabiting mesic environments, have adapted to night activity when temperatures decrease, humidity is high, and dew is present and/or to activity dependent on microhabitat that facilitates ideal conditions for refuge from predators, breeding, foraging, and potentially dispersal (Heatwole and Lim 1961; Spotila 1972; Feder 1983; Petranka 1998).

We found factors affecting detectability of *U. brucei* larvae and adults differed. Our results show larval detection is heavily influenced by survey method, while available refugia is the principal driver of detection for adults. Considering that in Chapter 2 we used a subset of data from Chapter 1, it is unsurprising that aquatic leaf litter bag surveys had the highest success rate associated with larval detection at a fine scale (p = 0.14 [0.07–0.24]) and commensurate with other studies that examined aquatic salamander detectability with aquatic leaf litter bag surveys (Waldron et al. 2003; Talley and Crisman 2007; Edwards et al. 2016). We found adult detections were associated with available refugia represented by deep leaf litter packs, which complements findings from Chapter 1. For example, one reason terrestrial area-unconstrained surveys (p = 0.13 [0.08–0.21]; Chapter 1), followed by terrestrial leaf litter bag surveys (p = 0.07 [0.04–0.14]; Chapter 1), had the highest adult detection probabilities for adults may be a result of the amount of leaf litter available to search. Given the exhaustive nature of the terrestrial area-unconstrained surveys (i.e., searching the entire 65-m riparian buffer), all leaf litter was examined without exception and terrestrial leaf litter bags were inherently representative of deep leaf litter. Crawford and Semlitsch (2007) had similar results with *D. ocoee*, *D. perlapsus*, Seal Salamanders (*Desmognathus* *monticola*), and Blue Ridge Two-lined Salamanders (*Eurycea wilderae*) being found more often in plots with deeper leaf litter.

Determining what influences microhabitat use of cryptic species is an essential step in understanding fine-scale distribution, efficacy of survey protocols, and informing management practices. Our findings suggest that stream depth and leaf litter depth influence *U. brucei* microhabitat use but may also be key factors affecting *U. brucei* occurrence across broader scales. In Chapter 1, we found occupancy estimates varied between streams, but we were unable to identify what was influencing occupancy at the 5-m segment scale. Our Chapter 2 findings indicate stream depth and leaf litter depth as two possible factors influencing occupancy at the segment scale and possibly occurrence from stream to stream. Each 5-m stream segment represented multiple habitat types that had varying stream flow velocities, water column depths, and leaf litter depths. However, GA2 and SC2 streams seemed to be more representative of ideal *U. brucei* microhabitat with most 5-m stream segments characterized by slow moving water, shallow water columns, and greater leaf litter availability and were also the two streams with the highest *U. brucei* occupancy estimates. Our SC1 stream, though still classified as a first-order stream, typically had faster stream flows, deeper water, and less leaf litter and seemed to be more vulnerable to high water events and feral hog (*Sus scrofa*) damage. Notably, in 2020, approximately 85 streams across northwest SC were sampled for *U. brucei* using eDNA, and only one new stream was discovered (SC1; C. Jachowski, unpublished data), suggesting *U. brucei* is rare in SC relative to GA for unknown reasons. Overarching biogeography, underlying geology, and the resulting forest composition may be why *U. brucei* populations are uncommon in SC compared to GA. For example, one of the shortcomings of our study was that we did not describe forest composition and the resulting leaf litter composition. Although, anecdotally, we did notice leaf litter composition in both SC streams was composed of more pine needles than the GA stream, and the SC1 stream gradient was much steeper than either SC2 or GA2.

MANAGEMENT IMPLICATIONS

Considering *U. brucei* occupy headwater streams and rely on leaf litter, they face multiple conservation threats. Mature, complex forest stands, especially those with tall canopies and vertical complexity, influence microclimate, have the potential to buffer against climate change, and are directly responsible for available leaf litter (Frey et al. 2016; Davis et al. 2018). For example, adverse environmental conditions (i.e., drought) can cause ephemeral water sources such as seeps to dry, whereas flooding events can wash away leaf litter. Moreover, forest structure alteration whether natural (i.e., tree die-offs from drought) or anthropogenic (i.e., timber harvesting) has the potential to alter stream quality (i.e., increase stream temperature, increase sedimentation) while simultaneously reducing available leaf litter. Collectively, all these events or alterations have been linked to abundance declines in plethodontid species (Crawford and Semlitsch 2007). Furthermore, invasive species such as feral hogs (*Sus scrofa*), Shovel-headed Garden Worms (*Bipalium kewense*), and exotic jumping worms (*Amynthas* species, especially *Amynthas agrestis*) have been documented to occur in the same geographic region as *U. brucei* (Callaham et al. 2003; Chang et al. 2021; pers. comm., Todd Pierson), including all three of our study streams (pers. obs., Chelsea Anderson). At the onset and throughout our study, we observed *S. scrofa* damage that directly impacted microhabitat quality due to extreme sedimentation and leaf litter disturbance from wallows and rooting (Figure 2.9). Concerningly, *B. kewense* is reported to have detrimental impacts on ecosystem processes when considering they are top predators of soil organisms (e.g., native earthworms, springtails), introduce new pressures to native species, alter nutrient cycling, and indirectly modify plant communities (Sluys 2016). Similarly, *Amynthas* species are known to transform soil structure and composition, though they do so by depleting organic matter (e.g., leaf litter) and excreting castings that result in a reduction of nutrient availability, soil stability, and shifts in soil temperatures; all of which impact soil and plant communities (Chang et al. 2021). Collectively, feral hogs and invasive annelids have negative ramifications on ecosystem function and structure, especially when considering microhabitat features (e.g., leaf litter); alarmingly, they have few to no successful management strategies in place to control populations (Ditchkoff et al. 2012; Sluys 2016; Chang et al. 2021). Additionally, prescribed burning, though necessary to maintain healthy forest ecosystems, may impact microhabitat quality when considering unexpectantly hot burns (i.e., leading to tree die-offs or complete reduction of organic layer) or burns that encroach on the stream (i.e., depletion of leaf litter adjacent to stream edge); thus, prescribed burns in adjoining areas should be carefully monitored. Generally, we recommend maintaining closed canopy and complex forest structures connected with *U. brucei* streams to sustain leaf litter buildup in accompanying microhabitats and limiting human access to prevent further disturbance that reduces microhabitat quality.

