

THE EFFECTS OF HABITATION ON BOLDNESS OF URBAN AND RURAL SONG  
SPARROWS (*MELOSPIZA MELODIA*)

A thesis presented to the faculty of the Graduate School of  
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## TABLE OF CONTENTS

List of Tables .....	iv
List of Figures .....	v
Abstract .....	vi
Introduction.....	1
Methods.....	4
Statistical Analyses .....	6
Results.....	8
Discussion.....	9
References.....	16

## LIST OF TABLES

Table 1. GPS Coordinates of Study Sites.....	6
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## LIST OF FIGURES

Figure 1. Map of Study Sites .....	7
Figure 2. Boxplot of Flight Initiation Distance (m) Across 5 Day Trials.....	8

## ABSTRACT

### THE EFFECTS OF HABITUATION ON BOLDNESS OF URBAN AND RURAL SONG SPARROWS (*MELOSPIZA MELODIA*)

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As urban development continues to dominate landscapes across the United States, wildlife species are changing their behavior in many ways. Urban animals are often bolder, or less fearful of new stimuli, than rural animals. While natural selection and adaptation can drive behavioral changes in urban animals, other factors, such as learning or habituation, can also lead to behavioral modifications. Habituation may play an important role, if urban individuals learn through repeated exposure that humans do not represent a threat. To determine how repeated human exposure affects song sparrow (*Melospiza melodia*) populations in an urban and rural habitat, I measured boldness as flight initiation distance (FID), after repeated exposure to a non-threatening human subject. FID measures the distance between human and bird, at which the bird flies away in fear of disturbance or predation. I collected FID data on male song sparrows in an urban and rural environment over 5 consecutive days. I found that after 5 days of successive trials, FID was individually repeatable in both urban and rural populations. Urban birds had consistently lower FID than their rural counterparts. I also found that FID went down over 5 days of repeated trials in the rural populations, but not the urban. These results suggest that habituation can occur quickly in rural birds and account for the greater boldness we typically see in urban populations.

## INTRODUCTION

Urbanization is an increasing problem for wildlife species across landscapes. Not only does urbanization disrupt ecosystems, but it also increases habitat fragmentation and increases the frequency of human and wildlife interactions. The impacts of urban development on wildlife communities and populations have been observed through various studies comparing wildlife behavior between urban and rural environments. Within urban communities, previous studies have noted behavioral changes in wildlife populations, including changes to foraging, mating, and predator avoidance (Robbins, 1993; Fleischer, Bowman, & Woolfenden, 2003; Ditchkoff, Saalfeld, & Gibson, 2006; Evans et al., 2010). Behavioral changes in response to urbanization might be selection for traits that allow for animals to tolerate disturbance and take advantage of resources (Fidler et al., 2007; Partecke & Gwinner, 2007; Møller, 2008; Evans et al., 2010; Atwell et al., 2012). While selection can drive behavioral changes in urban species, other factors, such as learning or habituation, can lead to further behavioral modifications, including changes in boldness.

Boldness defines the level of risk an animal will take in the presence of a predator or intruder. Animals that populate urban, developed communities typically show higher levels of boldness (Fernandez-Juricic et al., 2002; Vuorisalo et al., 2003; Bejder et al., 2006; Gonzalez et al., 2006; Møller, 2008; Evans et al., 2010). One way that researchers can assay boldness in birds is to measure flight initiation distance (FID), which measures the distance at which a focal bird flees from an approaching threat, in this case a human (Blumstein, 2003; Blumstein, 2006). A study in burrowing owls (*Athene cunicularia*) suggested that there is consistent individual variation in FID, where the distance at which an individual flees when a human approaches is

consistent, thus boldness is repeatable among individuals (Carrete & Tella, 2009). Previous studies have also found that urban species often have shorter FIDs than those in rural habitats, with urban animals expressing little fear behavior while in close proximity to humans, and with repeated exposure to human disturbances (Uchida et al., 2019; Scales et al., 2011; Møller & Tryjanowski, 2014). Increased boldness in urban species could have obvious benefits, if it allows urban animals to survive and reproduce, even in the face of high levels of disturbance.

