4-1-2024

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Amy Q. Friemoth
University of Dayton

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Abstract

Human modified landscapes can provide challenging new environments that can lead to rapid changes in species traits. In temperate forest ecosystems, salamanders play a critical role in forest floor dynamics, but populations are now faced with rapid habitat change, which can lead to local extinction events unless species can adapt behaviorally. The Eastern Red-backed Salamander, *Plethodon cinereus*, is the most common salamander species in the northeastern United States and can serve as a model species for examining the effects of anthropogenic change. Further, the Eastern Red-backed Salamander poses two common color morphs (striped and unstriped) that appear to differ in their responses to environmental change. Here, I investigate how proxies for urbanization differentially influence behavioral responses of the two color morphs of the Eastern Red-backed Salamander. I collected 20 striped and 20 unstriped individuals from Caesar Creek State Park, Ohio. I exposed individuals to a novel substrate (cement paver) and novel objects (anthropogenic litter items) to test exploration and neophobia behaviors. Based on previous studies, I predicted striped morphs would display more bold behaviors and be more willing to interact with novel objects. Using linear mixed-effects models, I found unstriped morphs were significantly bolder when exploring a novel substrate. Neophobia behavior was similar between both morphs. This work highlights how the distinct color morphs may respond to rapid habitat modification. These results have important implications for other North American salamanders as many species share the same color phenotypes and may be responding similarly.

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Introduction

Increased human activity has steadily altered natural communities, resulting in negative consequences for organisms that inhabit these areas. Habitat degradation is currently the leading cause of biodiversity loss as it fragments continuous stretches of natural land and introduces novel, and sometimes inhospitable environments for local wildlife (Haddad et al., 2015). Near urban centers, natural areas typically remain in a patchwork layout, separated by vast stretches of roadways, preventing movement of many small organisms. Habitat patches in close proximity to denser human population sizes also typically have higher amounts of anthropogenic garbage (e.g., Beckmann & Lackey, 2008). These litter products present novel stimuli for organisms and may alter their behavioral strategies for forging, mating, or defending territories (Trombulak & Frissell, 1999; Plaza & Lambertucci, 2017).

Novel variations in landscapes can impact the spatial distribution of species by challenging individuals and altering their movement patterns or behaviors (Alberti et al., 2017; Baxter-Gilbert et al., 2019; Lapiedra et al., 2017; Lowry et al., 2013). Studies suggest animals residing in urban environments such as birds (Atwell et al., 2012), reptiles (Peterman & Ryan, 2009; Ryan et al., 2014), and mammals (Ritzel & Gallo, 2020) exhibit behavioral changes. Modified behavioral traits such as boldness and neophilia may prove to be advantageous in navigating and exploiting urban environments (Kralj-Fiser et al., 2017). Boldness is characterized by an individual's willingness to take risks. Bolder individuals tend to show decreased anti-predatory responses for both novel and natural threats (Myers & Hyman, 2016). This can benefit an individual by increasing time spent foraging and defending a territory (Sol et al., 2013). Neophobia is

characterized by an individual's avoidance to interact with novel objects or stimuli whereas neophilia reflects attraction (reduced fear response). Neophilia can be beneficial because with no fear of new objects, individuals are not limiting their expansion of resource use, such as food and shelter acquisition (Miranda et al., 2013). Another key behavioral trait that may be altered by novel environments is thigmotaxis. Thigmotaxis, often referred to as wall-hugging behavior, is the tendency of an animal to remain close to vertical surfaces, such as walls or edges of open spaces (Kallai et al., 2007; Sharma et al., 2009). Both vertebrates and invertebrates have been shown to perform thigmotactic behaviors as an antipredator response and exploratory strategy (Grossen & Kelley, 1972; Kallai et al., 2007). This behavior serves as an index of anxiety for many species such as rodents (Harris et al., 2009), cockroaches (Salazar et al., 2018), fish (Colwill & Creton, 2011), and humans (Walz et al., 2016) with increased thigmotactic behaviors in stressful environments. Thigmotaxis can also serve as a proxy for behavioral traits such as boldness or shelter use (Burns, 2008; Webster & Laland 2015).

Woodland Salamanders (genus *Plethodon*) represent a key group for examining the effects of novel urban environments on behavioral modifications because these species demonstrate a suite of behaviors (e.g., territoriality and elaborate mating interactions) that may be disrupted by novel stimuli (Semlitsch et al., 2012; Garner et al., 2022). In addition, members of this genus are lungless and breathe entirely through their skin (cutaneous respiration). To facilitate cutaneous respiration, these species require cool, moist conditions, rendering this group highly sensitive to environmental change (Fisher-Reid et al., 2021). Indeed, a number of studies have shown that *Plethodon* presence and abundance is correlated with increases in vegetation and moisture

(Peterman & Semlitsch, 2013). The responses of these salamanders to habitat degradation are important to understand because these species play critical roles in forest floor dynamics, and their presence and abundance is suggested to be a key indicator of ecosystem health (Welsh & Droege, 2001).