Finally, consistent with detection results from Chapter 1, when the goal is to capture *U. brucei* larvae, we recommend using aquatic leaf litter bags and placing them in shallow portions of the stream where leaf litter has built up. In our experience, such conditions are often associated with seepages and spring heads. If the goal is to capture *U. brucei* adults, we suggest using terrestrial leaf litter bags placed in a riparian area characterized by deep (~ 111 mm) leaf litter.

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TABLES

Table 2.1. Seasonal windows and associated range over which surveys were conducted for Patch-nosed Salamanders (*Urspelerpes brucei*) in three occupied streams in Georgia and South Carolina, USA, 2021–2022.

	Survey date by stream		
Seasonal window	**GA2**	**SC1**	**SC2**
Summer 2021	4-Sep-21	21-Aug-21	19-Aug-21
Fall 2021	21-Nov-21	2-Dec-21	20-Nov-21
Winter 2021	13-Feb-22	n/a	17-Feb-22
Spring 2022-A	14-Mar-22	27-Apr-22	19-Mar-22
Spring 2022-B	23-Apr-22	18-May-22	22-Apr-22
Spring 2022-C	3-May-22	13-Jun-22	31-May-22

Table 2.2. A description of stream-specific, quadrat-specific, and survey-specific variables measured at three Patch-nosed Salamander (*Urspelerpes brucei*) occupied streams in Georgia and South Carolina, USA, 2021–2022.

Variable	**Description**
Stream-specific	
Canopy closure (%)	Continuous variable describing percent of shade at the top of each segment
Wetted stream width (m)	Continuous variable describing wetted width of stream channel for each segment
Quadrat-specific	
Leaf litter depth (mm)	Continuous variable describing average depth of leaf litter for each quadrat
Leaf litter moisture (%)	Continuous variable describing average percent of leaf litter saturated for each quadrat
Stream depth (mm)	Continuous variable describing average depth of water column for each quadrat
Distance to flowing water (m)	Continuous variable describing quadrat distance from segment characterized as flowing water
Survey-specific	
Survey method	Categorical variable describing each survey method employed to detect *U. brucei* during a seasonal window
Cloud cover (%)	Continuous variable describing percent of cloud cover at the start of each survey
Soil temperature (°C)	Continuous variable describing soil temperature at the start of each survey
Total rainfall in past week (cm)	Continuous variable describing total local rainfall amounts for the week prior to each survey
Stream depth (mm)	Continuous variable describing depth of water column for each quadrat
Leaf litter depth (mm)	Continuous variable describing depth of leaf litter for each quadrat

Table 2.3. *A priori* models for hypotheses regarding covariates associated with larval Patch-nosed Salamander (*Urspelerpes brucei*) occupancy during a study on microhabitat use at three occupied streams in Georgia and South Carolina, USA, 2021–2022.

Hypothesis	Model structure	Predictions
Occupancy is constant (null model)	$\Psi(.)$	Covariates have no effect on occupancy
Occupancy is influenced by leaf litter accumulation	$\Psi = \beta1$(leaf litter depth) + $\beta2$(stream depth)	Leaf litter depth (+), stream depth (-)
Occupancy is influenced by stream characteristics	$\Psi = \beta1$(distance from flowing water)	Distance from flowing water (+)

Table 2.4. *A priori* models for hypotheses regarding covariates associated with larval Patch-nosed Salamander (*Urspelerpes brucei*) detection during a study on microhabitat use at three occupied streams in Georgia and South Carolina, USA, 2021–2022.

Hypothesis	Model structure	Predictions
Detection is constant (null model)	$p(.)$	Covariates have no effect on detection
Detection is influenced by weather condition	$p = \beta1$(cloud cover) + $\beta2$(weekly rainfall totals)	Cloud cover (-) and weekly rainfall totals (-)
Detection is influenced by available refugia	$p = \beta1$(leaf litter depth) + $\beta2$(stream depth)	Leaf litter depth (+), stream depth (+)
Detection is influenced by larval emergence	$p = \beta1$(day of year survey conducted) + $\beta2$(day of year survey conducted^2)	Day of year survey conducted (+ with spring and fall)
Detection is influenced by competition	$p = \beta1$(other species presence)	Other species presence (-)
Detection is influenced by survey method	$p = \beta1$(aquatic constrained) + $\beta2$(aquatic leaf litter bag)	Aquatic constrained (-), aquatic leaf litter bag (+)

Table 2.5. *A priori* models for hypotheses regarding covariates associated with adult Patch-nosed Salamander (*Urspelerpes brucei*) occupancy during a study on microhabitat use at three occupied streams in Georgia and South Carolina, USA, 2021–2022.

Hypothesis	Model structure	Predictions
Occupancy is constant (null model)	$\Psi(.)$	Covariates have no effect on occupancy
Occupancy is influenced by leaf litter accumulation	$\Psi = \beta 1$(leaf litter depth)	Leaf litter depth (+)
Occupancy is influenced by stream characteristics	$\Psi = \beta 1$(distance from flowing water)	Distance from flowing water (+)

Table 2.6. *A priori* models for hypotheses regarding covariates associated with adult Patch-nosed Salamander (*Urspelerpes brucei*) detection during a study on microhabitat use at three occupied streams in Georgia and South Carolina, USA, 2021–2022.

