Personality in the City: Relationship Between Animal Behavioral Traits And Urbanization in a Fragile, Human-impacted Desert Ecosystem

by

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ABSTRACT

Human-inhabited or -disturbed areas pose many unique challenges for wildlife, including increased human exposure, novel challenges, such as finding food or nesting sites in novel structures, anthropogenic noises, and novel predators. Animals inhabiting these environments must adapt to such changes by learning to exploit new resources and avoid danger. To my knowledge no study has comprehensively assessed behavioral reactions of urban and rural populations to numerous novel environmental stimuli. I tested behavioral responses of urban, suburban, and rural house finches (*Haemorhous mexicanus*) to novel stimuli (e.g. objects, noises, food), to presentation of a native predator model (*Accipiter striatus*) and a human, and to two problem-solving challenges (escaping confinement and food-finding). Although I found few population-level differences in behavioral responses to novel objects, environment, and food, I found compelling differences in how finches from different sites responded to novel noise. When played a novel sound (whale call or ship horn), urban and suburban house finches approached their food source more quickly and spent more time on it than rural birds, and urban and suburban birds were more active during the whale-noise presentation. In addition, while there were no differences in response to the native predator, rural birds showed higher levels of stress behaviors when presented with a human. When I replicated this study in juveniles, I found that exposure to humans during development more accurately predicted behavioral differences than capture site. Finally, I found that urban birds were better at solving an escape problem, whereas rural birds were better at solving a food-finding challenge. These results indicate that not all anthropogenic changes affect animal populations equally and that determining the aversive natural-history conditions and challenges of taxa may help urban ecologists better understand the direction and degree to which animals respond to human-induced rapid environmental alterations.

DEDICATION

I would like to dedicate this dissertation to all of the wonderful women in my life: Graham, Zoe, Jenny, Elise, Rashel, Karla, Grace, Chelsea, Elizabeth, my mom, my amazing sister Jessica, and, of course, Kelvie, who has always been the light in all the darkness. Your strength, determination, compassion, and inner beauty inspire me every day. Thank you for always being, as Zoe would say, “100% team Melinda Weaver.” I love you all.

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PREFACE

The world in which we live is complex, often harsh, and always changing. Thus, animals often modify their behavior to be successful in their specific environment, namely by surviving to pass along their genes and by finding a mate with which to reproduce. To do this, they must make decisions about how to best utilize resources, such as nesting sites and potential food sources, while still protecting themselves from predators and competition from other animals vying for similar resources. Some behaviors are steadfast, working in multiple environments for a variety of species, such as how prey animals can be seen congregating in big groups to reduce the chances of being hunted by a predator (Hamilton, 1971), a behavior seen in many taxa of mammals, fish, and birds. However, other behaviors are more suited for a particular environment, such as desert animals seeking shelter from predators hiding underground when no vegetation is available (Noy-Meir, 1974). If the environment doesn't change throughout an animal's lifetime, even behaviors adapted for a specific ecosystem won't change, allowing the animal to consistently and predictably respond to a given situation each time it encounters it. However, when that environment changes, will the animal be able to change its behavior quickly enough to survive?

This is exactly the challenge that animals are facing as human populations expand across the globe. Humans can modify the environment in a variety of ways, such as agriculture, housing developments, and recreational parks, and they can do so quickly, reducing the timeframe that animals have to respond to changes. Perhaps the largest impact of humans on animal populations is urbanization. For the first time in human history, more people reside in cities (defined by the U.S. Census Bureau as areas consisting of 50,000 or more people) than rural areas, and this is expected to increase to 60% by 2030 (United Nations Population Fund, 2007), including the projected doubling of urban areas in this timeframe (U.N. DESA, 2003). Urban areas drive out many native species, due to habitat loss and modification (Grimm et al., 2008) or exposure to nonnative competitors (Shochat et al., 2010), predators (e.g., cats; Loss et al., 2013), pollution (Isaksson, 2015), and pathogens (Bradley and Altizer, 2007), resulting in reduced biodiversity in cities (McKinney, 2006). However, some species thrive in urban areas, taking advantage of resources such as availability of anthropogenic foods (Newsome et al., 2015; Tryjanowski et al., 2015), urban-provided cover and nesting sites (Isaac et al., 2014; Møller, 2009), year-round water supplies (Fokidis et al., 2009), and moderate weather (Shochat et al., 2006). An important question in conservation biology is – what makes some species more tolerant than others to urban development?

