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Nicholas Chang April 10, 2024

Thinking Like a Salamander:
Evaluating habitat use of the Talladega seal salamander (*Desmognathus cheaha*) in metro-Atlanta headwater streams.

by

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

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Abstract

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Understanding the habitat use of aquatic biota is necessary to predict how species respond to changes in environmental conditions, as well as to identify suitable habitats and restore degraded ones. The Talladega seal salamander (*Desmognathus cheaha*) of Georgia, Alabama, and Florida, USA, was recently split from the well-studied common seal salamander (*Desmognathus monticola*) found in Appalachia. While extensive studies have evaluated the microhabitat use and community ecology of *D. monticola,* most of this work did not evaluate what is now known as *D. cheaha*. We evaluated the habitat use of *D. cheaha* in 11 metro-Atlanta headwater streams to identify the role of substrate composition, cover object size and position, and the co-occurrence of other salamanders and crayfish, in predicting within-stream occupancy. In addition, we compared temperature, stream morphology, dissolved oxygen, and watershed features to examine salamander occupancy with varying levels of urbanization. Our microhabitat analyses suggest cover object size and the presence of another species to be strong predictors of fine-scale occupancy, but that these factors may be dependent on each other. We did not detect an effect of substrate composition on fine-scale distribution. Larger seal salamanders tended to occupy larger cover items and more aquatic habitat, and we found evidence suggesting shifts in habitat use of co-occurring species in the presence of *D.* cheaha. While no significant association was found between any stream-level variable and the presence of *D. cheaha*, statistical models that included declivity or stream temperature variability performed better than those where watershed urbanization or dissolved oxygen were predictors. Our data reduces knowledge gaps about habitat suitability for this species and improves our ability to appropriately address conservation needs. However, further analyses of microhabitat use across wider urban and geographic gradients are needed to better understand the habitat usage and conservation needs of this species.

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Acknowledgments

I am indebted to my advisor, Carolyn Keogh, for her generosity and support throughout my undergraduate journey, her patience as I agonized over study design, and her enthusiasm for all things aquatic (even when not crabs or snails). Gonzalo Vazquez-Prokopec, Chris Beck, and Michael Martin offered invaluable assistance and feedback as members of my committee.

This project would not have been possible without Will Hutchinson, who spent countless hours wrangling salamanders and measuring rocks with me. We were joined in the field by Bella Roeske, Jack Kagan, Iris Chen, Will Heilmann, Taryn Smith, Amanda Wolf, Abigail Peña, and Maddi Chen. Gabe Andrle taught me how to think like a salamander. Todd Pierson and Leah Rittenburg shared their expertise and HOBOs. Nick and JP trusted me with albino salamander secrets. Melissa Ivey, Jerry Byrd, Heather Waringa, Bobbi Hohmann, Sandy Kruger, and David Flaherty provided logistical support. Leah Thomas, Kim Awbrey, Murray Sternberg, Yulia Gu, Taylor Cyle, and Carl Brown shared many waffles and productive conversations with me in the Lek. Thank you all—I am forever grateful for your assistance and/or friendship.

I am grateful for the salamanders and crayfish that participated in this study, whether willingly or not. I am thankful for the forest and for those who have stewarded and protected it, and for the headwater streams and north-facing slopes that have taught me love, wonder, and awe. This project was supported by a James G. Lester Grant from the Department of Environmental Sciences and was conducted under IACUC protocol 202200162. This research was performed on the ancestral homelands of the Mvskoke (Creek) People.

I dedicate this thesis to John Wegner. Thank you for introducing me to this wonderful place, and for your mentorship in science and in life.

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Introduction

Global patterns in land use change have resulted in profound alterations in the structure and function of fluvial and riparian ecosystems through alterations to stream geomorphology and hydroperiod, the introduction of chemical contaminants, and biogeochemical shifts due to runoff and erosion (Carpenter et al. 2011, Booth et al. 2016, Cantonati et al. 2020). Such changes in the quality of stream habitat correspond with declines in freshwater biodiversity (Stendera et al. 2012), which has suffered from the conversion of land for both agricultural (Harding et al. 1998) and urban (Booth et al. 2016) uses. One geographic region that faces challenges with freshwater biodiversity loss stemming from land use change is the Southeastern United States, which is an area of incredibly unique and imperiled freshwater biodiversity (Elkins et al. 2019). Unfortunately, fluvial and riparian habitats in the Southeast have experienced widespread historical alteration (Nagy et al. 2011), which be exacerbated by projected future urbanization throughout this region (Martinuzzi et al. 2014, Terando et al. 2014, Metre et al. 2019).

Among the freshwater taxa potentially threatened by alterations to the structure and function of lotic habitats in the Southeastern US are salamanders (Amphibia: Caudata), which coincidentally reach their greatest levels of species richness in this region (Barrett and Price 2014). Globally, salamanders and other amphibians are the subject of a conservation crisis, and while there are a variety of factors influencing the loss of amphibian biodiversity, habitat destruction from land use change is one of the leading drivers (Luedtke et al. 2023). Southeastern stream salamanders are negatively impacted by watershed alterations (Barrett and Price 2014), with the effects of such alterations apparent at both the population (Orser and Shure 1972) and community levels (Barrett et al. 2010b, Surasinghe and Baldwin 2014, 2015, Rittenburg 2023). However, responses of stream salamanders to landscape disturbance vary spatially, with the same species exhibiting different tolerances to disturbance across different physiographic provinces (Surasinghe and Baldwin 2015). These responses also vary functionally across body type, with larger-bodied salamanders typically more sensitive to disturbance (Barrett et al. 2010b, Surasinghe and Baldwin 2015). While the exact drivers of salamander declines resulting from urbanization are difficult to tease out, contributing factors likely include genetic bottlenecks due to habitat fragmentation (Munshi-South et al. 2013, Fusco et al. 2021), expulsion of animals during high-flow events (Barrett et al. 2010a), changes in water quality or temperature regime (Bernardo and Spotila 2006, Woods et al. 2010), changes in the abundance of predators, prey, or competitors (Lowe and Bolger 2002), pollution (Bank et al. 2006, Tornabene et al. 2023), and shifts in substrate or microhabitat availability (Lowe and Bolger 2002, Barrett et al. 2010a, Surasinghe 2013).

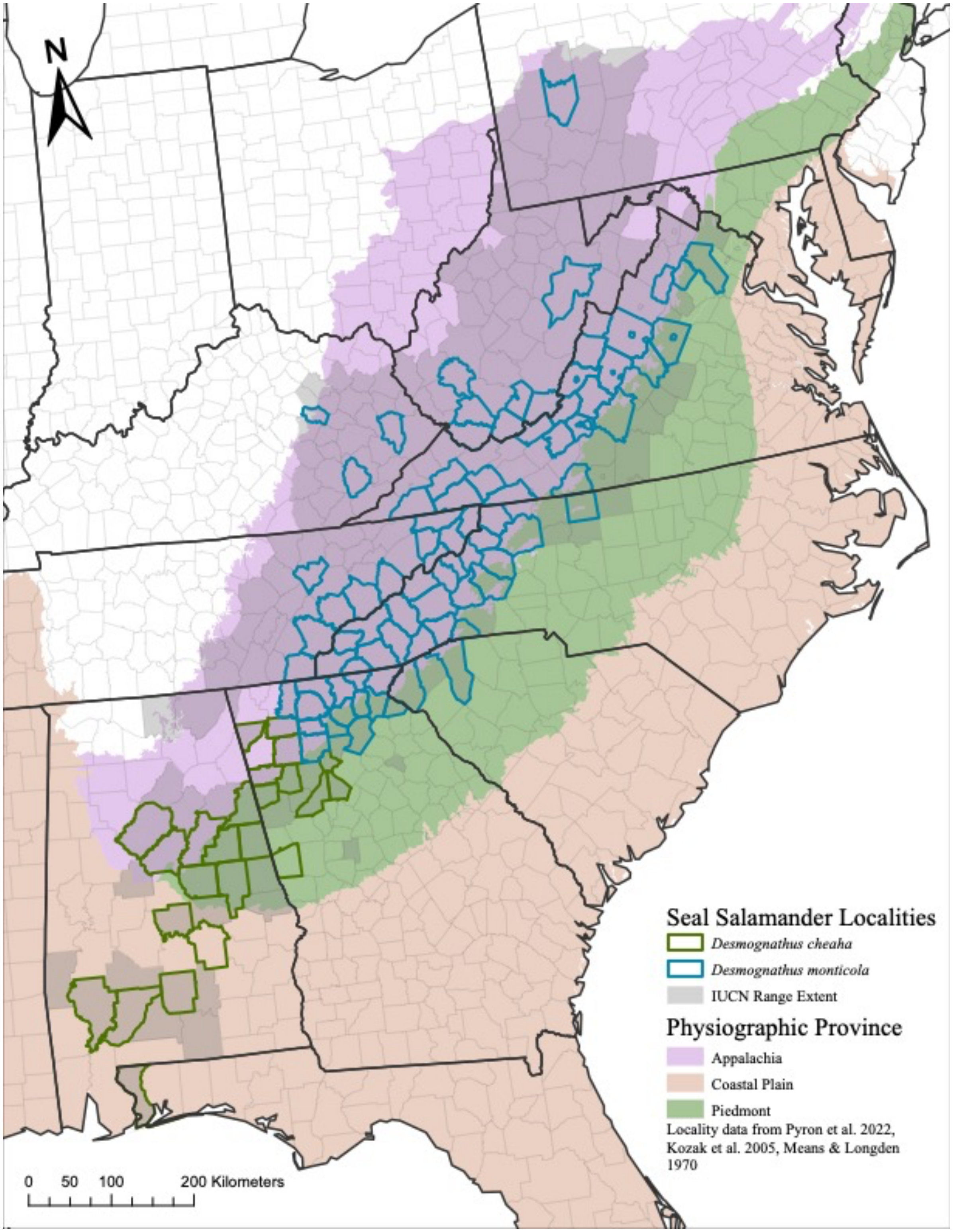
In relatively intact watersheds, stream microhabitat availability plays an important role in understanding the fine-scale distributional patterns of salamanders. Prior field studies have suggested that patterns in the distribution of stream salamanders may be linked to substrate type (Southerland 1986c, Diller and Wallace 1996, Smith and Grossman 2003, McIntyre et al. 2006), and both smaller plethodontids and larger stream-dwelling species have demonstrated active selection of substrate type in experimental trials (Southerland 1986b, Unger et al. 2020). Additionally, Southerland (1986c) found that densities of *Desmognathus sp.* salamanders in streams are limited by the availability of suitable cover objects. Though microhabitat patterns may seem trivial due to their fine spatial scale, understanding such associations can be important to understanding larger-scale meso-to-macrohabitat associations (Yeiser and Richter 2015), which may have more implications for management.

However, our understanding of fine-scale microhabitat selection by salamanders is confounded by community interactions between different species. Numerous studies have identified the phenomenon of microhabitat partitioning in stream salamander communities (Southerland 1986a, 1986b, Camp et al. 2013, Cudmore and Bury 2014), and have even found that fine-scale microhabitat partitioning may play a role in reducing gene flow in secondary contact zones when sympatric species occur in the same stream (Pierson et al. 2021). Much of the microhabitat research in stream salamanders has focused on members of the genus *Desmognathus,* which sort by size along a terrestrial-aquatic gradient in habitats where multiple species co-occur, with the larger species tending to occur in more aquatic areas while the smallest members of this assemblage have evolved to be fully terrestrial and undergo direct development (Hairston 1980, Bruce 2011).

Urban streams generally experience a deposition of fine substrates and homogenization of benthos (Violin et al. 2011), which impacts salamanders due to their reliance on structurally complex stream habitat and larger substrates (i.e., cobbles and coarse woody debris), which provides refuge areas (Barrett et al. 2010a, Barrett and Price 2014). While few studies have evaluated shifts in salamander microhabitat use across an urbanizing gradient, Surasinghe (2013) experimentally evaluated microhabitat partitioning between larger-bodied black-bellied salamanders (*Desmognathus sp.*) and smaller-bodied northern dusky salamanders (*Desmognathus fuscus*) in mesocosms simulating streams under different land use conditions. This study found evidence that, while black-bellied salamanders are competitively dominant in forested streams, they shift their microhabitat use to share cover items with *D. fuscus* in urban streams due to a loss of available in-stream cover availability. Thus, understanding the microhabitat usage of understudied species is important to inform whether such matters should be accounted for in conservation interventions.

The Talladega seal salamander (*Desmognathus cheaha;* ***Fig. 1***) is a plethodontid salamander native to the Southeastern United States which was recently split from the seal salamander (*Desmognathus monticola*; Pyron et al. 2022; ***Fig. 2***). In contrast with *D. monticola, D. cheaha* tends to be larger-bodied and occurs predominantly in the Piedmont, Ridge and Valley, and Coastal Plain eco-physiographic provinces of the US States of Georgia and Alabama, (Pyron et al. 2023). Its specificity of habitat use appears to vary across the range of this species, with authors reporting that it can be widespread throughout rocky headwater streams in the Piedmont and Ridge and Valley (Pyron et al. 2023) but restricted to microhabitats in direct contact with rock and flowing water in the Southeastern Plains (Folkerts 1968, Pyron et al. 2023).

Figure 1: Adult Desmognathus cheaha*. Dekalb Co., GA. Photo: Nick Chang*



Locality data from Pyron et al. 2022, Kozak et al. 2005, Means & Longden 1970

Figure 2: County-level identifications for both species of seal salamanders overlain over the IUCN Range Extent and physiographic provinces. Identifications from Pyron et al. 2022, with reference to Kozak et al. 2005, Means & Longden 1970.