The Eastern Red-backed Salamander, *Plethodon cinereus* has a widespread distribution in northeastern North American forests and is considered one of the most abundant terrestrial vertebrates in the United States (Mathis, 1991). Many *P. cinereus* populations are known to exist in small habitat patches across their range, suggesting some form of resilience to fragmentation (although they are sensitive to edge effects; Noel et al., 2007). *Plethodon cinereus* exhibits a color polymorphism with a striped morph, characterized by a red-orange dorsal stripe extending from the head to tail, and an unstriped morph, characterized by a uniformly black dorsum (Petranka, 1998). Color morph frequency varies greatly across the range of *P. cinereus*, but striped morphs are generally more common than unstriped morphs (Moore & Ouellet, 2014). These two color morphs appear to be ecologically distinct and respond differently to environmental pressures within a shared habitat. For example, a number of studies have associated the unstriped morph with warmer, drier climates, while striped morphs are more associated with cooler and wetter conditions (Lotter & Scott, 1977; Moreno, 1989; Anthony et al., 2008; Fisher-Reid et al., 2013; Anthony & Pfingsten, 2013). Previous work has also indicated that the two color morphs differ in their behavioral responses to predation attempts. These studies found that unstriped morphs experience higher rates of predation and respond to predator exposure by fleeing, whereas striped morphs more frequently display aggressive postures and remain immobile (Moreno, 1989; Venesky & Anthony,

2007). In addition, a study by Davis and Milanovich (2010) found baseline stress levels were higher in the unstriped morph than in the striped morph. Overall, these previous studies indicate that the striped morph displays more bold tendencies than the unstriped morph.

To date, no studies on the color morphs of *P. cinereus* have examined behavioral responses to urban environments. Thus, we know little about how rapid, human-mediated land use change affects the plastic and adaptive responses of these ecologically important salamanders. This study aims to examine how the two color morphs of *P. cinereus* respond to novel urban environments. To accomplish this, I will examine boldness and neophobia behavioral traits of striped and unstriped salamanders, which represent proxies to novel urban substrate (e.g., concrete) and anthropogenic litter (e.g., common garbage items). Based on previous studies, I expect that striped morphs, with their increased aggressive and territorial behaviors, will be more bold, and, therefore, less affected by novel urban substrates (Reiter et al., 2014). In addition, as previous work has demonstrated that striped morphs have lower baseline stress levels compared to unstriped morphs (Davis & Milanovich, 2010), I predict that striped morphs will exhibit less thigmotaxis-related tendencies during exploration trials. Lastly, I predict that striped morphs will display more neophilic behaviors compared to unstriped morphs and will be more willing to interact with novel human objects.

Methods

Field Collection Site

In October and November of 2023, I collected 20 striped and 20 unstriped adult individuals of *P. cinereus* from Caesar Creek State Park, Ohio. Adults were assessed

based on a minimum size of 32 mm snout-vent length (SVL; using NEIKO digital calipers), measured from the tip of the snout to the anterior portion of the cloaca. Within each color morph, we collected equal numbers of each sex (10 males and 10 females of each color morph). Sex information was captured by examining the shape of the salamander snouts. During the breeding season, males have broad, square snouts, while female snouts are more round. Females were also determined by the presence of eggs that can be seen through their semi-translucent abdomens. Collected salamanders were transported to the University of Dayton in 50 mL falcon tubes and re-examined to determine sex, mass (in grams using a MAXUS scale), and SVL (in mm). Each salamander was individually housed in a 470 mL Pyrex bowl (6.1 cm height, 12.4 cm diameter) with a moist paper towel. Salamanders were housed in a temperature and light controlled room at the University of Dayton and maintained at a constant temperature of 18°C with a 11 h light:13 dark cycle (08:00 – 19:00 h). Once a week, the salamanders were fed approximately 25 wingless fruit flies and their moist paper towel substrate was replaced. Salamanders acclimated to their new housing conditions for at least two weeks before the behavioral assays commenced.

Behavioral Assays

I ran two sets of behavioral assays (exploration and neophobia) involving salamander exposure to a novel urban landscape and novel human objects between October 2023 and February 2024. The behavioral assays were conducted in the same room in which salamanders were housed. When possible, both behavioral assays were run concurrently. On testing days, individual salamanders were randomly assigned to an assay and a treatment group (described below). All assays were conducted between 12:30

– 18:30. Salamanders were acclimated for at least one week between each trial. Each behavioral assay was video recorded and scored by a single researcher (AF) to minimize variation. The software program BORIS v. 7.13.8 (Friard & Gamba, 2016) was used to facilitate scoring behavioral traits. Experimental arenas were thoroughly washed between trials to avoid potential effects from residual pheromones.

