This paper is firstly dedicated to Dr. Tammy Windfelder who inspired me to go into this field, and who without, this paper and my never-ending passion for animal behavior and conservation would not exist.

Special thanks to my family, my partner, my suitemates, and my research partners who would listen to me ramble on for hours about the swamp.

Incredibly special thanks to Chipmunk 15 who kept me on my toes.

Drew University

College of Liberal Arts

Stress Responses in Eastern Chipmunks (*Tamias striatus*) at Two Locations – Drew University and the Great Swamp Watershed Association's Conservation Management Area (GSWA-CMA)

A Thesis in Biology

by

Sierra Joy Williams

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Abstract

I conducted this research over a period of eight weeks from June to July of 2021 at Drew University and the Great Swamp Watershed Association's Conservation Management Area (GSWA-CMA). These two locations were used due to the assumption that the resident animals on Drew University's campus experience a high amount of human interaction, while the resident animals at the GSWA-CMA experience a lower amount of human interaction. It was hypothesized that the chipmunks caught at Drew University's campus would be less stressed on average than the GSWA-CMA chipmunks due to their increased human interaction, which would lead to habituation associated with reduced stress responses. In order to test this hypothesis, I used two different tests – a hanging mesh bag test and a hole board test. From those two tests, eight different behaviors were analyzed: amount of time immobile, latency to enter the hole board test, scanning, grooming, locomotion, rearing/jumping/climbing, head-dipping, and time spent in the periphery of the hole board test.

My hypothesis was statistically significantly supported by most the tests I conducted. I used the hanging mesh bag test to see whether the chipmunks exhibited a flight response, which is associated with increased stress levels. I used the hole board test to observe the stress associated with a novel environment using freeze responses and exploratory behaviors as measurable units by testing how long their latency was to enter the test, analyzing their high or low stress behavioral responses, and how much time they spent in the periphery versus the center of the test apparatus. From these tests, I found that the GSWA-CMA chipmunks spent more time exhibiting behaviors associated with high stress; they spent more time moving in the hanging mesh bag test (thus exhibiting a flight response), spent more time engaging in high stress and

exploratory behaviors (locomotion, rearing/jumping/climbing, and head dipping), spent less time engaging in low stress behaviors (grooming and scanning), and spent more time in the periphery of the hole board test as a means of anti-predation protection. There is also evidence of habituation over time from a GSWA-CMA chipmunk that was captured six separate times, as she went from moving often in the hanging mesh bag test, exhibiting a stressful flight response, to slowly becoming completely immobile by her third capture, which is a sign of habituation.

This research is unique and important, as research focusing on chipmunks and urbanization has been steadily increasing over time. My project will build on that growing amount of published knowledge by sharing valuable information about how small mammals are affected by human interaction, which can be explored further to determine whether human interaction is negatively affecting their ability to thrive in a world that is constantly urbanizing.

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Introduction

Animals and Urbanization

As humans take over land that was previously untouched and cities became larger and louder, many animals begin to suffer due to anxiety and stress. According to Plumptre et al. (2021), only 3% of terrestrial ecosystems are ecologically still intact, meaning that there is only 3% of land where there has been no "anthropogenic habitat conversion [or] transformation" (Plumptre et al. 2021). Most species on Earth are living in an area that is no longer considered ecologically intact, so to survive they either must adapt or find an area that is intact enough for them to thrive. Adapting is difficult, as urban areas have detrimental issues such as noise, light, and chemical pollution.

Some species can adapt to urban life, specifically those with a "broad environmental tolerance," which would allow them to survive and reproduce in many different environments (Bonier et al. 2007). In order to adapt to urban life, most species have to change their behaviors in a variety of ways, such as changing their home range, their diet, their activity budget, and their nocturnal activity (Ritzel & Gallo 2020).

Those that successfully adapt to urban life thrive because they have adapted different behaviors. One example of different behaviors can be seen in the urban great tit (*Parus major*) in Leiden, Netherlands; the great tits sing higher notes to be heard above noise pollution caused by highways and airplanes (Slabbekoorn & Peet 2003). Although this species of bird successfully adapted in terms of their communication, they seem to be unable to adapt to their urban food resources. The urban great tit has plumage differences depending on if the bird forages in an urban area or a rural area due to differing levels of carotenoids in their diet. It was found that the carotenoid concentrations were lower in urban caterpillars, the urban great tit's main source of protein, meaning that their food is lower quality, and their naturally bright plumage has been unable to adapt to this change and has become duller (Isaksson & Andersson 2007).

In some urban areas, humans tend to put out resources, such as bird seed, all year round for animals to consume. This helps with food insecurity commonly seen in urban animals, but has been found to cause some physiological differences. The Florida scrub-jays (*Aphelocoma coerulescenes*) that live in suburban areas have lower stress levels than rural Florida scrub-jabs. These differences in stress levels are correlated with the availability of food. The year-round human provided food sources allow those in suburban environments to reproduce sooner and have lower stress levels, thus creating measurable physiological differences (Schoech et al. 2004).

It is generally accepted common knowledge that urbanization leads to a loss in biodiversity, but in some cases wildlife can still be diverse in urban areas due to the fact that "anthropogenic resources outweigh the costs associated with urbanization," (Narango et al. 2017) specifically supplemental feeding (bird feeders, compost, gardens, etc.), as mentioned above, and the fact that yards with fences protect animals from predators. Although fences may protect prey animals from predators, the abundant food sources do cause prey populations to increase in abundance, leading predator animals to also thrive in urban areas (Hansen et al. 2020). On the other hand, in urban areas where it is not common for individuals to put out food year-round, it has been found that due to a lack of natural and native food sources, bird populations can decline over time (Narango et al. 2017).

An extreme example of how animals in urban environments have adapted to thrive is the green anole (*Anolis carolinensis*). Those that live in urban environments are shaped differently than those in rural environments, as those in urban environments tend to have shorter hindlimbs as they are only able to perch on leaves as opposed to branches and tree trunks, as leaves are more readily available in urban environments (Irschick et al. 2005). Behaviorally, those in urban environments are less wary and display more frequently than their rural counterparts, experience lower predation, but have higher intrasexual competition (Lailvaux 2020).

Although some animals have adapted over time to survive in the highs and lows of urban life, it is important to understand how urban environments are negatively affecting animals. There is little research on how urban environments affect wildlife; Magle et al. (2012) estimates that only 2% of all publications consist of urban wildlife studies. These studies are incredibly rare in animal behavior and ecology journals, but most highly published in landscape ecology, wildlife biology, and conservation journals. These published articles and journals are mainly focused on birds and mammals, but Magle et al. (2012) pointed out that future research should be more focused on fish, arthropods, and herpetiles (Magle et al. 2012).

This low number of publications is shocking, as within urban behavioral studies, 93% of animal's behaviors were different in an urban environment than in a more naturalistic setting (Ritzel & Gallo 2020). Behavioral changes were seen in 10 orders: Artiodactyla, Carnivora, Chiroptera, Didelphimorphia, Diprotodontia, Eulipotyphla, Lagomorpha, Peramelemorphia, Primate, and Rodentia. Behavioral changes that are associated with an acclimatory response to the urban environment are changes in activity, diel, diet use, mating and reproduction, social, and spatial. The most common of these behavioral changes are a decreased home range, followed by increased nocturnality, diet changes, and resource selection changes. Along with acclimatory responses, the most common change in regulatory responses is a change in vigilance, while the most common changes in developmental responses is increased boldness and exploratory behaviors (Ritzel & Gallo 2020). All of this goes to show that urbanization is drastically changing how a variety of animals function, and it is greatly important to study them to learn more not only about their changes in behavior, but how we can help them thrive in their ever-urbanizing environments.

Stress in Urban Environments

In natural environments, most stressors that wild animals experience are due to the sounds of other animals (Flower et al. 2014), social living dynamics (Koolhaas et al. 1997; Abbott et al. 2003), maternal separation (Yeoman 2018), predation (Psychological Stress in Wild Animals 2021), and food stress (Moshkin et al. 2003). In areas where humans are present, on the other hand, they experience entirely different stressors known as "human-induced rapid environmental change." Some of those human-induced changes include activities such as habitat fragmentation, habitat destruction, hunting, urbanization, and pollution (Ferguson 2016).

Although some animals, like the rock pigeon found many American cities, are considered habituated, which is when an animal is well-adapted to their urban lifestyle and human-induced environmental change (Rose et al. 2006), many wild animals are not and experience some level of stress in non-naturalistic environments where humans are present. Even though urban stress is a common occurrence in their lives, it is important to understand what the actual effect of the stress is, so the animal can thrive. In some cases, extreme stress can affect an animal's ability to reproduce (Einarsson et al. 2008), their foraging behaviors (Tu et al. 2019), and can increase disease and injury (Psychology Stress in Wild Animals).