Though Pyron et al. (2023) report finding this species in extensively human-modified streams in the Piedmont and Ridge & Valley, an attempt to characterize salamander communities using eDNA in the metro-Atlanta area (located in the Piedmont) detected *D. cheaha* in three of 25 streams, all with low impervious surface cover, in contrast with other species (*D. perlapsus* and *Eurycea cirrigera*), which were detected with much more frequency (Rittenburg 2023).

Since much of the research on the microhabitat use and community dynamics of *Desmognathus sp.* salamanders has taken place in Southern Appalachia (Bruce 2011), to our knowledge no prior work that studied these aspects of *D. monticola* has included populations of what is now considered *D. cheaha.* Because body size is an important variable in predicting habitat use in *Desmognathus sp.* salamanders (Bruce 2011), and because *D. cheaha* can be larger-bodied than *D. monticola* (Pyron et al. 2022), these prior studies on *D. monticola* may not accurately describe the habitat or community ecology of *D. cheaha.* Furthermore, due to physiographic differences in these species' distributions, stream morphology is likely to differ substantially between their ranges. Based on anecdotal observations from other authors, we expect Piedmont populations of *D. cheaha* to be habitat generalists within occupied streams (Pyron et al. 2022). However, formal data collection is required to either support or refute anecdotes and inform conservation policy for understudied species (Bodinof Jachowski et al. 2016). Additionally, while urbanization is generally understood to have negative impacts on salamander communities and populations, comparatively little work has evaluated the proximate and specific alterations in stream habitat quality that lead to these declines, creating a data gap that limits the ability of conservation practitioners to protect these species (Barrett and Price 2014).

Therefore, we aim to improve our understanding of the ecology of *D. cheaha* by addressing the following questions:

- How does the stream-level occupancy of *D. cheaha* correlate with environmental parameters associated with urbanization?
- In streams where *D. cheaha* is present, what microhabitat characteristics determine within-stream habitat use?
- Does the presence of *D. cheaha* in streams result in shifts in the microhabitat usage of its congener *D. perlapsus*?

To improve our understanding of spatial variation in the abundance and distribution of *D. cheaha,* relative to other members of headwater stream assemblages, conducted surveys of 11 headwater streams. We described the habitat characteristics of stream reaches and extracted drainage-level data from existing datasets. Additionally, we collected morphometric and microhabitat use data for our focal species, as well as other salamander species and crayfish, in eleven streams in the metro-Atlanta area. While we do not assess patterns in microhabitat along an extensive urbanizing gradient, we hope that by using within-stream microhabitat measurements of *D. cheaha* presence and absence, we can shed more light as to the potential proximate and specific alterations in stream habitat quality which result in the exclusion or extirpation of the species from certain streams. Given that the microhabitat use of stream-dwelling salamanders is important in understanding the ecology of these species, we hope that this information may assist conservation practitioners in identifying specific restoration or management opportunities that may conserve the species, whether *in* or *ex situ.*

Methodology

Study Area

We selected eleven (n = 11) headwater streams within a 1.5-mile radius of Emory University's Atlanta campus, located within the South Fork Peachtree Creek subbasin (HUC 12: 031300011202) of the Chattahoochee River drainage in the Piedmont eco-physiographic province of Georgia, USA (***Fig. 3***). The exact location of these metro-Atlanta area study sites is withheld here to protect these locations from the threat of poaching and habitat degradation associated with recreational access. The selected streams are all located within mixed pine-hardwood or mature hardwood forests that have experienced varying levels of historical and contemporary disturbance. The selected streams also experience varying impacts from stormwater and development (Orser and Shure 1972, Jordan et al. 2005, Read 2023, Rittenburg 2023). We focused our sampling on streams with prior records of *D. cheaha* (n = 7), and sampled an additional n = 4 streams where we suspected *D. cheaha* might occur based on visual habitat quality. This project was constrained by transportation limitations, and we therefore restricted sampling to streams with relatively easy bike or pedestrian access within 1.5 miles of Emory's campus.

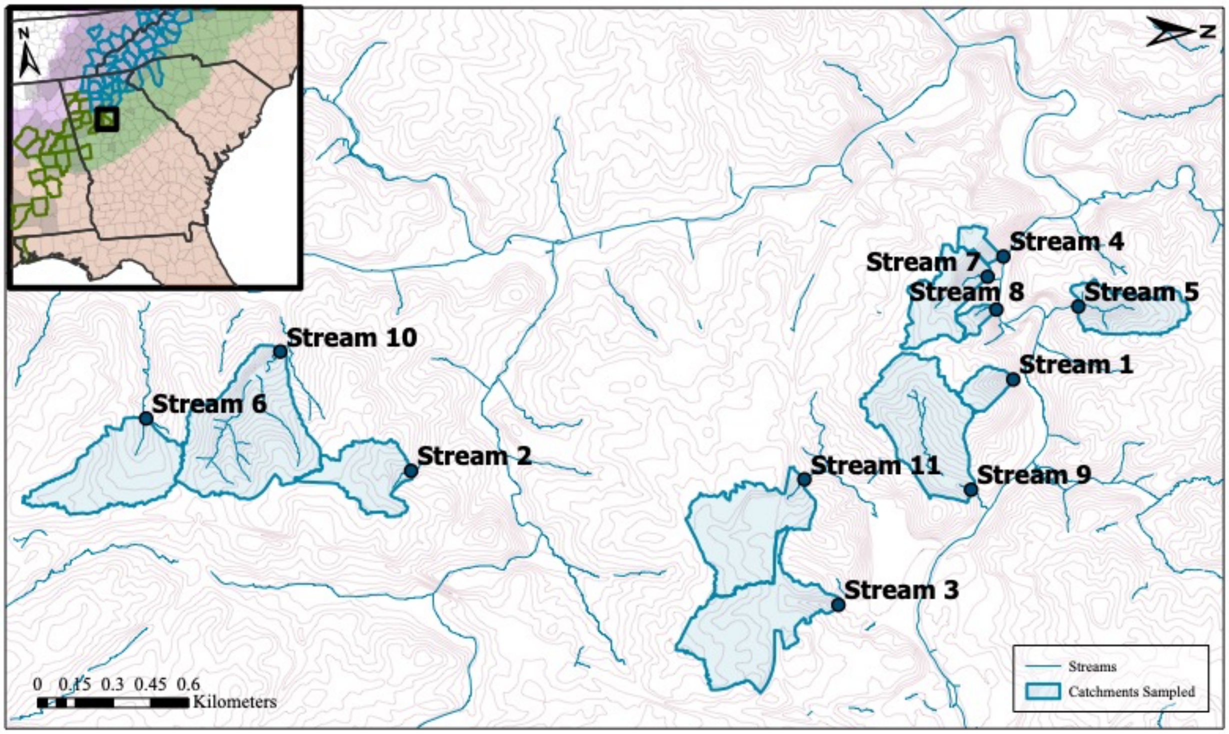


Figure 3: Position of study area within the range of D. cheaha*. Study sites were located in the South Fork Peachtree Creek subbasin. The study site map is intentionally vague to protect sensitive habitat.*

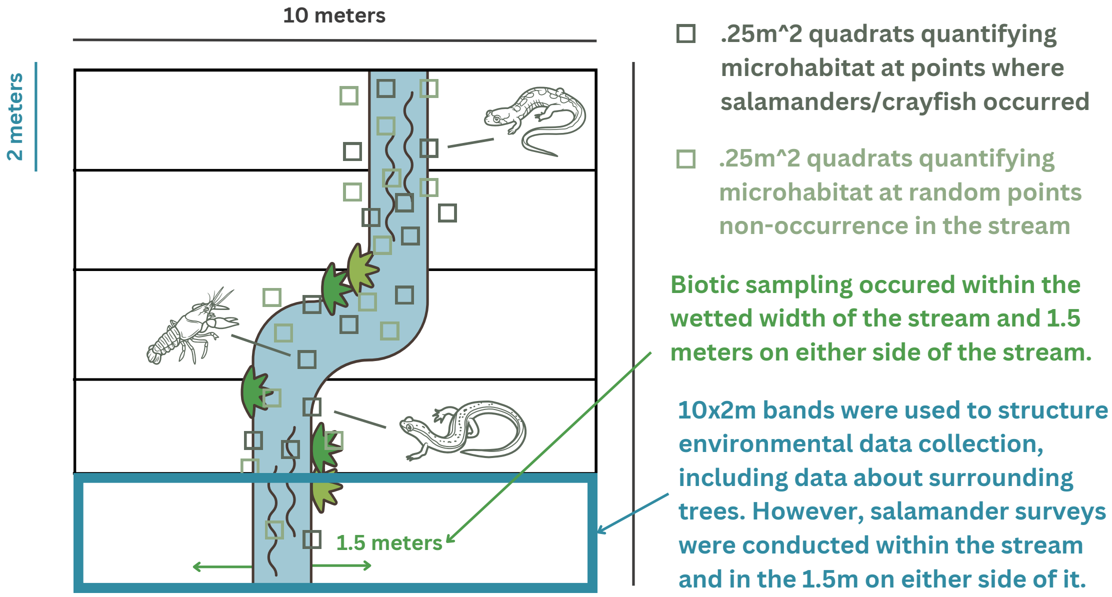
Plot Selection & Delineation

At each site, we selected a 10-meter stream reach to sample. When possible, each reach was selected based on having relatively abundant cover items compared to other reaches of the stream, to maximize our likelihood of finding our focal species (Southerland 1986c), and we hoped to eliminate this as a confounding variable when comparing streams. For the purposes of orientation and structuring abiotic data collection, we subdivided each reach into five 2m bands which were delineated with pin flags (***Fig. 4***). Reaches and bands were delineated with pin flags. We measured the declivity of each 10m reach with a Suunto PM-5 /360 PC clinometer (Suunto, Vantaa, Finland). In addition, we measured channel heights and water depths in each band. Two replicate measurements of channel height were taken in the middle of the band (i.e., 1 meter from either border of the band running perpendicular to the stream) on each side of the channel, and a single water depth measurement per band was taken in the center of the wetted width of the stream.

We also visually estimated the percent cover of bank leaflitter and vegetation for each band from the wetted width of the stream extending five meters on either side. Additionally, we identified, measured, and recorded the diameter at breast height (DBH) for each tree located within five meters of our sampled stream reach with a DBH of greater than 3 cm. Immediately prior to salamander surveys, we obtained dissolved oxygen readings with a YSI ProSolo Dissolved Oxygen Probe (Yellow Springs Instruments, Yellow Springs, OH); these readings were averaged to give a single reading for the entire stream reach. We also collected one conductivity reading using a HoneForest TDS Meter (HoneForest, honeforest.net) per reach.

Within each plot, we also deployed a HOBO Pendant Temperature/Light 64K Data Logger (Onset Computer Corporation, Bourne, MA) set to log water temperature every two hours. To obtain measures of daily stream temperature mean and amplitude from each site, we extracted temperature data from deployed HOBO loggers. We visually inspected time series data and clipped our dataset to time periods where all loggers were functioning properly. Though loggers were deployed for nearly ten months, there was variation in their functionality, and ultimately three relatively brief time periods were extracted: March 18-27, April 30-May 12, and October 9-18 2023. We summarized data by calendar date to calculate daily means and amplitudes.

In October 2023, after the completion of our salamander surveys (see below), we conducted habitat availability surveys to characterize the abundance of suitable cover items and the substrate composition of each stream. Within each two-meter band of each stream, we sampled two 1x1 meter quadrats; one quadrat was placed fully within the stream, and the second was placed straddling the margin of the stream. Based on data about the dimensions of cover objects utilized by *D. cheaha* from our spring field surveys, we counted the number of cover objects that fell completely or mostly within the quadrat that were greater than 7 cm in length. This minimum length was obtained by calculating the square root of the area of the smallest cover item found occupied by a salamander during microhabitat surveys earlier in the field season. Additionally, we visually estimated the percent cover of silt and clay (composited), sand, leaf litter, gravel, cobble, bedrock, wood, and vegetation within each quadrat.



☐ .25m^2 quadrats quantifying microhabitat at points where salamanders/crayfish occurred

☐ .25m^2 quadrats quantifying microhabitat at random points non-occurrence in the stream

Biotic sampling occured within the wetted width of the stream and 1.5 meters on either side of the stream.

10x2m bands were used to structure environmental data collection, including data about surrounding trees. However, salamander surveys were conducted within the stream and in the 1.5m on either side of it.

Figure 4: Figure showing band layout for structuring abiotic and biotic data collection for this project.

Salamander Surveys

Field surveys were performed from 17 March to 26 April 2023 based on our understanding that seal salamanders in South Carolina tend to be most surface active in April (Petranka 1998). We surveyed one to two sites per survey date, and no more than 17 days elapsed between sequential surveys. Prior to entering each site, we disinfected all boots and nets with a 10% bleach solution to mitigate the risk of transmitting pathogens between sites (Declining Amphibian Task Force n.d.). At the start of each survey, we deployed, and allowed to acclimate for the duration of the study, a Kestrel 3000 Weather Meter (Kestrel Instruments, Boothwyn, PA) to obtain air temperature, relative humidity, and wind speed. We obtained barometric pressure from a YSI ProSolo Dissolved Oxygen Probe (Yellow Springs Instruments, Yellow Springs, OH). These micrometeorological variables were measured to assess whether conditions during sampling affected our ability to detect *D. cheaha*.

To standardize survey effort and minimize the impacts of having multiple people instream, the same two searchers (Nick Chang and Will Hutchinson) conducted time- and area-constrained surveys encompassing the wetted width of each stream and 1.5 meters on either side of the stream for a period of one hour. We wore nitrile gloves while searching for and handling salamanders and crayfish. Crayfish were included in our study because they are comparable in size to our salamander species of interest and may compete with salamanders for cover objects (Cragg et al. 2021). We considered all rocks, coarse woody debris, and anthropogenic debris that were in contact with the substrate to be potential cover objects.

