The colour polymorphism and social behaviour of the Eastern Red-backed Salamander (*Plethodon cinereus*) in New Brunswick

BY

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Abstract

The Eastern Red-backed Salamander (*Plethodon cinereus*) is a terrestrial salamander well known for their striking variation in colouration. *Plethodon cinereus* has two common colour morphs: the striped and unstriped morph. However, a variety of other colour morphs also occur. These salamanders exhibit complex social behaviour and are often found co-habituating natural cover objects in pairs/groups, with a variety of factors influencing the way in which they aggregate on the forest floor. In this study, I aimed to increase our understanding on *P. cinereus* color polymorphism and social behaviour in New Brunswick by surveying 23 forests in New Brunswick and extracting provincial observations from iNatualist - a web-based community science platform - to collect data on these aspects of their natural history. I was able to document the Eastern Red-backed Salamanders color variation in New Brunswick from 542 observations, while also identifying two novel color morphs within New Brunswick (e.g., amelanistic and leucistic salamanders). I found that observations reported to iNaturalist are biased towards reporting uncommon/rare color morphs (e.g., unstriped and other), that salamanders in pairs/groups occupied cover objects with greater soil moisture which may be explained by mating groups/pairs requiring a moist environment to care for their eggs, and that mating pair/groups were more likely to be observed in the early spring and late fall. This work contributes valuable information regarding the natural history of *P. cinereus* in New Brunswick, yet more work is still needed to understand variation in their colour polymorphism and social behaviour in Canada.

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Acknowledgments

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1. Introduction

Colour polymorphism—multiple discrete colour phenotypes maintained within a population—is a type of phenotypic variation widely studied in ecology and evolution (Brock et al., 2022). Colour polymorphism occurs across a variety of taxa and ecological contexts (White and Kemp, 2016). Cases have been documented across most terrestrial and aquatic animals, and for many gymnosperm and angiosperm plants (White and Kemp, 2016). A classic example of a polymorphic species is the Peppered Moth (*Biston betalaria)*, which has a light- and dark-coloured morph and wherein directional selection during industrialization increased the occurrence of the traditionally rarer, dark-coloured morph (Bishop and Cook, 1975). Another example is the Land Snail (*Cepaea nemoralis*) that are polymorphic in their shell colour and banding, with numerous interacting factors contributing to the maintenance of their polymorphism (Cook, 1998). Functionally, polymorphism has been documented for colour traits involved in thermoregulation and aposematism in organisms like the Orb Web Spider (*Gasteracantha cancriformis*) (Ximenes and Gawryszewski, 2020) and Wood Tiger Moth (*Arctia plantaginis*) (Henze et al., 2018). Colour polymorphism has also been identified in various forms of deceptive anti-predator signaling including Batesian and Müllerian mimicry in two neotropical butterflies (*Heliconius erato* and *H. melpomene*) (Flanagan et al., 2004). Thus, various factors select for colour polymorphisms in a diversity of flora and fauna globally, yet there is still uncertainty regarding its function in many taxa.

In amphibians, colour polymorphism is generally expressed by differences in skin and eye colour, as well as dorsal patterning (Beukema et al., 2016). One group of amphibians in which colour polymorphism is notable are the terrestrial, woodland salamanders (*Plethodon* spp.) in which 14% (8/56 species) exhibit at least two colour morphs (often striped and unstriped). One of the most abundant and widespread species in this group is the Eastern Red-backed Salamander (*Plethodon cinereus*) that are well known for their striking variation in colouration. Its two main colour morphs were originally described as distinct species, but are now universally recognized as variants of the same species (Anthony et al., 2008). The most abundant, the striped morph, is characterized by a red dorsal stripe (Anthony et al., 2008), however chromatophores in the dorsal stripe are variable in colour. So, dorsal stripe shades of brown, pink, gray, white and yellow have also been observed in *P. cinereus* (Moore and Ouellet, 2014). In contrast, the unstriped morph, also referred to as the ‘lead’ or ‘lead-backed’ morph, lacks a dorsal stripe and is characterized by a uniformly pigmented black/gray dorsum (Anthony et al., 2008). There are six additional documented colour morphs of *P. cinereus*, including albino, amelanistic, erythristic, iridistic, leucistic, and melanistic. The erythristic morph (i.e., all red/orange and unstriped) is hypothesized to be a Batesian mimic of the toxic Eastern Newt (*Notopthalmus viridescens*) at the eft life stage (Moore and Ouellet, 2014). This is one of the numerous hypotheses about the function of *P. cinereus's* varied colouration, yet the function of their colour polymorphism still largely remains a mystery.

The proportion of *P. cinereus* colour morphs within a population varies greatly across the species’ range which extends throughout northeastern North America (Jaeger et al., 2016), with the striped morph being consistently more common than the unstriped morph (Table 1). There are various hypotheses as to why colour morph frequencies in this species may vary, for example dietary and behavioural differences (Otaibi et al., 2017; Anthony et al., 2008). Covariation between colour morph and other traits could also reflect among-morph variation in their suitability for different habitat types. A number of studies have hypothesized that the frequencies of *P. cinereus* colour morphs vary among populations due to contrasting environmental preferences of the morphs, and adaptation to different thermal niches (Petruzzi et al., 2006; Anthony et al., 2008). The unstriped morph has been associated with warmer, drier conditions compared to the striped morph (Lotter and Scott, 1977; Gibbs and Karraker, 2006). However, the degree to which the striped and unstriped morph vary in terms of environmental preferences is not always consistent. For example, Lotter and Scott (1977) found striped morphs less frequently in warm climates (i.e., a higher average daily temperature); Test (1955) found that the observation rate of the unstriped morph was related to increasing temperatures through the summer; and Petruzzi et al (2006) found that unstriped morphs of *P. cinereus* are found in warmer thermal microhabitats (i.e., varying temperature within a cover object) relative to striped morphs. In addition, Gibbs and Karraker (2006) suggests the probability of finding striped morphs increases with altitude, latitude, and longitude, which are all factors related to temperature. Understanding how color morphs of *P. cinereus* vary across their range is important for understanding their sociality and, in particular, how they form mating pairs and groups.

Table 1. The percentage of Eastern Red-backed Salamander (*Plethodon cinereus*) colour morphs reported across their geographic range. Data was acquired from a non-systematic search of literature. Color morphs include, striped, unstriped and other, and *n* indicates the sample size of salamanders surveyed.

		% of *P. cinereus* colour morphs				
Location	**Latitude, Longitude**	**Striped**	**Unstriped**	**Other**	***n***	**Reference**
Odell Park, Fredericton, New Brunswick, Canada	45.572260°N, 66.395448°W	83	7	10	103	5
Point Pleasant Park, Nova Scotia, Canada	44.626067°N, -63.573166°W	61	39	0	48	8
Point Pleasant Park, Nova Scotia, Canada	44.626067°N, -63.573166°W	60	40	0	20	8
Mount Mégantic, Montreal, Quebec, Canada	45.450000°N, -71.150000°W	99	1	0	101	7
Mount Royal, Montreal, Quebec, Canada	45.500000°N, -73.583333°W	97	3	0	120	7
Central Ontario, Canada	----	78	22	0	2144	6
Ringwood, New York, USA	----	100	0	0	161	4
Cuyahoga valley National Park, Ohio, USA	41.228931°N, 81.518511°W	78	22	0	224	1
Cuyahoga Valley, National Park, Ohio, USA	41.229617°N, 81.518825°W	77	23	0	188	2
Central Pennsylvania, USA	----	68	32	0	51	3

[1] Acord et al., 2013; [2] Anthony et al., 2008; [3] Brown, 1965; [4] Davis and Milanovich, 2020; [5] Jongsma, 2012; [6] Morneault et al., 2004 ; [7] Noël et al., 2007; [8] Russell et al., 2011

Research that relates to complex social behaviour is often focused on birds and mammals, while amphibian social systems are often viewed as being relatively simple (Liu et al., 2016). Yet, this is a misconception for many species (Furness and Capellini, 2019). For example, Eastern Red-backed Salamanders have evolved complex social systems that involve many intraspecific and interspecific behaviours (Jaeger et al., 2016). For example, they have complex communication systems (e.g., visual and chemical signals are used to convey information about aggression and submission) (Jaeger et al., 1982), they use sexual coercion to prevent mating partners from being socially polyandrous (Jaeger et al., 2016), and they also maintain territories via threat displays and biting (Jaeger et al., 1982). Their social behaviour is strongly influenced by pair formation during the breeding season in early spring and autumn. *Plethodon cinereus* is often found co-habiting cover objects (rocks and logs) in male-female pairs wherein they exhibit preferential behaviour toward their partner (Gillette et al., 2000). Thus, the mating system of *P. cinereus* is described as social monogamy (e.g., a long term or sequential living arrangement between an adult male and an adult female) (Jaeger et al., 2016). However, it has also been suggested that both members of a partnership may engage in extra-pair associations and that *P. cinereus* can be found defending territories in groups of three or more (Jaeger et al., 2016). The Eastern Red-backed Salamander's complex social behaviour not only has the ability to influence their occurrence across the landscape, but also the way in which they choose mates.