Hypothesis	Model structure	Predictions
Detection is constant (null model)	$p(.)$	Covariates have no effect on detection
Detection is influenced by weather condition	$p = \beta 1$(cloud cover) + $\beta 2$(weekly rainfall totals) + $\beta 3$(soil temperature)	Cloud cover (-), weekly rainfall totals (-), and soil temperature (-)
Detection is influenced by available refugia	$p = \beta 1$(leaf litter depth)	Leaf litter depth (+)
Detection is influenced by breeding activity	$p = \beta 1$(day of year survey conducted) + $\beta 2$(day of year survey conducted^2)	Day of year survey conducted (+ with spring and fall)
Detection is influenced by competition	$p = \beta 1$(other species presence)	Other species presence (-)
Detection is influenced by survey method	$p = \beta 1$(aquatic constrained) + $\beta 2$(aquatic leaf litter bag) + $\beta 3$(terrestrial constrained) + $\beta 4$(terrestrial leaf litter bag)	Aquatic constrained (-), aquatic leaf litter bag (-), terrestrial constrained (+), and terrestrial leaf litter bag (+)

Table 2.7. Summary of Patch-nosed salamander (*Urspelerpes brucei*) captures for each survey method (ACS: aquatic area-constrained survey, ALLB: aquatic leaf litter bag survey, TCS: terrestrial area-constrained survey, and TLLB: terrestrial leaf litter bag survey) across all seasonal windows at three occupied streams in Georgia and South Carolina, USA, 2021–2022. Values reported indicate the no. larvae detected / no. adults detected for each survey.

		Seasonal window						
Stream	**Survey method**	Summer2021	Fall2021	Winter2021	Spring2022-A	Spring2022-B	Spring2022-C	**Grand total**
GA2	ACS	**2/-**	-	-	-	-	**1/-**	**3/-**
	ALLB	**2/-**	-	**1/-**	**1/-**	**-/2**	**8/1**	**12/3**
	TCS	-	-	-	-	**-/2**	-	**-/2**
	TLLB	-	-	-	**-/3**	**-/3**	-	**-/6**
SC1	ACS	-	-	n/a	-	-	-	-
	ALLB	-	**2/-**	n/a	-	-	-	**2/-**
	TCS	-	-	n/a	-	-	-	-
	TLLB	-	-	n/a	-	-	-	-
SC2	ACS	**1/-**	-	**1/-**	-	**-/1**	-	**2/1**
	ALLB	**1/1***	**1/-**	**1/-**	**3/-**	-	**1/-**	**7/1***
	TCS	-	-	-	-	-	-	-
	TLLB	-	-	-	**-/2**	**-/4**	**-/2**	**-/8**
Grand total		**6/1***	**3/-**	**3/-**	**4/5**	**-/12**	**10/3**	**26/21* = 47***

n/a Survey method not conducted

*One metamorph included in adult count

Table 2.8. Ranking of confidence set of models from most supported to least supported that are hypothesized to influence detection (step one) and probability of use (step two) of larval Patch-nosed Salamanders (*Urspelerpes brucei*) at three occupied streams in Georgia and South Carolina, USA, 2021–2022.

Model	Model structure	K^b	AICc	ΔAICc	Wt
Detection (step one) confidence set					
Survey method	Ψ(.) *p*(method)	3	158.91	0.00	0.70
Null	Ψ(.) *p*(.)	2	162.39	3.48	0.12
Competition and predator avoidance	Ψ(.) *p*(other species present)	3	162.65	3.74	0.11
Available refugia	Ψ(.) *p*(leaf litter depth + stream depth)	4	164.72	5.81	0.04
Larval emergence	Ψ(.) *p*(day of year survey conducted + day of year survey conducted^2)	4	166.27	7.36	0.02
Weather condition	Ψ(.) *p*(cloud + rainfall + soil temperature)	4	166.53	8.32	0.01
Probability of use (step two) confidence set					
Leaf litter accumulation	Ψ(leaf litter depth + stream depth) *p*(method)	5	152.44	0.00	0.93
Null occupancy	Ψ(.) *p*(method)	3	158.91	6.47	0.04
Stream characteristics	Ψ(distance from stream flow) *p*(method)	4	159.33	6.88	0.03
Null	Ψ(.) *p*(.)	2	162.39	9.95	0.01

[b] Number of parameters in the model.

Table 2.9. Best supported occupancy model parameters, estimates, standard errors (SE), and 95% confidence intervals (CI) for factors hypothesized to influence detection and probability of use of larval Patch-nosed Salamanders (*Urspelerpes brucei*) at three occupied streams in Georgia and South Carolina, USA, 2021–2022. Detection and occupancy parameter estimates are scaled.

Parameter	**Estimate**	**SE**	**Lower 95% CI**	**Upper 95% CI**
Detection (*p*)				
Aquatic area-unconstrained survey (Intercept)	-2.63	0.60	-3.80	-1.46
Aquatic leaf litter bag survey	0.79	0.63	-0.45	2.03
Probability of use (Ψ)				
(Intercept)	-0.65	0.73	-2.09	0.79
Leaf litter depth	1.95	1.09	-0.20	4.09
Stream depth	-2.58	1.26	-5.05	-0.10

Table 2.10. Ranking of confidence set of models from most supported to least supported that are hypothesized to influence detection (step one) and probability of use (step two) of adult Patch-nosed Salamanders (*Urspelerpes brucei*) at three occupied streams in Georgia and South Carolina, USA, 2021–2022.