Although many studies have found urban individuals to be bolder than their rural counterparts, how urban populations become more bold still remains unclear. If boldness has a genetic basis, then selection may favor bolder individuals in urban habitats. Observations that urban individuals learn through repeated exposure that humans do not represent a threat (Rodríguez-Prieto, Fernández-Juricic, Martín, & Regis, 2009; Clucas & Marzluff, 2012), suggest that habituation may play an important role to explain differences in boldness between urban and rural animals. Thus, behavioral plasticity and habituation could be beneficial in urban environments. However, few studies have examined if habituation can play a role in causing greater boldness in urban animals (Cavalli, Baladrón, Isacch, Biondi, & Bó, 2018). In this study, I examine the role of habituation in the expression of boldness in urban and rural song sparrows.

Song sparrows (*Melospiza melodia*), are a common songbird species that have successfully invaded urban habitats. Urban populations of song sparrows show higher levels of boldness compared to rural populations (Evans et al., 2010; Scales et al., 2011; Foltz et al., 2015). In this study, I examined variation in risk perception across urban and rural populations of song sparrows to determine if habituation influences FID; more specifically, I measured how FID changed after repeated exposure to a non-threatening human subject. I hypothesized that both urban and rural song sparrows' FID will be influenced by repeated exposure to human



disturbance. However, I predicted urban song sparrows' FID would decrease significantly less than rural individuals, while I also predicted rural song sparrows to still have an overall longer FID than urban birds at the conclusion of the study. I also predicted that levels of boldness would be higher in urban song sparrows at the beginning of the study and that both urban and rural individuals would become bolder as the study persisted. The goal of this research was to test the degree in which habituation alters behavior within urban and rural landscapes in song sparrow populations.

## METHODS

For this study, I surveyed song sparrow territories in both urban and rural locations. I mapped urban sites on the campus of Western Carolina University in Cullowhee, NC, and Bridge Park located in Sylva, NC. Urban sites were locations frequented by humans, within a close proximity to buildings, parking lots and sidewalks, and landscaped with bushes, grass lawns, and ornamental trees. The rural sites were located at Kituwah Mound, near Bryson City, NC, as well as various Christmas tree farms located within a 25-mile radius of WCU (Table 1, Figure 1). Rural study sites consisted of hay fields, Christmas tree farms, and agricultural fields that were infrequently visited by humans. I identified twenty individuals and their territories in both the urban and rural sites. I began this study on May 14, 2019 and completed the study on July 20, 2019.

During breeding season, I mapped urban and rural song sparrow territories 1-2 days before each new set of 5-day FID trials began. Male song sparrows typically remain in the same territory throughout the breeding season (Hughes & Hyman, 2011), which allowed for territory mapping to be a sufficient distinguishing feature between individual males within a population. Only males were included in this study, and as song sparrows are not sexually dimorphic, males were differentiated from females by their song.

At each territory, I conducted flight initiation distance (FID) trials to assay boldness. I began all trials approximately 25 meters from the singing male, where I approached the bird at a consistent walking speed. Next, I recorded the distance at which the male bird flushed or took cover. Then I recorded the starting distance from the subject, as well as the distance at which the bird took flight, and the approximate height of the bird at the beginning of the trial (Myers &

Hyman, 2016). The distance at which the bird took flight, and the height of the bird at the beginning of the trial are used to calculate the hypotenuse of the triangle formed by these collected measurements, which equals FID scores (Blumstein, 2006). I measured all distances with a measuring tape.

Each day I began trials at a different territory, to ensure randomization, and that FID of an individual was not measured the same time every day (Cavalli et al., 2018). To maintain a consistent, recognizable appearance, I wore the same style and color of clothing during each 5-day trial. Subjects with a shorter FID are considered bolder than individuals with a longer FID.

To measure the effects of habituation on FID, I performed separate trials over a multi-day span. On each song sparrow, I performed successive trials, with 1 trial per individual, per day for 5 consecutive days. All methods were approved by Western Carolina University IACUC, permit # 2019-013.