Mate selection in *P. cinereus* is influenced by a variety of factors, for example familiarity (Guffey et al., 1998) and morphology (Chouinard, 2012). Mate selection in *P. cinereus* is also influenced by colouration - adults are often found in pair associations (i.e., mating pairs or groups) in a non-random way. There is considerable evidence that they choose mates who are more similar to themselves in phenotype (i.e., colouration), which is also known as assortative mating (Acord et al., 2013). Across taxa, many phenotypic traits can drive assortative mating behaviour (Accord et al., 2013), and the Eastern Red-backed Salamander is specifically known to mate assortatively by their colour polymorphism. In a study conducted at the Cuyahoga Valley National Park near Peninsula, Ohio, USA, 71% of *P. cinereus* mating pairs consisted of the same colour morph (Accord et al., 2013). This is similar to the findings of Anthony et al (2008), which also surveyed Cuyahoga Valley National Park during *P. cinereus'* breeding season and found that 72 % of mating pairs matched in colour. The way in which *P. cinereus* color morphs mate assortatively, in addition to their preference for different habitats, makes this species an excellent model to ask question about reproductive isolation.

For my Honours research, I aimed to examine the effect of environment on the assortative mating behaviour of *P. cinereus*. As the unstriped morph is more common in drier, warmer microhabitats, whereas the striped morph is more likely to occur in wetter, colder microhabitats (Petruzzi et al.,2006), the number and degree of interactions between morphs has the potential to greatly vary across this species' range. In areas of great environmental variation, there may be fewer interactions between individuals of opposing morphs thereby increasing the incidence of assortative mating and restricting gene flow through reproductive isolation (Acord et al., 2013). I predicted that the occurrence of unstriped mating pairs would be low in wet/cool habitats compared to striped mating pairs, and that non-assortative mating pairs will be most common at intermediate moisture and temperature levels. However, due to the low proportion of unstriped *P. cinereus* I found in the field in New Brunswick, I re-directed my research objectives to be as followed:

(1) **Document colour polymorphism of Eastern Red-backed Salamanders in New Brunswick and investigate whether microclimate factors differ between the habitats selected by each morph.** I hypothesize populations of *P. cinereus* in New Brunswick will include the main colour morphs of this species, and that striped and unstriped morphs will be associated with different microclimates (i.e., the environment under their cover object) and macroclimates (i.e., seasonal temperature and precipitation trends). I predict that striped morphs will be more common than the unstriped morph, as typically seen across their range (Table 1), and that striped salamanders will be found in areas associated with a moister and cooler microclimate and macroclimate relative to unstriped morphs.

(2) **Curate a community science dataset of Eastern Red-backed Salamander colour polymorphisms in New Brunswick from iNaturalist to broaden the geographic scope of my study and compare this dataset to my field observations.** Much information regarding species' colour polymorphism exists in web-based community science platforms, like iNaturalist, in which participants can upload photographs of organisms to be identified by other participants (http://www.inaturalist.org/). As of 2022, this platform holds more than 9,000,000 species observations, including almost 10,000 research grade observations of the Eastern Red-backed Salamander. I will test the reliability of community science data by statistically comparing estimates of colour morph percentage in New Brunswick on iNaturalist to my own observations from the field. I predict there will not be a difference between community science and field observations of striped salamanders, but that the less common colour morphs (e.g., unstriped, albino, amelanistic, erythristic, iridistic and leucistic) will be over-represented in the community science data. This is because participants may be more likely to upload

unique colour morph observations of *P. cinereus* than the common colour morphs (striped) due to their rarity.

(3) **Document the social behaviour of *P. cinereus* in New Brunswick and how it changes over time. I hypothesize that the grouping behaviour of *P.cinereus* will vary with their mating season.** I predict that salamanders will be found in mating pairs and mating groups during the breeding season (early spring and autumn), whereas salamanders will spread out over a wider spatial distance over the summer months.

(4) **Study habitat selection of *P. cinereus* found solitary and in pairs/groups. I hypothesize that habitat quality will affect the grouping behaviour of *P. cinereus***. First, I predict that *P. cinereus* in groups will occupy higher quality habitats, which will be determined by larger cover object dimensions, a higher moisture level, a greater canopy coverage, and a cooler temperature. These predictions are similar to the findings of Quinn and Graves (1999) who conducted a field study on social aggregation in *P. cinereus* in Michigan and found that there was a significant difference between habitat quality of lone individuals and groups of *P. cinereus* under cover boards.

I achieved these study aims by surveying for the Eastern Red-backed Salamanders in New Brunswick across their active season (May to October 2022) and by extracting community-science data from iNaturalist. The social behaviour and colour polymorphism of *P. cinereus* has been extensively studied in the United States (Anthony et al., 2008; Highton, 1959), however little information exists regarding Canadian populations. More specifically, little information concerning *P. cinereus* behaviour and colour polymorphism exists for New Brunswick populations, thus this research will provide knowledge regarding local Eastern Red-backed Salamander natural history.

2. Methodology

2.1 Study species

Plethodon cinereus is a lungless plethodontid salamander commonly found across eastern North America, specifically from southeastern Canada southward to northern North Carolina and from the Atlantic Coast northwestward to the northern Mississippi River (Jaeger et al., 2016). In Canada, they occur from the Great Lakes region in Ontario, east through Quebec and into the Maritime Provinces (Powell et al., 2016). This species is fully terrestrial and can be found on the forest floor where they forage for invertebrates within the leaf litter and often take refuge under cover objects like rotting logs and rocks (Jaeger et al., 2016). Male and female *P. cinereus* defend areas together under these cover objects to maintain exclusive access to feeding areas and courtship sites (Acord et al., 2013). Breeding for this species occurs in the fall or spring (Jaeger et al., 2016).

2.2 Study Sites

I surveyed for the Eastern Red-backed Salamander in 23 New Brunswick forests from May to August 2022 and then resampled 7 of these sites from September to October 2022 to look at how pair/group formation changes seasonally (Table 2). Study sites consisted of naturally protected areas, public walking trails and parks, and private property. Sites were mostly mixed forests that contained both deciduous and coniferous trees, yet some were predominately deciduous forests. Elevations of sites ranged from -56 m to 190 m and soil type was either mineral or peat mix (http://www.snb.ca).

2.3 Field Methods

At each site, I moved through the forests haphazardly looking for areas with many shelter objects for salamanders. Once potential habitat was located, I used a circular transect method with a radius of 25 m (Richter et al., 2013) and walked from the center point of the circle along a transect while flipping cover objects until I covered the entire area of the circle (1962 m^2). Across all surveys, I flipped 3,345 cover objects with an average ± standard deviation of 111 ± 75 cover objects per survey. Only 195 of the 3,345 cover objects had *P. cinereus* underneath. These habitats (*n* = 195) ranged from 3 cm x 3.5 cm x 3.5 cm (length x width x height) to 32 cm x 223 cm x 770 cm, and were on average 172 cm x 17 cm x 12 cm. This is a surface area of 2,924 cm^2 or 0.29 m^2. The cover objects *P. cinereus* used consisted of 18% rocks and 82% logs. If a salamander was found under a cover object, it was captured. I then determined whether the individual was an adult based on the snout-to-vent length (i.e., > 32 mm) (Quinn and Graves, 1999). For adult salamanders, I measured and recorded snout-to-vent length (SVL; mm) (i.e., the distance from the snout to the end of the cloacal opening), total length (mm) (i.e., the distance from the snout to the end of the tail), mass (g) using a digital pocket scale, and then determined the sex by candling (i.e., examination of the venter for the presence of testes in males and eggs in gravid females as described in Gillette and Peterson 2001). Salamanders were handled with nitrile gloves and measures to prevent disease spread were implemented in our data collection protocols (Canadian Herpetofauna Health Working Group, 2017).