Animals can respond to urbanization in three distinct ways: disperse to a new environment, adjust through phenotypic plasticity, or adapt through genetic changes (Wong & Candolin, 2014). Because human-induced changes happen so rapidly, there is not often time for genetic adaptation (Chevin & Lande, 2010), so animals plastically adjust their behavior, such as by modifying their response to predators (McCleery, 2009; Kitchen et al., 2010), competitors (Hasegawa et al., 2014), or mates (Lane et al., 2011), or their choice of habitat (Miller et al., 1998, Yeh et al., 2007) and feeding tactics (Møller, 2008; Liker & Bokony, 2009). Those who cannot behaviorally respond often perish, which is why many scientists predict that the majority of the earth's species will be extinct in the next 50-100 years (Stork, 2010).

Often, the way that animals endure human-induced rapid environmental change is through behavioral responsiveness to novelty (i.e. neotolerance; Reale et al., 2007). Humans introduce many new abiotic stimuli or biotic challenges into the environment, ranging from new food sources (Robb et al., 2008) and predatory threats (Kauhala et al., 2015) to noise pollution (Perillo et al., 2017), and species who thrive near humans may show superior ability to problem-solve and acclimate/adapt to such novelty (Greenberg & Mettke-Hoffman, 2001; Kark, 2007; Møller, 2008; Liker & Bokony, 2008; McCleery, 2009; Kitchen et al., 2010). In fact, a meta-analysis of animal behavioral responses to urbanization found that phenotypic changes were happening at a greater rate in cities than natural areas and other areas impacted by humans (Alberti et al., 2017). However, when and how these changes occur is less well-understood. Thus, a comprehensive set of studies testing a suite of behavioral responses to diverse urban stressors, in an animal that exists in both urban and natural settings, is needed to understand contexts in which urban animals may benefit from either pursuing or avoiding novelty.

To address this, I chose to investigate urban impacts on behavior in a bird species that is ubiquitous in the southwestern United States, the house finch (*Haemorhous mexicanus*). Birds are excellent subjects for comparative studies of urban wildlife because many species survive well in both urban and rural environments, while some thrive in only in the presence or absence of human developments (Blair, 1996). Unlike many species that persist in cities, many species of birds, especially songbirds, are not considered pests but instead are drawn to yards with feeders and bird baths, allowing the population density of urban birds to more than double compared to that of their rural counterparts (Tryjanowski et al., 2015).

Specifically, house finches are an excellent model organism for studying behavioral responses to urbanization because, in their native range in the desert southwestern United States, they inhabit urban, suburban, and native rural ecosystems. Utilizing the extensive greater Phoenix land use area data from the Central Arizona-Phoenix Long-Term Ecological Research Project, I was also able to choose sites that differed in many known urban variables, such as population density and land-use features (Giraudeau et al., 2014), in which to study these birds. House finches also readily display behaviors like foraging and aggression in open environments (e.g. feeders, backyards), are abundant and easily captured, and are amenable to captive behavioral experimentation. I captured house finches at six sites, ranging from urban Phoenix to rural Estrella Mountain Park (see Appendix 3 for map) to examine behavioral differences among three distinct populations (urban, rural, and suburban). Specifically, I investigated three behavioral metrics that would probe their responsiveness to novelty: activity behavior (defined by flights; Ditchkoff et al., 2006), stress behavior (defined by bill wipes; Clark, Jr., 1970), and latency to approach/time spent on an aversive or rewarding novel stimulus (Toms et al., 2010).

In a series of behavioral tests and experiments conducted both in the field and lab during two seasons across four years in both juveniles and adults, I exposed house finches to multiple novel anthropogenic stimuli and stressors and recorded behavior and measured stress hormone levels to determine what differences, if any, existed between urban, suburban, and rural populations. Because urban areas have more human-created buildings, food sources (e.g. bird feeders, trash cans), and noises (e.g. car alarms, construction, traffic), I predicted that adult city birds would show greater behavioral resilience and decreased stress response toward the stimuli, whereas juveniles would acclimate to urban stimuli based on exposure to these stimuli during development.