Model	**Model structure**	***K*** ^{b}	**AICc**	**ΔAICc**	**Wt**
Detection (step one) confidence set					
Available refugia	Ψ(.) *p*(leaf litter depth)	3	137.98	0.00	0.99
Survey method	Ψ(.) *p*(method)	5	148.53	10.55	0.01
Weather condition	Ψ(.) *p*(cloud + rainfall + soil temperature)	5	151.09	13.12	0.00
Breeding activity	Ψ(.) *p*(day of year survey conducted + day of year survey conducted^2)	4	151.64	13.66	0.00
Null	Ψ(.) *p*(.)	2	153.45	15.48	0.00
Competition and predator avoidance	Ψ(.) *p*(other species present)	3	154.43	16.46	0.00
Probability of use (step two) confidence set					
Leaf litter accumulation	Ψ(leaf litter depth) *p*(leaf litter depth)	4	131.73	0.00	0.94
Null occupancy	Ψ(.) *p*(leaf litter depth)	3	137.98	6.25	0.04
Stream characteristics	Ψ(distance from flowing water) *p*(leaf litter depth)	4	139.50	7.77	0.02
Null	Ψ(.) *p*(.)	2	153.45	21.72	0.00

^{b} Number of parameters in the model.

Table 2.11. Top ranked occupancy model parameters, estimates, standard errors (SE), and 95% confidence intervals (CI) for factors hypothesized to influence detection and probability of use of adult Patch-nosed Salamanders (*Urspelerpes brucei*) at three occupied streams in Georgia and South Carolina, USA, 2021–2022. Detection parameter estimate is scaled but occupancy estimate is not.

Parameter	**Estimate**	**SE**	**Lower 95% CI**	**Upper 95% CI**
Detection (*p*)				
(Intercept)	-2.78	1.54	-2.87	-1.04
Leaf litter depth	0.06	0.03	0.10	1.24
Probability of use (Ψ)				
(Intercept)	-1.96	0.72	-7.81	-1.75
Leaf litter depth	0.67	0.29	0.01	0.12

FIGURES

Figure 2.1. Photo of typical Patch-nosed Salamander (*Urspelerpes brucei*) stream habitat located in South Carolina, USA, 2022.

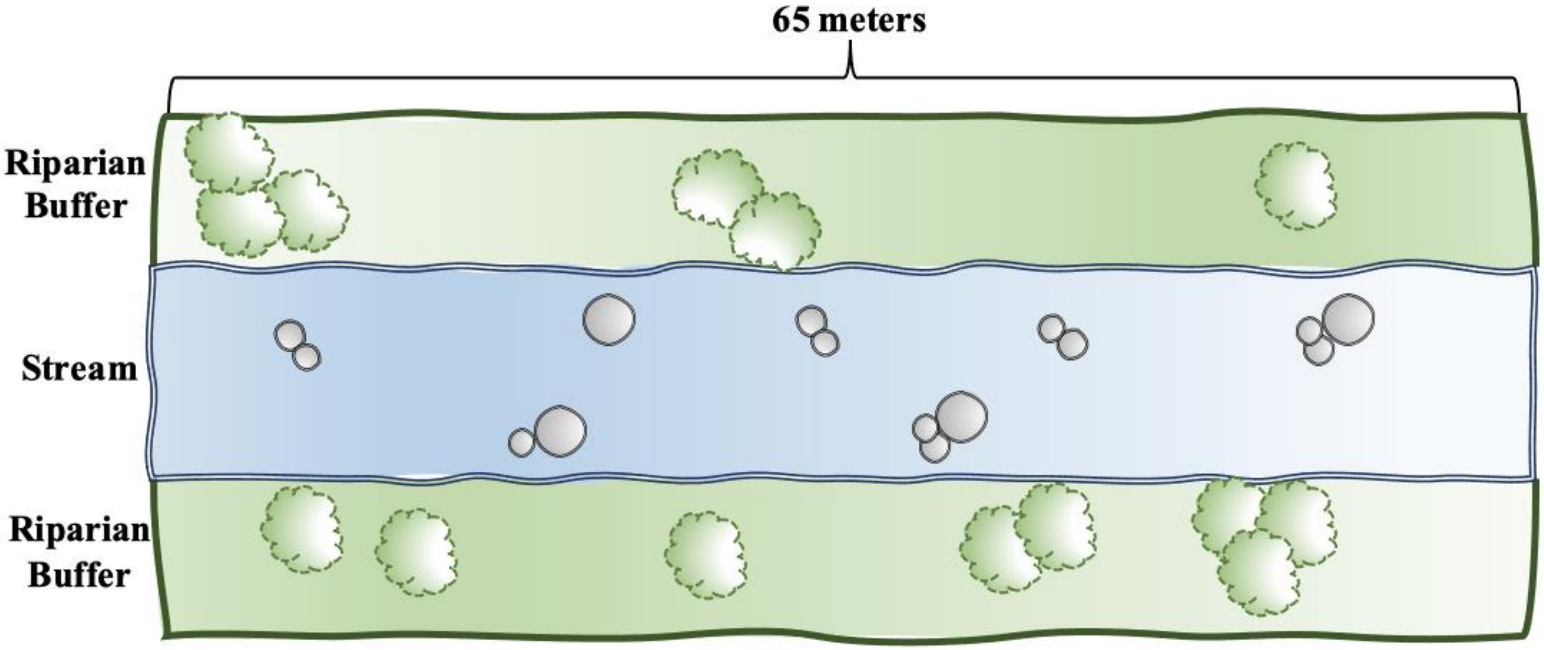

Figure 2.2. Stratum used in experimental design for evaluating Patch-nosed Salamander (*Urspelerpes brucei*) microhabitat use. Riparian buffer represents the terrestrial strata, and the stream represents the aquatic strata.