Table 2. Study sites where surveys were completed in New Brunswick. I also detail the type of forest (deciduous, coniferous, or mixed), number of surveys completed at each site, and general location of the site. If multiple surveys were completed at a site (including re-sampling), surveys began at different center points and were at least 50 m away from each other.

Study Site	Forest Type	No. of Surveys	Latitude (°N)	Longitude (°W)
Atlantic Wildlife Institute, Cookville	Mixed	2	46.019056	-64.312141
Beach Hill Park, Sackville	Mixed	2	45.947305	-64.401771
Claude D. Taylor School, Riverview	Mixed	2	46.050628	-64.818338
Irishtown Nature Park, Riverview	Mixed	3	46.142254	-64.772009
Lower Walker Rd trail system	Mixed	4	45.931778	-64.406939
Mapleton Park, Moncton	Mixed	1	46.125254	-64.834051
Millcreek Nature Park, Riverview	Mixed	2	46.054798	-64.766586
NCC Reserve # 22, Cookville	Deciduous	2	46.110723	-64.572065
Odell Park, Fredericton	Deciduous	1	45.949589	-66.678509
Private Property 1, Sackville	Deciduous	1	45.962104	-64.408868
Private Property 2, Sackville	Deciduous	1	45.962132	-64.408856
Private Property 3, Sackville	Mixed	2	45.975515	-64.470876
Private Property, Shediac	Mixed	1	46.112490	-64.583635
Private Property 1, Summerville	Mixed	2	45.352821	-66.089468
Private Property 2, Summerville	Mixed	2	45.353310	-66.088226
Private Property, Lakeside	Mixed	2	45.502690	-65.790055

If more than one salamander was under a cover object, I noted their location relative to one another before capture. Specifically, I categorised mating pairs and groups of *P. cinereus* using a maximum intrapair distance of 30 cm, which has been commonly used in previous studies (Acord et al., 2013). Two sexually mature salamanders within 30 cm from one another were defined as a mating pair, whereas three or more sexually mature salamanders all within 30 cm from one another were defined as a mating group. I also noted the total number of individuals (adults and juveniles) located under cover objects together, as a measure of aggregation tendency of salamanders at each site.

Lastly, for each cover object that adult salamanders were found under, I measured environmental factors associated with these sites. Once the cover object was flipped and the salamander(s) were removed, I measured the length, width, and height dimensions of the log or rock using a measuring tape. I also measured soil moisture (%) using a SM150 Soil Moisture Sensor (Delta-T Devices) and soil temperature (^{o}C) using an infrared thermometer. Cover objects were placed back in their original location and canopy cover was measured 30 cm directly above using a spherical convex densiometer (43887 Model A, Universal Field Supplies). After data was collected on the salamanders and environmental factors were measured, the salamanders were released back under cover and their shelter object was flagged to prevent resampling.

I conducted this research under authorization from The Department of Natural Resources and Energy Development, Government of New Brunswick (Scientific Permit No. SP22--002) and my protocols were approved by the Mount Allison University Animal Care Committee (ACC # 103181).

2.4 Data Extraction from iNaturalist

I downloaded iNaturalist data on occurrences of *P. cinereus* in New Brunswick on 23 September 2022. This data included 315 ‘research-grade’ observations of Eastern Red-Backed Salamanders from 2008 to 2022. To be considered research grade, the observation must be a 2/3 consensus on the identification of the species, as well as include a photograph and location for sighting. Each observation in my dataset included a digital photograph and the time, date, and location of the occurrence all submitted by a community member (http://www.inaturalist.org/). I then reviewed this data and checked data regarding location of the observation, contributor identity, the date and time the contributor uploaded the observation, and then identified the colour polymorphism of the salamander in the uploaded photo. Low-quality photographs in which colour polymorphism could not be confidently determined (e.g., they were heavy shaded or zoomed-in) were not included (n = 16) in the final dataset.

2.5 Data Extraction from Environment Canada and Natural Resources

I downloaded climate data from the Government of Canada's Environment and Natural Resources website (weather.gnc.ca) on average daily temperature and precipitation across the duration of my study. I determined the nearest weather station to each study site by measuring the distance between them using Google Earth Pro (version 7.3.6) and then I download data from the appropriate station. This included data from 21 weather stations in New Brunswick and 1 weather station from Nova Scotia. After I downloaded the data, I then calculated the average and standard deviation of the daily temperature (^{o}C) and daily precipitation (mm) from this past year (2022) across the *P. cinereus* active season (from May to October).

2.6 Statistical Analyses

All analyses, which consisted of a χ^2-test and a variety of mixed effect models (see details below), were performed in R version 4.22 (R Core Team, 2023). Before the analyses, I explored the data to ensure they met all model assumptions, like no unexplainable outliers or collinearity between predictor variables (Zuur et al., 2010). For all statistical tests, α was set at 0.05. After tests were run, I ensured they fulfilled their assumptions (e.g., normality of residuals for fixed and random effects, homogeneity of variance, overdispersion) by using the *'check_model'* function from the R package *'performance'* (Lüdecke et al., 2021).

2.6.1 *Plethodon cinereus* Colour Polymorphism in New Brunswick

I summarized the percentage of striped, unstriped, and other (i.e., any morphs other than striped or unstriped) *P. cinereus* colour morphs separately for those that I observed in the field and that were documented on iNaturalist. I then tested whether my field surveys and the community-science data resulted in different percentage estimates of *P. cinereus* colour morphs in New Brunswick using a χ^2-test (Turhan, 2020). I performed this test using the R function *'chisq.test'* from the *'stats'* R package (R Core Team, 2023) and results from the χ^2-test were visualised for interpretation using the R function *'ggbarstats'* from the R package *'ggstatsplot'* (Patil, 2021).

2.6.2 Testing for Color Morph Environmental Preferences

I examined whether macroclimate conditions differed between striped and unstriped morphs of *P. cinereus* using my iNaturalist dataset. I linked the location of the reported iNaturalist observation to the nearest New Brunswick weather station, and distance between them ranged from <1 km and 29 km. The final dataset included 299 observations of salamanders and weather data from 22 stations. I ran two linear mixed effect models (LMM) using the function *'lmer'* from the '*lmerTest'* R package (Kuznetsova et al., 2017). Each LMM separately examined two response variables, average daily temperature (^{o}C) and daily precipitation (mm), and included the fixed effect of colour morph (categorical variable: striped or unstriped) and the random effect of weather station.

In addition, using my field dataset, I examined whether microclimate conditions differed between striped and unstriped morphs of *P. cinereus*. This included adult males and females (*n* = 143) captured in the field across 20 different sites. To accomplish this, I ran two generalized linear mixed effect models (GLMM) using the function *'glmer'* from the *'lmerTest'* R package (Kuznetsova et al., 2017). Each GLMM separately examined two response variables, soil moisture (%) and canopy cover (%), and included the fixed effects of colour morph (categorical variable: striped or unstriped), sex (categorical variable: female or male), SVL (mm; continuous variable) and the random effect of study site. I also initially included an interaction between colour morph and sex in both models, but it was removed if not significant to allow for interpretation of main effects. If interactions were significant, I created contrasts between the relevant fixed effects and corrected comparisons with a post-hoc Tukey HSD multiplicity adjustment using the *'emmeans'* R function from the R package *'emmeans'* (Lenth, 2022). Soil percent and canopy cover are percentages (between 0 and 100), so I converted these data to proportions and used a binomial distribution for the models (Crawley, 2012). I also ran a LMM using the function *'lmer'* from the '*lmerTest'* R package (Kuznetsova et al., 2017) to test whether soil temperature (^{o}C) differed between salamander color morph, sex, and SVL. In this model I also included study site as a random effect. I used the same protocol for inclusion of an interaction between color morph and sex, as well as for post-hoc multiple comparisons as above.