## STATISTICAL ANALYSES

I calculated repeatability of individuals by following Lessells and Boag (1987). I used a repeated measures ANOVA to determine if song sparrow FID went down after 5 days of repeated trials. I used a Welch Two Sample t-test to test for differences in FID between urban and rural habitat on day 5. I used a linear mixed effect model (LMEM) to test for differences in FID of urban and rural sites, and the effect of trial by habitat on FID, with ID as a random effect and habitat as a fixed effect. To account for outliers, I ranked the data.

Table 1. GPS coordinates and locations of nine rural and three urban and song sparrow (*Melospiza melodia*) territories and study sites.

<b>Rural Locations</b>	<b>Latitude and Longitude</b>	<b>Urban Locations</b>	<b>Latitude and Longitude</b>
Kituwah Mound	35°26'01.5"N, 83°24'18.8"W	Western Carolina University	35°18'23.76"N, 83°11'1.44"W
Balsam Lake	35°16'14.3"N, 82°58'11.0"W	Little Savannah Road	35°18'22.8"N, 83°11'38.7"W
Neddie Mountain Road Tree Farms	35°15'42.6"N, 83°00'11.6"W	Bridge Park	35°22'29.5"N, 83°13'28.1"W
Pine Creek Road	35°11'04.5"N, 83°11'01.4"W		
Salt Rock Road	35°11'26.6"N, 83°11'03.0"W		
Windy Gap Road	35°09'15.1"N, 83°12'36.5"W		
Cullowhee Mountain Road	35°12'22.2"N, 83°11'22.4"W		
Wolf Creek Tree Farm	35°17'05.9"N, 83°07'55.0"W		
Hornet Drive	35°23'47.5"N, 83°18'09.6"W		