During the surveys, we methodically overturned all cover objects. Upon encountering a salamander or crayfish, we attempted to capture the organism with aquarium nets, and when successful, we placed the captured organism in a clean plastic bag with water. These plastic bags were sealed such that there was air within them, and then were floated in a cooler filled with stream water to maintain a stable temperature for the animals. In many cases, we were not able to successfully capture the organism. When possible, we identified escapees to species or genus, and recorded these individuals in our dataset. We then marked the cover object within the stream for all individuals, including escapees, with a pin flag labeled with a code corresponding to the individual. In a few instances, we encountered salamanders and crayfish in the open (not under a cover object) or in burrows. In these cases, we captured the individuals for morphometric data and flagged the location for microhabitat data collection, but noted that the individual was either in a burrow or in the open. To collect salamanders in burrows, we paused the timer and attempted to "fish" salamanders by using a pin flag to mimic the movement of prey and lure the salamander out of its burrow and into a net.

Upon the completion of the exhaustive survey, we measured the snout-vent (from the tip of the snout to the distal end of the cloaca) and the total length of all captured salamanders with a ruler and mass in grams with an electronic scale. Each salamander also received a tail score (1-3) based on tail fullness. We measured carapace length, total length, and mass in grams of all captured crayfish. Length measurements were completed in the plastic bags to reduce stress to the animals.

We attempted to identify all salamanders to species. Though our field site is located near the contact zone between *D. cheaha* and *D. monticola* and surrounding areas are modeled to be suitable for both species (Pyron et al. 2023), specimens from our county and in the immediate vicinity of our sampling area, including from one of our sampled streams, have been genetically identified as *D. cheaha* (Max Seldes & R. Alex Pyron, unpublished data). Prior work using eDNA has also identified *Desmognathus cheaha,* but not *D. monticola,* in the subbasin where our field sites were located (Rittenburg 2023). Thus, we assumed that all seal salamanders captured were *D. cheaha*.

Dusky salamanders in this area have been previously identified as *Desmognathus fuscus fuscus* (Orser and Shure 1972), and subsequently as *D. conanti* (Huang and Wilson 2013, Dymit 2019, Read 2023), but we identify them here as *D. perlapsus* following recent taxonomic revisions (Pyron and Beamer 2022b) and genetic identifications of populations from our area (Rittenburg 2023). Thus, we assumed that all dusky salamanders captured were *D.* We were able to distinguish most dusky and seal salamanders from each other through morphological gestalt. When we encountered difficulties identifying captured salamanders, we examined diagnostic characteristics for identifying each species (***Supp. Table 1***). In n = 5 instances, a salamander escaped and we did not feel comfortable identifying it to species without further information. These observations were excluded from our analysis. Though we often had field assistance in the form of scribes, all handling of animals was performed by individuals who had completed appropriate training through Emory IACUC.

For each point-of-capture in each stream reach, we documented the cover object type (rock, coarse woody debris, burrow, open), distance to water, water depth, and cover object dimensions. Distances were measured from the center of each cover object when the salamander or crayfish was found underneath a cover object. For each occupied cover object, we then randomly selected an additional unoccupied cover object by spinning a pencil over the cover object and selecting the nearest cover object that it pointed to that was large enough to cover a salamander (3 cm), similar to Rossell et al. (2018). We then recorded the same data (cover object type, distance to water, water depth, and cover object dimensions) for unoccupied cover objects. Additionally, for every occupied and vacant point, we overturned the cover object, placed a 0.25 x 0.25 meter quadrat around the center of the cover object's footprint, and photographed the quadrat. We visually estimated the percent cover of sand, silt and clay (pooled), gravel, rock, leaf litter, wood, and vegetation from each quadrat using these photos.

Upon the completion of microhabitat data collection in a plot, all salamanders and crayfish were released at their respective flagged cover objects or points of capture (for individuals in the open). Salamanders and crayfish were not held in captivity for greater than 1.5 hours, were kept out of sunlight, and were monitored for signs of distress. Animal handling was performed under Emory University IACUC protocol 202200162.

Watershed Data

We obtained a 1 m^2 digital elevation model (DEM) raster dataset from the National Elevation Dataset (USGS n.d.), and used this dataset as an input to delineate watersheds for each focal stream using the Watershed function in ArcGIS Pro (ESRI, Redlands, CA). The coordinates of the lower bound of each stream reach were used to place pour points for these watersheds so that only the area upstream of the sampled area was included in the analysis. We then converted watershed rasters into polygons and calculated the area of each polygon. When available, these output watershed shapefiles were visually compared with subbasin maps for Emory's 2005 Stormwater Management Plan (Jordan et al. 2005) to ensure that they were similar in shape and extent to prior analyses. We obtained the National Landcover Database Urban Imperviousness raster dataset for 2021 from the Multi-Resolution Land Characteristics Consortium at a 30 m^2 resolution (Dewitz 2021), and calculated the percent impervious surface cover in each drainage using the Zonal Statistics tool in ArcGIS Pro.

Statistical Analysis – Stream-level Occupancy by *D. cheaha*

Due to the relatively low variation in *D. cheaha* counts between streams, we used logistic regression to analyze the presence and absence of *D. cheaha* across streams (n=11). Due to our low sample size, we were greatly constrained in the number of predictors we were able to include in each model. Therefore, we fit a set of logistic regression models describing occupancy based on *a priori* hypotheses of factors influencing *D. cheaha* presence or absence from streams ***(Table 1)***. These were then ranked using AIC to determine which predictor(s) best described our data.

Table 1: Stream-level models with a priori hypotheses for relevance

Predictor/Model	***a priori* Hypothesis**
Daily mean stream temperature (°C)	Salamander presence will be negatively associated with higher mean stream temperatures (Bernardo and Spotila 2006)
Daily mean stream temperature amplitude (°C)	Salamander presence will be negatively associated with higher daily temperature variation (Bernardo and Spotila 2006, Cecala et al. 2018)
Dissolved Oxygen	Salamander presence will be positively associated with higher dissolved oxygen levels (Folkerts 1968)
Water depth (cm)	Salamander presence will be positively associated with deeper water (Southerland 1986a)
Cover object density (item/m^2)	Salamander presence will be positively associated with a greater abundance of cover objects (Folkerts 1968, Southerland 1986c)
Channel height (cm)	Salamander presence will be negatively associated with higher average channel height (Orser and Shure 1972, Surasinghe 2013)
Mean diameter at breast height of trees within the plot (cm)	Salamander presence will be positively associated with forest age (Lowe and Bolger 2002)
Stream declivity (°)	Salamander presence will be positively associated with higher declivity (Folkerts 1968)
Watershed area (m^2)	Salamander presence will be negatively associated with larger watershed area (Cecala et al. 2018)
% impervious surface cover in the watershed	Salamander presence will be negatively associated with a higher proportion of upstream impervious surface cover (Barrett et al. 2010a)
Null Model	Salamander presence is not dependent on measured environmental variables

Statistical Analysis – Microhabitat Use by *D. cheaha*

To conduct microhabitat analyses, we first subset our microhabitat data only to include cover objects from streams where *D. cheaha* was detected (n = 7, out of n = 11 total streams), to account for the potential that other streams had cover objects which were hypothetically suitable for *D. cheaha* based on physical characteristics, but where the species is otherwise excluded due to the physiochemical water quality characteristics of the stream. We further removed observations of *D. cheaha* and other salamanders and crayfish from the open (i.e., not under cover) or in burrows (n = 10). Our final sample size for analysis included n = 30 cover items occupied by *D. cheaha* and n = 218 cover items which did not shelter *D. cheaha.* We treated each cover item examined as a replicate, and used mixed-effects logistic regression with a binomial response of *D. cheaha* present (1) or absent (0). Importantly, cover items that did not shelter *D. cheaha* but did shelter either other species of salamanders or crayfish were treated as absences. We accounted for site-level variation in microhabitat use and availability by including stream identity as a random effect in our model. Due to our relatively limited statistical power, we fit a series of models containing unique subsets of explanatory variables based on *a priori* hypotheses about habitat use, listed in ***Table 2***. We subsequently ranked these models using AIC to determine which one was most descriptive of our data.

Table 2: Mixed-effects models of D. cheaha *presence/absence under individiual cover objects (with site as a random effect) with a priori hypotheses for why this combination of variables would be predictive*

Predictors	***a priori* Hypothesis**
% cover rocky substrate + Habitat Type (stream/margin/terrestrial) + (1|Stream)	Cover items with rockier substrates have greater flow and cavity space, potentially making them harder territories to defend. Pyron et al. (2023) also suggest that the species is only found in contact with bare rock and flowing water in the Coastal Plain, which makes it interesting to see whether there is an association in the Piedmont. As the largest commonly-encountered member of the stream salamander assemblage, we expect *D. cheaha* to utilize more aquatic habitat as opposed to marginal habitat (Southerland 1986a).
% cover leaf litter + (1|Stream)	Prior study of other *Desmognathus sp.* found leaf litter to be important in predict salamander presence (Southerland 1986c). Cover items surrounded by leaf litter have more surface area to hide and forage in.
Cover Object Area + Habitat Type (stream/ margin/terrestrial) + (1|Stream)	We expect seal salamanders will select larger cover items closer to the stream, given our understanding of habitat use in other large *Desmognathus sp.* (Grover 2000). As the largest commonly-encountered member of the stream salamander assemblage, we expect *D. cheaha* to utilize more aquatic habitat as opposed to marginal habitat (Southerland 1986a).
Cover Object Area * Count of Non-Seal Occupants + (1|Stream)	We expect seal salamanders will select larger cover items closer to the stream, given our understanding of habitat use in other large *Desmognathus sp.* (Grover 2000). Refuge sharing is uncommon between members of headwater stream assemblages 4/8/24 1:02:00 PMWe expect that since larger cover objects should be more suitable habitat, they will be more likely to shelter salamanders. However, since refuge-sharing is uncommon, we expect that the effect of cover object size on *D. cheaha* presence will be dependent on whether this cover object is already occupied by another occupant. Therefore, we include an interaction parameter for these two terms.
Null Model (1|Stream)	Habitat selection is not dependent on sampled environmental variables

In addition, based on our understanding that larger-bodied desmognathan salamanders tend to exhibit different habitat use than smaller-bodied ones (Bruce 2011), we tested for differing habitat usage by individuals of *D. cheaha* of different sizes. Only captured individuals from under cover objects with associated cover object and morphometric data are included in this analysis (n = 22). Finding strong correlations between mass in grams and both total length ($R^2 = 0.913$) and snout-vent length ($R^2 = 0.905$), and identifying only n = 3 salamanders with a tail score less than 3 (the maximum score), we used mass in grams as the measure of salamander size for these tests. We used mass as a measure of body size as it may be a better predictor of salamander health than length alone (Southerland 1986a, Hoffacker et al. 2018). We used mixed-effects linear regression, with stream as a random effect, to fit two different models for salamander mass as a function of habitat use (***Table 3***). While we initially sought to fit a single model with salamander mass as a function of cover object area, habitat type, and an interaction between the two, we lacked the statistical power to do so.

Table 3: Mixed-effects linear regression models, with a priori hypotheses for their importance, describing salamander mass as a function of microhabitat features.

Model	***a priori* Hypothesis**
Cover Object Area (cm^2) + (1|Stream)	Larger salamanders will be more likely to utilize larger cover items (Moore et al. 2001)
Habitat Type (stream/marginal) + (1|Stream)	Larger salamanders will be more likely to occupy within-stream habitat (Camp and Lee 1996, Bruce 2011)

Additionally, we examined whether the size of available cover objects differed between in-stream and margin habitats. We used mixed-effects linear regression to model cover object area for all data points (regardless of occupancy) in seal-occupied streams (n = 236) as a function of habitat type, with stream as a random effect.

Mixed-effects logistic and linear regression models were fitted using the package *glmmTMB* (Brooks et al. 2017) in R version 4.3.1 (R Core Team 2023).

Statistical Analysis – Shifting Microhabitat Use by *Desmognathus perlapsus*

Finally, we tested whether the Chattooga dusky salamander, *Desmognathus perlapsus,* utilizes different habitat in the presence of *D. cheaha.* We analyzed microhabitat points where we observed *D. perlapsus* in either stream or marginal habitat which were sheltering under either rocks or coarse woody debris (n = 67). We used mixed-effects logistic regression to model the occupancy of *D. perlapsus* in marginal (0) or stream (1) habitat as a function of *D. cheaha* presence in the stream and the density of cover objects within the band that this observation occurred in. The inclusion of cover object density as a fixed effect allowed us to control for the role of habitat availability in determining within-stream habitat usage (Southerland 1986c). Both stream and band were considered random effects, with band nested within stream.

This model was fit using the package *glmmTMB* (Brooks et al. 2017) in R version 4.3.1 (R Core Team 2023).

Results

Field Surveys

During our surveys, we identified the following salamander species: the Talladega seal salamander (*Desmognathus cheaha*), Chattooga dusky salamander (*Desmognathus perlapsus*), southern two-lined salamander (*Eurycea cirrigera*), three-lined salamander (*Eurycea guttolineata*), and northern red salamander (*Pseudotriton ruber ruber*).

In addition, we encountered at least two crayfish species. We were able to identify the state-threatened Chattahoochee crayfish (*Cambarus howardi*) in the field due to its unique coloration (Hobbs 1981). We were able to identify other crayfish species to the genus *Cambarus,* but were not able to identify them to species in the field. Subsequent surveys of a subset of the study streams were able to identify the variable crayfish (*Cambarus latimanus*) as a widespread species within the study area, but we are unable to say that this is the only additional species encountered during surveys. Since salamanders are the focal taxon in this study, we pooled counts of crayfish for all analyses.