Chipmunks

A common animal present in both urban and rural deciduous forests in the eastern United States and in southeastern Canada is the eastern chipmunk (*Tamias striatus*). They have a reddish-brown coat with a black stripe that runs down the center of their back surrounded by a white stripe lined in black on either side. They tend to be from 20-25cm (8-10in) long and weigh between 57-142g (Wildlife in Connecticut 2020). Despite their abundance in all areas from urban to rural environments, not much research has been done focusing on their behaviors in the wild, specifically their temperament around people. Looking into their stress responses caused by humans would be vital in learning more about other similar species that require conservation in similar environments.

In order to properly study the effects of human interaction on chipmunks, I caught chipmunks from two separate locations. One location, Drew University in Madison, New Jersey, has regular visitors present in a small arboretum where many urban animals call home. The other location, the Great Swamp Watershed Association's Conservation Management Area (GSWA-CMA), has fewer regular visitors to their large nature preserve.

Drew University

Drew University is a liberal arts college located in Madison, New Jersey. As of Fall 2020, Drew University had a total enrollment of around 1,600 students. Students that live on and off campus, faculty and staff, and Madison residents regularly use the sites found within Drew University's campus.

Drew University has approximately 84 acres (33.99 hectares (ha)) of public woods, and 45 of those acres (18.21 ha) are known as the Drew Forest Preserve. It is called the preserve because starting in 2008 and continuing today, Drew University students, faculty, and volunteers have been removing invasive plants and reintroducing native plants. Over 26 native species of trees, 85 different species of wildflowers and ferns, and hundreds of native shrubs have been planted. In order to preserve the newly planted native species within the wooded areas, the Forest Preserve is surrounded by 3m (10ft) high deer exclosure fencing which was put up in 2011. This fencing allows the replanted native plants to thrive, protecting rich biodiversity unseen in other parts of New Jersey. Within the Drew Forest Preserve, there have been approximately 120 species of bird observed, and plenty of amphibians, turtles, small mammals, and insects (Biodiversity and Ecological Restoration at Drew 2022). Within the Forest Preserve, there are two separate wooded areas; one known as the Zuck Arboretum, and one known as the Hepburn Woods, both of which are open to the public.

The Zuck Arboretum is an arboretum with a self-guiding nature trail that goes around two ponds. This section of woods is the most popular among students, faculty and staff, and town residents due to its beautiful scenery, well-kept path, and labeled plants. Across from the Zuck Arboretum is the Hepburn Woods. The Hepburn Woods is similar to the Zuck Arboretum, as it also has deer exclosure fencing, but it is slightly less popular due to its less well-kept paths. Both areas have had long-term small mammal population studies examining how the small mammal populations have changed due to the restoration work. These studies have been ongoing since Ian

DeStefano, a Drew University student at the time, initiated the project in 2009 (DeStefano 2010). Various courses also use these areas of woods to learn about the environment and conduct small-scale projects.

Among the non-preserved acres of woods, is a site called the President's House Woods, which is located in the southeast corner of Drew University's campus. This site has no deer fencing, little to no restoration work, and no specific trails. Despite being less popular overall, this site still has some long term small-mammal population studies, and some courses use this area to learn about the environment.

All of these sites have similar resident animals such as the eastern gray squirrel (*Sciurus carolinensis*), the raccoon (*Procyon lotor*), the eastern chipmunk (*Tamias striatus*), the white-footed mouse (*Peromyscus leucopus*), the deer mouse (*Peromyscus maniculatus*), the Virginia opossum (*Didelphis virginiana*), the red fox (*Vulpes vulpes*), the groundhog (*Marmota monax*), the striped skunk (*Mephitis mephitis*), the southern flying squirrel (*Glaucomys volans*), and the Northern short-tailed shrew (*Blarina brevicauda*), the white-tailed deer (*Odocoileus virginianus*), and many of the common New Jersey birds such as the blue-jay (*Cyanocitta cristata*) and northern cardinal (*Cardinalis cardinalis*).

The Great Swamp Watershed Association's Conservation Management Area (GSWA-CMA)

The Great Swamp Watershed Association's Conservation Management Area (GSWA-CMA) is a conservation site whose goal is to protect and improve the "health of the Passaic River through science, education, land preservation, stewardship, and advocacy" (Great Swamp

Watershed Association(c) 2022). The GSWA-CMA first acquired 73 acres (29.54 ha) of land from Harding Township, New Jersey, in 1996, and that land is known as the Conservation Management Area. All of their locations combined have incredibly important ecosystems such as wetlands, freshwater marshes, vernal pools, forests, and the Silver Brook. The Silver Brook is a tributary of the Great Brook and the Passaic River, and it contains many state and federally threatened species, such as the wood turtle (*Glyptemys insculpta*) and barred owl (*Strix varia*). As of August 2017, the GSWA-CMA was designated an accredited land trust by the National Land Trust Accreditation Committee, which made it one of only eight land trusts in New Jersey (Great Swamp Watershed Association(a) 2022).

Similar to Drew University's Forest Preserve, the GSWA-CMA also has a network of trails and deer exclosure fences, although on a much larger scale. The GSWA-CMA has about three miles of boardwalk and paths that are open to the public and schools (Great Swamp Watershed Association(a)). Although open to the public, it is estimated that it has less visitors overall than the amount of people that use the Drew Forest Preserve on a daily basis.

The GSWA-CMA has similar resident animals as the Drew University sites, but some additional residents such as the American mink (*Neogale vison*), the black bear (*Ursus americanus*), the long-tailed weasel (*Neogale frenata*), and the meadow jumping mouse (*Zapus hudsonius*).

Project Overview

My project was funded by the Mellon Grant and conducted at Drew University's research program, Drew Summer Science Institute (DSSI). It was conducted over a period of eight weeks from June to July of 2021.

This is a behavioral research project focused on comparing the temperament and stress levels of the eastern chipmunks at the two locations described above, Drew University and the GSWA-CMA. These two locations were used because Drew University's campus has more people who are present in the small arboretum, while the GSWA-CMA has less people who are present in the large land preserve. It should be noted that the vegetation is different at these two sites, but that should have had no effect on my research, as the natural vegetation does not affect the animal's responses to human interaction due to the fact that we trap the animal, therefore removing them from any vegetation. By using these two different locations, I was able to form an understanding of whether there was a difference in stress responses from those chipmunks due to human interaction.

My research will help build upon current knowledge on how chipmunks, and other comparable small mammal populations are being affected by human interaction and whether that interaction is negatively affecting their ability to thrive. I predicted that the chipmunks caught on Drew University's campus would be less stressed on average than the chipmunks caught at the GSWA-CMA due to habituation from the increased human interaction. The chipmunks caught at the GSWA-CMA would be more stressed on average due to the fact that they rarely interact with humans, and therefore were not as habituated or docile. Looking at their temperament and stress responses relates to their responses to human interaction because it has been shown that an animal's temperament can play a role in its habitat use, predation avoidance, dispersal, and social behavior (Martin & Reale 2008), aspects of fitness (Dingemanse & Réale 2005).

In order to collect data to support my hypothesis, I conducted two different tests; a hanging mesh bag test and a hole board test. With these two different tests I analyzed nine different behaviors: amount of time immobile, latency to enter the hole board test, scanning, grooming, locomotion, rearing and jumping, head-dipping, and time spent in the periphery of the hole board test.

Methods

Setting up the Traps

Traps and Bait

I used thirty traps total (10 traps per grad, and 3 grids per site) to catch the animals in the field, all from Live Trap (livetrap.com): one extra-large Tomahawk trap (raccoon/feral cat collapsible trap #207) measuring 32x10x12 inches, three large Tomahawk traps (squirrel/muskrat collapsible trap #202) measuring 19x6x6 inches, three medium Tomahawk traps (chipmunk/rat collapsible trap #201) measuring 16x5x5 inches, and three small Sherman traps (rigid mouse/vole trap #101) measuring 10x3x3 inches. The Tomahawk traps were metal barred cages with a weight-triggered platform that shut one front door, while the Sherman traps were either a solid or perforated metal cage with a weight triggered platform that shut one front door.

While setting up the Tomahawk traps, we placed an appropriately sized stick in the entrance of the front door to prevent any of the animal's tails from becoming trapped or broken when the door closes; the stick did not prevent the door from closing, but created a small gap so their tail would not get stuck. When each trap was placed, we tested them to make sure they were functioning properly and that the stick was big enough to create the small gap. We also put cardboard over the top of each of the traps for shade from the sun and leaf litter inside of each trap for some cover for the animal. Figure 1 has images of each of the traps with cardboard overtop, sticks in the entrance, and leaf litter in the traps.

I had permission to conduct this trapping research from the New Jersey Department of Environmental Protection's Division of Fish and Wildlife (SC 2021108 for exotic non-game species and SC 2021-02 for game species), and all procedures were approved by Drew's Institutional Animal Care and Use Committee (Approval #19-01).