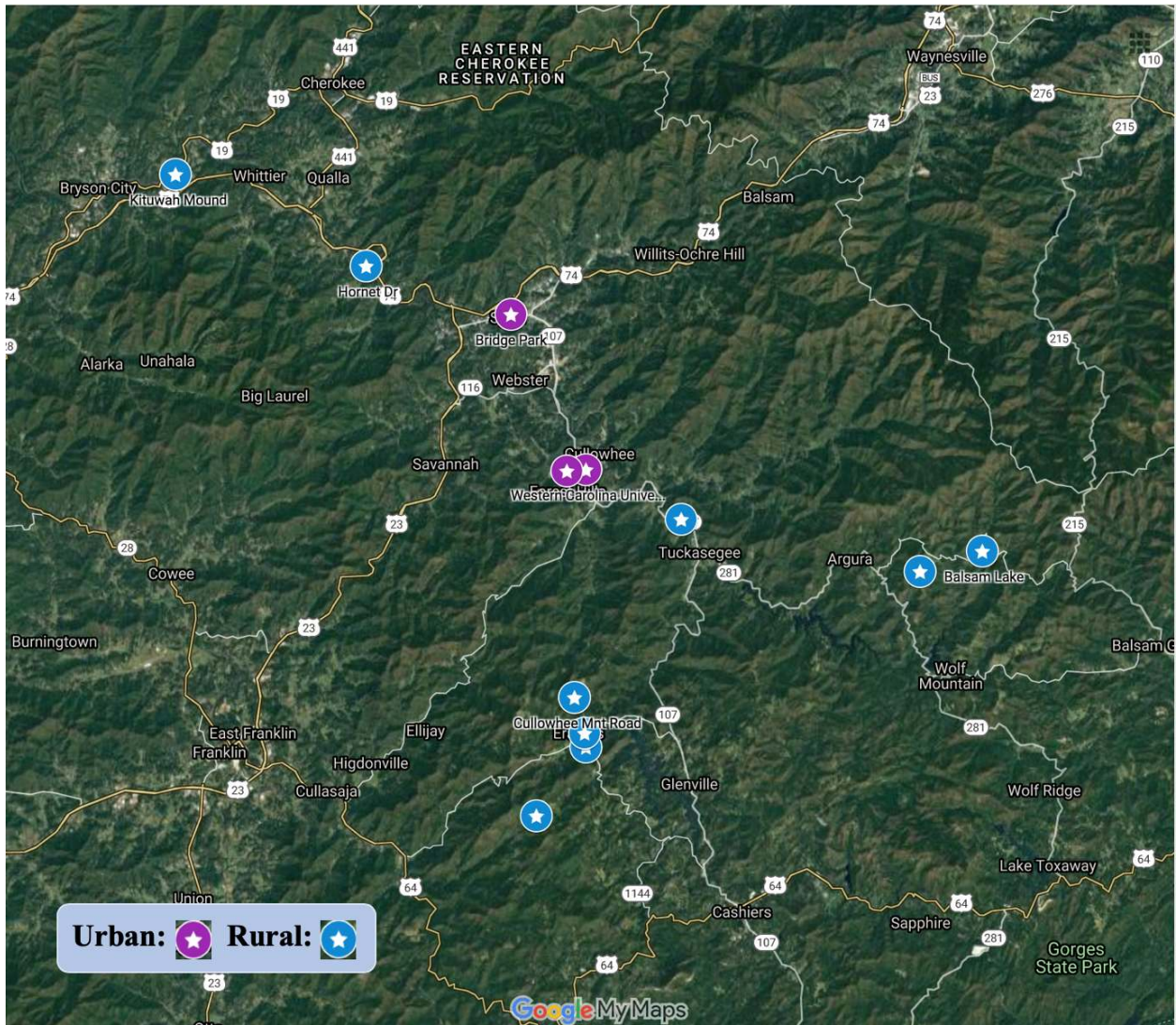


Figure 1. Map of urban and rural song sparrow study sites across western North Carolina, created using Google Maps.

## RESULTS

After 5-days of successive trials, FID was individually repeatable (Urban:  $r= 0.237$ ; ANOVA:  $F= 2.522$ ,  $p= 0.00195$ ; Rural:  $r= 0.242$ ; ANOVA:  $F= 2.598$ ,  $p= 0.00162$ ). There was a significant difference in FID between habitats, (ANOVA:  $p= 0.003$ ;  $df= 1$ ; Fig. 1), where urban birds had lower FID than the rural birds.

FID significantly declined over the course of 5-days of repeated trials (ANOVA:  $p< 0.0001$ ;  $df= 4$ ; Fig. 1), where I found no evidence of a difference in rural and urban individuals after 5-days of trials. ( $t= -0.40171$ ,  $df= 32.548$ ,  $p= 0.6905$ ). There was a significant interaction between trial and habitat (ANOVA:  $p= 0.024$ ;  $df= 4$ ; Fig. 1), as FID declined after successive trials in rural, but not in urban habitats (ANOVA: Rural:  $p< 0.00001$ ; Urban:  $p= 0.799$ ;  $df= 4$ ).