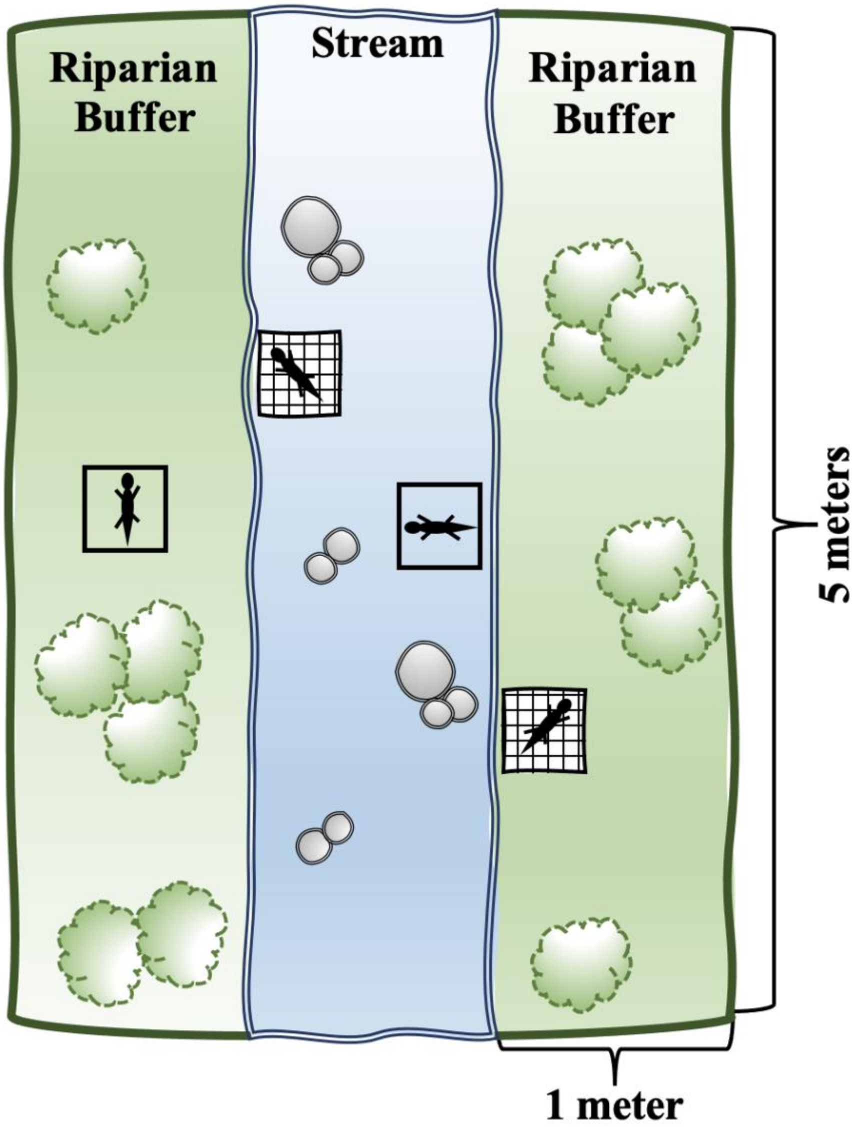

Figure 2.3. Experimental design for evaluating detectability and probability of use of Patch-nosed Salamanders (*Urspelerpes brucei*) using four unique quadrat types, which were randomly placed in a five-meter-long stream segment. Gridded squares represent leaf litter bag survey quadrats (one aquatic and one terrestrial), and open squares represent area-constrained survey quadrats (one aquatic and one terrestrial).