Figure 1. Images of each of the trap sizes with visible leaf litter inside and cardboard over top. In the large trap image, you can clearly see the stick coming out of the entrance to prevent any animal tails from getting stuck in the trap door. (a) is a small Sherman trap, (b) is a medium Tomahawk trap, (c) is a large Tomahawk trap, and (d) is an extra large Tomahawk trap.

Each trap had a different amount of bait correlated to the size of the trap. The bait consisted of peanut butter, peanuts, apple slices, and oats on a leaf placed just behind the trigger platforms. Figure 2 shows an image of three large baits.

- Extra large trap: one large scoop of peanut butter, 1/2 of an apple slice, 5 peanut halves, some oats.
- Large trap: one large scoop of peanut butter, 1/3 of an apple slice, 4 peanut halves, and some oats.
- Medium trap: one medium scoop of peanut butter, 1/4 of an apple slice, 3 peanut halves, and some oats.
- Small trap: one small scoop of peanut butter, 1/5 of an apple slice, 2 peanut halves, and some oats.

Figure 2. An image of three large baits on a log.

Sites and Grids

At the two different locations (Drew University and the GSWA-CMA), we trapped at four different sites. Three of those sites were on Drew University's campus: the Zuck Arboretum (6/7/2021-6/11/2021), the Hepburn Woods (6/14/2021-6/18/2021), and the President's House Woods (6/20/2021-6/25/2021). The last site was located at the GSWA-CMA (7/14/2021-7/19/2021). Each site had two trail cameras placed near the extra-large traps. We were at each site for five 24-hour periods, and the trail cameras were placed at the sites three days prior to trapping and remained three days post trapping to collect data regarding which animals were present in the area.

Each of those four sites had three different grids which were numbered for ease. A grid is a section within the site where traps were set up in rows, which can be seen in Figure 3. Each grid had all ten traps placed via alternating sizes. At the Zuck Arboretum and the Hepburn Woods, one grid was outside of the deer exclosure fencing with a trail camera, while the other two were located within the deer exclosure fencing with another trail camera; Table 1 offers a more in-depth explanation of the site system.

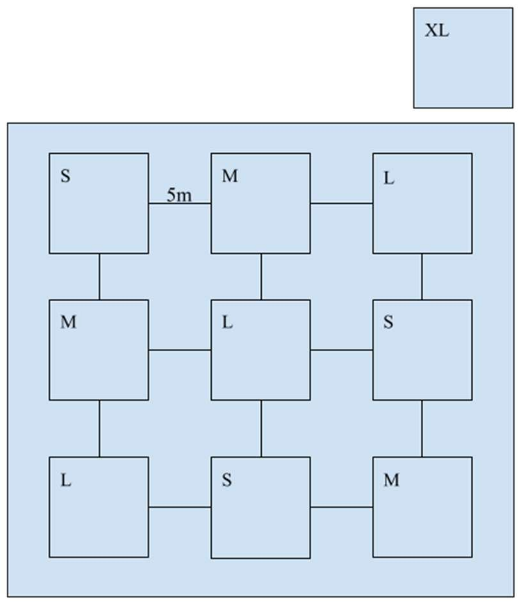


Figure 3. Sample diagram of a grid per site, inspired by Ilianna Anise’s (2017) thesis.

Table 1. Site and grid organization, inspired by Iliana Anise's (2017) thesis.

Location	Site	Dates	Grids within deer exclosure	Grids outside deer exclosure
Drew University	Zuck Arboretum	6/7/2021-6/11/2021	#1, #2	#3
	Hepburn Woods	6/14/2021-6/18/2021	#10, #11	#12
	President's House Woods	6/20/2021-6/25/2021	--	#4, #5, #6
Great Swamp Watershed Association (GSWA)	Conservation Management Area (CMA)	7/14/2021-7/19/2021	#7, #8, #9	--

Within two of the grids per site, we would place a hole board test for easy access. Figure 4 shows an image of the hole board test taken from a videoclip.

Figure 4. A screenshot looking down into the hole board test from a video that was analyzed. From this angle, you can clearly see the wooden board with the PVC pipe holes, the small trap's door that allows the chipmunk to enter the trap, the Plexiglas preventing the chipmunk from jumping out, and the reflection of the camera that was used to record the videos.

Checking the Traps

Times and Weather

The traps were first checked around sunrise, which was generally around 6 am. Our last trap check was at sunset, generally around 8:30 pm, to make sure that they were all empty before we left, and then the traps were left overnight to catch any nocturnal or early rising animals.

During the day, we checked the traps at different intervals depending on the current heat index (in Fahrenheit) found on the Weather App to prevent the animals from becoming dehydrated or getting heat stroke. We checked them:

- At least every 3 hours for a heat index in the 70s.
- At least every 2 hours for a heat index in the 80s.
- At least every hour for a heat index in the 90s.
- At least every thirty minutes for a heat index over 100.

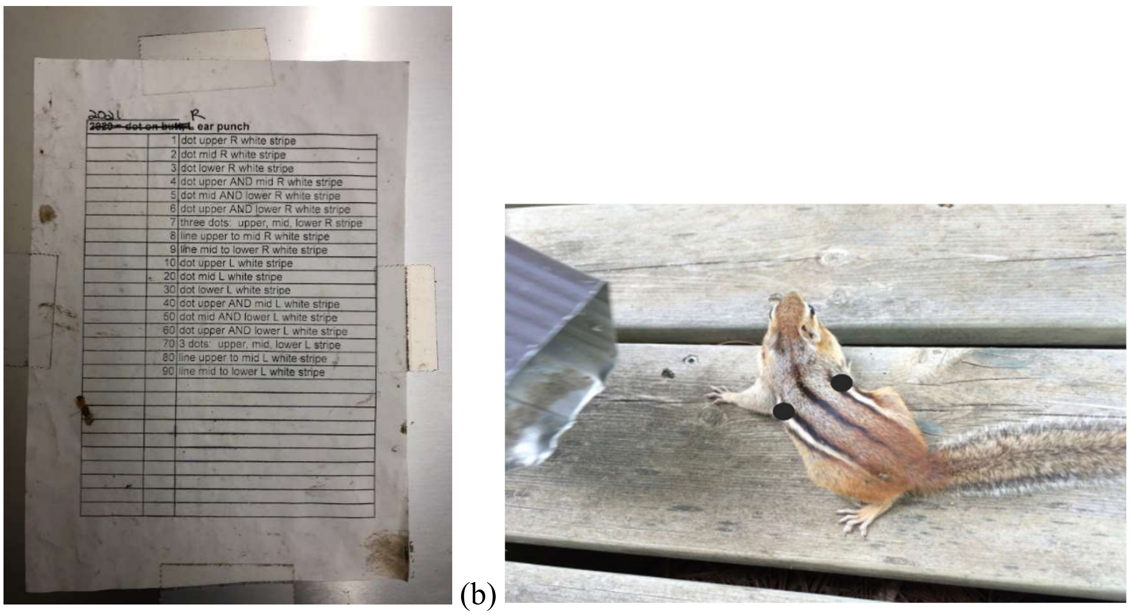
If the weather consisted of storms that would lead to flooding, we would check the traps right before the heaviest portion of the storm and close them for the night. If the rain was not going to lead to flooding, we would check the traps at the normal time intervals.

Chipmunks

The chipmunks were either found in a medium trap or a small trap throughout the day. In both size traps, we would first check if the chipmunk was visually stressed (biting the bars, banging into the bars, panting, etc.), if so, we would cover the trap with a towel until we were ready to process them.

If the chipmunk was in a small trap, we would transfer them into a medium trap in order to look at them properly using leather gloves. If the chipmunk was not previously marked with dye, we would mark a pattern on the white back stripes according to a dyeing key while within the medium trap. The dyeing key can be seen in Figure 5, if the chipmunk was the 1st caught we would only dye one dot in the upper portion of their right white stripe, while if they were the 11th chipmunk caught, we would dye both the dot in the upper portion of their right white stripe (this dot signifies 1) and one dot in the upper left stripe (this dot signifies 10). The dye we used was Just for Men Mustache and Beard Dye (Jet Black), as that is a non-toxic and also dark hair dye that has worked well for similar projects in previous years. The chipmunks were dyed to identify them, as we did not pierce them with identifying ear tags. At this point we would also treat any obvious wounds with alcohol or water as appropriate and count any visible ticks.

(a) (b)



2021 R
~~2020 – dot on butt, L~~ ear punch

	1	dot upper R white stripe
	2	dot mid R white stripe
	3	dot lower R white stripe
	4	dot upper AND mid R white stripe
	5	dot mid AND lower R white stripe
	6	dot upper AND lower R white stripe
	7	three dots: upper, mid, lower R stripe
	8	line upper to mid R white stripe
	9	line mid to lower R white stripe
	10	dot upper L white stripe
	20	dot mid L white stripe
	30	dot lower L white stripe
	40	dot upper AND mid L white stripe
	50	dot mid AND lower L white stripe
	60	dot upper AND lower L white stripe
	70	3 dots: upper, mid, lower L stripe
	80	line upper to mid L white stripe
	90	line mid to lower L white stripe

Figure 5. (a) Picture of dyeing chart used to dye and identify chipmunks for 2021. (b) Example of a chipmunk dyed, according to our dyeing key, this chipmunk would be chipmunk #11, as it has one dot in its upper right white stripe and one dot in its upper left white stripe. Image source: https://inaturalist-open-data.s3.amazonaws.com/photos/1904062/large.jpg.