Specifically, in Appendix B, I exposed birds to the presence of both humans and a model predator (Cooper's hawk, *Accipiter striatus*), to which birds at all sites are exposed. In Chapter 1, I presented birds with a suite of novel abiotic stimuli: novel environment, novel objects, novel food, and novel noise. In Chapter 2, I replicated the study in Appendix B but with juveniles during a common-garden experiment, in which I randomly assigned birds from both urban and rural sites to a low human exposure treatment or a high human exposure treatment and repeated the human-presence trials at various time points during development. In Chapter 3, I gave birds two problem-solving challenges, one aversive (escape from confinement) and one rewarding (food-finding). Finally, in Appendix A, I took blood samples of birds before and after human-presence and novel-environment trials to measure stress hormones to understand the link between behavioral and hormonal responses. Across these studies, I predicted that urban and suburban house finches would show fewer signs of behavioral and hormonal stress in response to novel stimuli and urban stressors (but not the native predator, which is a known predator to birds at all sites) and would approach food and solving problems more quickly than rural birds in the presence of these novel stimuli.

CHAPTER 1

URBAN HOUSE FINCHES ARE LESS AVERSE TO NOVEL NOISES, BUT NOT OTHER NOVEL ENVIRONMENTAL STIMULI, THAN RURAL BIRDS

Abstract

Human-inhabited or -disturbed areas pose many unique challenges for wildlife, including reacting to novel environmental stimuli like car traffic, buildings, and anthropogenic noise. Animals inhabiting these environments must adapt to such changes by overcoming such novelty (i.e. neotolerance, neophilia), and either exploiting new resources or avoiding danger. Although many studies have tested animal responses to individual forms of novelty (e.g., human objects, food, urban noise), to our knowledge no study has comprehensively assessed behavioral reactions of urban and rural populations to numerous novel environmental stimuli. We tested exploratory behavior of urban, suburban, and rural house finches (*Haemorhous mexicanus*) in response to four different types of novelty (novel structural environment, novel object, novel noise, and novel food) in separate captive experiments, while also recording each bird for 30 minutes before exposure to determine baseline activity level. Although we found few population-level differences in behavioral responses to novel objects, environment, and food, we found significant differences in how finches from different sites responded to novel noises. When played a novel sound (whale call or ship horn), urban and suburban house finches approached their food source more quickly and spent more time on it than rural birds, and urban and suburban birds were more active during the whale-noise presentation. These results indicate that, in comparison with

other types of novel stimuli, anthropogenic noise may be a key driver of urban adaptation in birds and its influence may vary depending on the type of sound.

Introduction

The growth of urban areas continues to pose a challenge to the planet's biodiversity. Since 2007, more people reside in cities than natural areas (United Nations Population Fund, 2007), and urban growth is projected to double in the next decade (U.N. DESA, 2003), resulting in a predicted 70% of the human population residing in cities by 2050. This poses many challenges to wildlife, such as destruction of native habitat, introduction of non-native predators and competitors, and air/water/noise pollution (Marzluff, 2001; Shochat et al., 2006). However, many species of animals thrive in the presence of humans, benefiting from increased availability of food (e.g., human refuse, bird feeders, agricultural sources), water (Fokidis et al., 2009), cover, and nesting sites (e.g., buildings, shade trees; Møller, 2009). A recent study shows that population density of urban birds has more than doubled compared to that of their rural counterparts, in large part because of the supplemental food sources provided by humans (Tryjanowski et al., 2015). The question of which traits allow some species to adjust to and flourish in urban environments while others are driven out is an important one facing urban behavioral ecologists.