2.6.3 *Plethodon cinereus* Grouping Behaviour and Habitat Selection

To examine the seasonality of aggregations in *P. cinereus*, I summarized the number of salamanders that were found solitary, in mating pairs (2 adult salamanders within 30 cm of one another), and groups (3+ adult salamanders within 30 cm of one another) for each month from May to October. Only 6 salamanders were found in groups, so mating pairs and groups were pooled together in further analyses.

I examined habitat selection of grouped and solitary *P. cinereus* in New Brunswick. To accomplish this, I ran two GLMMs using the function *'glmer'* from the *'lmerTest'* R package (Kuznetsova et al., 2017) separately to analyse soil moisture (%) and canopy cover (%), which were converted to proportions and modelled using a binomial distribution (Crawley, 2012). Each GLMM contained the fixed effects of aggregation status (categorical variable: grouped and solitary), sex (categorial variable: female and male), and SVL (mm; continuous variable), as well as the random effect of study site. I also ran two LMMs using the function *'lmer'* from the *'lmerTest'* R package (Kuznetsova et al., 2017) separately to analyse soil temperature (^{o}C) and cover object volume (cm^{3}). These LMMs contained the same fixed and random effects as the GLMMs.

3. Results

3.1 *Plethodon cinereus* Colour Polymorphism in New Brunswick

During my 6-month field study, I found 243 Eastern Red-backed Salamanders (164 adults (>32mm), 73 juveniles, and 7 neonates). A total of 233 salamanders were classified as the striped or red-backed morph, 8 were classified as the unstriped or lead-backed morph, and 2 were classified as the erythristic morph (i.e., categorised as "other" in statistical analyses). The iNaturalist dataset contained 315 reports of the Eastern Red-backed Salamander in New

Brunswick, which I reviewed and found there were 263 observations of the striped morph, 21 observations of the unstriped morph, and 15 observations of other morphs (i.e., categorised as "other" in statistical analyses). The other morphs that were reported included 12 erythristic salamanders, 1 albino salamander, 1 amelanistic salamander, and 1 leucistic salamander. The remaining 16 observations were unidentifiable due to issues with the photographs (i.e., too unfocused), so they were removed from the dataset.

Figure 1. Colour variation in the Eastern Red-backed Salamander (*Plethodon cinereus*) in New Brunswick. All photos were reported on iNaturalist (http://www.inaturalist.org/) and assessed in this study. The top left individual is a leucistic morph (photo credit: Bradley Doiron), the top middle (photo credit: Alexis Godin) and top right individuals (photo credit: Sébastien Benoit) are considered to be red-backed or striped morphs, the bottom left individual is an amelanistic morph (photo credit: Damien Mullin), the bottom middle individual is a lead-backed or unstriped morph (photo credit: David Robichaud), and the bottom-right salamanders is an erythristic morph (photo credit: Dani Landry).

In a comparison of the colour morphs observed during field surveys *versus* reported on iNaturalist in New Brunswick, I found a significant relationship between the method of observation (e.g., field observations versus a community-science approach) and colour morph frequencies ($\chi^2_2 = 11.92$, $p < 0.01$) (Figure 2). Both methods were similar in how likely they would observe/report striped morphs ($p = 0.18$), but community-scientists were more likely to report the less common colour morphs (unstriped: $p = 0.02$, and other: $p < 0.01$).

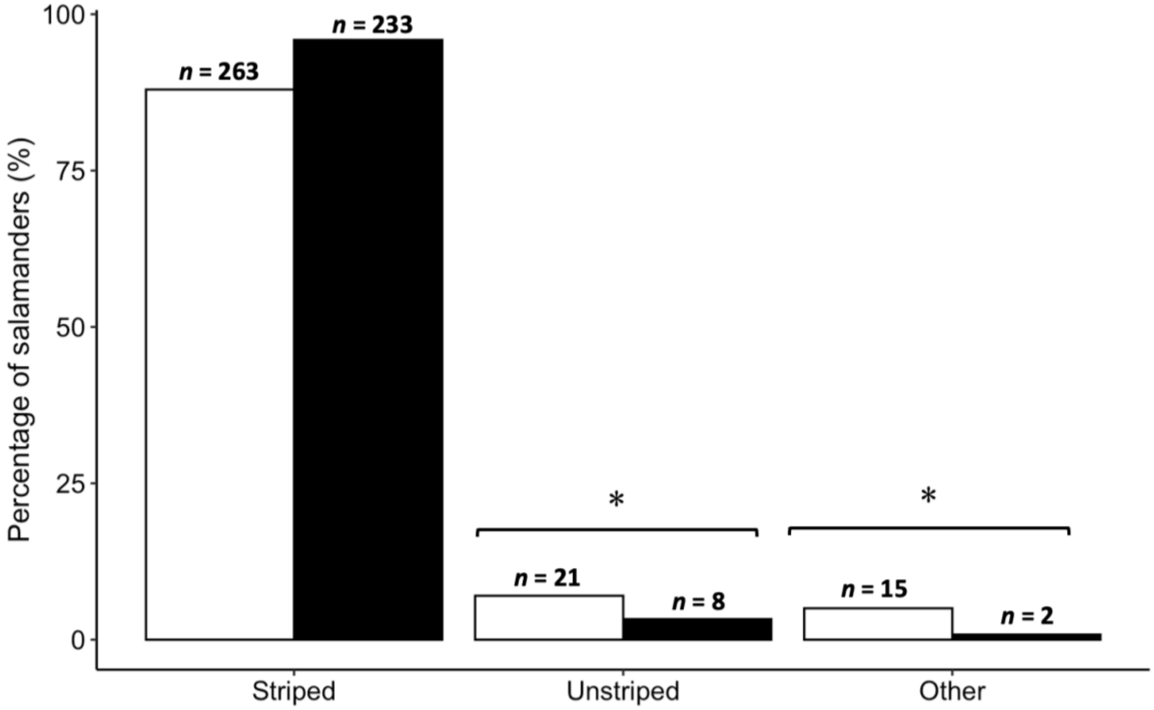

Figure 2. The percentage of Eastern Red-backed Salamanders (*Plethodon cinereus*) colour morphs observed during field surveys (black bars) and reported on iNaturalist (white bars). Data is presented by colour morph, which includes the striped or red-backed morph, the unstriped or lead backed morph, and any other color morphs observed (including erythristic, albino, amelanistic, or leucistic). In the graph, *n* denotes sample size.

3.2 Testing for Color Morph Environmental Preferences

All summaries of data in this section are presented with average +/- standard deviation unless specified. Neither of the macroclimate variables, average daily temperature (^{o}C) or daily precipitation (mm), significantly differed between *P. cinereus* colour morphs (Table 3; Figure 3). I also tested for differences between microclimate variables selected by *P. cinereus* colour morphs using my field data. I found a significant interaction between colour morph and sex for soil moisture (Table 4) and a significant difference between unstriped males and unstriped females (*p* = 0.04); specifically, unstriped males occurred under cover object with, on average, 10% moister soil than unstriped females (*z* = -3.160, *p* = 0.010). All other contrasts between sex and colour morphs were not significant (*p* > 0.050). On average, striped females and males were found under cover objects with 20 ± 2 % soil moisture. Whereas unstriped females and males were found under cover objects with 16 ± 3 % and 26 ± 4 % soil moisture, respectively (Figure 4A). In addition, there was a significant positive relationship between soil moisture and salamander SVL; larger salamanders occurred under cover objects with a higher soil moisture (Table 4). For the remaining microclimate variables, soil temperature (°C) and canopy cover (%), there was no significant effects of colour morph, sex, or SVL (Table 4; Figure 4). On average, striped morphs occurred under cover objects with a soil temperature of 16.2 ± 0.8 °C and canopy cover of 69 ± 0 % (Figure 4) and unstriped morphs occurred under cover objects with a soil temperature of 16.5 ± 1.2 °C and canopy cover of 70 ± 0 % (Figure 4).