After they were dyed, while wearing leather gloves over our nitrile gloves, we would transfer them into a mesh bag to conduct the hanging mesh bag test, where we held them suspended in the air for one minute (further information regarding the test can be found on page 30).

Next, we would weigh the chipmunk in the mesh bag by attaching a 300g Pesola to the bag and holding it in the air. The bag was tared after the chipmunk was transferred into a small trap for the hole board test.

If the chipmunk's right ear was not previously pierced, we would record the dye markings in our field notes and pierce the right ear after conducting the hanging mesh bag test, while if their right ear was already pierced, we would record their dye markings in our field notes. If the chipmunk's left ear was pierced, we would pierce their right year, as a piercing on the left ear corresponded with even numbered years, while a piercing on the right ear corresponded with odd numbered years. If both of their ears were pierced that meant that the chipmunk had been captured now three years in a row, and we would process as normal without piercing their ears. This was done as the piercing would stay present throughout multiple years so we can calculate when, if they were ever, first captured, while the dye would only stay present for the summer, so we could uniquely identify them.

To pierce their ears, we securely held the chipmunk against the ground while they were still in the mesh bag. When it is immobile, we would pull their right ear out of the bag and pierced their ear quickly using an ear piercer (1539 compound action punch from National Band and Tag Co., Newport, KY). After the ear was pierced, we would then squeeze gauze against the new hole to reduce bleeding and if after 60 seconds the ear was still actively bleeding, we would use a styptic pencil to stop it.

Before the hole board test, we recorded the general age (juvenile, subadult, adult), sex, weight, which trap they were found in, any markings (dye, injury, and/or unique natural patterning), location of ear piercing, and their hanging mesh bag test results in our field notes.

For the hole board test, we transferred the chipmunk from the mesh bag into an available and empty small trap to allow them to enter the hole board test. Finally, once the hole board testing was complete, we encouraged them to leave the test within 2-5m of their original trapping location so they can easily find their burrows.

Non-Chipmunk Animals

Raccoon and Opossum

The raccoons and opossums were found in the extra-large traps most commonly during the first check of the day, as they are nocturnal. We would first check if the animal was visually stressed (biting the bars, banging into the bars, panting, etc.), if so, we would cover the trap with a towel until we were ready to process them.

Once ready to process, we first checked if we previously marked them with dye on their body or on their ears. If they were marked, we recorded the pattern in our field notes. If they were not marked, we created a new unique marking, and again record the pattern in our field notes. The dye we used was Just for Men Mustache and Beard Dye (Jet Black), as that is a non-toxic and also dark hair dye that has worked well for similar projects in previous years. We also recorded the general age (juvenile, subadult, adult), sex, trap number, any markings (dye, injury, and/or unique natural fur patterning) in our field notes. If there were any obvious wounds, we treated them with water or alcohol as appropriate.

To release the animals from the trap, those who had an up to date rabies vaccine would wear Kevlar gloves over nitrile gloves, and using sticks to keep distance from the opening of the trap, would open the door and encourage the animal to exit. As the animal walked away, we would confirm their sex if possible.

Squirrel

The squirrels were found in the extra-large traps, large traps, and medium traps throughout the day. We would first check if the squirrel was visually stressed (biting the bars, banging into the bars, panting, etc.), if so, we would cover the trap with a towel until we were ready to process them.

Once ready to process, we first checked if the squirrel was previously marked with dye on their head or body and/or if they were previously tagged with an identifying ear piercing.

If the squirrel was in an extra-large trap and had neither dye nor tag, we would dye their body with a new unique marking and record the pattern in our field notes, as it is impossible to pierce the squirrel's ears in an extra-large trap. The dye we used was Just for Men Mustache and Beard Dye (Jet Black), as that is a non-toxic and also dark hair dye that has worked well for similar projects in previous years. If they were marked with dye previously, we would record the pattern in our field notes and identify them, if they were tagged we would attempt to record the unique ear tag ID. If the squirrel was not cooperating and was visibly stressed, we would just create a new unique mark on their body and record the pattern in our field notes.

If the squirrel was in a large or medium trap and had no ear tag, we would pierce the left ear if female and the right ear if male. To pierce the ear, while wearing leather gloves, we would stuff the trap with towels until the squirrel was completely immobile with the correct ear available to grab. We would then pull the ear into an appropriate position to tag using an ear tag piercer and new ear tag ID (Monel small ear tags 1005-1 from National Band and Tag Co., Newport, KY), and pierce the ear quickly. Once the ear was pierced, we would remove the towels and record the new ear tag ID number. If the squirrel already had an ear tag ID, we would record the ID number.

After dyeing or tagging the squirrels, we would record the general age (juvenile, subadult, adult), sex, trap number, and any markings (dye, injury, and/or unique natural fur patterning) in our field notes. If there were any obvious wounds, we would treat them with water or alcohol as appropriate and remove any large ticks.

If the squirrel was in an extra-large trap, at this point we would open the trap while wearing leather gloves and encourage the squirrel to exit. If the squirrel was in a large or medium trap, we would weigh them.

To weigh the squirrel, we placed a nylon bag around the opening of the cage door while wearing leather gloves over nitrile gloves. We would then open the cage door and encourage the squirrel to enter the bag. Once the squirrel was in the bag, a person wearing Kevlar gloves over their nitrile gloves would hold the squirrel in place at the end of the bag so another person could close the bag and attach a 1kg Pesola to the bag. The person wearing the Kevlar gloves would then hold the bag in the air via the Pesola to record the weight of the squirrel. Then, the bag would be opened while the squirrel was held secure in the bottom of the bag, and the squirrel would be released by holding the open bag close to the ground. Once the squirrel was out of the bag, we would tare the bag to get the total weight of the squirrel and record the weight in our field notes.

Bird

On the rare occasion a bird was in trapped in either an extra-large or large trap, we would immediately open the door and encourage the bird to exit to reduce the likelihood of injury. Afterwards, we would record the species, sex, and trap number in our field notes.

Mouse

Mice were found in small traps most commonly during the first or last checks of the day. As the small traps are entirely enclosed without bars, we did not check if the animal was visually stressed before processing.

To process them, we would transfer them into a mesh bag while wearing leather gloves over nitrile gloves. At this point, we would treat any obvious wounds with alcohol or water as appropriate, and count any large ticks.

If the mouse was not previously marked with dye, we would mark a unique pattern on their body and record the pattern in our field notes. The dye we used was Just for Men Mustache and Beard Dye (Jet Black), as that is a non-toxic and also dark hair dye that has worked well for similar projects in previous years. We would then record the length of its right hind foot and tail. To do so, we would hold the mouse securely by the base of its tail with its body supported on the person’s hand with a leather glove protecting them, while their other hand would be wearing nitrile gloves and measure the foot and tail with a ruler. If the mouse started biting the measurer, an additional person distracted the mouse by holding a leather glove for it to bite.

After they were dyed and measured we would conduct the hanging mesh bag test, where we held them suspended in the air for one minute (further information regarding the test can be found on page 30). Next, we would weigh the mouse in the bag by attaching a 100g Pesola to the bag and holding it in the air. The bag was tared after the mouse was transferred back into an empty available small trap for the hole board test. While the mouse was being transferred, we would record the general age (juvenile, subadult, adult), sex, weight, trap number, any markings (dye, injury, and/or unique natural patterning), their measurements, and their hanging mesh bag test results).

For the hole board test, we transferred the mouse from the mesh bag into an available and empty small trap to allow them to enter the hole board test. Finally, once the hole board testing was complete, we encouraged them to leave the test within 2-5m of their original trapping location so they can easily find their burrows.

Behavioral Tests

Hanging Mesh Bag Test

A hanging mesh bag test is when a chipmunk is suspended in a bag for one minute, and the amount of time the chipmunk spent immobile is recorded. If the chipmunk spent more time immobile than not, that meant that they were not as stressed in the presence of humans, as inspired by Martin & Reale (2008)'s study where they used high static time (immobility) to show that the animal was more docile, and therefore less stressed.

To conduct the hanging mesh bag test, we put the small animal (chipmunk or mouse) into a mesh bag while wearing leather gloves over our nitrile gloves. We then held the mesh bag in the air, and with a stopwatch on our phones, recorded when it was moving and not moving for 60 seconds. We then transferred the animal into an available and empty small trap for the hole board testing.