Animals face many survival threats, such as predators, territorial conspecifics, and harmful food sources, in their natural environments, but relaxed selection on neophobia is one potential driver of adjustments to urban areas that has recently gained traction in urban ecological studies (Tryanjowski et al., 2016; Griffin et al., 2017). In particular, increased availability of anthropogenic food sources (43% of people in the United States and 75% in the UK feed birds in urban areas; reviewed in Robb et al., 2008) may permit the adoption of novel feeding tactics, such as feeding from bird feeders, agricultural sites, or trash cans/dumps (Kark, 2007; Møller, 2008; Liker & Bokony, 2009). Thus, urban individuals may be more willing to approach novel objects because they may contain food and pose little threat, and thus locate and exploit novel food sources more than their rural counterparts (Greenberg & Mettke-Hoffman, 2001). This propensity to take risks in response to an unknown situation has been described as ‘exploration,’ ‘risk-taking,’ and ‘neophilia/neotolerance’ (Reale et al., 2007). Studies of neophilia/neotolerance have implications for behavioral innovation, stress responses, competitive ability, aggression, and fitness of animals residing with or near humans (reviewed in Greggor et al., 2015). However, neophobia could still be valuable in urban areas because neophilic animals may face increased exposure to toxins, predators, and parasites that they should learn to avoid (Greenberg & Mettke-Hoffman, 2001).

Studies on behavioral responses of animals in urban areas to novel/anthropogenic stimuli have provided conflicting results. Captive mynas (*Acridotheres tristis*) in urban areas show higher levels of exploration in novel foraging tasks and in the face of predators than their rural counterparts (Sol et al., 2011). However, wild populations of house sparrows (*Passer domesticus*) and brown-headed cowbirds (*Molothrus ater*) show lower levels of exploration than rural conspecifics when approaching novel objects and structures (Echeverria & Vassallo, 2008). A breadth of research has been done on house sparrows, a well-known urban adapter, in captivity, also producing conflicting results. Bokony et al. (2012) found no difference in latency to approach novel food or objects between urban and rural house sparrow populations, though Martin and Fitzgerald (2005) found that populations of house sparrows who had more recently invaded an urban area were more likely to approach novel food, though not novel objects, than established populations, and Liebl and Martin (2014) found that sparrows in edge populations were more likely to approach novel food sources than established populations. Thus, additional studies are needed to understand contexts in which urban animals may benefit from either pursuing or avoiding novelty.

In addition to the aforementioned forms of visual stimuli, urban animals face another unique challenge near humans: anthropogenic sound, which can come in novel forms (e.g., frequencies, locations, daily timing, etc.) and be considerably louder than natural ambient sound (Barber et al., 2009). For example, in the United States, more than 88% of the population is exposed to anthropogenic noise louder than 55 dB(A) (Mennitt et al., 2013), which is roughly equivalent to the sound of constant rainfall (Chepesiuk, 2005) and at the point at which humans begin to show elevated stress hormones (Babisch, 2003). In the face of anthropogenic noise, which rarely serves as a rewarding stimulus, animals must either modify behavior (e.g., alter vocalizations, tune out) and/or stress physiology to persist in noisy human environments or risk being extinguished from human-impacted areas.

Many studies have measured behavioral responses of animals to single novel anthropogenic stimuli (e.g., novel environment, objects, noise, food; reviewed in Griffin et al., 2017), but to our knowledge no one to date has investigated responsiveness to multiple stimuli in the same study system. Given that prior studies on different taxa using different stimuli have revealed mixed results, such an investigation is needed to better evaluate consistency of neoresponsiveness in urban v. rural animals. We tested the exploratory behavior of several populations of a common North American bird species (the house finch, *Haemorhous mexicanus*) sampled across a gradient of urbanization in the desert of southwestern U.S.A. (Phoenix, Arizona). Specifically, we exposed captive urban, suburban, and rural finches to four types of novel anthropogenic stimuli: (1) structural environment (Escheverria & Vassallo, 2008), (2) objects (Drent et al., 2003), (3) noise, and (4) food. To thoroughly explore site- and stimulus-specific differences in behavior, we also recorded each bird in its cage for 30 minutes to determine the baseline activity level since general movement around the cage (also a relatively new environment for the birds) could affect exploratory behavior. Urban areas have more human-created structures, food sources (e.g., bird feeders, trash cans), and noises (e.g., car alarms, construction, traffic) than non-urban areas, so we predicted that urban and suburban birds would show more exploratory behavior toward and tolerance of these novel anthropogenic stimuli (i.e., that they would show less behavioral stress, be less active, and approach food more quickly than rural birds in the presence of these novel stimuli).