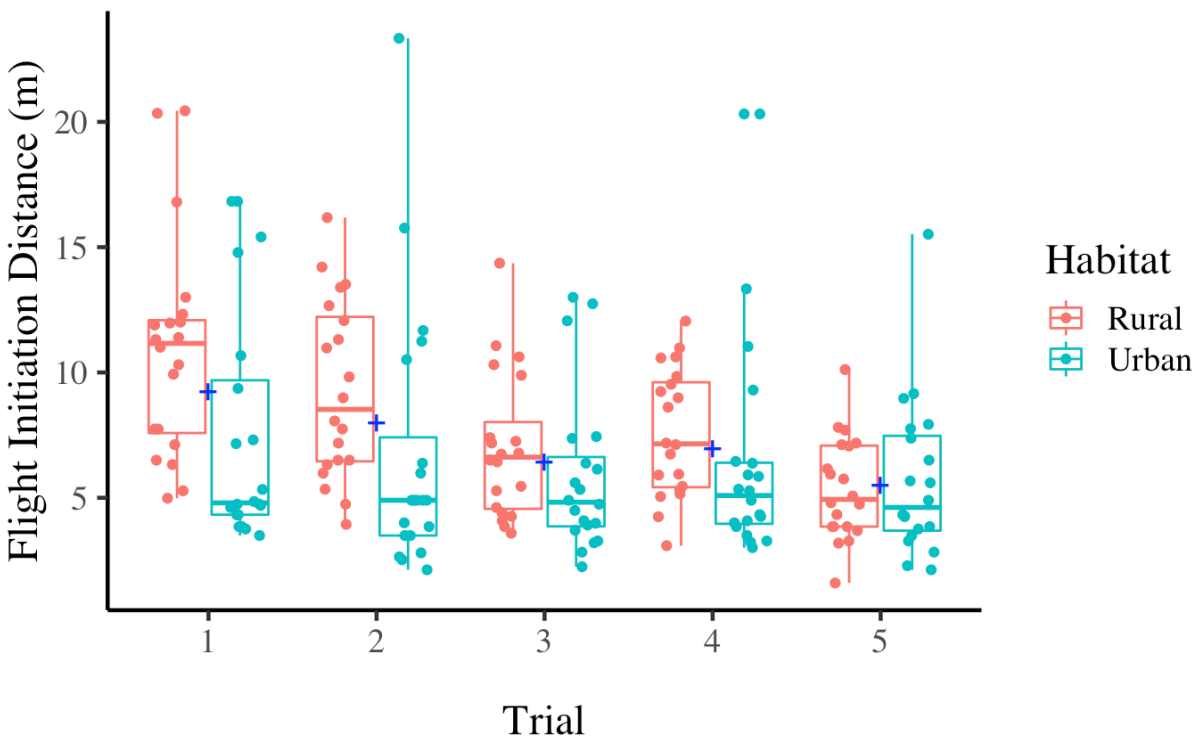


Figure 1. Boxplot of flight initiation distance in response to a human observer taken over a course of 5 days, with one trial per day, from 20 males in urban habitat and 20 males in rural habitat. The blue crosses represent the mean FID between urban and rural individuals.

## DISCUSSION

I found that FID is individually repeatable, which suggests that there was consistent individual variation in levels of boldness. Across the 5-day treatment, repeatability of FID was low; with an  $r$  value of 0.24 in urban and rural territories. Similar studies suggest that low repeatability could be due to low sample size ( $n=20$ ) and duration of the study (Runyan & Blumstein, 2004; Carrete & Tella, 2009). Another explanation of low repeatability after successive trials could be due to habituation (Carrete & Tella, 2009), where repeatability in FID would decline as individuals become habituated to the approaching observer. It has been suggested that individuals might habituate to a behavioral assay when measured successively, which could enable the individual to become desensitized to the approach or, in contrast, become more responsive to the assay (Martin & Reale, 2008). A study on Sable Island horses (*Equus caballus*), found that repeatability of FID was high among individuals when tested on the same day, but decreased along trial days (Cabrera et al., 2017). I did not measure repeatability within days, but habituation across days could explain our results of low repeatability of FID. While few studies look at the repeatability of FID, it is important to note repeatability of behavior, as repeatability can help determine whether there is a genetic basis to a behavior, including increased boldness observed in urban song sparrow populations (Bell et al., 2009). Although repeatability is likely to decrease across trials, I still found that there is repeatable individual variation in FID in both urban and rural populations.

Repeatability of FID was similar between urban and rural populations, but urban populations of song sparrows are bolder than rural ones. Previous studies have found that urban individuals are bolder than their rural counterparts (Cavalli et al., 2018; Evans et al., 2010;

Engelhardt & Weladji, 2011; Scales et al., 2011; Rebolo-Ifrán et al., 2015; Samia et al., 2015; Myers & Hyman, 2016; Mikula et al., 2018). A study of various bird species indicated that some species are more likely to have shorter FID in urban landscapes, especially when the species is synanthropic (Bjørnvik et al., 2015). Song sparrows do not rely entirely on utilizing human resources; however, they do take advantage of human resources, if they are available, suggesting they are a synanthropic species.

I also found that FID declined over 5 days; however, there was a significant interaction between trial and habitat, where FID declined over trials in the rural populations, but not in the urban. Similarly, over consecutive, 9-day trials, rural burrowing owls decreased their FID but urban owls did not decrease their FID (Cavalli et al., 2018). Finding that rural song sparrows decrease their FID over 5 days indicates that habituation can occur quickly. Habituation occurs when an individual learns, through repeated exposure, that an external stimulus does not pose a threat, thus reducing their behavioral response to the stimulus (Mirza et al., 2006), and such learning could have a significant impact on song sparrows' ability to deal with disturbance from humans. Although I found that urban song sparrows did not decrease their FID over time, these urban populations have been exposed to heightened levels of human disturbance, thus FID could have already decreased due to human interactions that occurred before this study was performed. Rural populations are not subject to daily and continuous human interaction; therefore, in our experimental treatment, rural birds' FID could decline more rapidly to repeated human exposure.