Hole Board Test

We also conducted a hole board test, which is an "open field test that measures the behavioural reaction of an animal in a novel environment from which escape is prevented by a surrounding wall" (Martin & Reale 2008). In my hole board test, I looked at eight different behaviors: latency to enter the hole board test, scanning, grooming, locomotion, rearing and jumping, head-dipping, and the time spent in the periphery of the test. I correlated each of these behaviors with a different level of stress, and the results were recorded after the test took place via video footage analysis. This test is less reliable than the hanging mesh bag test as certain behaviors the chipmunks exhibited (specifically scanning, grooming, locomotion, rearing and jumping, and head-dipping) was up to interpretation and could have been biased by the recorder. Also, the camera quality at times was not perfect. The hanging mesh bag test is more reliable as there is no interpretation of whether the chipmunk is immobile or not, and the results were directly recorded in the field, not afterwards via video footage analysis.

While analyzing the data, I did not take into account recaptures even though most of the chipmunks had at least one recapture. This is because in each recapture, there was a chance that the individual chipmunk could have habituated to humans or accumulated more stress overtime, which could lead to skewed results (Martin & Reale 2008). One chipmunk, Chipmunk 15 from the GSWA-CMA, is mentioned due to the fact that she was captured a total of six times, more than any chipmunk captured, and she does show signs of habituation over time, specifically in the mesh bag handling test.

My hole board test is made out of an industrial tote box with a wooden board at the bottom that has eight alternating and equally spaced holes made out of cut PVC piping (which can be seen in Figure 4). There was a small rectangular hole near the bottom cut out of the side of the box so the small trap could be easily placed inside of the box and removed quickly; this hole will be referred to as the small trap entrance. For recording, we put two pieces of plexiglass on top of the box and set up a recording camera above the hole board test, or had another person hold a recording phone above the test. We wrote down the animal's ID on an index card and put it on the plexiglass in view of the recording for identification for the videos, and removed the ID card once the animal was within the box.

Once the small animal was in an empty small trap, we put the backside of the trap into the small trap entrance and while wearing leather gloves over our nitrile gloves, we then opened the trap door by reaching our hand into the box, closed the plexiglass, and removed the ID index card. We did not encourage the animal to exit the small trap until 180 seconds passed. Once the animal exited the small trap, we removed it and put a plastic square over the test's entrance so the animal could not escape. If the animal needed encouragement to exit the small trap, we gently banged on and shook the small trap for 120 seconds until the animal exited. If the animal did not exit after 120 seconds of encouragement, we opened the plexiglass, closed the small trap, closed the entrance, and released the animal directly into the test by opening the small trap, and then closed the plexiglass once the animal was within the test. If the animal left the small trap quickly, meaning their latency to enter the hole board test was low, that was evidence that they were less stressed as opposed to if they took a long time to exit the small trap.

When the animal was in the hole board test, we left the animal in the test with a camera and phone recording for 300 seconds. After 300 seconds passed, we removed the plastic closing the entrance, and encouraged the animal to exit the test. We then transferred the video files to a computer and analyzed the recordings for specific behaviors as listed in Table 2.

Table 2. Definitions of analyzed hole board test behaviors. Definitions based off Martin & Reale (2008).

Behavior	Definition
Latency to enter the hole board test	The amount of time it takes the animal to leave the small trap and enter the hole board test with a maximum of 180 seconds before the animal was encouraged to enter the test via gentle banging and shaking of the small trap.
Scanning	The amount of time the animal spent moving its head while its body was still.
Grooming	The amount of time spent grooming.
Locomotion	The amount of time the animal was moving, but not jumping or rearing; the amount of time the animal spent running and walking.
Rearing, jumping, and climbing	These three terms are combined as they looked incredibly similar when analyzing the video footage and are all considered to be similar in terms of amount of stress.

	Rearing is the amount of time the animal was standing on its hind legs. Jumping is the amount of time the animal was jumping into the walls or plexiglass of the trap. Climbing is the amount of time a chipmunk spent trying to a climb the wall, but not necessarily jumping to do so.
Head dipping	The amount of time the animal spent putting its head into the PVC pipe holes at the bottom of the hole board test.
Periphery and center of the hole board test	The amount of time the animal spent either near the walls (the periphery) of the test and within the center of the hole board test.

Results

Hanging Mesh Bag Test

While hanging in a mesh bag during their first capture, the Drew University chipmunks spent significantly more time immobile than the GSWA-CMA chipmunks on average (Fig. 6; t-test: t(2)=1.24E-05, p<0.025). This indicates that the GSWA-CMA chipmunks may have been more stressed and exhibited more of a flight response around humans than the Drew University chipmunks.

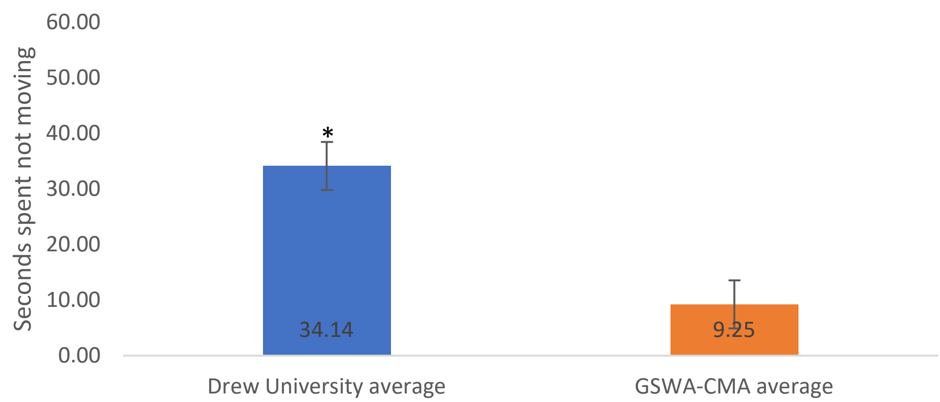


Fig 6. Average amount of time (seconds) each chipmunk from Drew University and the Great Swamp spent not moving in a mesh bag handling test on their first capture

Hole Board Test

Latency to Leave

While in a small trap that opens to the hole board test, the Drew University chipmunks and GSWA-CMA chipmunks did not have a significant difference regarding exiting the trap into the test (Fig. 7; t-test: $t(10)= 0.753$, $p<0.05$).

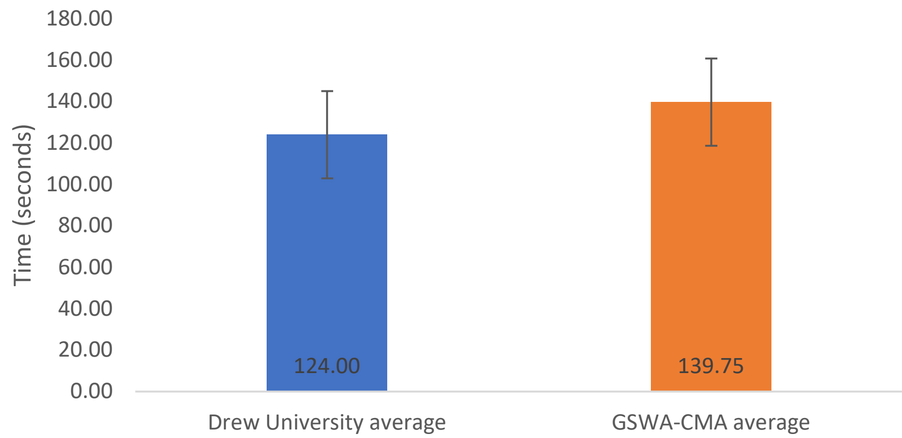


Fig 7. Average amount of time (seconds) it took Drew University and Great Swamp chipmunks to leave the trap into the hole board test

Behavioral Analysis

Five separate defined behaviors were analyzed for each chipmunk within a 120 second time frame, in order to account for escapes or methodological differences that occurred at the beginning of this research (Fig. 8a). Rearing, jumping, and climbing were combined to reduce bias as all of those behaviors look incredibly similar when analyzing the video footage. The five behaviors were then averaged together as either a high stress or a low stress behavior (Fig. 8b). Locomotion, rearing/jumping/climbing, and head dipping were considered high stress behaviors while grooming and scanning were considered low stress behaviors. It can be seen, and was statistically supported, that the GSWA-CMA chipmunks spent more time, on average, exhibiting behaviors considered high stress, thus showing that they were more stressed than the Drew University chipmunks (Fig. 8b; t-test: t(2)=0.00934, $p<0.025$).