Using logistic regression at the level of the stream, we did not find a significant relationship between detection of *D. cheaha* and sampling date or detection (***Supp. Table 2***), suggesting that there was not a seasonal trend in our detection of our focal species.

We detected *D. cheaha* in the seven (n = 7) streams identified *a priori* to support the species, but did not detect *D. cheaha* in the four (n = 4) streams that were included without a prior record of their presence. In streams containing *D. cheaha,* counts during our exhaustive 10m reach surveys ranged from 1 to 13, with a total of 34 individuals documented across all sites. All observed individuals of *D. cheaha* utilized rocks as cover objects, except for n = 2 which were fished from burrows and n = 1 which sheltered under a piece of wood. Of the n = 34 individuals observed, n = 22 were successfully captured.

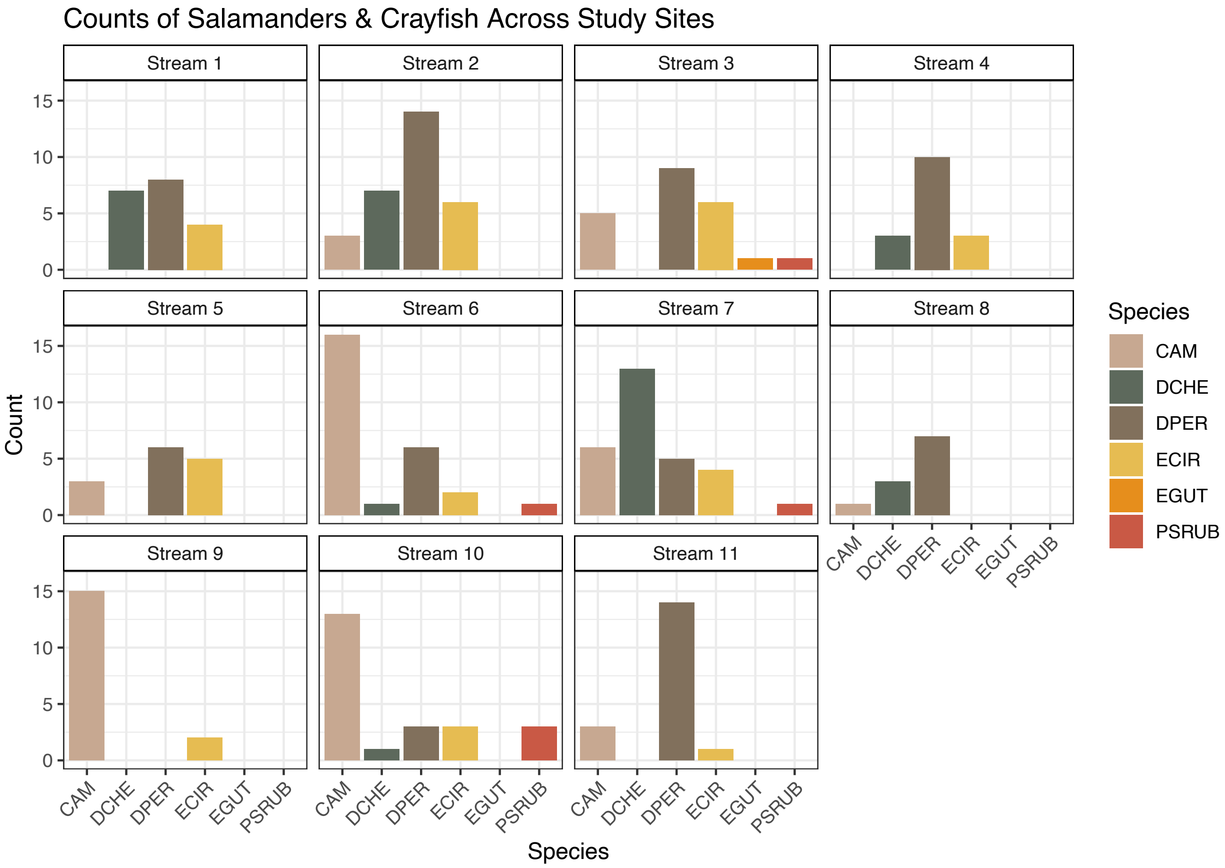


Figure 5: Counts of salamanders and crayfish encountered by site. CAM: crayfish (pooled), DCHE: Talladega seal salamander, DPER: Chattooga dusky salamander, ECIR: southern two-lined salamander, EGUT: three-lined salamander, PSRUB: northern red salamander.

Table 4: Table of field sites including date sampled, count of D. cheaha *observed, cover object density across the reach, and drainage area.*

Stream #	**Date Sampled**	**Count of *D. cheaha***	**Cover Obj. Density (count/m^2)**	**Drainage area (m^2)**	**Prior Records of *D. cheaha***	**Prior Urbanization Research**
1	2023-03-17	7	0.87	23297.22	Chang pers. obs.	No Record
2	2023-03-18	7	0.64	75267.95	Rittenburg 2023	Rittenburg 2023
3	2023-03-19	0	0.70	161288.47	No Record	Orser & Shure 1972
4	2023-04-05	3	1.26	15232.80	Chang pers. obs.	No Record
5	2023-04-07	0	0.34	69891.67	No Record	No Record
6	2023-04-09	1	0.88	136199.15	Rittenburg 2023	Rittenburg 2023
7	2023-04-12	13	0.99	76164.00	Read 2023	Orser & Shure 1972
8	2023-04-16	3	1.64	11648.61	Chang pers. obs.	No Record
9	2023-04-16	0	0.70	159496.37	No Record	Read 2023
10	2023-04-21	1	0.52	214155.24	Rittenburg 2023	Rittenburg 2023
11	2023-04-26	0	1.04	146055.67	No Record	Read 2023

Stream-level Analysis

None of the models that we evaluated found a significant predictor for reach-level detections of our focal species at the $\alpha = 0.05$ level. However, after comparing the outputs of our models, including the null model, with AIC, we determined that the model incorporating declivity was the most descriptive (***Table 4***). After performing a likelihood ratio test, we found that the declivity model performed significantly better than the null model ($p = 0.0356$), though we caution that this test may not be reliable due to our sample size (Bolker et al. 2009). The second-best performing model, which included daily temperature amplitude, performed marginally better than the null model (p = 0.069) which, given our sample size, suggests further investigation may be warranted.

Table 5: Outputs from logistic regression models describing the association of the stream-level presence/absence of Desmognathus cheaha *in relation to different environmental predictors. Models are arranged in descending AIC order, with the null model highlighted.*

Model	**AIC**	**Predictor Statistics**			
		Predictors	*Odds Ratios*	*CI*	*p*
Daily mean temperature	18.1	dailyMeanTemp	0.40	-4.894 – 2.205	0.583
% impervious surface cover	18.056		1.03	-0.065 – 0.164	0.563
Conductivity	17.884		0.99	-0.051 – 0.022	0.469
Dissolved Oxygen	17.686		1.23	-0.273 – 0.765	0.401
Channel height	17.6		0.97	-0.112 – 0.034	0.383
Watershed area	16.582		1.00	$-4.142\text{x}10^{-5}$ – $5.73\text{x}10^{-6}$	0.210
Mean DBH of surrounding trees	16.54		1.19	-0.068 – 0.507	0.211
Cover object density	16.503		22.64	-1.105592 – 10.007817	0.240
Null model	16.421				
Water depth	15.247		0.67	-1.154 – 0.033	0.154
Daily mean temperature amplitude	15.122	dailyMeanAmpl	0.01	-12.099 – 0.315	0.124
Declivity	14.004		1.83	0.017 – 1.962	0.257

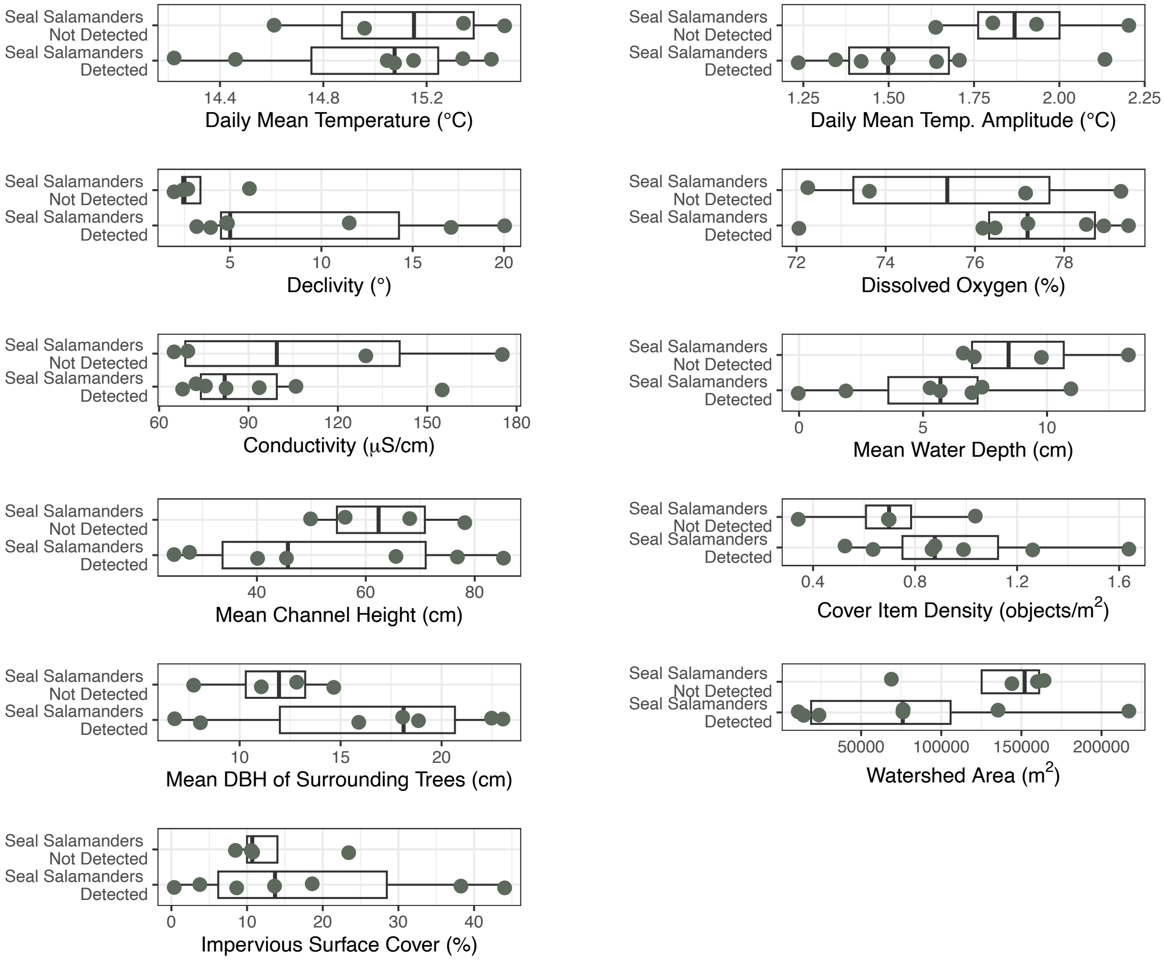


Figure 6: Boxplots comparing stream-level predictors of environmental parameters in streams where D. cheaha *was and was not detected.None of these relationships are statistically significant.*

Microhabitat Analysis

After comparing the outputs of the five *a priori* models and the null model using AIC, we determined that the model incorporating cover object area, the presence of another species of salamander or crayfish, and an interaction parameter between the two, to be our best model for describing patterns in microhabitat use by *D. cheaha* in our study **(*Fig. 7, Table 5*).** Using a likelihood ratio test, we found that this model performed significantly better than the null model

($p < 0.0001$); however, we caution that due to our low sample size, this output may not be a reliable metric of model performance (Bolker et al. 2009).

While cover object area is the only significant predictor in this model at $\alpha = 0.05$, the interaction parameter between cover object area and the presence of another species is marginally significant ($p = 0.63$). Due to our relatively small sample size, we suspect these results suggest that the effect of cover object area on cover object suitability is dependent on the presence of an additional community member. While this model has a similar AIC to the model with the same variables but without the interaction parameter (ΔAIC = 2), We found that the model with the interaction parameter performed significantly better than the simpler model using a likelihood ratio test ($p = 0.045$), though again, this test should be interpreted carefully due to our sample size (Bolker et al. 2009).

Cover object area was also a significant predictor in the third-best performing model, underscoring its importance. Neither substrate nor cover item position were determined to be significant predictors in our models.