Methods

Field Methods

We trapped adult house finches during the winter (pre-breeding season; December 2013 - February 2014); we selected this time of year for testing because in our prior work (Weaver et al., 2018) we saw greater urban-rural differences in behavior during the winter season than the summer molt season. Because of the length of the season, we used day trapped as a covariate in statistical analyses; however, this was not significant so we removed it from the model. Based on an a priori power analysis and similar prior phenotypic work by our group on these birds (Giraudeau et al., 2015), we aimed to trap ~20 birds per site using basket traps baited with sunflower seeds at each of six sites across an urban gradient: two considered urban (n = 55 birds captured in total), two considered suburban (n = 39), and two considered rural (n = 53), based on urban land-use parameters (e.g. population density, landscape type) measured by the Central Arizona-Phoenix Long-Term Ecological Research program (Giraudeau et al., 2014). The urban sites consisted of the Arizona State University (ASU) – Tempe campus aviary and a neighborhood near downtown Phoenix, the suburban sites were a suburban park (Gilbert, AZ) and neighborhood (Chandler, AZ), and rural sites were located at natural desert areas in South Mountain and Estrella Mountain Regional Parks (see map in Appendix C).

At capture, each bird was fitted with a numbered United States Geological Survey metal identification band and transported to ASU in a paper bag. Because of financial costs associated with captively housing the birds, we trapped finches at one site, brought them to captivity for eight days (see below for test procedures), returned them to the wild, and then trapped at the next site. Each bird went through one trial per day, beginning the day after capture. While birds might have experienced some captivity-related stress, no birds decreased in weight during the study, and we exposed all birds to the same conditions. We randomized site order and found no significant site differences in trapping date in our analysis.

Captive housing and initial testing

We individually housed birds for 8 days in small wire cages (60cm x 40cm x 30cm) in a climate-controlled indoor vivarium on the Arizona State University – Tempe campus.

All birds were provided with a diet of black sunflower seeds and tap water *ad libitum* during daylight hours and housed in the same room, which was kept at a temperature of 25º C on a natural outdoor light cycle. Each bird went through four trials (described below), in which they were separately exposed to a novel environment, novel objects, novel noises, and novel food. Trial order was randomized among birds and sites. Also, on the day after capture, each bird was filmed for 30 minutes with a video camera in its home cage to establish baseline activity levels that may explain variation in behavioral performance during the subsequent trials. From each video, two independent observers scored several behaviors using the software program Cowlog (Hanninen & Pastell, 2009): the number of flights (defined as any time bird moves using its wings; used as a general measure of activity level), number of bill wipes (proposed as an indicator of stress and displacement behavior; Tinbergen, 1940; Clark, 1970), and time spent on the feeders. Data collected by each observer were tested for repeatability (Table 1.4; Lessells & Boag, 1987), and behavioral scores were averaged for final analyses. Observers were pre-screened on training videos and expected to reach 0.75 repeatability with each behavior before participating in official data collection. If this did not occur, a third observer watched a subset of videos, and we selected the data from the observer with which the third person's repeatability was > 0.75. All observers were blind to site to avoid observer bias.

Novel Environment Trials

Each bird was released for one hour into a large, novel aviary cage (Figure 1.1) equipped with a large storage box with holes carved into it, a picnic table, a large branch, and two zebra finches (*Taeniopygia guttata*, a male and female). The box, table, and branch contained sunflower seeds in, on, or next to it. The large storage box was used to create a novel feeding structure that birds had never experienced, the table represented a human-associated object that could appear at urban, suburban, and rural sites, and the branch was included as an object from the natural environment. Because birds in urban areas may be exposed to non-native competitors introduced by humans (e.g., house sparrows, rosy-faced lovebirds *Agapornis roseicollis*), we included zebra finches (which are native to Australia and thus foreign to house finches) as novel biotic stimuli. Each trial was recorded by two video cameras arranged to capture as much of the large aviary space as possible. In addition to the aforementioned three behaviors, we also quantified how much time subjects spent on each of the box, table, and branch (or within 1 m of the zebra finches) as well as their latency to approach each. Prior to the trial, birds were food-deprived overnight to ensure motivation to feed. For all trials in this study, trials were conducted between 0700 and 1400 hours, but we found no significant effect of time of day tested on our analysis and so did not include this factor in the final analyses.