Exploratory Assay

For exploratory assays, salamanders were placed in an experimental arena (508 x 65 mm) made of plexiglass. The arena was covered with brown opaque paper on the external surface to minimize outside stimuli. Four cement pavers (300 x 300 mm) were placed in a square arrangement to form the base of the arena. Depending on the treatment, the cement pavers remained exposed to serve as the novel urban environment (mimicking paved roads or walkways) or were completely covered with the same paper towels placed in their housing to serve as the control. Before each assay, the substrate (paver or paper towels) was well-moistened with spring water to ensure normal breathing conditions for salamanders. Two black shelters (140 x 45 mm) were centered against the left and right wall of the arena with spring water spritzed inside to retain moisture (see Fig. 1). Prior to trials, individual salamanders were acclimated in a closed, black shelter centered along the side of a wall for 600 seconds. After the acclimation period, the shelter was lifted, and each salamander was free to explore the experimental arena for 1800 seconds. A large sheet of plexiglass with a 508x508 mm grid in the middle was placed on top of the arena to prevent the salamanders from escaping. Exploratory assays were recorded using a GoPro11 suspended from above the arena. A total of 80 assays were conducted with each salamander undergoing both treatments (control and paver) in a

random order. From video recordings, I scored several behaviors, including the total time a salamander spent moving within the arena, total time spent performing thigmotaxis, and total time spent hiding in the shelter or between the back of the shelter and wall.

Neophobia Assay

For neophobia assays, we placed salamanders in an experimental arena (508 x 405 mm) that was constructed in the same manner as the exploratory arena. A large sheet of plexiglass with a 508x508 mm grid made of 25.4 mm squares in the middle served as the base of the arena. The arena was placed over the grid so that each corner lined up exactly with the grid. To evaluate the level of neophobia expressed by salamanders to common human litter items, individuals were exposed to three novel objects (8 oz plastic water bottle, 12 oz soda can, and 8 oz styrofoam cup) and a control (rock). These objects were selected because they all represent common litter items found in urban wildlife areas. The experimental arena was divided into 5 zones (wall, zone 3, zone 2, zone 1, zone 0) with a novel object placed at the center of the arena prior to the start of the assay. Assignment of the zones was based on the gridded base where zone 0 was the object plus any additional squares the object touched, zone 1 was the three grid squares outside of zone 0, zone 2 was the three grid squares outside of zone 1, zone 3 was the residual gridded squares, and wall was full contact between the front half of the salamander's SVL and any side of the arena wall (see Fig. 2). Before each assay, the arena, object, and shelter were sprayed with spring water. Salamanders acclimated in a closed, black shelter centered along the side of a wall for 600 seconds. After the acclimation period, the shelter was lifted, and each salamander was left to interact with the given object for 1800 seconds. A large sheet of plexiglass was placed on top of the arena to prevent the

salamanders from escaping. The assays were recorded on either a Wyze Camera or

GoPro11 suspended from above. A total of 160 assays were conducted with each

salamander exposed to all four objects (water bottle, soda can, Styrofoam cup, and rock)

in a random order. From the videos, I scored the proximity of the salamander to the object

using the zones (outermost ring = 203.2 mm and inner-most ring = 76.2 mm) where a

closer distance to object (zone 0) was considered a neophilic response and a further

distance from object was considered a neophobic response (zone 3). The closest distance

a salamander approached an object was determined by the closest zone it crossed over.

The zones were converted into numerical data using the gridded base. The closest

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over. The zones were converted into numerical data using the gridded base.

Statistical analyses

Exploratory and neophobia behavior were examined separately and several

aspects of their behavior were modeled. I ran three separate models to examine

exploratory behavior. These separate responses were: total moving time, total hiding

time, and total thigmotaxis time. For each of these exploratory behavior trials, the

predictor variables were the same and included: salamander color morph

(striped/unstriped), treatment (control/cement paver), sex (male/female), and SVL.

Salamander SVL and mass were highly correlated (R^2=0.81), thus only SVL was used as

a predictor variable in each model. In each analysis, color morph and treatment were

modeled as an interaction. For neophobia behavior, I ran a linear mixed effect model

which analyzed the response of distance to the treatment object. For this model, the

predictor variables included: salamander color morph (striped/unstriped), treatment (rock

control, water bottle, styrofoam cup, or soda can), sex (male/female), and SVL. In the analysis, color morph and treatment were modeled as an interaction.

To examine the effects of the predictor variables on exploratory and neophobia behavior, I ran linear mixed effect models using the *lmer* function within the 'lme4' (Bates et al., 2015) and 'lmertest' (Kuznetsova et al., 2017) packages using RStudio Software version 4.3.2 (R Core Team, 2023). Within each model, salamander identity was included as a random intercept.