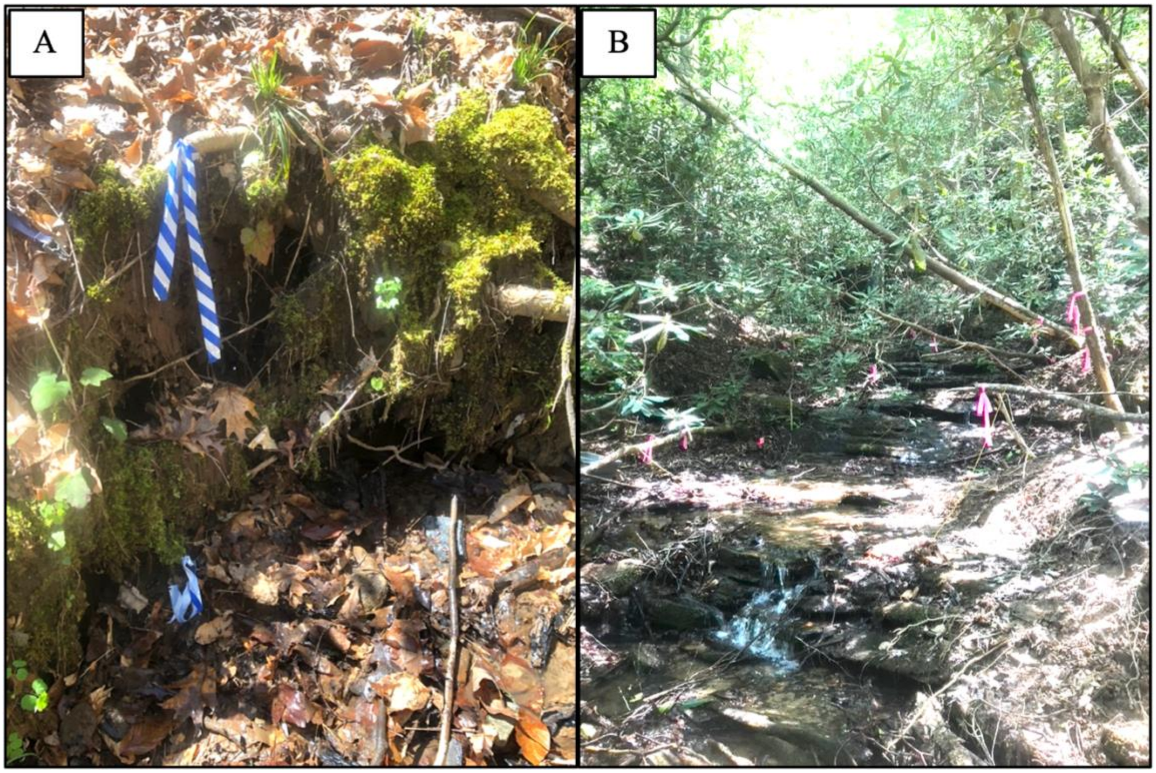

Figure 2.4. Photo of Patch-nosed Salamander (*Urspelerpes brucei*) stream flow characteristics (A: classified as spring head/seep, B: classified as flowing water) located in Georgia (A) and South Carolina (B), USA, 2022.

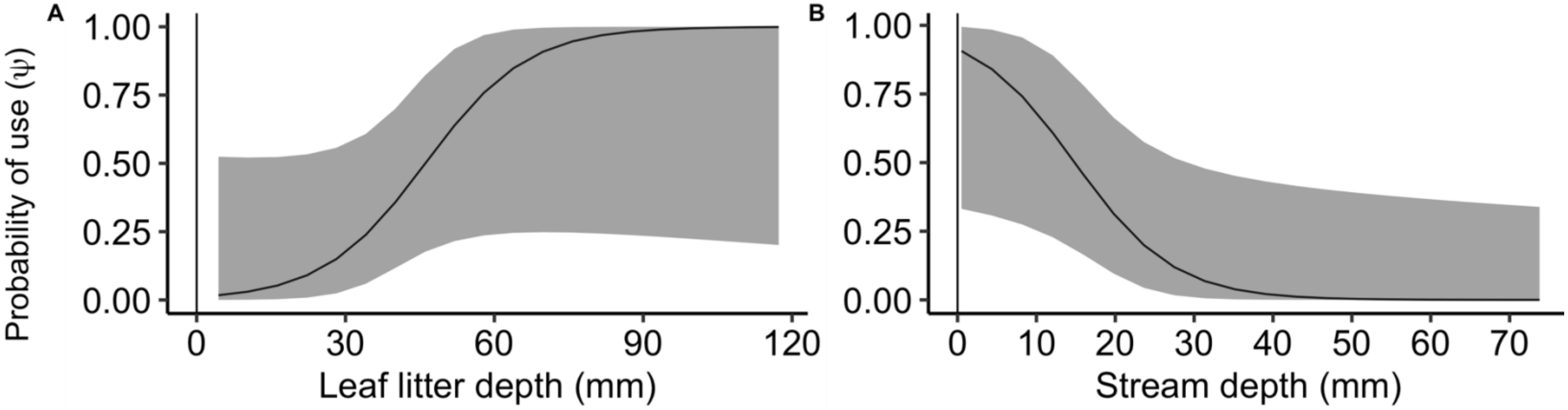

Figure 2.5. The effect of leaf litter accumulation (A: leaf litter depth (mm), B: stream depth (mm)) on larval Patch-nosed Salamander (*Urspelerpes brucei*) probability of use at three occupied streams in Georgia and South Carolina, USA, 2021–2022. The solid line represents the predicted use estimates, and the shaded areas represent 95% confidence intervals.