Novel Object Trials

Each bird participated in three different hour-long, novel-object trials on three separate days in our campus courtyard aviary (Figure 1.1) and was exposed to three objects (one per trial): a flashing glow stick, a camouflage flag, and a tree branch of a size similar to the glow stick and base of the flag (sham control). The two novel objects were selected because they might appeal to different visual sensitivities, as the glow stick flashed different colors (while remaining stationary) and the flag moved unpredictably with wind (while not exhibiting striking color). Each object was placed on a perch next to the only food dish in the aviary cage prior to an overnight food deprivation period to ensure motivation to feed. We used four of each different type of object and randomly assigned different objects to each bird to avoid pseudoreplication. As above, testing time of day was used as a co-variate in analyses, order of object presentation was randomized, and each trial was recorded with video cameras mounted on tripods placed outside of the test arena. In addition to quantifying flights, bill wipes, and time spent on the feeder, we also measured latency to approach the food dish. Immediately before and after each trial, we also measured a physiological response, breath rate, by counting number of breaths in one minute as the bird was held in hand, a method of measuring stress that is less invasive than taking a blood sample and previously was shown in great tits (*Parus major*) to increase following stressful situations (Carere & van Oers, 2004).

Novel Noise Trials

Each bird participated in two 30-min. novel-noise trials on successive days. Trials were again held in our campus courtyard aviary with a food and water dish and after birds had experienced overnight food deprivation. For the first 10 minutes of every trial, we played back white noise, to determine how birds generally reacted to a standard sound being played through the speakers at maximum volume in order to see if they reacted differently to the novel sound based on just responding to the speaker. Finches were then exposed to 10 minutes of either humpback whale vocalizations or ship sounds (order of noise trial was again randomized among birds), both of which should be novel to desert house finches. These sounds were compiled from a looped recording of online sounds (https://drive.google.com/drive/folders/1ixWK1U0wgGYc_wimfhoLoGeAvs4dWL3q) played throughout the 10-minute trial, selected because one (ship) is a monotonous, lower-frequency sound and the other (whale) is a variable, higher-frequency sound, representing different types of novel noise types to inland populations of house finches from Arizona. While both overlap in frequency range with both house finch song and urban noise (see Figure 1.1 for spectrograms), the dominant ship-sound frequencies overlap more with those of urban noise, and the same is true whale noise with house finch song. We played no sound for the final 10 minutes of each trial. To maintain consistency in sound quality across trials, the sound was played from the same location, behind the aviary three meters from the food dish with no person present, and the same speaker was used on maximum volume (32 dbM) with a first-generation iPod shuffle. Because the sounds were synthetically recorded and not designed to how the range of songbird repertoire is affected (as critiqued in Kroodsma, 1990), just to present birds with novel sounds, we used only one ship recording and one whale recording during the trials (Van Donselaar et al., 2018). All trials were video-recorded, and we scored the same bird behaviors described above for the novel-object trials. We also measured breath rate before and after each trial by counting how many breaths each bird took in one minute.

Novel Food Trials

Each bird participated in one 30-minute novel-food trial, which occurred in its home cage after an overnight food-deprivation period. Because these trials were all conducted at the same time of day (0700 hrs.), time of day was not included as a co-variate in the statistical analyses. For these trials, we added diced papaya, rather than the typical sunflower seeds, to each bird's food dish. We chose this food because it is unlikely that desert house finches are exposed to this food source in the wild, but it is known to be one of the primary food sources of Hawaiian populations of this species (Hirai, 1974). Each trial was recorded on a video camera. Birds were scored for latency to land on the feeder after the food was introduced to the cage and for time spent feeding. Trials were limited to a half hour because birds were left in their home cage and did not need to explore the environment to find the food.