Table 3. Outcomes of the linear mixed-effects models testing if macroclimate (i.e., annual daily precipitation and annual daily temperature) differed between iNaturalist observations of striped and unstriped Eastern Red-backed Salamanders (*Plethodon cinereus)*. Reference levels for each variable are shown in parentheses following variable names. For each model, I present coefficient estimates (*β*) and their corresponding standard error (*SE*), t-values (t) and p-values (*p*) for fixed effects, as well as variance (σ^2) and *SE* of random effects and residuals. N_{obs} refers to the number of observations and $N_{weather}$ station refers to the number of weather stations. Significant values are bolded.

	Average daily temperature (°C)				**Average daily precipitation (mm)**			
	N_{obs} = 283, $N_{weatherstation}$ = 22				**N_{obs} = 283, $N_{weatherstation}$ = 22**			
Fixed effects	***β***	***SE***	***t***	***p***	***β***	***SE***	***t***	***p***
Intercept (unstriped)	**15.502**	**0.128**	**121.00**	**<0.001**	**3.014**	**0.087**	**34.56**	**<0.001**
Color morph (striped)	0.000	0.014	0.00	0.997	-0.000	0.007	-0.010	0.995
Random effects	**σ^2**	***SE***			**σ^2**	***SE***		
Weather station	0.360	0.600			0.167	0.409		
Residuals	0.004	0.061			0.001	0.030		

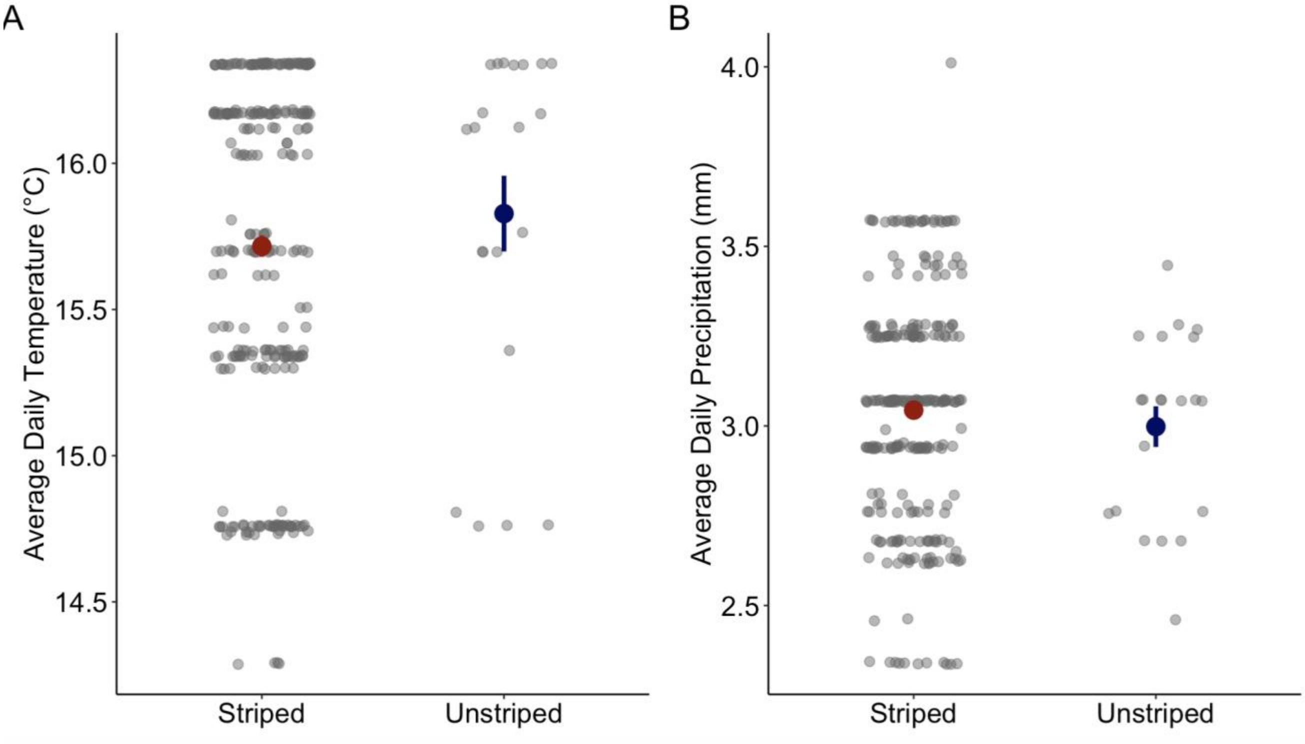

Figure 3. Average daily temperature **(A)** and average daily precipitation **(B)** of Eastern Red-backed Salamander (*Plethodon cinereus*) color morphs (striped and unstriped) across their active season (May to October) as reported on iNaturalist in New Brunswick (*n* = 299 observations). The circle and line ("red" and "purple") represent the mean and standard error of each color morph and the black/grey circles represent the raw data points. Neither macroclimate variable significantly differed between colour morphs.

Table 4. Outcomes of the generalized linear mixed-effects models (GLMM) and the linear mixed effect model (LMM) testing if microclimate (i.e., soil moisture, canopy cover, and soil temperature) differ between field locations where striped and unstriped Eastern Red-backed Salamander (*Plethodon cinereus*) occur. Model outputs are shown on the latent scale for GLMMs. Reference levels for each variable are shown in parentheses following variable names. An interaction between colour morph and sex was initially included in all models, but if not significant it was removed (denoted by --- below). For each model, I present coefficient estimates (β) and their corresponding standard error (*SE*), *z*-values for GLMM (*z*) or *t*-values for LMM (*t*), and *p*-values (*p*) for fixed effects, as well as variance (σ^2) and *SE* of random effects and residuals. N_{obs} refers to the number of observations where N_{site} refers to the number of sites surveyed. Significant values are bolded.

	Soil moisture (%)				**Canopy cover (%)**				**Soil temperature (°C)**			
	N_{obs} = 143, N_{site} = 20				**N_{obs} = 143, N_{site} = 20**				**N_{obs} = 143, N_{site} = 20**			
Fixed effects	***β***	***SE***	***z***	***p***	***β***	***SE***	***z***	***p***	***β***	***SE***	***t***	***p***
Intercept (unstriped, female)	**-2.498**	**0.240**	**-10.41**	**< 0.001**	**-0.635**	**0.195**	**-3.257**	**0.001**	**16.113**	**2.151**	**7.490**	**<0.001**
Color morph (striped)	-0.233	0.147	-1.579	0.114	0.037	0.075	0.497	0.619	-0.294	0.853	-0.344	0.731
Sex (males)	-0.009	0.041	-0.225	0.822	0.356	0.300	1.186	0.235	0.211	0.328	0.643	0.521
SVL	**0.021**	**0.005**	**3.941**	**<0.001**	0.006	0.005	1.210	0.226	0.008	0.044	0.175	0.862
Color morph : Sex	**0.505**	**0.195**	**2.598**	**0.009**	---	---	---	---	---	---	---	---
Random effects	**σ^2**	***SE***			**σ^2**	***SE***			**σ^2**	***SE***		
Study site	0.178	0.422			0.046	0.215			11.490	3.390		
Residuals	---	---			---	---			3.570	1.890		