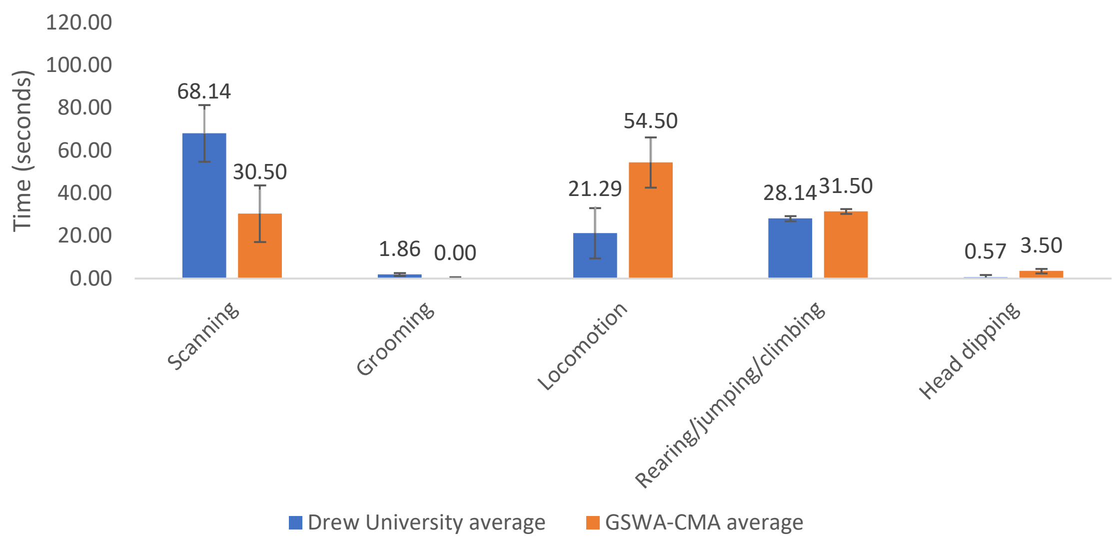


Fig 8a. Average amount of time (seconds) individuals from Drew University and the Great Swamp spent exhibiting specific behaviors within the first 120 seconds after entering hole board test

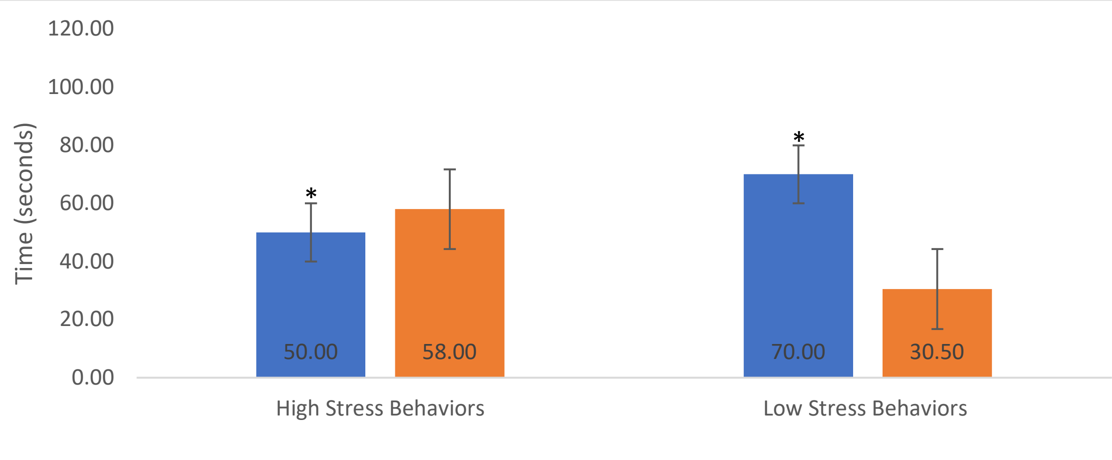


Fig 8b. Total averages of chipmunks exhibiting either high stress or low stress behaviors. High stress behaviors consist of head dipping, locomotion, and rearing/jumping/climbing. Low stress behaviors consist of scanning and grooming.

Periphery Test

While within the hole board test, average times were calculated regarding how often the chipmunks were in the periphery of the test and the center of the test. The GSWA-CMA chipmunks spent statistically significantly more time in the periphery of the hole board test compared to the Drew University chipmunks (Fig. 9; t-test: $t(2)=1.38E-04$, $p<0.025$). This indicates that the GSWA-CMA chipmunks may have been more stressed than the Drew University chipmunks.

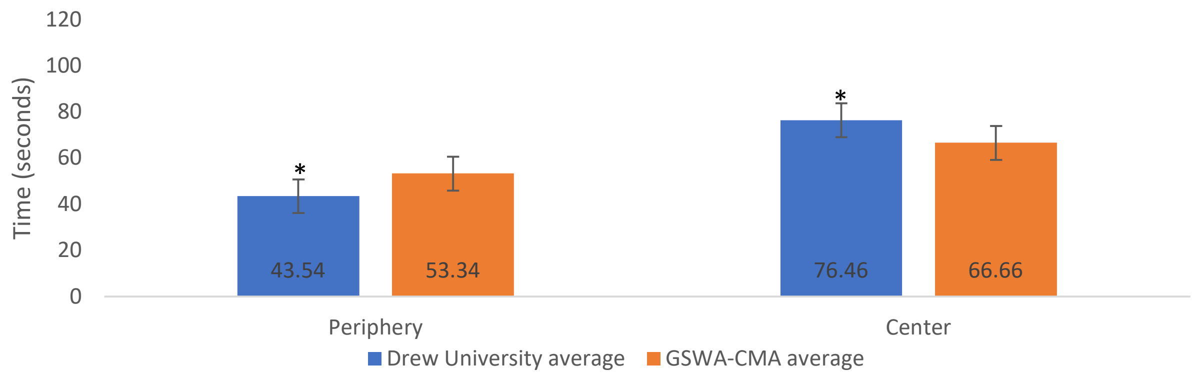


Fig 9. Average amount of time (seconds) chipmunks were at the periphery of the hole board test or in the center of the hole board test

Discussion

To reiterate, I predicted that the chipmunks caught on Drew University's campus would be less stressed on average than the chipmunks caught at the GSWA-CMA due to habituation from the near constant human interaction, while the chipmunks at the GSWA-CMA would be more stressed since they rarely interact with humans, and therefore would not be as habituated or docile.

I based this prediction off of the fact that Drew University has 84 acres (33.99 ha) of woods and hundreds of resident students and faculty, commuters, and town residents that regularly use the woods in all seasons and who sometimes venture off path into the woods. The GSWA-CMA has 73 acres (29.54 ha) of woods, but in total the Great Swamp National Wildlife Refuge itself has over 7,500 acres (3035.14 ha) of habitat (Great Swamp Watershed Association(b) 2022) and has the most visitors in the spring and summer months who tend to only stay on the designated paths and who are directly told to not interact with the wildlife or litter. Due to the fact that the chipmunks on Drew University's campus regularly experience human interaction either directly or indirectly (via food, noise pollution, litter, etc.), I assumed that those chipmunks would be more habituated.

Also, looking at our trail camera footage, it seems that the Drew University sites had more chipmunks who were around our traps pre, during, and post trapping (Fig. 10). This increased amount of chipmunks seen on video could be evidence that the chipmunks were used to these trail cameras and traps being around and therefore were more willing to investigate them, or they did not view humans as a threat, and were more willing to investigate the area. The

Zuck Arboretum on Drew University's campus may have also had a larger population size compared to all of the other sites leading to more chipmunks seen on the trail camera footage.

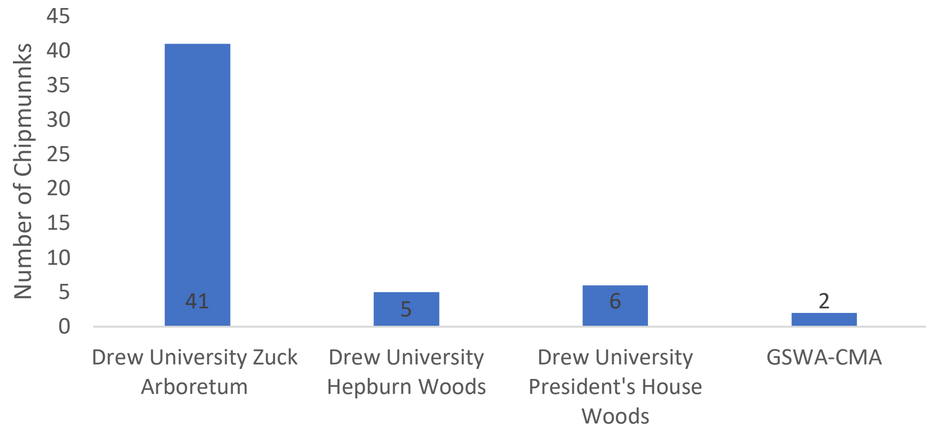


Fig 10. Total numbers of chipmunks seen on trail camera footage pre, during, and post trapping

Hanging Mesh Bag Test

I predicted that the GSWA-CMA chipmunks would have been more stressed when handled by a human than the Drew University chipmunks, and therefore would have exhibited a flight response to get away from the potential predator.

One explanation for why the GSWA-CMA chipmunks did indeed spend more time moving than not could be explained by Hernández et al. (2018). In their study of wild wood mice (*Apodemus sylvaticus*), they found that the mice that were ready to breed had a faster flight response than mice that were not ready to breed, perhaps due to their need to survive to reproduce and take care of their young (Hernández et al. 2018).