Statistical Methods

We ran all statistical analyses in the R computing environment (Ver. 2.15.1). Values were square-root- or log-transformed to normalize them when necessary. We selected behaviors that we found in previous studies to measure different traits (Weaver et al., 2018), allowing us to analyze each behavior separately rather than combine them using principal components analysis. Also, as we believe each of these behaviors captures unique information because they are not correlated (Weaver et al., 2018), we ran separate analyses of variance (ANOVAs) rather than a multivariate ANOVA (MANOVA; Huberty & Morris, 1989). We applied sequential Bonferroni corrections (Rice, 1989) to account for multiple testing (i.e. four novel-stimulus tests, so starting p-value was 0.0125 in each model). Flights represent general activity in an environment, bill wipes capture stationary stress/displacement behavior, latency to approach object/feeder measures exploration of a specific object in the environment, and time spent feeding represents acclimation to the novel object/food. To determine whether the behavioral responses of house finches to novel stimuli differed based on degree of urbanization, we ran a series of

ANOVAs using capture site (urban, suburban, rural), sex, and their interactions as the predictors/covariates and breath rate, flights, bill wipes, time spent on feeders, and latency to approach objects/feeders as the separate response variables. In addition, we entered individual as a random effect to account for the fact that birds were tested in multiple trials. In the novel-object and -noise trials in which there was more than one treatment, we also used treatment as a predictor. When necessary, we used Tukey's honest-significant-difference tests for post-hoc comparisons. Because there were three novel object trials, we also calculated repeatability (Stoffel et al., 2017) using the protocol established in Lessells and Boag (1987) for each bird to measure temporal consistency in behavioral responses.

Results

Home Cage Activity Levels

During the first day in captivity, we found that urban and suburban birds were significantly more active (i.e. greater number of flights; Figure 1.2) than rural birds. In addition, urban birds displayed more stress behaviors (i.e. greater number of bill wipes; Table 1.1) than rural birds, but this was not significant after Bonferroni correction. There were no capture-site differences in time spent on the feeder, and overall we found no significant effects of sex or the site*sex interaction on finch behavioral measures (Table 1).

Novel Environment Trials

Urban, suburban, and rural birds did not significantly differ in latency to approach or time spent on the box, table, or branch (Table 1.1). However, urban birds spent less time on the feeder and took longer to approach it than did suburban and rural birds (Figure 1.3). Interestingly, rural birds approached the zebra finches significantly faster and spent more time near them than did urban and suburban birds (Figure 1.3). There were no site differences in activity or stress behaviors and no significant effects of sex or any interaction terms on finch behavior in this experiment (Table 1.1).

Novel Object Trials

We found no effect of urbanization on finch behaviors or breath rate in the novel-object trials, regardless of stimulus type (glowstick, control stick, or flag; Table 1.2). There were also no significant effects of sex or the site*sex interaction on finch behaviors or breath rate (Table 1.2). Overall, rural birds were most repeatable in their behavior, and urban birds were least repeatable, with the biggest differences appearing in the time spent on feeder and latency to approach feeder, where rural birds were highly repeatable but urban birds were not (Table 1.3). Regardless of capture site, initial breath rate was most repeatable, and flights and bill wipes were least repeatable (Table 1.3).

Novel Noise Trials

Regardless of noise type (ship vs whale), urban and suburban birds approached the feeder significantly more quickly during the sound playback than did rural birds (Figure 1.4). We also found that urban, suburban, and rural birds differed significantly in the amount of time spent on the feeder (Figure 1.4); suburban birds spent the most time on the feeder, regardless of time or noise, whereas rural birds spent the least. Noise exposure significantly affected how much time birds spent on the feeder, with birds from all sites spending the least amount of time on the feeder after the noise presentation (Figure 1.4), regardless of noise type. Because they spent the most time on the feeder during the white noise (Figure 1.4), we believe that birds were responding to the noise rather than the exposure of the speaker in captivity.

We found no significant site differences in finch activity level, but there was a site*noise interaction, such that suburban birds were less active than both urban and rural birds during the ship-noise presentation, but not during the whale trials (Figure 1.4). Urban, suburban, and rural birds did not differ in bill wipes during these trials, but there was an effect of noise type on bill wiping; birds bill-wiped more during the whale sound (Figure 1.4), regardless of site. There were no effects of sex, site, noise types, or any interactions on breath rate (Table 1.2).