Table 6: Microhabitat models for predicting the presence of Desmognathus cheaha *under cover items, with models arranged vertically by descending AIC values. The null model is highlighted.*

<table>
<tr><th>Model</th><th>AIC</th><th colspan="4">Predictor Statistics</th></tr>
<tr><td rowspan="2"><i>Leaf litter</i>
% cover leaf litter + (1|Stream)</td><td rowspan="2">182.8</td><td>Predictors</td><td>Odds Ratios</td><td>CI</td><td>p</td></tr>
<tr><td>per litter</td><td>1.00</td><td>-0.02 – 0.02</td><td>0.863</td></tr>
<tr><td rowspan="2"><i>Null Model</i>
(1|Stream)</td><td rowspan="2">180.9</td><td>Predictors</td><td>Odds Ratios</td><td>CI</td><td>p</td></tr>
<tr><td>(Intercept)</td><td>0.12</td><td>-2.89 – -1.42</td><td><0.001</td></tr>
<tr><td rowspan="4"><i>Rocky substrate and cover item position</i>
% cover rocky substrate + Habitat Type (stream/ margin/ terrestrial) + (1|Stream)</td><td rowspan="4">180.6</td><td>Predictors</td><td>Odds Ratios</td><td>CI</td><td>p</td></tr>
<tr><td>rocky</td><td>1.00</td><td>-0.01 – 0.02</td><td>0.754</td></tr>
<tr><td>hab[stream]</td><td>2.09</td><td>-1.587x10^{-1} –1.632</td><td>0.107</td></tr>
<tr><td>hab[terrestrial]</td><td>7.77 e-09</td><td>-1.990x10^{4} –1.987x10^{4}</td><td>0.999</td></tr>
<tr><td rowspan="4"><i>Cover object area and position</i>
Cover Object Area + Habitat Type (stream/ margin/ terrestrial) + (1|Stream)</td><td rowspan="4">172.1</td><td>Predictors</td><td>Odds Ratios</td><td>CI</td><td>p</td></tr>
<tr><td>covArea</td><td>1.00</td><td>3x10^{-4} – 2.01x10^{-3}</td><td>0.005</td></tr>
<tr><td>hab[stream]</td><td>1.97</td><td>-0.24 – 1.59</td><td>0.147</td></tr>
<tr><td>hab[terrestrial]</td><td>0.00</td><td>-2.13x10^{4} – 2.12x10^{4}</td><td>0.999</td></tr>
<tr><td rowspan="3"><i>Cover object area and occupancy</i>
Cover Object Area + Presence of non-Seal Occupants + (1|Stream)</td><td rowspan="3">160.4</td><td><i>Predictors</i></td><td><i>Odds Ratios</i></td><td><i>CI</i></td><td><i>p</i></td></tr>
<tr><td>roommate [+1]</td><td>0.11</td><td>-3.607 – -0.860</td><td>0.001</td></tr>
<tr><td>covArea</td><td>1.002</td><td>0.001 –0.003</td><td><0.001</td></tr>
<tr><td rowspan="4"><i>Cover object area and occupancy with interaction parameter</i>
Cover Object Area * Presence of non-Seal Occupants + (1|Stream)</td><td rowspan="4">158.4</td><td><i>Predictors</i></td><td><i>Odds Ratios</i></td><td><i>CI</i></td><td><i>p</i></td></tr>
<tr><td>roommate[1+]</td><td>0.36</td><td>-2.684 – 6.197e-01</td><td>0.221</td></tr>
<tr><td>covArea</td><td>1.003108</td><td>0.002 – 4.712e-03</td><td><0.001</td></tr>
<tr><td>roommate[1+] × covArea</td><td>0.9981147</td><td>-0.004 –9.865e-05</td><td>0.063</td></tr>
</table>

Table 7: Full regression output for the best performing model for microhabitat occupancy by D. cheaha, *which included community member, cover area, and an interaction between the two.*

***D. cheaha* Occupancy**

Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	0.06	0.02 – 0.15	**<0.001**
roommate [roommate]	0.36	0.07 – 1.86	0.221
Cover object area (cm^2)	1.00	1.00 – 1.00	**<0.001**
roommate [roommate] × Cover object area (cm^2)	1.00	1.00 – 1.00	0.063
Random Effects			
σ^2	3.29		
$\tau_{00\ \text{PlotCode}}$	0.50		
ICC	0.13		
N_{PlotCode}	7		
Observations	248		
Marginal R^2 / Conditional R^2	0.224 / 0.327		

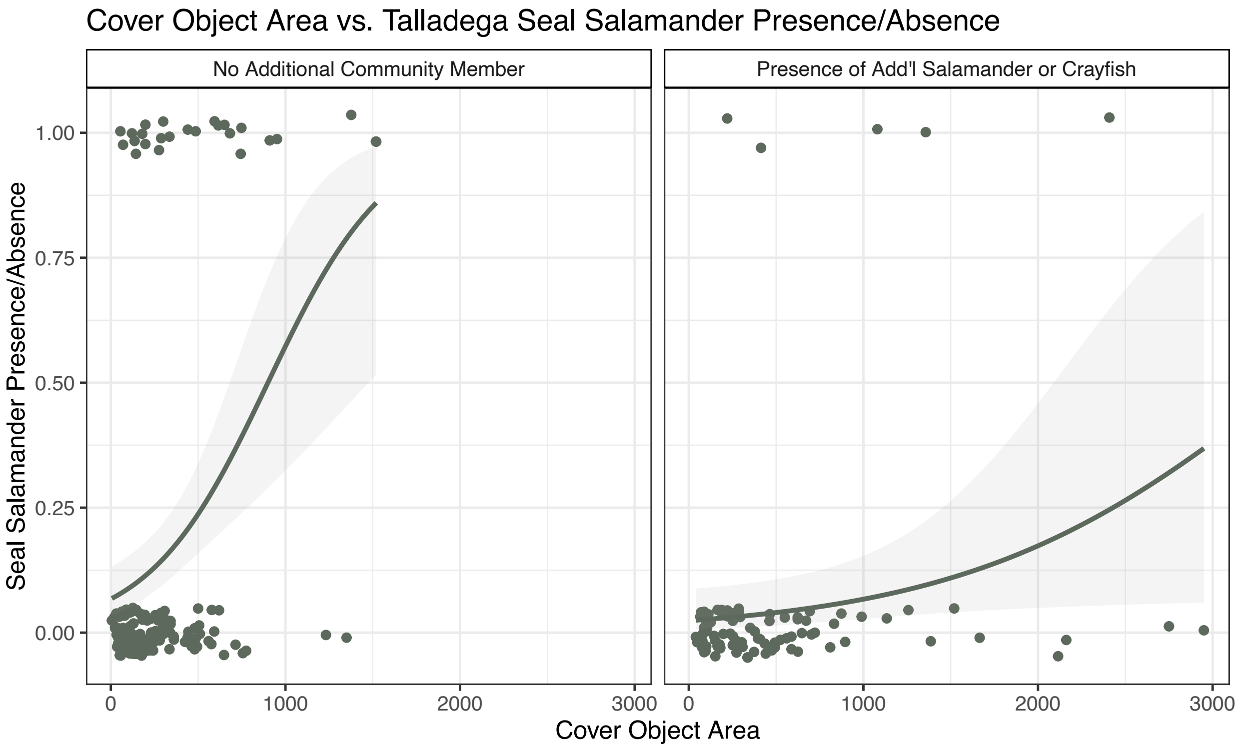


Figure 7: Logistic regression plot showing an association between cover object area and the presence/absence of seal salamanders. The left panel shows this association for cover items that did not shelter an additional species of salamander or crayfish; the right panel shows this association for cover items that did shelter an additional species of salamander or crayfish.

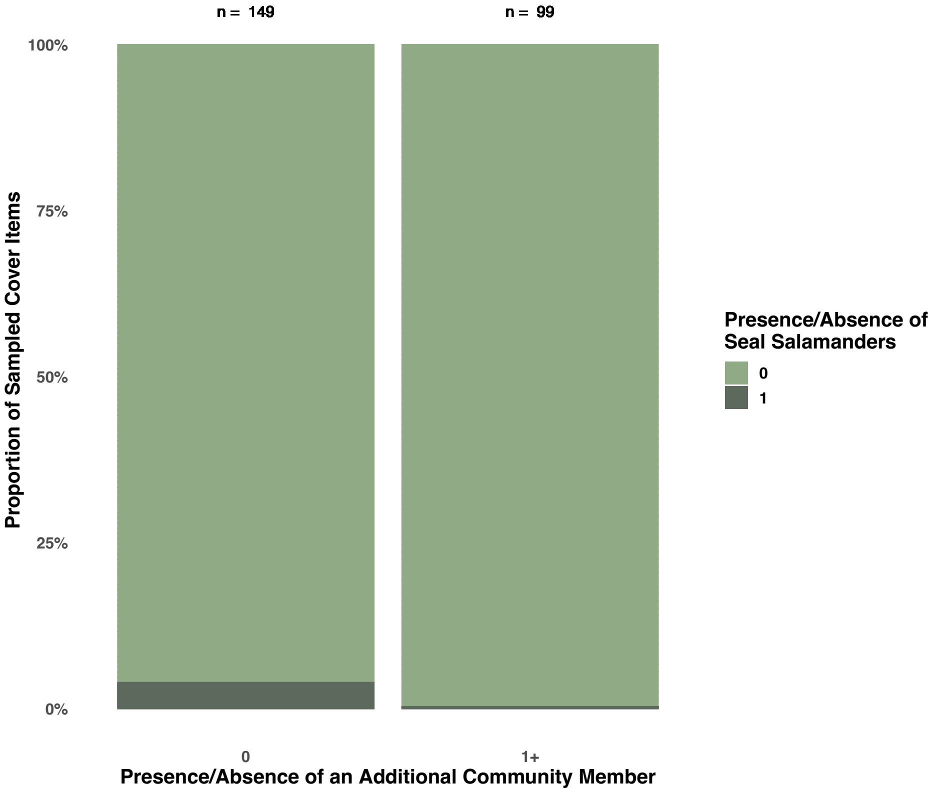


Figure 8: Stacked barplots comparing the presence of seal salamanders under cover objects with and without other community members. The left bar shows cover items where there was no additional species of salamander or crayfish. The right bar shows cover items where there was an additional species of salamander or crayfish.

In our analysis of *D. cheaha* habitat use based on body size, we found a significant relationship between cover object area and mass in grams of *D. cheaha* ($\beta = 0.004$, $p < 0.001$; ***Table 8***). For a 1 cm^2 increase in cover object area, we expected salamander mass to increase by 0.004 grams (***Fig. 9***). This model performed significantly better than the null model ($p < 0.001$). Additionally, we found a significant relationship between stream habitat (compared to marginal) and mass in grams ($\beta = 3.57$, $p = 0.010$; ***Table 9***). We expect that, moving from marginal habitat to stream habitat, salamander mass would increase by 3.571 grams (***Fig. 10***). This model performed significantly better than the null model using ($p = 0.016$).

Table 8: Regression output for model characterizing D. cheaha *mass as a function of cover object area*

	***D. cheaha* mass**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1.97	-0.18 – 4.13	0.073
Cover Object Area (cm^2)	0.004	0.00 – 0.01	**<0.001**
Random Effects			
σ^2	6.21		
$\tau_{00\ \text{PlotCode}}$	1.13		
ICC	0.15		
N_{PlotCode}	5		
Observations	22		
Marginal R^2 / Conditional R^2	0.543 / 0.613		

Table 9: Linear regression output for model characterizing D. cheaha *mass as a function of habitat type*

	***D. cheaha* mass**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	3.61	1.24 – 5.98	**0.003**
habitat [stream]	3.57	0.87 – 6.27	**0.010**
Random Effects			
σ^2	10.18		
$\tau_{00\ \text{PlotCode}}$	1.35		
ICC	0.12		
N_{PlotCode}	5		
Observations	22		
Marginal R^2 / Conditional R^2	0.223 / 0.314		

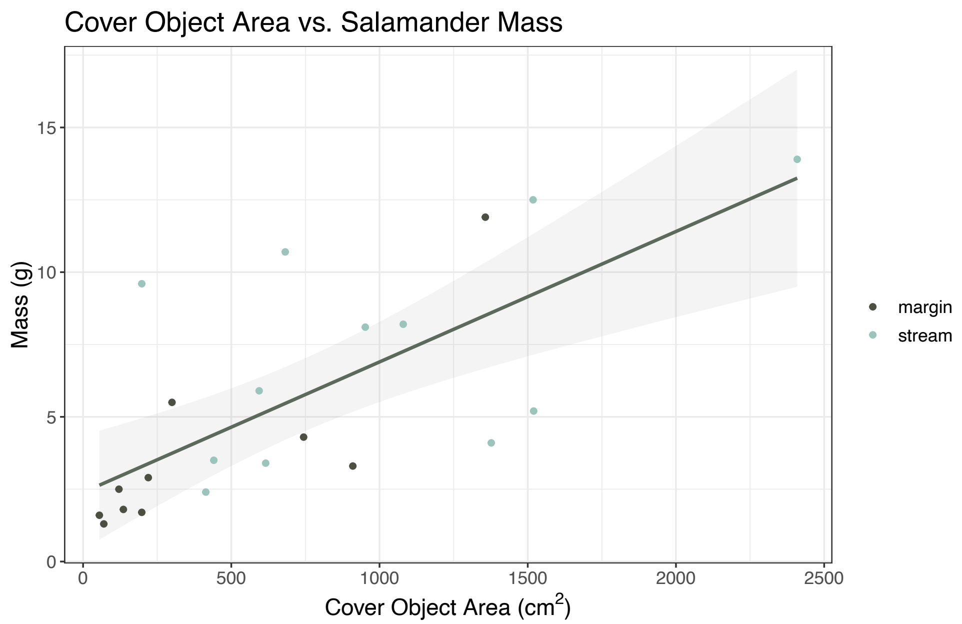


Figure 9: Linear regression plot of cover object area vs. salamander mass in grams. Note a potential outlier in the right hand side of the plot. The removal of this outlier had no qualitative effect of the significance of cover object area as a predictor.