Greater boldness in urban populations of song sparrows could arise through several mechanisms. It is possible that natural selection favors individuals who are bolder in urban habitats, or that bolder individuals are more likely to select urban habitat. Alternatively, individuals who are less bold must become bolder, through learning, to acclimate to a constantly



changing urban environment and reside in close proximity to humans. Each of these hypotheses has some support.

Several studies have linked adaptive urban phenotypes to specific genetic changes (Harris & Munshi-South, 2017). True adaptation with a genetic basis has been described in tomcod (*Microgadus tomcod*), where deletion of a specific gene allowed for increased pollution tolerance of polychlorinated biphenyls (PCBs) (Wirgin et al., 2011). Reid et al. (2016) and Whitehead et al. (2011) also found genetic adaptation in killifish (*Fundulus heteroclitus*), where urban killifish evolved a tolerance to PCB pollution. Konorov et al. (2017) provided evidence that suggests black garden ants (*Lasius niger*) exhibit genomic exaptation in order to adapt to urban environments, which indicates that individuals who demonstrate phenotypic plasticity are less likely to be excluded from specific habitats (Badyaev, 2005; Lowry et al., 2013). It is possible that selective pressures of urban habitat drive genetic changes that confer a more advantageous phenotype, which could potentially result in increased levels of boldness in urban populations. Our analysis also suggests that rural song sparrows can also become bolder with repeated human stimulus; therefore, we must consider that habituation plays an important role in successful colonization of an urban environment.

Urban populations of wildlife have also been suggested to be habituated to human activity (Clucas & Marzluff, 2012; Blumstein, 2014; Polich & Barazowski, 2016; Cavalli et al., 2018; Avilés-Rodríguez & Kolbe, 2019), which could explain the consistent decline in FID of rural populations, but not urban. Previous studies have suggested that transfer of habituation may occur in urban populations of wildlife, where populations habituate to humans, and then transfer that habituation from humans to predators, and as a result, show greater boldness towards predators (McCleary, 2009; Geffroy et al., 2015). While transfer of habituation may contribute to

an overall increase in boldness of urban populations, it has also been suggested that reduced vigilance could be an underlying factor to increased boldness (Uchida et al., 2019). According to Uchida et al. (2019), Eurasian red squirrels (*Sciurus vulgaris*) have reduced vigilance in urban habitat compared to rural individuals in their habitat. With potentially fewer threats of predation, urban wildlife can weigh the cost of risk allocation and responding to a threat (Beauchamp, 2004; Bell, 2005; Blumstein & Daniel, 2005; Dingemanse et al., 2007; Rodriguez-Prieto et al., 2009). Though habituation could have occurred in urban song sparrow populations, previous studies have found that transfer of habituation or reduced vigilance do not seem to be occurring. Myers and Hyman (2018) found that urban song sparrow populations actually show stronger reactions to alarm calls, which could indicate the presence of a predator, and increased vigilance. Greater boldness in urban animals, such as song sparrows, could involve a combination of inherent boldness and behavioral flexibility.

Behavioral flexibility enables reduced vigilance in individuals and has been noted as a driving factor to successful colonization of wildlife in urban habitat (Sol et al., 2002; Sol et al., 2013; Griffin et al., 2017; Cavalli et al., 2018). This plastic behavior allows the individual to quickly respond to a rapidly changing environment, and increased tolerance to humans could be extremely beneficial to urban wildlife, where individuals must alter their behavior and learn to tolerate human disturbance (Uchida et al., 2019). The unpredictability that comes with living in urban habitat is why it is pertinent for individuals to remain plastic and could explain why bolder individuals occupy urban landscapes. If bold individuals are also more plastic, selection could favor bolder individuals in urban habitat, while selecting for less-bold individuals in rural habitat. If individuals are less responsive to human approach in urban habitat than rural, it could suggest habituation, which seems likely, as learning to tolerate disturbance can be advantageous