Results

Exploratory behavior models

Total time moving was influenced by the interaction between color morph and treatment type. Striped morphs spent less time moving when they were placed in paver treatments compared to the control treatment, while unstriped morphs spent more time moving during paver treatments compared to the control (Estimate = 179.407, SE = 66.642, P = 0.011; Table 1; Fig. 3a, Fig. 4a). Salamander sex (Estimate = 52.244, SE = 53.292, P = 0.333) and mean SVL (Estimate = 3.124, SE = 7.772, P = 0.690) did not influence total moving time. Total hiding time was not influenced by color morph (Estimate = 27.860, SE = 169.660, P = 0.870), treatment (Estimate = 67.520, SE = 141.790, P = 0.637), or the interaction between color morph and treatment type (Estimate = -172.80, SE = 200.53, P = 0.394). Salamander sex (Estimate = -65.88, SE = 131.90, P = 0.620) and mean SVL (Estimate = -15.34, SE = 19.24, P = 0.394) were also not important predictors of salamander hiding time. Total thigmotaxis time was influenced by the interaction between color morph type and treatment type. Striped morphs spent less time performing thigmotaxis behaviors in the paver treatment compared to the control

treatment, while unstriped morphs spent more time performing thigmotaxis behaviors in the paver treatment compared to the control (Estimate = 327.692, SE = 163.437, P = 0.049; Fig. 3c, Fig. 4b). Salamander sex (Estimate = 44.389, SE = 87.867, P = 0.615) and mean SVL (Estimate = -2.025, SE = 12.814, P = 0.875) did not influence total thigmotaxis time.

Neophobia behavior models

When exposed to different trial treatments (rock, plastic water bottle, soda can, and styrofoam cup), there were significant effects of treatment type, but not color morph. Closest distance to object was influenced by individual treatment, where striped and unstriped morphs kept a significantly greater distance from the cup treatment (Estimate = 77.470, SE = 24.243, P = 0.002), soda can treatment (Estimate = 48.260, SE = 24.243, P = 0.048), and water bottle treatment (Estimate = 55.880, SE = 24.243, P = 0.023) compared to the control (Table 2; Fig. 5, Fig 6). Color morph (Estimate = 7.466, SE = 24.974, P = 0.765) nor the interaction between color morph and treatment type had influence on the proximity to object. Salamander sex (Estimate = 6.605, SE = 13.034, P = 0.613) and mean SVL (Estimate = -1.159, SE = 1.901, P = 0.543) were also not important predictors of how close a salamander approached the object.

Table 1. Exploratory behavior model results for (A) total moving time (B) total hiding time, and (C) total thigmotaxis time. Bold effects are significant.

Model	Estimated	SE	P-value
Explanatory			
(A) *Total Moving Time*			
(Intercept)	212.405	346.649	0.544
Treatment paver	-148.765	47.123	**0.003**
Color unstriped	-18.826	64.562	0.772
Sex male	52.244	53.292	0.333
Mean SVL	3.124	7.772	0.690
Treatment paver:color unstriped	179.407	66.642	**0.011**
(B) *Total Hiding Time*			
(Intercept)	1271.270	858.910	0.147
Treatment paver	67.520	141.790	0.637
Color unstriped	27.860	169.660	0.870
Sex male	-65.880	131.900	0.620
Mean SVL	-15.340	19.240	0.430
Treatment paver:color unstriped	-172.800	200.530	0.394
(C) *Total Thigmotaxis Time*			
(Intercept)	785.722	573.143	0.175
Treatment paver	-200.597	115.567	0.087
Color unstriped	-151.741	122.437	0.219
Sex male	44.389	87.867	0.615
Mean SVL	-2.025	12.814	0.875
Treatment paver:color unstriped	327.692	163.437	**0.049**

Table 2. Neophobia behavior model results for closest distance to object. Bold effects are significant.