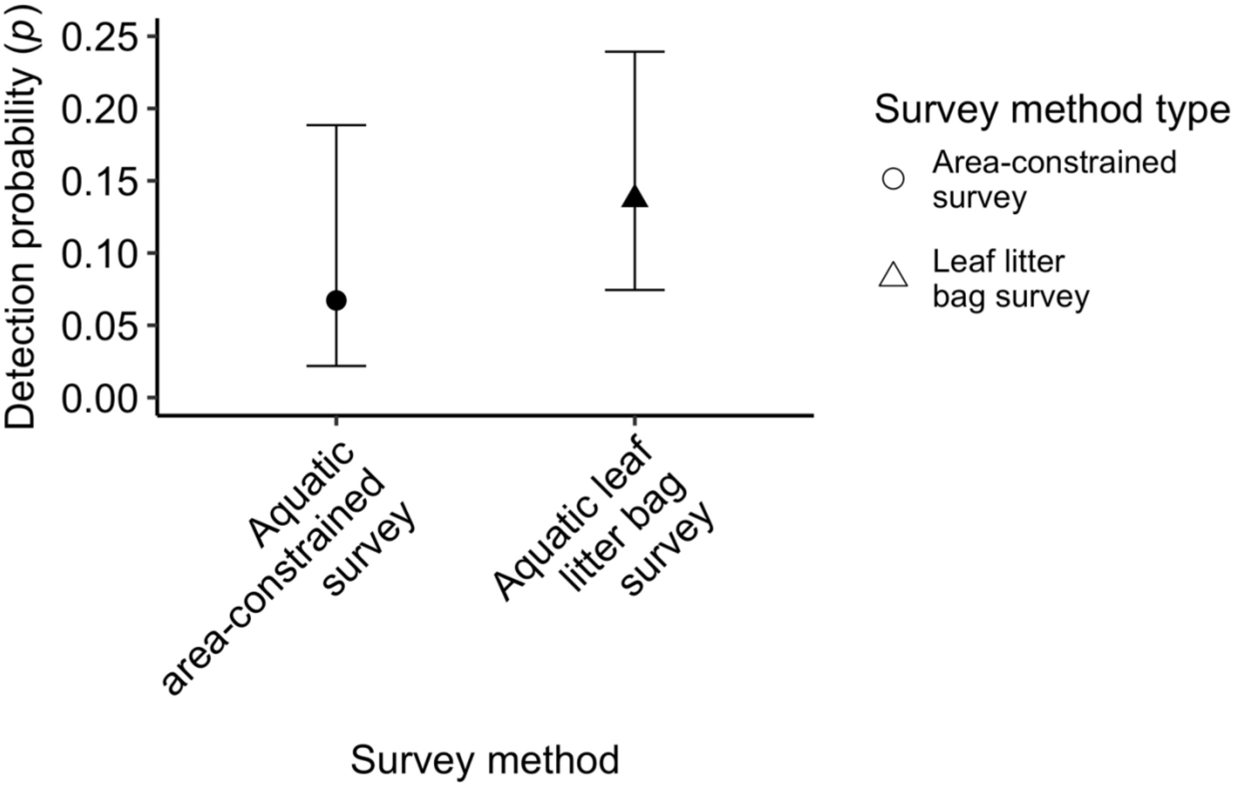

Figure 2.6. The effect of survey method on larval Patch-nosed Salamander (*Urspelerpes brucei*) detection probability at three occupied streams in Georgia and South Carolina, USA, 2021–2022. The solid point represents predicted detection probability, and the bars represent 95% confidence intervals.

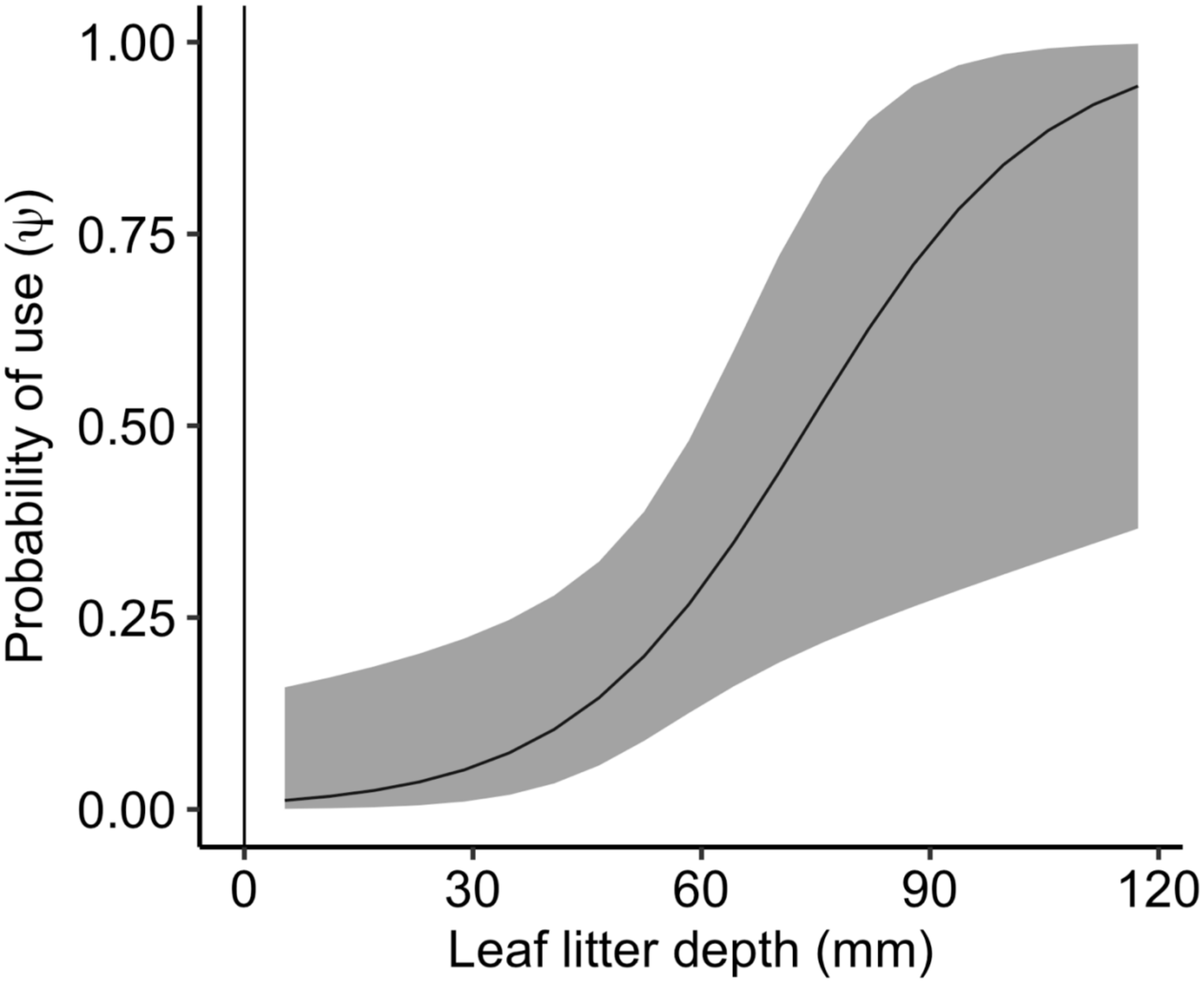

Figure 2.7. The effect of leaf litter accumulation (leaf litter depth) on adult Patch-nosed Salamander (*Urspelerpes brucei*) probability of use at three occupied streams in Georgia and South Carolina, USA, 2021–2022. The solid line represents the predicted use estimates, and the shaded areas represent 95% confidence intervals.