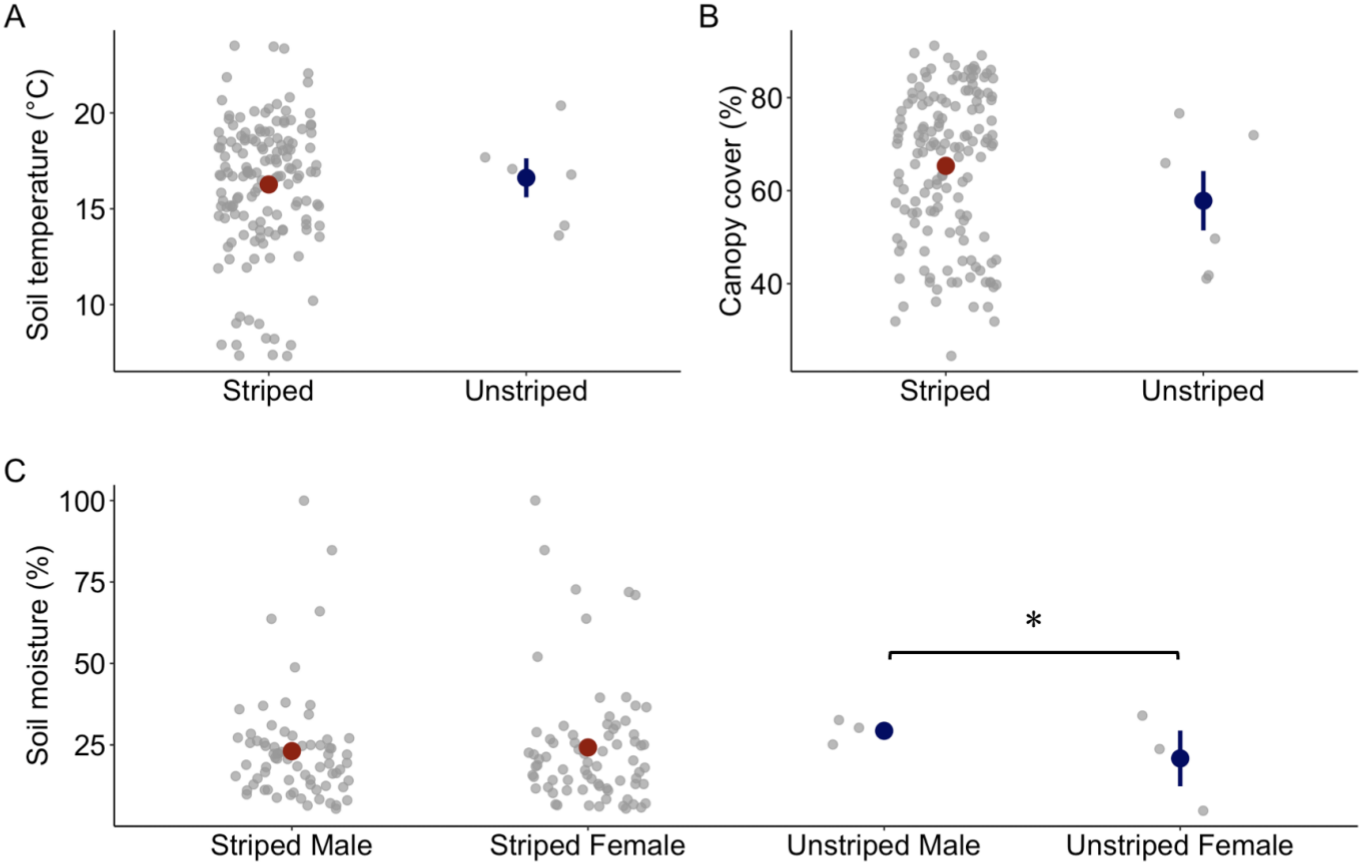

Figure 4. Microclimate variables including **(A)** soil temperature, **(B)** canopy cover, and **(C)** soil moisture at sites where Eastern Red-backed Salamanders (*Plethodon cinereus)* were observed in the field. The circle and line ("red" and "purple") represent the mean and standard error of each colour morph and the black/grey circles represent the raw data points. I visualise each microclimate variable for striped and unstriped morphs, and due to a significant interaction between colour morph and sex for soil moisture I also visualise data by sex. I found no significant difference between color morph and soil temperature or canopy cover, and a significant difference between unstriped males and unstriped females for soil moisture.

3.3 *Plethodon cinereus* Grouping Behaviour and Habitat Selection

Of the 164 adult salamanders found during my field surveys, 26 were found in mating pairs (12 striped-striped pairs, 1 striped-unstriped pair, and 0 unstriped-unstriped pairs) and 6 were found in two mating groups of three. One of these mating groups contained all striped individuals and the other contained two striped and an unstriped individual. All mating pairs were male-female mating pairs, and the two groups of three each contained two males and a single female. The number of salamanders in mating/groups was greatest in May (*n* = 6), August (*n* = 8), and October (*n* = 7) (Figure 5).

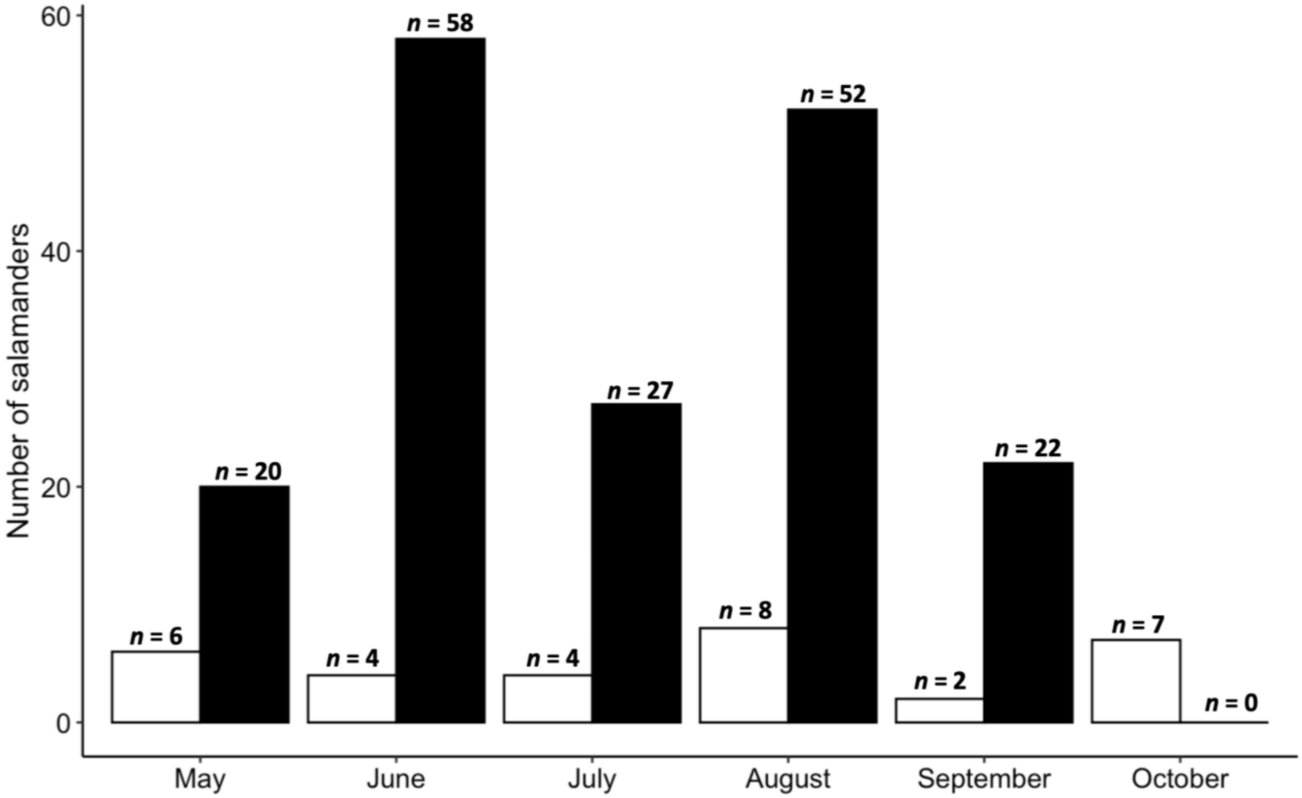

Figure 5. The number of adult Eastern Red-backed salamanders (*Plethodon cinereus*) found during field surveys in pairs/groups (white bars) and alone (black bars) from May to October 2023, where *n* indicates the sample size.

Salamanders in pairs/groups were found under cover objects with a higher soil moisture (Table 5; Figure 6) and salamander SVL was also related to soil moisture (Table 5). I did not find a relationship between salamander social status and soil temperature, cover object volume, or canopy cover (Table 5; Figure 6). Salamander SVL was significantly, positively related to cover object volume, but not the other two other environmental variables (Table 5).