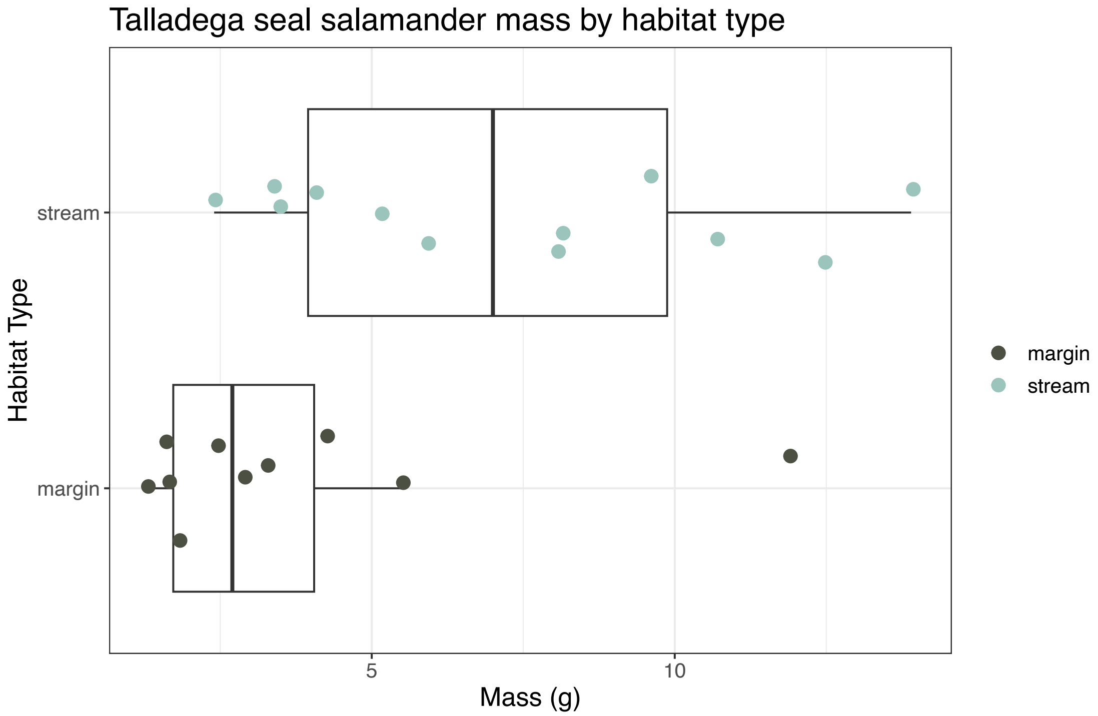


Figure 10: Boxplot showing D. cheaha *body size by microhabitat type.*

We hypothesized that a potential observed trend in marginal habitat usage by smaller salamanders (***Fig. 9;*** note the observed clustering of salamanders using marginal habitat to the bottom left corner of the plot**)** might caused by a greater availability of larger cover objects within the stream channel. After fitting a model of cover object area as a function of habitat type in streams where *D. cheaha* is present, with stream as a random effect, we did not find as significant relationship between cover object area and habitat type (p = 0.110, ***Table 8***). We were unable to explicitly test for an interaction effect between habitat type and cover object area due to constrained sample size (Bolker et al. 2009).

Table 10: Regression output for a mixed effects model of cover object area by habitat type.

	Cover Object Area (cm^2)		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	321.53	232.50 – 410.56	**<0.001**
hab [stream]	94.00	-21.19 – 209.18	0.110
Random Effects			
σ^2	196034.64		
$\tau_{00\ \text{PlotCode}}$	0.02		
ICC	0.00		
N $_{\text{PlotCode}}$	7		
Observations	236		
Marginal R^2 / Conditional R^2	0.011 / 0.011		

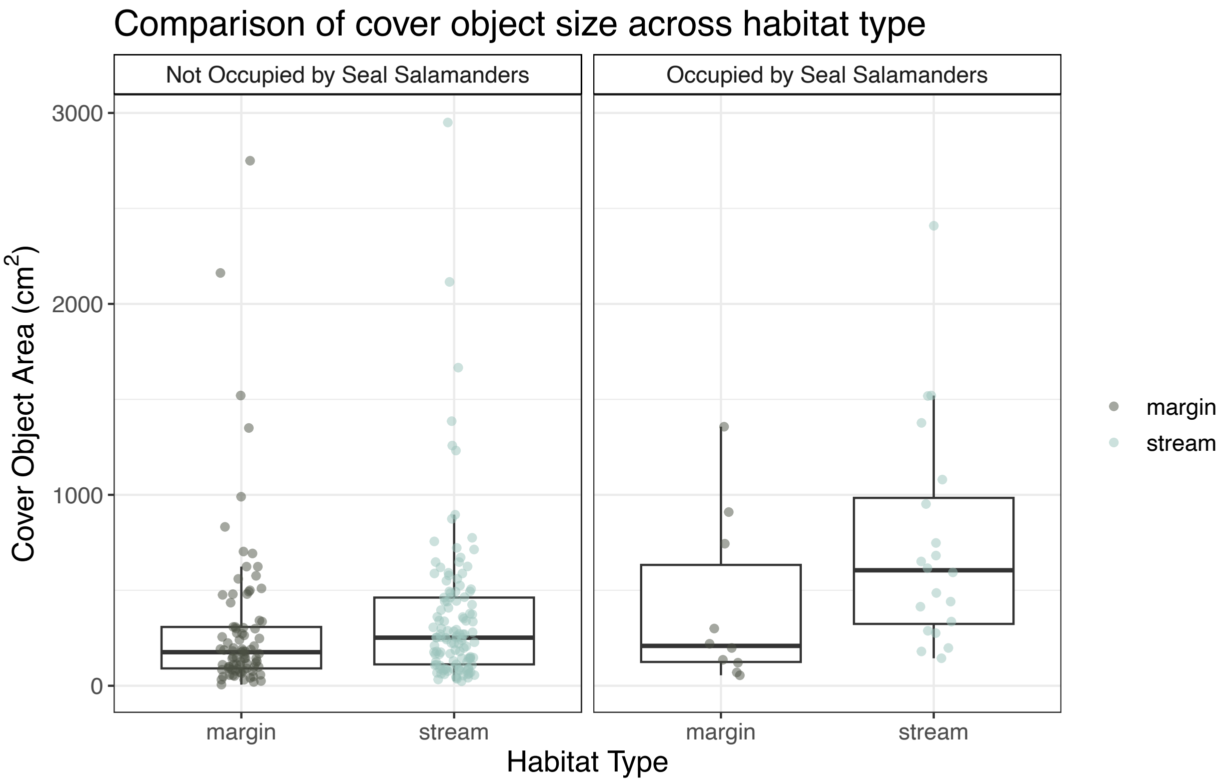


Figure 11: Boxplot showing comparison of cover item size across stream and marginal habitat.

<u>Shifting Microhabitat Use by *D. perlapsus*</u>

In our analysis of shifting habitat use by *D. perlapsus* in the presence of *D. cheaha,* we found a significant association between stream habitat use by *D. perlapsus* and the presence of *D. cheaha* in the stream (p = 0.022). In the presence of *D. cheaha,* the likelihood of *D. perlapsus* occupying stream habitat is 1/5 of that in streams without *D. cheaha* (***Fig. 11***). In addition, the density of cover objects adjacent to the sampled point may be considered marginally significant (p = 0.065) given our sample size. The model suggests that as the density of cover objects increases by one object per meter squared, the likelihood of finding *D. perlapsus* in stream habitat decreases by around ¾, though further data collection is needed to validate whether a relationship exists here. This model performed significantly better than both the null (p = 0.009) and a model fit without cover object density as a predictor (p = 0.034).

Table 11: Regression output for model testing relationship between D. perlapsus *habitat use and both the presence of seal salamanders in the stream and the density of cover items surrounding the salamander's refuge object.*

Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	7.43	1.22 – 45.34	**0.030**
Seal Stream	0.20	0.05 – 0.79	**0.022**
Cover Object Density (object/m^2)	0.78	0.61 – 1.02	0.065
Random Effects			
σ^2	3.29		
τ_{00} bandNum:PlotCode	0.39		
τ_{00} PlotCode	0.00		
N bandNum	5		
N PlotCode	10		
Observations	67		
Marginal R^2 / Conditional R^2	0.235 / NA		

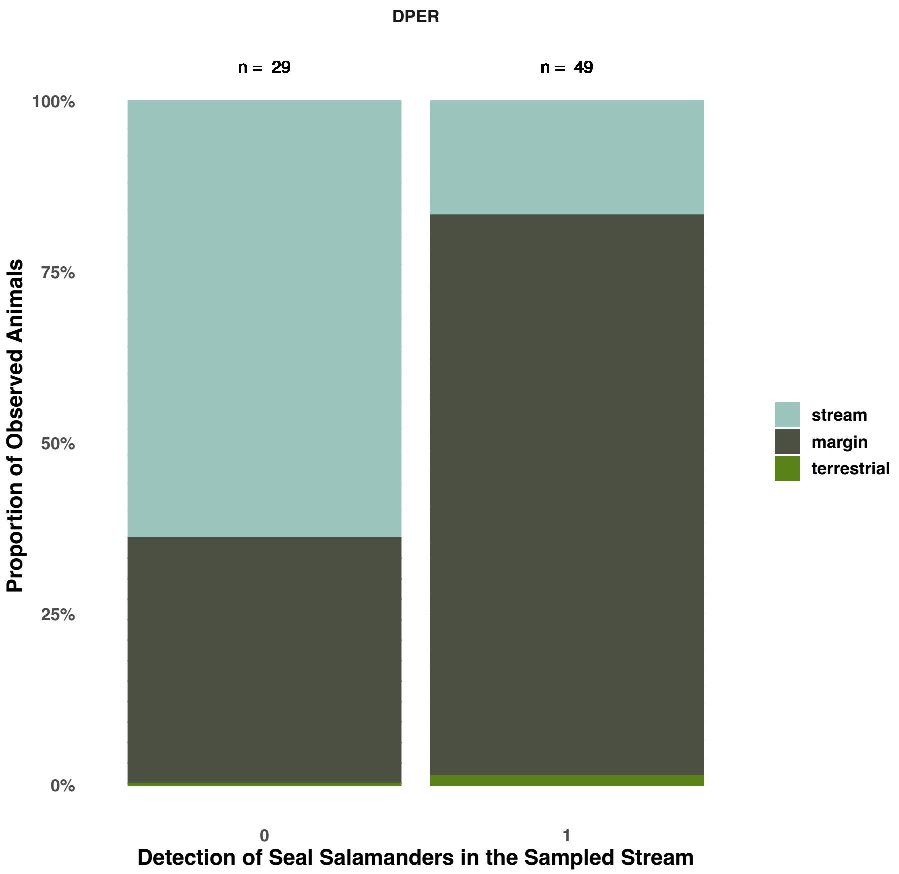


Figure 12: Stacked bar plot showing the distribution of Desmognathus perlapsus *observations and vacant points in streams where seal salamanders were and were not detected.*

Discussion

Collecting foundational natural history information about the habitat use of *Desmognathus cheaha* is important to address key knowledge gaps in the ecology of the species. These data are particularly important due to the apparent sensitivity of *D. cheaha* to anthropogenic disturbance in some portions of its range (Holzheuser and Means 2021, Pyron et al. 2023). At the level of a stream, we did not detect a significant association between any of the environmental variables we analyzed and the detection of *D. cheaha*. At the microhabitat level, we found a significant association between the presence of *D. cheaha* and both cover object area and the presence of additional community members*,* but did not find an association for cover item position or substrate composition. Additionally, we found a significant relationship between cover object area and *D. cheaha* mass. Finally, we found evidence to suggest that habitat usage by *D. perlapsus,* a co-occurring congener, is dependent on the presence of *D. cheaha*. While our ability to draw broad conclusions from this project is constrained by our sample size and the geographic scope of our work, we hope that this project can provide a baseline to facilitate future research into the ecology of this enigmatic and poorly understood species.

Stream-level Interpretation

At the stream level, no predictors were found to have a significant association with the presence or absence of *D. cheaha*. However, we had a relatively small sample size (n = 11), and we sampled over a relatively restricted geographic area. Additionally, because the main aim of this project was to understand microhabitat usage, we specifically sampled streams that we knew or predicted to support our focal species. Thus, we specifically avoided heavily urbanized streams that we believed would not support our species, and therefore did not sample across a wide urban gradient. We predict that if we had, a clearer signal suggesting an impact of urbanization on *D. cheaha* presence and absence would have emerged. This is particularly apparent for upstream impervious surface cover and density of cover objects, both of which are supported in the literature as predictors influencing the abundance of stream salamanders (Southerland 1986c, Barrett and Price 2014, Cecala et al. 2018). Additionally, while *D. cheaha* was detected at seven of our eleven streams, *D. perlapsus* occurs in all of them (though there was one stream where *D. perlapsus* was not detected during our surveys). We suspect that *D. cheaha* is more sensitive to urbanization than smaller congeners such as *D. perlapsus*, which would align with prior work (Barrett et al. 2010b, Surasinghe and Baldwin 2015), though more research is needed to understand the impacts of urbanization on this species.

While not significant, our model ranking process found declivity to be the best predictor of *D. cheaha* detection within a stream. This finding is interesting given anecdotes from the Coastal Plain, where others have suggested that the species may be associated with high-gradient, fast-flowing streams (Folkerts 1968, Pyron and Beamer 2022). Folkerts (1968) suggested this utilization of fast-flowing streams might relate to dissolved oxygen content. While our model incorporating dissolved oxygen performed worse than our null model, dissolved oxygen levels were generally uniform across sites. Additionally, while our streams are located in the Piedmont, Folkerts' work included populations in the Coastal Plain. In the Coastal Plain, which sits at a lower elevation and therefore has warmer streams than the Piedmont (Utz et al. 2011), dissolved oxygen may be a more significant limiting factor in stream systems (Nagy et al. 2011, Allan et al. 2021). Higher gradient streams with more topographic complexity may also generally support cooler water temperatures (White et al. 1987) and provide a refuge for populations living near the edges of their physiological tolerances (see Future Directions). As mentioned in Folkerts (1968), these conditions are likely also related to the flow of water in streams. While we sought to measure flow rates in our streams, two of our sites had sluggish flow and may be considered seepages or intermittent streams, and we were not able to obtain flow readings from these sites. Alternatively, steeper streams may simply be located in areas that were protected from disturbance by complex topography that precluded logging or development.