| Model	Estimated	SE	P-value
Neophobia			
Closest *Distance to Object*			
(Intercept)	80.687	85.877	0.349
Treatment cup	77.470	24.243	**0.002**
Treatment soda can	48.260	24.243	**0.048**
Treatment waterbottle	55.880	24.243	**0.023**
Color unstriped	7.466	24.974	0.765
Sex male	6.605	13.034	0.613
Mean SVL	-1.159	1.901	0.543
Treatment cup:color unstriped	0.000	34.285	1.000
Treatment soda can:color unstriped	-11.430	34.285	0.739
Treatment waterbottle:color unstriped	-1.270	34.285	0.971
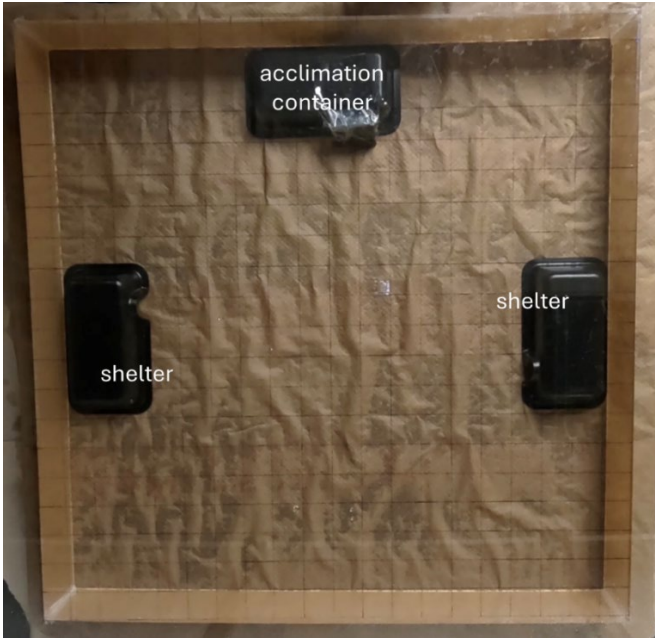
Figure 1. Experimental set up of the exploratory assay arena. Shelters remained in the arena for the duration of the trials for salamanders to hide in. Salamanders were initially left under the acclimation containers for 300 seconds to adjust to the new settings. After the 300 second period, these containers were removed from the arena.
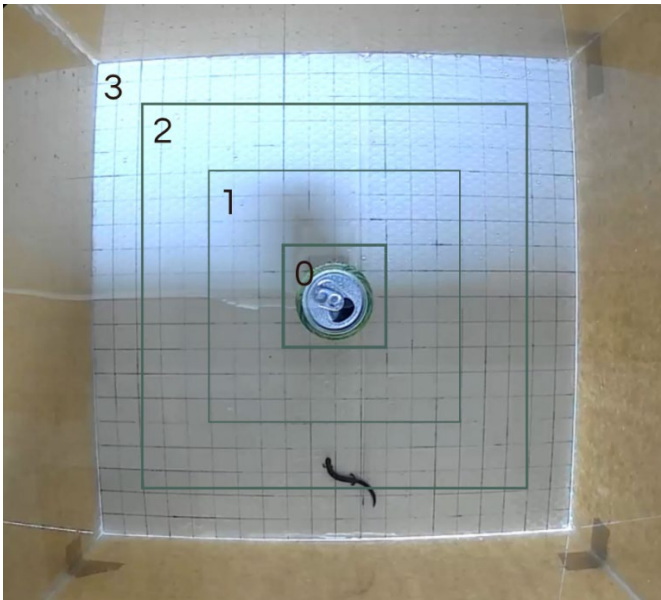
Figure 2. Experimental set up of the neophobia assay arena with numbers representing the zones. Zone 0 was the object plus any additional squared the object touched, zone 1 was the three grid squares outside of zone 0, zone 2 was the next three grid squares outside of zone 1, and zone 3 was the residual gridded squares.
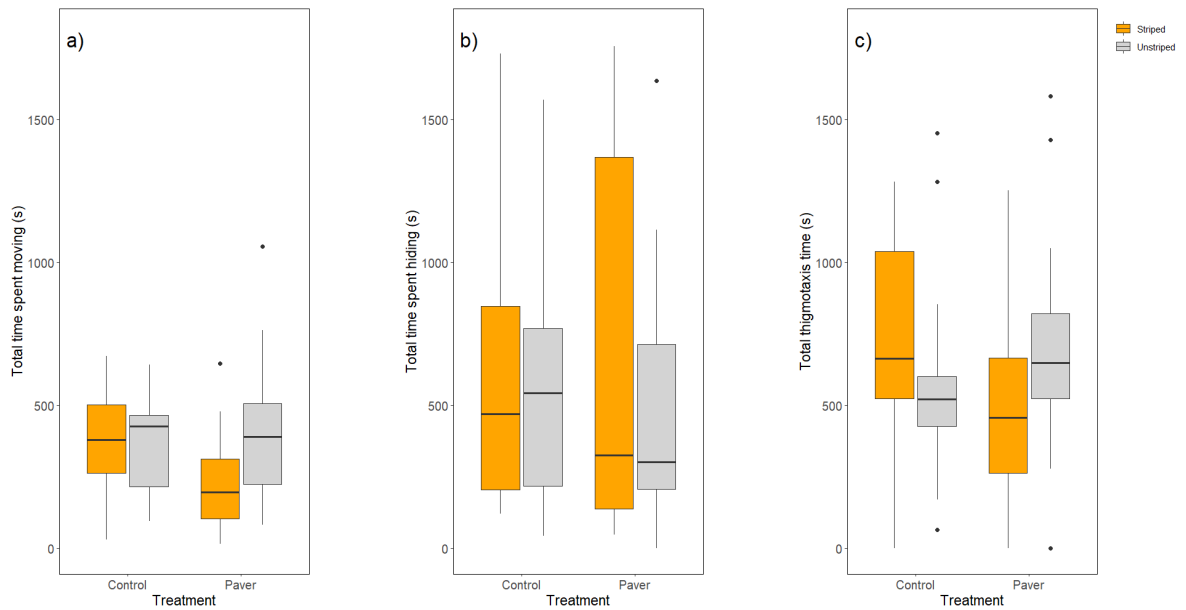