We caught our chipmunks during the summer, which is when their second breeding season is. Eastern chipmunks specifically breed in early spring, and then again in July, and have a thirty-one day gestation period (Eastern Chipmunk 2022). We caught the GSWA-CMA chipmunks mid-July, which is when they would have been gestating, and caught the Drew University chipmunks in June, so they were not quite yet mating or pregnant at that point. Because of the specific time we caught the GSWA-CMA chipmunks, it makes more sense that the breeding age chipmunks would have a flight response instead of a freeze response, as they would have been gestating at that point and viewed humans as a potential predator that could negatively affect their gestation.

In regards to freezing versus flight responses, Eilam (2005) mentions that a freeze response is only efficient if used “before the prey is spotted by the predator,” once the prey is spotted, or captured in this case, it is most efficient to flee in order to attempt to evade the attack to survive (Eliam 2005). Due to the fact that the chipmunks may have been in breeding season and the GSWA-CMA chipmunks viewed humans as potential predators, the flight response would have been the logical response for them to take to try to escape the stressful situation.

Also, previous studies, such as Martin & Réale (2008), used the handling mesh bag test to measure docility in chipmunks. Docility “has been defined as the reaction of an animal towards humans.” It is apparent that the Drew University chipmunks spent more time not moving, which could be evidence that they were more docile towards humans, as they are more often around humans that can be associated as non-threatening predators.

Hole Board Test

Latency to Leave

I predicted that the GSWA-CMA and Drew University chipmunks would be equally as wary to enter a new environment due to stress, and this was the case, as the majority of both chipmunk populations did not leave the trap during the initial 180 seconds, perhaps due to a freeze response.

Regarding flight versus freeze stress responses, in the hanging mesh bag test, the chipmunks were forced into a flight response, as they were now captured by a potential predator and a freezing response would no longer be efficient (Eliam 2005). In this case however, the chipmunk was not only in an environment it was familiar with, but in an enclosed environment, meaning that the potential predator could no longer see them. Because the potential predator could not see them in the small trap, but the possibility of being seen in a new novel and seemingly open-topped environment was high, the chipmunks exhibited a freeze response in order to survive.

I believe the novel environment plus potential predator threat created a high risk for both the Drew University and GSWA-CMA chipmunks, causing neither of them to enter the test faster than the other. In the future, I recommend perhaps increasing the time period to 300 seconds, to see if those additional 120 seconds would allow the chipmunk to become comfortable with the novel testing space and enter it willingly.

Behavioral Analysis

I recorded five different chipmunk behaviors which were defined in the methods section of this paper; locomotion, jumping/climbing/rearing, head dipping, scanning, and grooming.

Scanning is considered low stress as the animal is in a vulnerable position, remaining completely still, looking around, and not making an attempt to hide. All of these combined possibly shows that the animal is relatively comfortable with its environment. The Drew University chipmunks spent the most time on average scanning, which supports the hypothesis that the Drew University chipmunks were the least stressed by human interaction due to their habituation from being around humans more often than the GSWA-CMA chipmunks.

Grooming is also considered low stress. According to Daniels et al. (2004), in rats, grooming was found to mean that the rats were stressed. But, Martin & Reale (2008)'s chipmunks had some grooming, but did not have "a complete cephalocaudal progression of grooming," which is a cycle of grooming associated with the rodents like in Daniels et al. (2004)'s study and also in Kalueff & Tuohimaa (2005)'s study. So perhaps in chipmunks, grooming is not a sign of stress, but is only seen when the animal needs to groom for cleanliness, as in my experiment the chipmunks were only seen to be grooming when it was raining outside. Instead of stress, it can be assumed that the chipmunks were comfortable enough to spend time cleaning the water and mud off of their fur while in the test. Unfortunately, because grooming was only seen when it was raining and not in any other type of weather, this behavior cannot be used to support or refute the hypothesis that the Drew University chipmunks are less stressed around humans in comparison to the GSWA-CMA chipmunks, but this behavior is still included in figure 8a as it is a behavior that was exhibited more than once.

Head dipping is considered high stress because it is a sign of exploratory behavior (File & Wardill 1975; Martin & Reale 2008; Lyons et al. 2017). In this case, perhaps the exploratory behavior would be a sign that the chipmunks are trying to find an easy way to escape that does not involve standing on their hindlegs, but instead involves a more natural action of digging and going into a hole. The GSWA-CMA spent more time head dipping than the Drew University chipmunks, which shows that they were more exploratory, and possibly more stressed as they were interested in finding ways to escape the box. Also, chipmunks from urban environments tend to have less exploratory behaviors, such as head dipping, due to the fact that urban environments often have predictable food sources (Lyons et al. 2017; Lowry et al. 2012) and less predation (Fischer et al. 2012). Lyons et al. (2017) also connects this reduced exploratory behavior to personality, as chipmunks from urban environments are found to be bolder and therefore more docile around humans. The connection of exploratory behavior and urban boldness relates to my data, as the chipmunks from urban areas (Drew University) were more docile and less stressed while the chipmunks from non-urban areas (GSWA-CMA) were more stressed as they were actively exhibiting exploratory behaviors.

Locomotion is also considered a high stress behavior due to the fact that it is connected to vigilance and exploratory behaviors. During locomotion, the animal often stops to look around, a moment of vigilance, which is used to detect attack and to improve their endurance (McAdam & Kramer 1998), therefore when the chipmunks are locomoting often, it can be a sign that they are trying to see if and where the possible dangers are. Also, locomotion is considered an exploratory behavior (File & Wardill 1975; Martin & Reale 2008; Lyons et al. 2017), which can be connected to stress levels and urbanization in the same way that head dipping is. Because the

Drew University chipmunks do not often need to spend a lot of time foraging for food, they have reduced locomotion levels compared to the GSWA-CMA chipmunks and are therefore less stressed.

Lastly, rearing/jumping/climbing is also considered a high stress behavior. These behaviors have been combined as they are all signs of a flight response and exploratory behaviors (Martin & Reale 2008), and because they are difficult to easily distinguish when analyzing video footage. Bringing in the previous statement that animals only exhibit a flight response if they know the predator has seen them or feel threatened, it makes sense that the GSWA-CMA chipmunks had higher exploratory behaviors and a flight response, as they were wary of the novel environment and potential human predator, who is around the test, and used those exploratory behaviors to try to escape the test.

It is evident that the GSWA-CMA chipmunks spent more time on average doing the high stress behaviors (head dipping, locomotion, rearing/jumping/climbing) instead of the low stress behaviors (scanning and grooming), showing that overall, the GSWA-CMA chipmunks were more stressed than the Drew University chipmunks.

Periphery Test

Lastly, I predicted that the GSWA-CMA and Drew University chipmunks would be equally as likely to be in the center or periphery of the hole board test due to the fact that neither group have ever been in a hole board test. It was statistically significantly seen that the GSWA-CMA chipmunks spent more time in the periphery of the hole board test than the center when compared to the Drew University chipmunks.

This could be due to the fact that a variety of prey animals prefer to be in areas of cover as opposed to areas of openness in order to prevent predation (Basille et al. 2015; Katz et al. 2010). For example, fox squirrels have increased perceived predation risk in areas with closed canopies but no wall coverage (Potash et al. 2018), similar to being in the center of my hole board test. Being against a wall allowed the chipmunks to have some sort of coverage, perhaps giving them a sense of safety against a perceived predator.

According to Ennaceur et al. (2006), who compared different strains of mice in a test that had open platforms and closed arms, the least anxious strain of mouse spent more time on the central platforms and less time in the closed arms. The least anxious mice also made more entries to the center and bridges of the platform compared to more anxious mice (Ennaceur et al. 2006). This can be connected to my data, which also shows that the least anxious chipmunks (the Drew University chipmunks) spent more time in the center of the hole board test and less time in the periphery.

These behavioral changes were not only seen in other chipmunk studies such as Martin & Reale (2008) and Lyons et al. (2017), but were also seen in Ritzel & Gallo's (2020) study, where they looked at behavioral changes found in 10 different orders of animals, including Rodentia. They found that the most common change in development responses is increased boldness in urban animals. This increased boldness can be seen in my study, as the Drew University chipmunks were more likely to be in the center of the hole board test instead of the periphery, showing increased boldness and less fear towards potential predators.

Chipmunk 15

At the GSWA-CMA, there was one chipmunk that we caught six separate times, while every other chipmunk we caught at most three separate times. None of the chipmunk recaptures were included in the data sets as to not skew them, but Chipmunk 15 offers some valuable insight to habituation of small mammals, specifically through the bag test.