Our second-best performing model had average daily stream temperature amplitude as a predictor. Though this predictor was not significant and the ΔAIC was low, this model (ΔAIC: 1.118) performed better than the model including daily mean stream temperature (ΔAIC: 4.096). Unfortunately, due to limitations with our loggers, we were unable to capture temperature profiles at the height of summer. This is important, because summer thunderstorms have been credited with inducing broad temperature fluctuations in urban streams as stormwater runoff absorbs heat from impervious surfaces before entering surface waters (Nelson and Palmer 2007), meaning that we have likely underestimated the temperature variation that sampled salamanders were exposed to.

Our model incorporating percentage cover with impervious surfaces had a positive estimate though it was ultimately non-significant and ranked worse than our null model. However, we note that this result may have been influenced by a stream that flows out from a parking garage, but where innovative stormwater management infrastructure was installed during the construction of the building (Wegner, pers. comm.). Notably, this stream had the second most stable temperature regime of our sites, which we would not expect from a site with high impervious surface cover, suggesting that some urban impacts may be mitigated here. Additionally, two of our sites where we did not detect *D. cheaha* are downstream of athletic fields which, though not impervious, may not serve the same role as forest in reducing the impacts of stormwater runoff. These fields may have underlying stormwater infrastructure that drains into streams.

Anecdotally, we observed higher abundances of our focal species in areas that are surrounded by mature hardwood forest, though there were also sites with mature forest that had low abundances. Interestingly, at one field site where prior surveys had returned a number of *D. cheaha,* we only observed one individual. This suggests that some other within-stream variables, potentially at a mesohabitat scale, may influence where in the stream these salamanders occur. This selected site, though it had abundant cover items, had a relatively low declivity and had the highest temperature variability despite having one of the lowest amounts of impervious cover upstream.

We also observed that three of the sites where *D. cheaha* was absent were clustered relatively close together and were located on property that historically supported cattle grazing, though the vegetation in these areas was primarily mature hardwoods or mixed pine-hardwoods and may not have been grazed directly. These streams were located in areas known to be the subject of historical disturbances and have been observed to experience flashy discharges during storm events, though we did not measure discharge and observed similar conditions at other sites where *D. cheaha* was detected. An additional site without detections of *D. cheaha* drains a residential area, is culvertized at its confluence, and had the lowest density of cover objects.

Microhabitat Interpretation

At the microhabitat scale, we found that occupancy of *D. cheaha* was positively associated with increasing cover object size, which aligns with previous findings from predominantly terrestrial *Desmognathus* (Rossell et al. 2018). Previous work has also found cover object area and temperature to correlate with salamander body size, and that larger salamanders also experienced slower rehydration rates, suggesting that microhabitat selection may be due related to physiological requirements of the salamanders related to balancing evaporative pressures (Grover 2000).

Additionally, we found a significant negative association between the presence of *D. cheaha* and the presence of another occupant. Because *D. cheaha* is the largest member of the stream salamander assemblage that we observed (aside from *Pseudotriton ruber,* which was observed infrequently and most commonly as larvae), they likely exclude smaller species from utilizing the same cover items (Southerland 1986b). This finding is similar to those of prior studies that suggest refuge sharing is generally uncommon both between congeners in *Desmognathus* (Southerland 1986b) and between salamanders and crayfish (Cragg et al. 2021). The inclusion of the interaction parameter allows us to account for the fact that, while *D. cheaha* may not be occupying these larger rocks, there are other community members utilizing this habitat, and the habitat is therefore not "unsuitable" for salamanders or crayfish.

Though the position of cover objects within the study area (coded as stream/ margin/ terrestrial) was ultimately not significant in our models, we note that no individuals of *D. cheaha* were detected in terrestrial habitat, and that the performance of this variable may have been affected by sample. In models where this variable was included, *D. cheaha* trended towards being observed more often in stream habitat than marginal habitat. Though this trend was not significant, greater use of within-stream habitat would align with our hypotheses of *D. cheaha*'s habitat use given its status as the largest frequently encountered member of the stream salamander assemblage **(*Fig. 12*)**.

Desmognathus monticola often co-occurs with larger salamanders in the black-bellied salamander complex (i.e. *Desmognathus gvnigeusgwotli, D. kanawha,* and *D. mavrokoilius*), and prior research has demonstrated a tendency of this species to utilize more aquatic habitat in the absence of larger-bodied congeners (Southerland 1986a, Rissler et al. 2004). In our study area, where *D. cheaha* is the largest commonly encountered member of the stream salamander assemblage, we would expect that the species would utilize aquatic habitat more frequently than *D. monticola,* but may also monopolize streambank habitat given its size compared to *D. perlapsus.* A comparison of habitat use by both *D. cheaha* and *D. monticola* across the geographic range of these two species could offer interesting insights into evolutionary patterns and the role of community dynamics in driving shifts in species morphology.

Our findings suggest that the substrate composition underneath cover objects does not play a strong role in the within-stream distribution of this species. This finding corroborates the anecdotal accounts of Piedmont habitat use for this species by Pyron et al. (2023), who observed that the species appears to be widespread in the Piedmont streams where it occurs and suggested that it be considered a habitat generalist in this geographic region. In this respect, these Piedmont populations of *D. cheaha* appear to be more similar to *D. monticola,* which also demonstrates a wide range of substrate preferences (Southerland 1986b). However, our surveys did not include areas with exposed bedrock, and one follow-up survey in one of our sampled streams failed to find *D. cheaha* in the area where we had originally detected it, but did detect the species in a previously unsampled area that had exposed bedrock. A potential explanation is that our surveys took place during a time when we expect high levels of surface activity from *D. cheaha,* potentially meaning that during these times salamanders are able to utilize a wide variety of microhabitats, but during other parts of the season exhibit habitat specialization. One of the initial motivations for this study was to evaluate patterns in substrate use given the apparent selectiveness of Coastal Plain populations of this species (Pyron et al. 2023). Data from Folkerts (1968) suggests that percent cover with rock could be an important factor in predicting the proportion of Alabama stream salamander communities composed of *D. cheaha* versus smaller congeners.

Prior work suggests that salamanders at lower elevations may be more sensitive to the loss of forest canopy, perhaps because such populations occur near the margins of their physiological temperature tolerances (Tilghman et al. 2012). Salamanders occurring near the edge of their physiological tolerances may consequently rely more on specialized microhabitats (Bernardo and Spotila 2006), which may vary in availability across elevational gradients (Camp et al. 2017), potentially explaining these anecdotal differences in habitat use between Piedmont and Coastal Plain populations. Hoffacker et al. (2018) found that exposure to warmer temperatures negatively impacted larger, more aquatic *Desmognathus sp.* salamanders, but did not detect an effect on smaller congeners; the authors also suggested that spatial niche partitioning broke down under warmer temperatures, leading to increases in refuge sharing. Folkerts (1968) provides preliminary data that suggests shifts in *Desmognathus* community composition in relation to rock cover (which may be correlated with higher-gradient, cooler streams), inviting further inquiry.

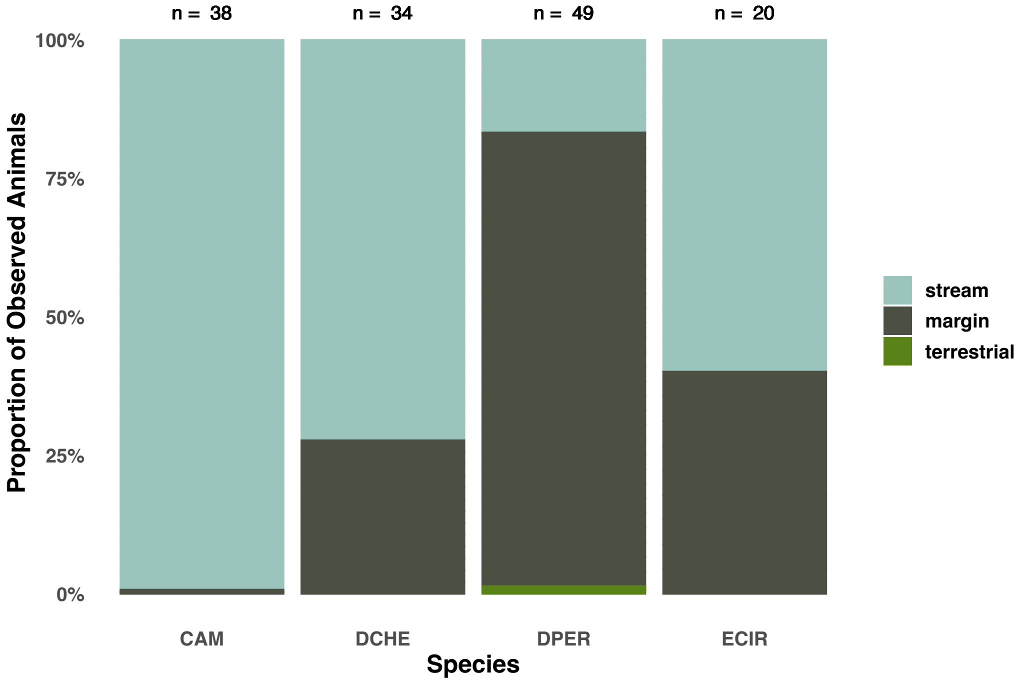


Figure 13: Figure showing the distributions of commonly-observed species in different habitat types. This data only includes animals from streams analyzed in microhabitat analyses (i.e. those where D. cheaha *was detected). Crayfish were pooled. CAM = crayfish, DCHE =* Desmognathus cheaha*, DPER =* Desmognathus perlapsus, *ECIR =* Eurycea cirrigera.

In addition to our analyses exploring the presence and absence of seal salamanders from different areas of the stream, we found evidence to suggest differences in habitat use between seal salamanders of different sizes. In particular, we found evidence that larger salamanders are more likely to occupy larger cover objects. Prior research on intraspecific interactions in southern black-bellied salamanders (*Desmognathus amphileucus*) identified differences in habitat use based on body size (Camp and Lee 1996). This study also experimentally evaluated aggression in the field and found not only that adults in all cases exhibited aggression towards juveniles, but also salamanders occupying cover objects reacted aggressively to "intruding" salamanders regardless of any differences in body size between the two (Camp and Lee 1996). Additionally, Dymit (2019) identified territoriality towards conspecifics in Chattooga dusky salamanders (*Desmognathus perlapsus*). We expect that similar mechanisms drive patterns in cover item selection in *D. cheaha,* where larger salamanders likely occupy the largest cover items, and successfully defend these cover items from smaller conspecifics.

Prior work with *D. amphileucus* suggested that larger-bodied salamanders were more likely to be found on the stream margin, while smaller salamanders were more likely to be found within the stream, though this may be attributed to the extensive sampling of streambank burrows in this study (Camp and Lee 1996). Additionally, prior work has noted that burrow usage was common in Pisgah black-bellied salamanders (*Desmognathus mavrokoilius*) and (*Desmognathus monticola*) (Southerland 1986c). While we were unable to identify a clear signal with respect to differential habitat use by differently sized salamanders, we only encountered two seal salamanders which sheltered within burrows, and did not notice abundant burrows along the streambank.

Community Interpretation

We found that Chattooga dusky salamanders (*Desmognathus perlapsus*) utilized stream habitat more frequently than terrestrial habitat in streams where they did not occur with *D. cheaha.* This finding aligns with previous research which has established that the wild distributions of smaller-bodied stream dwelling salamanders shift to become more aquatic in the absence of larger assemblage members (Rissler et al. 2004). While more research is required to understand how a combination of community interactions and urbanization influence habitat usage by salamanders, such shifts in habitat use in response to the loss of larger assemblage members may influence the predation intensity experienced by prey of *D. perlapsus*, as well as nutrient transport by *D. perlapsus* along the terrestrial-aquatic interface.

Future Directions

This study did not find clear signals suggesting microhabitat specialization in our sampled populations of *Desmognathus cheaha*, which aligns with previous accounts suggesting that the species utilizes a diversity of available habitats within occupied Piedmont streams (Pyron et al. 2023). However, information on the microhabitat use of this species in the Coastal Plain, where they are predicted to be reliant on heavily specialized habitat (Pyron et al. 2022), is scarce.

We recommend that future studies evaluate the microhabitat use of populations within the Coastal Plain, as well as compare the stream characteristics of occupied streams in the Coastal Plain and Piedmont, to better understand the constraints related to habitat use by this species in different geographic areas. Additionally, future work characterizing this species' physiological responses to heightened temperatures could provide insight into any observed differences in habitat use and community interactions between the Piedmont and Coastal Plain. Such data may be alsothat seek to forecast the response of this species to climate change, which may have important implications for its conservation (Milanovich et al. 2010, Luedtke et al. 2023). Comparing the ecology of this species across different eco-physiographic regions may inform differing management objectives across distinct parts of its range, including through the designation of a Distinct Population Segment, which has important implications for the protection of imperiled populations under federal law.

Crucially, our evaluation of reach-level occupancy does not account for differences in the abundance or population size between sampled streams, which are important metrics of the health of a population. While we have anecdotally noticed differences in the abundances of salamanders in these streams, our ability to account for this was constrained by our limited sampling area (a 10m stream reach). Additionally, due to temporal constraints which limited our ability to resample sites several times, we did not account for the imperfect detection of salamander species through our surveys, as in Cecala et al. (2018). Future work should examine the influence of environmental features on the occupancy and abundance of *D. cheaha* on streams over a wider urban gradient. Given information on other species of *Desmognathus* (Orser and Shure 1972, Surasinghe and Baldwin 2014, Cecala et al. 2018) and the differences in detection between *D. cheaha* and other salamanders (Rittenburg 2023), we expect that *D. cheaha* are likely more sensitive to urbanization than other salamanders. Future work should address the role of diverse urbanization-related variables in influencing stream salamanders, and should incorporate population estimates into such analyses to improve our understanding of how urbanization affects population viability.