Table 5. Outcomes of generalized linear mixed-effects models (GLMMs) and linear mixed effect models (GLMMs) testing if microclimate variables (i.e., soil moisture, canopy cover, and soil temperature) and cover object volume differ between field locations where solitary and grouped Eastern Red-backed Salamanders (*Plethodon cinereus)* occur*.* Reference levels for each variable are shown in parentheses following variable names. For each model, I present coefficient estimates (β) and their corresponding standard error (*SE*), *z*-values for GLMM (*z*) or *t*-values for LMM (*t*), and *p*-values (*p*) for fixed effects, as well as variance (σ^2) and *SE* of random effects and residuals. Model outputs are shown on the latent scale for GLMMs. N_{obs} refers to the number of observations where N_{site} refers to the number of sites surveyed. Significant values are bolded.

	Soil moisture (%)				**Canopy cover (%)**			
	N_{obs} = 143, N_{site} = 20				**N_{obs} = 144, N_{site} = 20**			
Fixed effects	***β***	***SE***	***z***	***p***	***β***	***SE***	***z***	***p***
Intercept (solitary, female)	**-2.745**	**0.249**	**-11.04**	**<0.001**	**-0.513**	**0.164**	**-3.12**	**0.002**
Status (grouped)	**0.3190**	**0.053**	**6.066**	**<0.001**	0.026	0.039	0.663	0.507
Sex (male)	0.0030	0.040	0.074	0.941	-0.021	0.028	-0.764	0.445
SVL	**0.025**	**0.005**	**4.69**	**<0.001**	0.002	0.004	0.664	0.506
Random effects	***σ²***	***SE***			***σ²***	***SE***		
Study site	0.217	0.466			0.047	0.217		
Residuals	---	---			---	---		
	Soil temperature (°C)				**Cover object volume (cm³)**			
	N_{obs} = 145, N_{site} = 20				**N_{obs} = 125, N_{site} = 19**			
Fixed effects	***β***	***SE***	***t***	***p***	***β***	***SE***	***t***	***p***
Intercept (solitary, female)	16.231	1.955	8.302	**<0.001**	-32434.503	26297.045	-1.233	0.220
Status (grouped)	-0.771	0.457	-1.689	0.094	7289.403	6875.654	1.060	0.291
Sex (male)	0.242	0.326	0.742	0.459	-4901.534	5248.490	-0.934	0.352
SVL	0.001	0.043	0.023	0.982	**1434.447**	**629.646**	**2.278**	**0.025**
Random effects	***σ²***	***SE***			***σ²***	***SE***		
Study site	11.233	3.352			34476530.589	5871.672		
Residuals	3.504	1.872			838249611.121	28952.541		

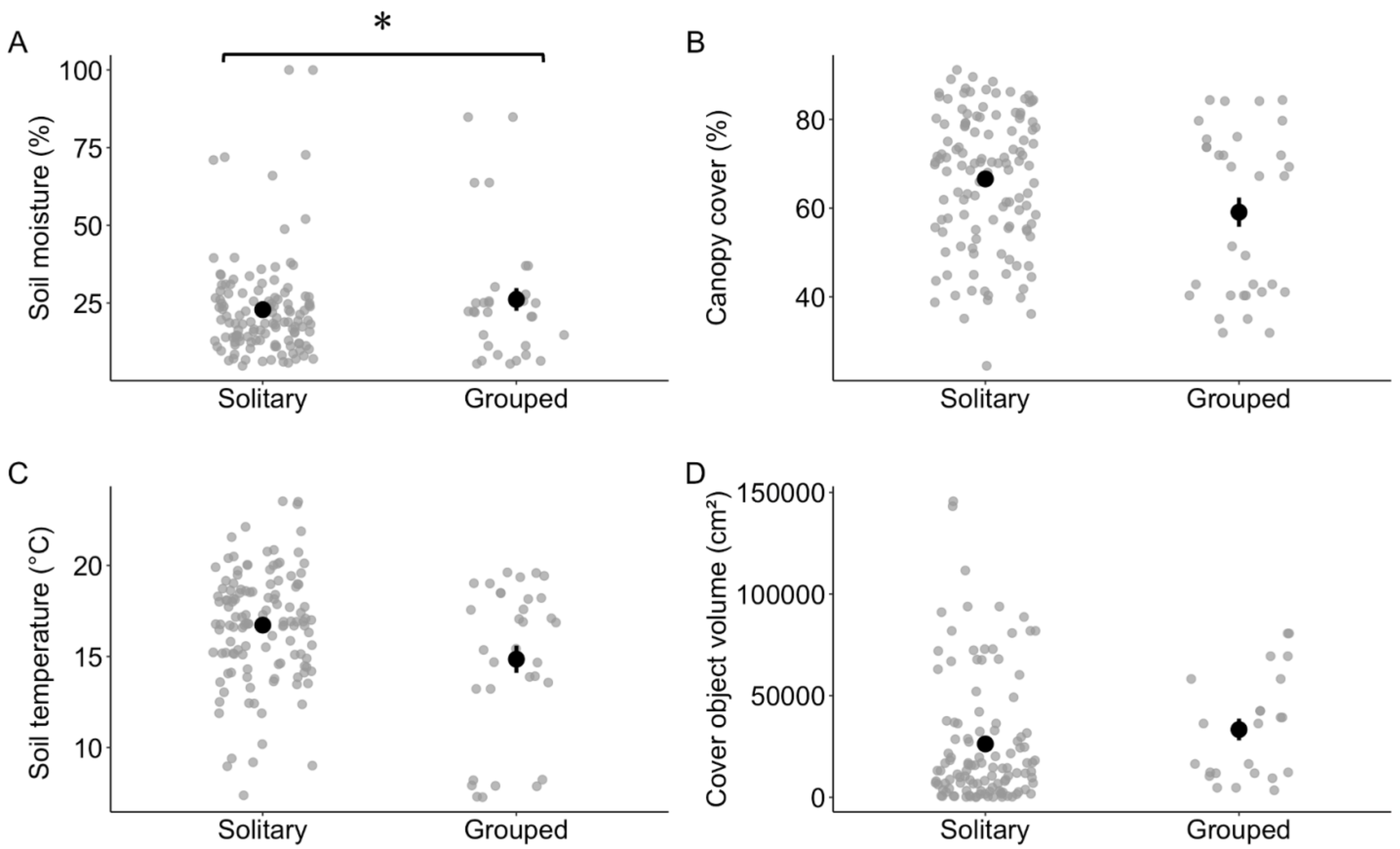

Figure 6. Habitat differences between Eastern Red-backed Salamanders (*Plethodon cinereus*) found under cover objects in groups *versus* found solitary. Environmental variables include **(A)** soil moisture, **(B)** canopy cover, **(C)** soil temperature, and **(D)** cover object volume. The circle and line ("black") represent the mean and standard error of solitary or grouped/paired salamanders and the black/grey circles represent the raw data points. I looked at the difference between pair status (solitary and grouped) and microclimate variables using a linear mixed model and generalized mixed models. I found no significant difference between social status (i.e., solitary or grouped) and canopy cover, soil temperature or cover object volume, and I found a significant difference between social status and soil moisture.

4. Discussion

A number of studies have suggested that the Eastern Red-backed Salamander's common colour morphs, striped and unstriped, are associated with contrasting environments, specifically that the unstriped morph is associated with warmer, drier conditions compared to the striped morph (Lotter and Scott, 1977; Gibbs and Karraker, 2006). In this research, I investigated whether microclimate variables differed between striped and unstriped colour morphs using field data, and whether macroclimate variables differed between striped and unstriped morphs using a dataset generated through community science observations (via iNaturalist). I did not find a difference in the microclimate (consisting of canopy cover, soil temperature and moisture at the cover object) selected by *P. cinereus* color morphs. I also did not find a difference between the macroclimate (consisting of the average daily temperature and precipitation at each survey site) selected by *P. cinereus* color morphs. However, my inability to detect a significant difference between the macroclimate and microclimate used by colour morphs may be due to the small sample size of unstriped salamanders in my iNaturalist dataset (*n* = 21) and field dataset (*n* = 6). Based on the machine-learning approach used by Hantak et al (2022) to predict occurrences of *P. cinereus* colour morphs across their range in Canada, unstriped morphs appear to be common at very specific, limited locales. Thus, future work that aims to compare the habitat preferences of *P. cinereus* colour morphs in New Brunswick may need to focus surveys at these particular sites. In addition, this research found that the morphs overlap by 87% in climatic niche space, which may suggest their habitat preference difference may not be consistent across their range (Hantak et al., 2022). Overall, more work is needed to determine how macroclimate and microclimate habitat preferences vary between *P. cinereus* colour morphs in New Brunswick.