Figure 3. Exploratory behavioral responses of: (a) total time spent moving in arena, (b) total time spent hiding in shelter, and (c) total time spent in thigmotaxis between striped (orange) and unstriped (gray) *P. cinereus* on control and novel paver surfaces.
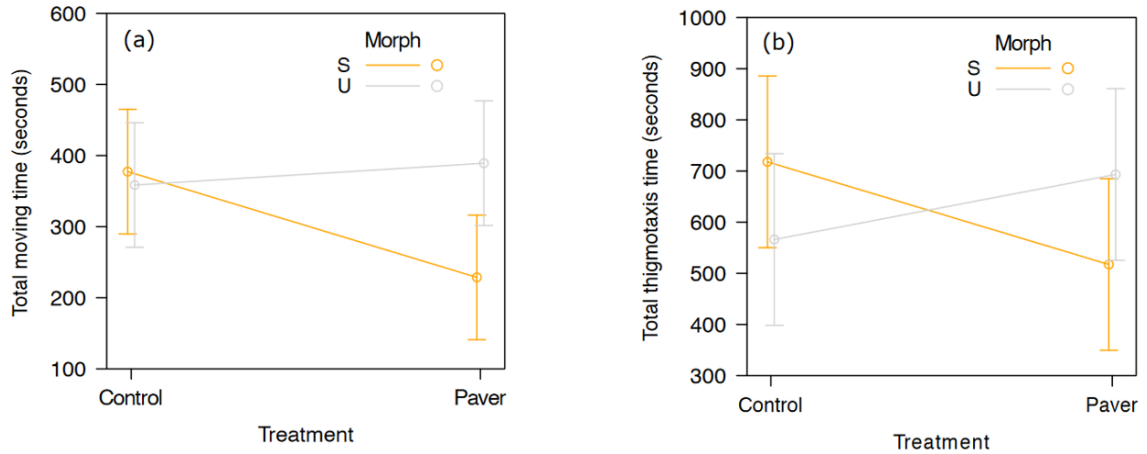
Figure 4. Model effect plots of significant interaction effects of exploratory behavior in *P. cinereus*. (a) Total moving time was influenced by the interaction between color morph and treatment. (b) Total time displaying thigmotaxis behavior was influenced by the interaction between color morph and treatment. Error bars in each plot represent 95% confidence intervals.
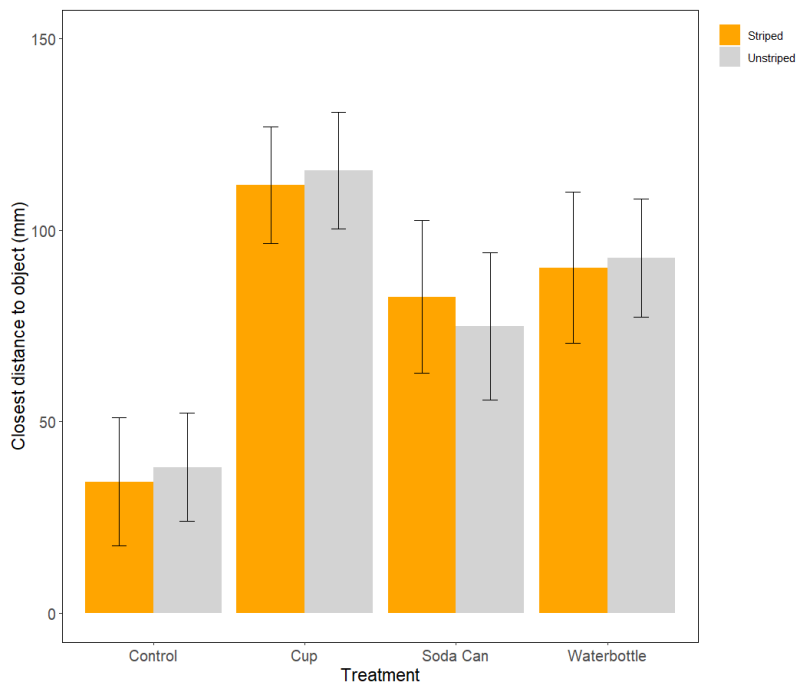
Figure 5. Neophobia behavioral response of closest distance to the novel object between striped (orange) and unstriped (grey) *P. cinereus* for control (rock), Styrofoam cup, soda can, and water bottle treatments. The level of neophilic behavior expressed by salamanders is presented by the closest distance a salamander approached the object treatment and the average time spent close to the object treatment (zone 0).
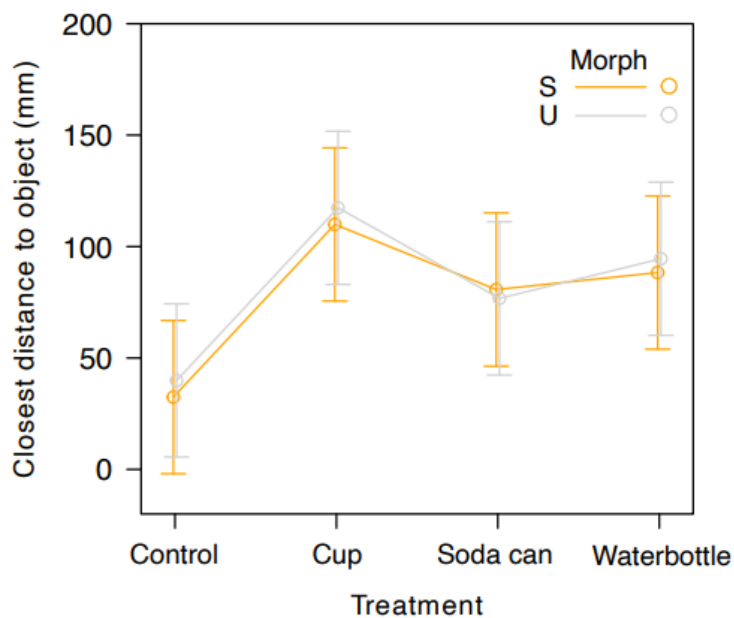
Figure 6. Model effect plot of the significant predictor of neophobia response in *P. cinereus*. The neophobic level of closest distance to object was influenced by treatment, but not color morph Error bars in each plot represent 95% confidence intervals.