Finally, future studies should evaluate shifts in the inter- and intraspecific interactions of salamanders along an urban gradient, as well as the consequences of such shifts on a population, assemblage, and ecosystem scale. Such research is needed to understand how urbanization influences salamander behavior, and whether there are changes that are governed by processes other than interspecific interactions. In addition, such research will help shed light onto the interactions stream salamanders play in broader forested and urban ecosystems.

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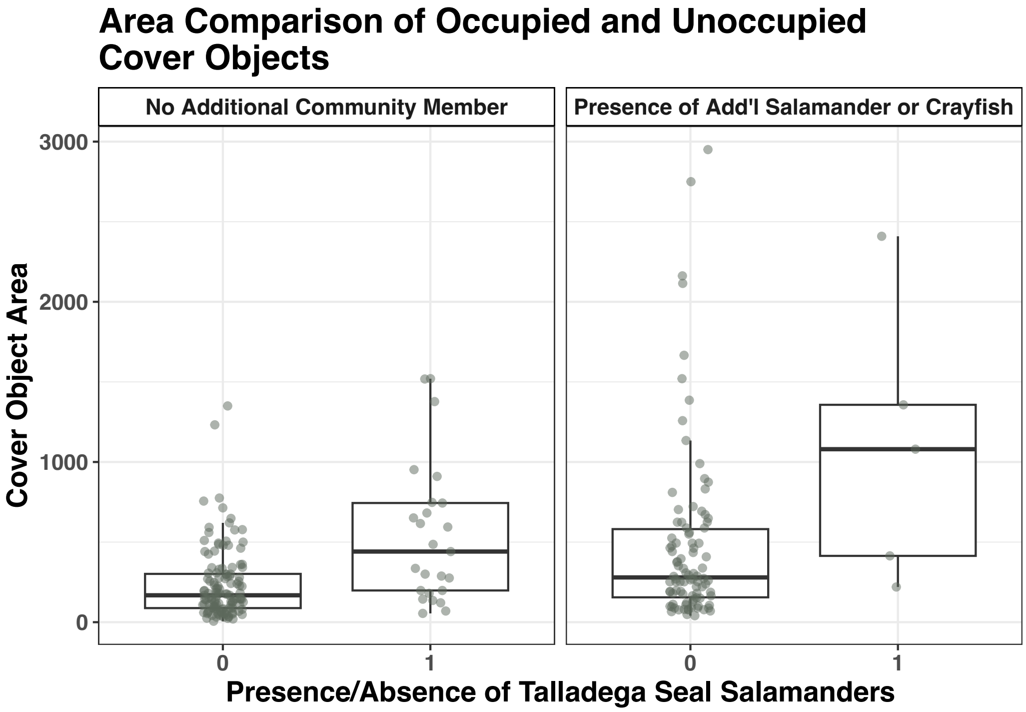
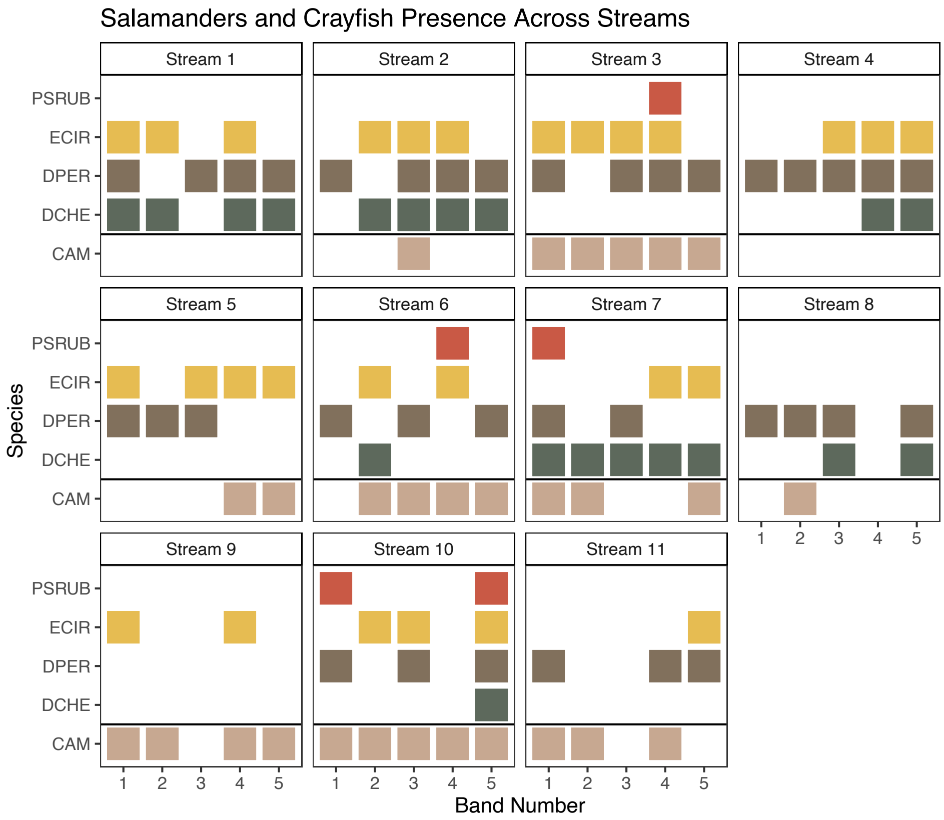
Appendix I: Supplemental Information

Supplementary Table 1: Characteristics for differentiating sympatric Desmognathus *species within the study area.*

Characteristic	***Desmognathus cheaha*** **(Pyron et al. 2023)**	***Desmognathus perlapsus*** **(Pyron & Beamer 2022)**
Toes	Has keratinized toe tips	Does not have keratinized toe tips
Tail	Heavily keeled tail	Round or weakly keeled tail
Body size	Larger bodied and more muscular	Smaller bodied and more slender
Head morphology	More pronounced jaw musculature and wider snouts; eyes tend to "bug out"	Less pronounced jaw musculature and narrower snouts
Dorsal pattern	Faded dorsal coloration resulting in scattered dark spots on a gray background	Variable dorsal pattern either well-defined (in younger individuals) or dark, but not gray with spots
Ventral pattern	"pearly translucent or cream-colored venter with dendritic or fractal brown pigmentation invading from the ventrolateral margin"	White spots / "flecking" on ventral surface



Supplemental Figure 1: Comparison of cover object area of objects occupied by Talladega seal salamanders. The left panel shows data from cover items which did not include other species of salamander or crayfish, while the panel on the right shows cover items which did have at least one salamander or crayfish.



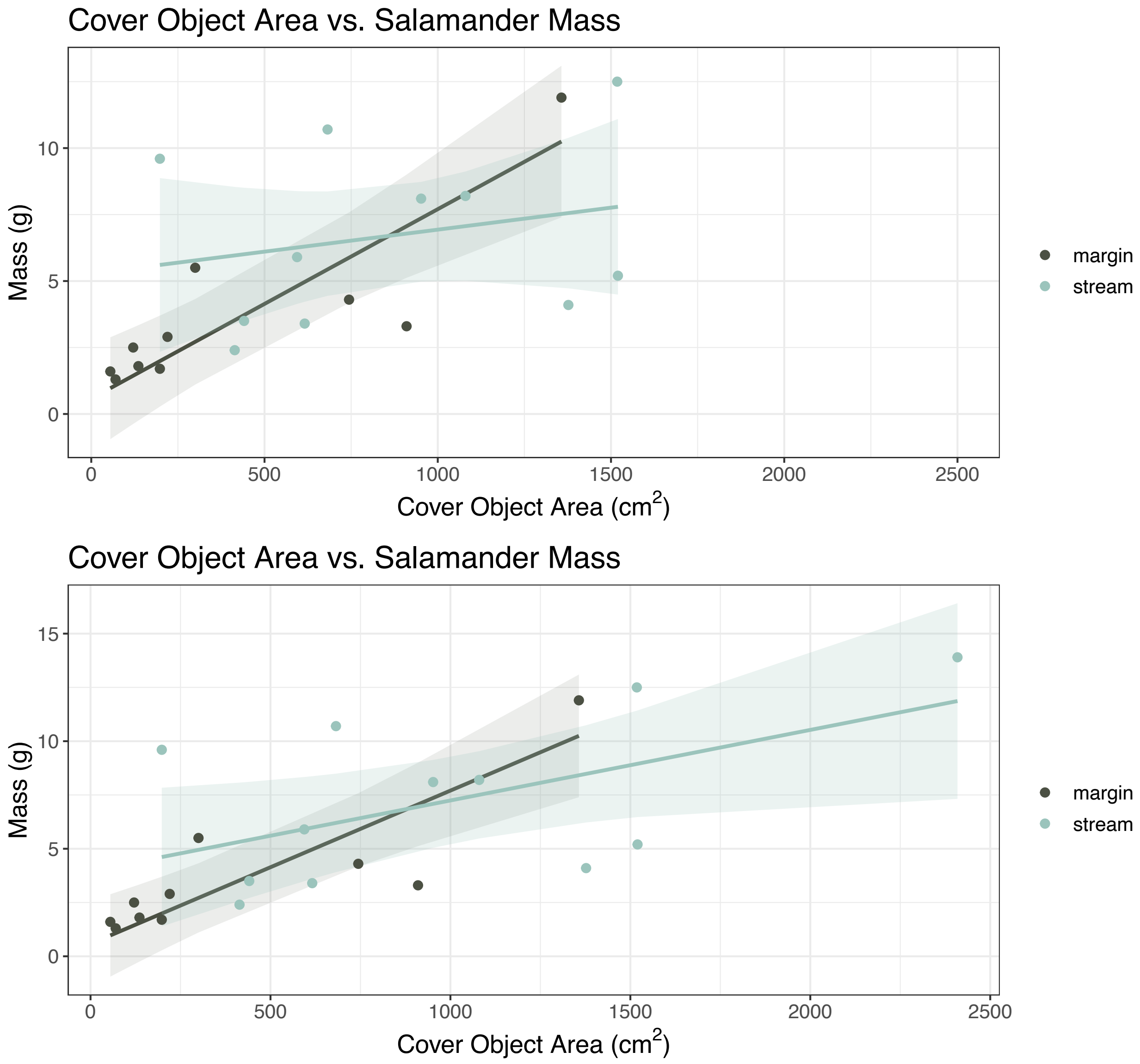
Supplemental Figure 2: Checkerboard Matrix of detections of salamanders and crayfish in bands of sampled streams. Each 10m stream reach was divided into five 2m long stretches (bands) to structure occurrence information. Colored squares indicate detections of a species within a band. Band numbers run from 1 at downstream to 5 at upstream. A single observation of Eurycea guttolineata *was excluded, as this species was only found at one site. Crayfish were pooled. PSRUB: red salamander, ECIR: southern two-lined salamander, DPER: Chattooga dusky salamander, DCHE: Talladega seal salamander, CAM: crayfish.*

Supplementary Table 2: Condition data from sampling events.

Stream #	**Date Sampled**	**Count of *D. cheaha***	**Air Temp. (°C)**	**Water Temp. (°C)**	**Pressure (mmHg)**	**Rel. Humidity (%)**
1	2023-03-17	7	14.4	13.5	735.2	97.6
2	2023-03-18	7	4.2	11.5	737.9	89.1
3	2023-03-19	0	1.4	8.2	744.5	45.2
4	2023-04-05	3	27.0	12.7	740.8	66.9
5	2023-04-07	0	18.9	NA	743.7	86.6
6	2023-04-09	1	12.3	13.5	744.5	78.8
7	2023-04-12	13	22.0	15.9	741.0	39.4
8	2023-04-16	3	18.6	16.0	737.3	94.9
9	2023-04-16	0	20.9	17.3	735.5	80.5
10	2023-04-21	1	21.2	16.4	739.1	63.1
11	2023-04-26	0	13.6	14.5	740.0	83.5

Supplementary Table 3: Regression models for logistic regressions modeling detection of seal salamanders and seasonal or weather-related factors. Each regression was a model run separately due to our sample size. We did not find a significant relationship between our detection of our focal species and any of these variables.

MODEL	**ODDS RATIO**	**CI**	**R^2 TJUR**	**P-VALUE**
Day of Year	0.98	-0.137 – 0.070	0.023	0.626
Air Temperature (°C)	1.06	-0.107 – 0.260	0.048	0.471
Water Temperature (°C)	1.14	-0.423 – 0.717	0.030	0.625
Atm. Pressure (mmHg)	0.86	-0.621 – 0.238	0.057	0.453
Relative Humidity (%)	1.01	-0.068 – 0.075	0.002	0.880



Supplemental Figure 3: Linear regression plot showing cover object area plotted against mass in grams of D. cheaha. *The top panel shows this potential interaction and data with a potential outlier removed; the bottom panel shows this outlier included. This figure shows a potential interaction effect between cover object area and habitat type, though we were not able to explicitly test for an interaction due to constrained sample size. Regression lines were estimated from separate regressions on stream and margin subsets of seal salamander morphometric data.*

Appendix II: Testimonials

“The coldest I have ever been.”

–Will Hutchinson

“First time putting salamanders in bags!”

–Iris Chen

“I enjoyed protecting salamanders from melanoma and eating bánh mì in soaked pants.”

–Bella Roeske

“Well spent time with the most beautiful slimy creatures!”

–Taryn Smith