Eastern Red-backed Salamanders colour polymorphism has been extensively documented, however there is a lack of information regarding the distribution of their colour polymorphism in certain regions; one of which being New Brunswick, Canada. I was able to document the color polymorphism of this species in considerable spatial detail, as well as compare methods of data collection (e.g., field observations versus a community-science approach) using 299 community-science observations from iNaturalist and 243 field observations collected across this species' 2022 active season. I found that striped salamanders are the most common colour morph, in both field and community science data, and that the unstriped morph is less common, as is typically seen across their range (Table 1). With the help of my iNaturalist data, I was also able to identify novel colour morphs to New Brunswick such as the amelanistic morph (observed by Damien Mullin) and the leucistic morph (observed by Bradley Doiron) which to the best of my knowledge, have yet to be formally documented in the province (Moore and Ouellet, 2014). Contrary to other animals in which atypical colouration is typically found in only young individuals because it is maladaptive, all of the rare colour morphs of Eastern Red-backed Salamanders encountered during my study were adults. The persistence of these rare colour morphs in the adults of *P. cinereus* may suggest that it is less detrimental due to their cryptic behaviour (i.e., spending the majority of their time under cover objects) and that they select a variety of different backgrounds in their natural environment (Moore and Ouellet, 2014). Although factors that influence *P.cinereus* color variation across their range largely remains a mystery, information on rare colour morphs in New Brunswick tells us that factors influencing their variation in color polymorphism is also influencing New Brunswick populations because we are seeing similar morphs (e.g., amelanistic, albino, leucistic) in other regions like Nova Scotia and Quebec (Moore and Ouellet, 2014). In addition to the occurrence of colour morphs in New Brunswick, I also investigated whether an individual's colouration impacted the likelihood of finding and reporting these salamanders using different data collection methods.

When comparing methods of data collection, I found that both methods (e.g., field survey *versus* community science) were similar in how likely they would observe/report the most common colour morph: the striped morph. However, data collection using a community-science approach was more likely to observe/report less common and unique colour morphs (e.g., unstriped and others). These results are similar to a study conducted by Lehtinen et al (2020) that investigated differences between community-science and field data on the color morph frequency of Eastern Gray Squirrels (*Sciurus carolinensis*). They found that community-science methods of data collection were more likely to observe/report the novel white phenotype of Eastern Gray Squirrels (e.g., leucistic and albino squirrels) (Lehtinen et al. 2020). Community-science data collection approaches are useful for many reasons, like studying colour polymorphism (Hantak et al., 2022b), climate association on colour patterns (Hantak et al., 2022a), and assessing species parasitism and predation rates (Putman et al., 2021). My dataset on *P. cinereus* colour polymorphisms was dramatically increased with the inclusion of community science data, making this a very powerful approach in research (Chandler et al., 2017). But it is important to know and identify potential biases in this type of data. Specifically, knowing that the likelihood of observing/reporting colour morphs on iNaturalist may be biased towards rare/unique morphs is an important consideration to using this data in research. It Is also important to consider the difference in the geographic range between iNaturalist observations and my field observations. The observations reported on iNaturalist were more widely distributed in the province whereas my field observations were much more restricted to southeastern parts of the New Brunswick, therefore it is possible that the high occurrence of rare morphs reported on iNaturalist is in fact reflective of natural populations but differs from my field observations because of the difference in distribution. In addition to observing/reporting rare and unique morphs, my community science dataset may also be biased towards locations that are easily accessible by people, like along roadways or hiking trails (Serniak et al., 2023). Of course, community-science data via online platforms, like iNaturalist, has the potential for a variety of other biases, for example differences in search effort, as well as over representing species in clusters/groups (Callaghan et al., 2021).

It is commonly known that Eastern Red-backed Salamanders form and resides within mating pairs and groups where they co-defend territories together under cover objects (Anthony et al., 2008), however the degree in which they socially interact varies seasonally across their range. For example, in Virginia, *P. cinereus* show an aggregated spatial distribution in the spring, and after territorial boundaries are established, maintain a uniform spatial distribution throughout the remainder of their active season (Jaeger, 1979). In contrast, a study conducted in northern Michigan suggested that *P. cinereus* exhibit an aggregated spatial distribution consistently throughout the active season (Quinn and Graves, 1999). I surveyed for *P. cinereus* across their active season (May to October) and recorded how occurrences of social aggregations under cover objects changed over time in New Brunswick. I found that the number of salamanders in mating pairs and groups was greatest in May (*n* = 6) with 30% of salamanders found in mating pairs/groups that month, August (*n* = 8) with 15% of salamanders observed in mating pairs/groups, and October (*n* = 7) with 100% of salamanders found that month in mating pairs/groups. These results are similar to Jaeger (1979) where the degree to which these salamanders socially interact is greatest in the spring, however I also found that pairs/groups are common in the early fall. Several explanations for these results are that *P. cinereus* typically mate in the spring and early fall, and are more likely to co-defend areas under cover objects during these times (Anthony et al., 2008), or that their social behaviour is influenced by their foraging behaviours, which vary seasonally because it is largely influenced by temperature (Heatwole, 1961). Understanding the Eastern Red-backed Salamander geographical variation in behaviour is important for understanding their adaptation to differing ecological conditions across their range (Jaeger et al., 2016).

A number of factors can influence the social behaviour of the Eastern Red-backed Salamander, particularly the way in which they arrange themselves on the forest floor. Examples of these factors include resource distribution, the number of cover objects available (Grover, 1998), as well as the quality of the cover object (i.e., habitat quality). An ideal salamander habitat has a cool temperature and moderate moisture level, which can be facilitated through the coverage of the forest canopy and the size of the cover object. Quinn and Graves (1999) studied how habitat quality influenced social aggregations of salamanders in Michigan using artificial cover boards and found that salamanders in groups occupied higher quality habitats. In my work, I also located solitary adult salamanders and those in pairs/groups, but under natural cover objects during my field surveys. Interestingly, I found that soil temperature, cover object size, and canopy cover did not differ between locations where I found solitary versus paired/grouped salamanders. Yet, I did find that salamanders in groups occupied cover objects with a higher soil moisture. Thus, my results suggest aggregation behaviour of *P. cinereus* may be influenced by soil moisture. Male and female salamanders in mating pairs/groups are likely defending territory for oviposition, and thus require a high soil moisture content to care for their eggs successfully. Females lay their eggs in clutches which are frequently in partial contact with surrounding material wherein they absorb water from damp substrates (Heatwole ,1961). Therefore, it is likely that out of all environmental variables measured (i.e., soil temperature, canopy cover, and soil moisture) that soil moisture is the most important for paired/grouped salamanders when selecting the habitat they will co-defend.

The colour polymorphism and social behaviour of *P. cinereus* has been extensively studied in the United States (Anthony et al., 2008; Highton, 1959), Ontario, Quebec, and Nova Scotia (Morneault et al., 2004; Noël et al, 2007; Russell et al, 2011), yet little information exists for New Brunswick populations. This study is the first in New Brunswick to include data from range of *P. cinereus* populations across 23 different forests, while also making use of community-science data to increase our knowledge about this species' natural history. This work also provides baseline knowledge for future Riley Lab research on Eastern Red-backed Salamander colour polymorphism and distribution, which is critical information that will be used to guide future research. My research highlights that more work is still needed to understand Eastern Red-backed Salamander distribution and color polymorphism in eastern Canada, and because of contrasting environmental preferences and assortative mating behaviour between colour morphs, to understand their potential to ecologically diverge from one another (Reiter et al., 2014).

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