Discussion

Color polymorphic species, which have co-adapted trait sets, are expected to respond differently to human-mediated habitat modification. Much work on the distinct color morphs of the Eastern Red-backed Salamander, *P. cinereus*, demonstrates clear morph differences, but their responses to urbanization pressures remain unknown. To

study how the striped and unstriped color morphs of *P. cinereus* behaviorally respond to novel urban environments, I exposed individuals to a novel urban substrate and common anthropogenic garbage to examine boldness and neophobia behavioral traits. Based on previous studies indicating that the striped morph is generally more territorial and bold compared to the unstriped morph (Anthony & Pfingsten, 2013), I predicted striped morphs would be bolder when exposed to a novel urban substrate and novel urban waste. The results of this study indicate that there are morph-specific differences in behavioral interactions with urban objects; however, the direction of the responses goes against my initial predictions. I found striped morphs were less bold when exploring novel substrates compared to unstriped morphs. In addition, I found the striped and unstriped morphs both displayed neophobic behaviors to all three novel objects.

Exploratory Behavior

Tests of exploratory behavior in novel environments indicate that color morphs demonstrate divergent responses to novel substrates, where striped morphs spent less time exploring the novel substrate, while unstriped morphs spent more time investigating the arena. This result goes against my initial predictions, where, based on previous studies, I expected striped morphs to be more bold and spend more time exploring new surfaces (Moreno, 1989; Reiter et al., 2014; Sih et al., 2004; Venesky & Anthony, 2007). My findings suggest unstriped morphs may be better suited for novel urban environments compared to striped morphs. One possible explanation for this finding may be related to how the two morphs exhibit different escape strategies after predator exposure. Past work has shown that striped morphs are more likely to display aggressive postures and remain immobile as a defensive strategy, while unstriped morphs are more likely to flee from

predators, thus, showing more mobility (Moreno, 1989; Venesky & Anthony, 2007). In addition, Moreno (1989) suggested unstriped morphs are generally more active on the forest floor compared to striped morphs based on increased observed predation attempts on the unstriped morph.

Another possible explanation for the increased movement of the unstriped morph and decreased exploration of the striped morph on the novel surface may be related to variation in moisture levels across treatments. Several studies have suggested unstriped morphs are better adapted to drier and warmer climates (Anthony et al., 2008; Evans et al., 2020; Moreno, 1989). For example, Fisher-Reid et al. (2013) found unstriped morphs of *P. cinereus* were more active in drier conditions than striped morphs in a Long Island, New York population. It is possible that the design of our exploratory behavior treatments may have led to differences in moisture levels across the treatment – where the paver surface likely retained less moisture than the control, which was lined with paper towels. Thus, my finding appears to corroborate all of the past studies that the unstriped morph is better adapted to drier conditions, which lends support to the idea that unstriped morphs may be better suited for urban environments.

In relation to exploratory behavior, my results also reveal that thigmotaxis tendencies of the two morphs differed. I found that unstriped morphs increased in thigmotaxis tendencies when exposed to the novel paver treatment, while striped morphs decreased in this behavior. This finding suggests that although unstriped morphs spent more time exploring the novel environment compared to striped salamanders, they also exhibited higher levels of stress. This interpretation aligns with a previous study that demonstrated baseline stress levels of unstriped morphs are higher than striped morphs

(Davis & Milanovich, 2010). Thus, although unstriped morphs may be more willing to explore new environments, there may be negative effects on their fitness. Future research examining baseline stress levels after exploring novel environments is a key next step in understanding the potential consequences of expressing bold behaviors in this color polymorphic species.