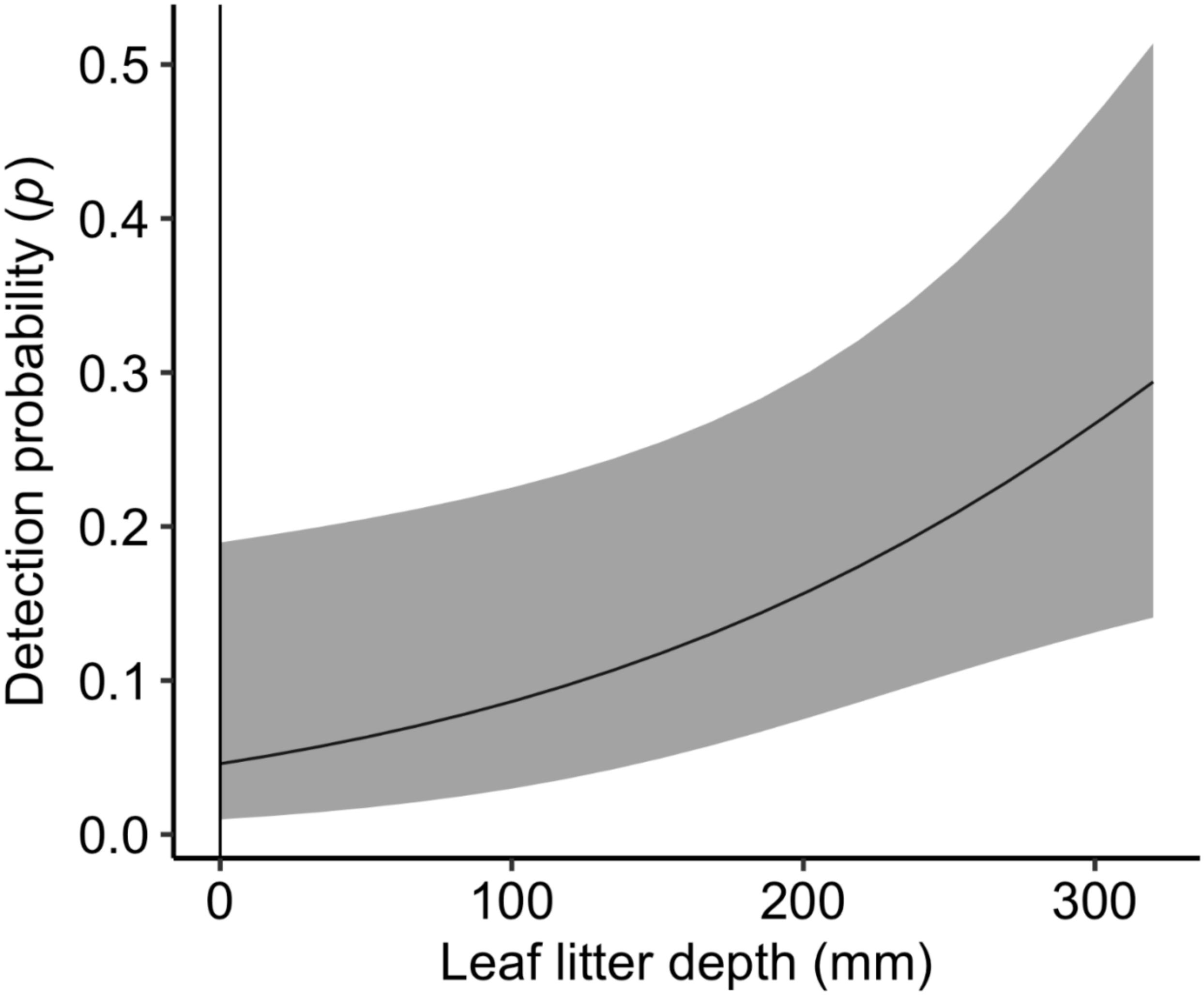

Figure 2.8. The effect of available refugia (leaf litter depth) on adult Patch-nosed Salamander (*Urspelerpes brucei*) detection probability at three occupied streams in Georgia and South Carolina, USA, 2021–2022. The solid line represents the predicted occupancy estimates, and the shaded areas represent 95% confidence intervals.

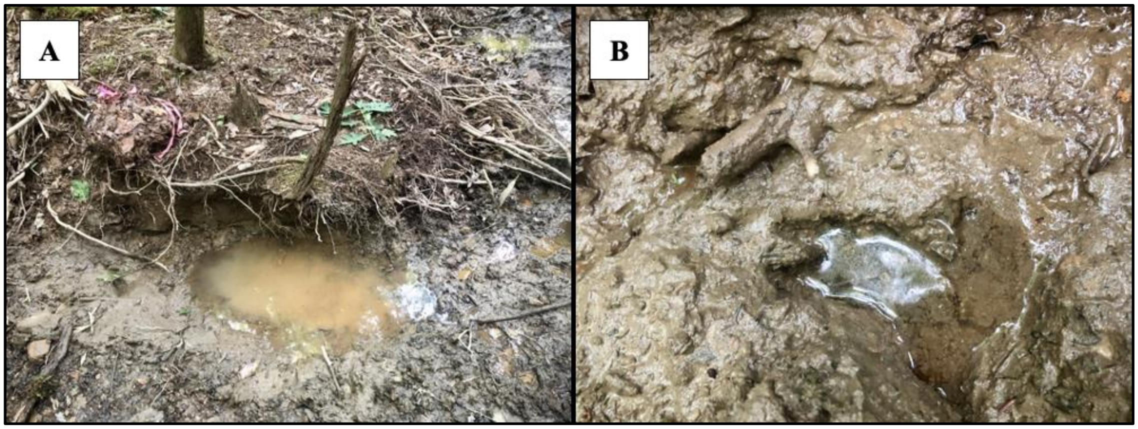

Figure 2.9. Photo evidence of feral hog (*Sus scrofa*) damage (A: hog wallow, B: hog track) at two Patch-nosed Salamander (*Urspelerpes brucei*) streams located in South Carolina, USA, 2022.