in urban environments. Furthermore, some areas of urban habitat have higher amounts of human traffic, which could also alter the FID of individuals residing in areas with more frequent pedestrian interaction (Rodriguez-Prieto et al., 2009). Individuals residing in more trafficked areas of urban habitat are likely more habituated than individuals residing in rural habitat. Rodriguez-Prieto et al. (2009) suggested that urban blackbirds (*Turdus merula*) had shorter FID during high human traffic times of day and had longer FID during times where human traffic was less frequent, suggesting plastic behavior and habituation to higher frequencies of human disturbance. In this study, I randomized the time of day that I performed FID trials; however, birds were more accessible during low-traffic time periods, where I was more likely to complete a trial. By reducing FID during high-volume pedestrian hours, urban individuals are likely weighing risk allocation, while also becoming habituated to humans, where the stimulus may be considered as low risk.

There are obvious benefits of reducing individual FID in urban habitat and becoming bolder. Polich and Barazowski (2016) noted that populations of urban painted turtles (*Chrysemys picta*) were bolder (lower FID) than their rural counterparts, and suggested that by becoming bolder, individuals minimized loss of basking opportunities. Previous studies have shown individuals who inhabit urban landscape have lower stress response to a human threat than their rural counterparts (Scoeck et al., 2004; French et al., 2008), potentially due to increased food availability, or repeated and frequent exposure to a stressful environment. French et al. (2008) also noted that tree lizards had increased immunocompetency when living in urban habitat compared to rural, resulting from less stress and more food abundance. By learning to tolerate human disturbance, individuals can better utilize urban resources and reap the benefits from colonizing urban landscapes.

If urban song sparrows can become habituated to a human approach, through learning, rather than adapting through genetic changes, then rural individuals must have the same capability of habituating. In our study, we found no evidence of a difference in rural and urban individuals after 5-days of trials. These results indicate that rural song sparrow boldness can become indistinguishable from urban birds after 5 consecutive days of repeated human stimulus. The question still remains, how far can rural and urban individuals reduce their FID over a period of time? According to Mikula et al. (2018) habituation is not a neutral state for animals, and fear response has been recorded in wildlife species after many years of human exposure (Williamson & Feistner, 2003; Jack et al., 2008; McDougall, 2012; Nowak et al., 2014). More specifically, after 15 years of human occupancy and observations, chimpanzees (*Pan troglodytes*) of the Taï Forest still showed fear response towards humans (Boesch-Achermann & Boesch, 1994). Similarly, capuchin monkeys (*Cebus capucinus*) and stumptail macaques (*Macaca arctoides*) were not completely habituated after 20 years of research and 14 years of observation (Rasmussen, 1991; Jack et al., 2008). These results suggest that an animal's evolved response to the risk of predation can be retained, where individuals remain fearful of humans post habituation (Frid & Dill, 2002; Mikula et al., 2018). If song sparrows also retain fear behavior, it is very likely that there is a limit to how much individuals reduce their FID over a given period of time. Further studies should be conducted to answer these questions.

Many studies indicate that urban species are inherently bolder than their rural counterparts, which can be driven by adaptation or habituation. In our study, I found that FID is individually repeatable in song sparrows, but it remains unclear whether there is a genetic basis, or if habituation is the driving factor. I also found that urban birds are bolder than their rural counterparts, which could be a result of adaptation, habituation, or both. I found suggestive

evidence that both urban and rural song sparrows habituate to a repeated human stimulus; however, rural song sparrows became bolder as trials persisted. If selection towards advantageous phenotypes in urban habitat is occurring, then it is possible that traits of lesser value in urban habitat will be lost over time, thus decreasing behavioral diversity within urban populations. Moreover, if habituation can drive behavioral modifications, including boldness, we can expect further loss of behavioral diversity, including fear behavior, in urban birds. The loss of diversity in urban bird populations over a long period of time has the potential to enable divergence of species (Unfried, Hauser, & Marzluff, 2013). While rural habitat becomes urbanized and habitat fragmentation increases, it is important that we continue to study the responses of wildlife populations to these changes. Through further investigation, similar to the study of Eurasian red squirrels (Uchida et al., 2019), FID and alert distance (AD), the distance at which a focal animal becomes aware, or alerts, to a human or predator approach, could be used to determine other factors that affect boldness in song sparrow populations.

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