Neophobia Behavior

Striped and unstriped morphs of *P. cinereus* did not differ in neophobic responses to common litter items. However, I did find strong support for all individuals of *P. cinereus* demonstrating aversion to approaching the novel objects (styrofoam cup, soda can, and plastic water bottle), while all salamanders were comfortable approaching the rock (control item). This finding suggests that regardless of color morphology, salamanders can interpret new objects within their environment and demonstrate fear towards approaching them. Indeed, their responses did not statistically differ when presented with the three distinct novel objects. The lack of behavioral plasticity may be problematic because flexible responses are critical for the survival and success of a species in dynamic environments (Wong & Candolin, 2014). Fear towards all novel objects will likely dictate ecological functioning of salamanders within their natural habitats. For example, increased garbage within a salamander's territory may lead to a decrease in foraging. A study by Chejanovski et al. (2017) found that anoles from urban habitats expressed greater unwillingness to forage compared to lizards from more forested populations. The authors postulated that this result may be due to anoles in more urbanized environments perceiving greater predation risk than more pristine habitats. It is also possible that increased anthropogenic trash will reduce mating opportunities for

these salamanders if they are making decisions to avoid areas with these objects. *Plethodon cinereus* demonstrates elaborate courtship interactions (Gergits & Jaeger, 1990), and female choice of males is, in part, related to the quality of male territory (Mathis, 1991). Thus, mating interactions may decline as a function of increased litter objects.

Future Work

More research is necessary to understand the impact of urban environments on the behavioral responses of *P. cinereus*. To date, there have been few studies on the effects of urbanization on *P. cinereus*, which is surprising as this species is very common and is known to inhabit small, urban, habitat patches. In addition, these past studies are all related to genetic diversity and population sizes of these salamanders in urban areas compared to rural forests (Noel et al., 2007; Wilk et al., 2020; Gade et al., 2023). No studies have yet examined behavioral responses of *P. cinereus* to increases in urbanization even though these rapid changes in habitat may directly affect their fitness. Further, color morph responses to urban environments largely remains an open question. In this study, color morphs demonstrated different exploratory behaviors in response to a paved surface, but we did not find any differences in neophobia behavior. A clear path forward for this type of work would be to increase the scale and examine the same behavioral traits, boldness and neophobia, on salamanders from multiple populations, including sites that are in well-forested versus more urbanized areas. The collection site for this study, Caesar Creek State Park contains numerous trails covering 43 kilometers and spans nearly 4,700 acres. Many of the trails do not contain paved walkways including the location where salamanders were collected for this work. In addition, there was very

little garbage in our sample area. In comparison, urban parks are typically very small and experience much more human traffic, increasing the number of paved surfaces and trash. Thus, it would be interesting to examine whether increased exposure to these novel surfaces and objects alters behavioral responses of the salamanders. For example, it is possible that if an individual salamander comes into contact with a water bottle multiple times within their habitat the fear response may decrease.

Conclusion

Urbanization is rapidly altering natural landscapes. These alterations impact native wildlife by providing new challenges that negatively affect organisms residing within these areas (McKinney, 2002). In my study, there was strong support for urbanization pressures altering salamander behaviors. Contrary to my initial predictions, unstriped morphs performed better in urban environments compared to striped morphs by spending more time exploring novel paved surfaces. Thus, it may be possible that unstriped morphs will become more common in urban areas as they express trait sets (e.g., higher tolerance of drier conditions; Moreno, 1989; Anthony & Pfingsten, 2013) that allow them to exploit these non-natural environments. Examining changes in color morph frequency over time is tractable through use of historical collections data (Hantak et al., 2021). Although my study found that unstriped morphs appear to be more willing to explore urban landscapes, it is possible that this morph expresses higher stress levels – due to their increased thigmotaxis behaviors. Increased stress might ultimately affect the unstriped morphs health and longevity rendering the fitness consequences unknown. Anthropogenic garbage had an overall negative impact on *P. cinereus*, regardless of color morph. These neophobic tendencies towards novel litter objects could result in

diminishing populations of salamanders as there will be a decrease in suitable or familiar habitats. Overall, my study provides the first experimental evidence of boldness and neophobic behavioral traits in a color polymorphic salamander and increases our understanding of the role of behavior in novel urban environments.

Acknowledgements

I would like to thank Dr. Maggie Hantak for all of her incredible guidance and support throughout this entire thesis. I am also incredibly grateful to the Hantak Lab including Brendan Enochs, Emerson Amy, and Cara Brensike for their assistance in the lab and in the field.

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