As seen in Figure 11, when Chipmunk 15 was first caught, she spent very little time remaining immobile in the hanging mesh bag test, meaning that she was stressed and exhibiting a flight response. Overtime, however, she spent less and less time moving, until by recapture 3 (her fourth time in a hanging mesh bag test), she was completely still for the entire 60 seconds. She remained to be completely still in her fourth, fifth, and sixth recaptures, showing that not moving is a sign of less stress, as she possibly became habituated and more docile by her fourth recapture.

Interestingly enough, Chipmunk 15, escaped the hole board test at recapture two and kept trying to escape the trap by getting through the door before it was closed at every recapture after that, fully escaping once more at recapture four.

Through her hanging mesh bag test, it can be assumed that Chipmunk 15 might have habituated to the testing and became calmer overtime. This calmer state paired with her escape attempts could show that overtime chipmunks may get used to human interaction and may even tolerate it to a degree once they know that the human is not going to harm them. This habituation was also seen in Martin & Reale (2008)'s study, as they mentioned that as chipmunks were tested more often, the intensity of their behaviors were reduced in both the hole board and handling bag test. Chipmunk 15 may have been tested so much, that her intensity of behavior was reduced to the point of not being present any longer, allowing her to no longer have a flight response at all.

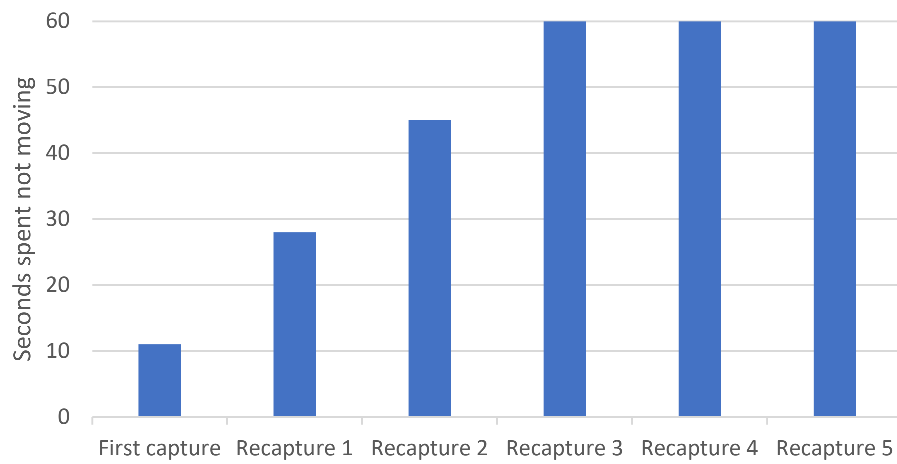


Fig 11. Amount of time (seconds) Chipmunk 15 spent not moving in a mesh bag handling test on all of her captures.

Future Work

This research as conducted over a period of a month with little to no preparations due to the pandemic, so there are a variety of things that could have been done to make this research experience go more smoothly and to yield more convincing results.

Firstly, communication with research partners beforehand is recommended. When we first started the experiment, almost none of us were on the same page in terms of methodology, so our first few captures in the Zuck Arboretum were incorrectly released into the hole board test, escaped, or were just not recorded properly. When we incorrectly released a chipmunk into a hole board test, we opened the trap door and encouraged the chipmunk to leave the trap too soon, in this case, when analyzing data footage, I did not include these chipmunks. When a chipmunk escaped the hole board test, we were not able to collect any behavioral data, as they were not in the hole board test for the 600 second period, and I once again, did not include these chipmunks in data analysis, but I did include their latency data as they did correctly enter the hole board test. This made looking at the data incredibly difficult, so discussing an action plan before doing the fieldwork is definitely necessary. This lack of communication only happened for the first few collections, so I excluded those results so it would not influence the rest of my data.

In some cases during testing, we were talking, moving, and making a lot of noise while in other cases we were silent and not moving. This could have led to different reactions in the animals as creating noise might have created a higher stress response, while not creating noise might have created a smaller stress response. In order to minimize confounding variables such as this, the research partners should agree beforehand to not speak at all and to minimize movement while a test is being conducted.

Along with clear communication and standards for the methodology, there should be clear communication in regard to notetaking and recording important information. In this experiment, we had a distinct coding system for reading field notes, and this should definitely be continued to avoid any confusion with analyzing the data.

In regard to the analyzing video footage, I recommend that only one individual is put in charge of doing so, which is what was done for this study. This is because behavioral data is up to interpretation of the viewer, so some may define the time frame of animal doing one behavior differently from another. So, to reduce the likelihood of overlapping times or differing definitions, only one individual should analyze the video footage. If more than one individual is conducting video footage analysis, there should be a training period of watching and coding previously analyzed videos to test their reliability. Once the individuals are all on the same level of reliability within a certain threshold, then more than one can analyze video footage.

Lastly, as mentioned earlier, this experiment was only conducted over the period of one month. Because of this short time frame, not many animals were captured and analyzed. If this experiment is repeated at a larger scale, more animals should be collected over time in order to get a more representative sample of the behaviors of the entire population.

Along with making the capturing and data analysis more streamlined, I believe it is important to look into hormonal levels via fecal or hair samples as well, which was attempted but not accomplished as none of the chipmunks provided fecal samples. By looking at the levels of cortisol in fecal or hair samples, we can see whether the behavioral responses affiliated with stress are actually stress behaviors, as if the chipmunk exhibited high stress responses (such as the GSWA-CMA chipmunks) then their cortisol levels would also be high. This type of result can be seen in Lyons' et al. (2017) paper, where they found that chipmunks captured in naturalistic environments had higher cortisol values than chipmunks captured in urban environments, which also correlated to their behavioral tests. This type of information would be incredibly valuable if this research would be continued.

Conclusion

This behavioral research project was conducted over a period of eight weeks from June to July of 2021 at two different locations – Drew University and the Great Swamp Watershed

Association's Conservation Management Area (GSWA-CMA). These two locations were used due to the assumption that Drew University's campus has more people present who interact with the resident animals more often than the lesser amount of people at the GSWA-CMA, and because people who do interact with the forest on Drew University's campus tend to leave signs of their presence, such as litter and noise pollution, while people at the GSWA-CMA do not due to training and strict rules.

I hypothesized that the chipmunks caught at Drew University would be less stressed on average than the chipmunks caught at the GSWA-CMA due to their increased human interaction, which would lead to habituation and increased docility. To test this, two different tests were conducted, a hanging mesh bag test and a hole board test. From these two tests, a variety of behaviors were analyzed: amount of time immobile, latency to enter the hole board test, scanning, grooming, locomotion, rearing and jumping, head-dipping, and time spent in the periphery of the hole board test. When analyzing the behaviors, only the first capture of each animal was used, as over time the animal may have become habituated or sensitized over time, thus skewing their behavioral results.

My hypothesis was supported by most of the tests conducted. In the hanging mesh bag test, the GSWA-CMA chipmunks spent statistically significantly more time moving, which I correlated with a flight response caused by stress. Both the GSWA-CMA and Drew University chipmunks were reluctant in entering the hole board test, showing high level of stress associated with a novel environment and a freeze response. Once the chipmunks were within the hole board test, they had significant behavioral differences; it was found that the GSWA-CMA chipmunks spent more time exhibiting behaviors that I associated with high stress levels, rearing/jumping/climbing, locomotion and head-dipping, while the Drew University chipmunks spent more time exhibiting behaviors that I associated with low stress, scanning and grooming. Lastly, the GSWA-CMA chipmunks statistically significantly spent more time in the periphery of the hole board test as opposed to the center of the test, once again showing higher stress levels associated with a novel environment and possibly the openness of the test. These results support my hypothesis that animals that rarely have human interaction are significantly more stressed when humans are present compared to animals that often have human interaction. These results are also supported in Martin & Reale (2008) where they found that chipmunks upon first capture exhibited high stress responses in both the hanging mesh bag test and hole board test. Also, Lyons et al. (2017) found that chipmunks from urban areas exhibited less stress, possibly due to the fact that they have less predators, more food sources, and few the human experimenters as less threatening than chipmunks from rural areas, and that result is seen here as well.

I also analyzed data for a chipmunk that was caught a total of six times, three times more than any other chipmunk captured. This chipmunk, Chipmunk 15, was from the GSWA-CMA, and in the hanging mesh bag test, she went from being mobile for almost an entire minute to being completely immobile by her last three captures. This result, combined with the knowledge that she was caught at the same site each time, shows evidence of habituation associated with reduced stress responses. Habituation was also seen in Martin & Reale's (2008) study, as they found that as chipmunks were tested more often, their behavior intensities were reduced, which was definitely seen here as Chipmunk 15 went from an intense flight response to no visible stress reaction at all. Unfortunately, we did not have enough video footage from the hole board test to see if her behaviors correlated with her hanging mesh bag test habituation over time, but it can be assumed that they did, as previous studies found those two correlated.

This research is incredibly important as it will build on the small amount of published knowledge associated with how small mammals are being affected by human interaction, and whether that human interaction is negatively affecting their ability to thrive in an ever-urbanizing world.

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