Novel Food Trials

Of the 143 birds that completed the trial, 121 of those approached the feeder and pecked at the food. Birds from the three sites did not differ significantly in time spent eating or latency to approach the novel food source (Table 1.2). There were also no significant effects of sex or the sex*site interaction on finch behaviors during these trials (Table 1.2).

Discussion

Because urban and suburban animals can be exposed to a variety of novel environmental stimuli, we tested whether or not urban, suburban, and rural house finches showed differential behavioral responses to anthropogenic noises, structures, food sources, and objects. We predicted that, given their presumed prior experience with such stimuli (some benign, others rewarding), urban birds would better behaviorally adjust to these stimuli, showing less behavioral stress, exhibiting lower activity levels in the cages, and approaching feeding sources more quickly than rural birds. We found support for these predictions in the novel-noise trials, as urban and suburban birds approached the feeder more quickly and spent more time on the feeder during noise presentations than did rural birds. However, urban, suburban, and rural birds showed few behavioral differences in response to presentation of novel feeding structures, objects, and food.

Most studies on response to anthropogenic novelty, regardless of context or species, have focused on response to human objects, environmental conditions, or supplemental food (Griffin et al., 2017) but rarely have included noise, though behavioral responses to noises have been studied extensively in birds and amphibians (Barber et al., 2010). Only one other study has included response to urban noise as compared with other urban stimuli. Van Donselaar et al. (2018) found that urban chickadees were more likely to approach a feeder during presentation of urban noise than rural but found no differences between latency to approach novel objects, though this study utilized more urban-specific stimuli rather than presentation of novelty. As latency to approach novel food or objects is the common measure in novelty tests (Griffin et al., 2017), our results indicate that rural birds exhibited a stronger aversive response to the novel noises, regardless of noise type. The fact that we found stronger behavioral differences in noise trials than the other novelty presentations is consistent with prior work in both birds (Bayne et al., 2008; Rheindt, 2003) and anurans (Eigenbrod et al., 2008), which show that species richness is higher on transects further from noisy traffic ways, indicating that many species tend to avoid anthropogenic noises, though these studies were not testing novelty or response to other stimuli. Moreover, anthropogenic noise negatively impacts animal hunting efficiency in mammals (Luo et al., 2015; Siemers & Schaub, 2011) and birds (Mason et al., 2016), body condition (Schroeder et al., 2012) and pairing success in songbirds (Bayne et al., 2008), foraging success in mammals (Brown et al., 2012; Shannon et al., 2014) and fish (Purser & Radford, 2011), and mating success in birds (Gross et al., 2010; Habib et al., 2007; Reijnin & Foppen, 1994).

Finches in our study also differentially responded to the varied noise treatments, such that birds showed a greater number of stress behaviors and spent less time on the feeder during whale sound trials but not during ship sound trials. This is particularly interesting because the whale sounds were more heterogeneous, high-frequency vocalizations (see Figure 1.1 for spectrograms), which are unlike lower-frequency, constant noises typically recorded in cities (e.g., traffic, construction; Wood & Yerezinac, 2006; Goodwin & Shriver, 2011), but more similar to the higher-frequency songs that they produce naturally and that they even modify to higher frequency ranges in response to city noise (Halfwerk & Slabbekoorn, 2009). Most studies to date focus on response to urban noise, but none that we know of have looked at novel noises and/or higher-frequency noises that may overlap with natural vocalizations.

Aside from significant habitat- and noise-type-specific behavioral responses by the finches to the noise stimuli, we found few other urban/rural behavioral differences in the other novel environmental-stimulus trials. Rural birds approached the novel birds (zebra finches) faster than did urban and suburban birds, but there were no population-level differences in latency to approach novel feeding structures, though urban birds did spend less time on the familiar feeder than rural birds and took longer to approach it, which could be affected by motivation to feed. That rural birds approached novel birds more quickly is not what we had predicted; however, this result paired with finding no differences in response to the other novel structures, objects, and food may indicate that rural birds are exposed to enough novelty (e.g. novel birds, structures, food types depending on season) in their natural environments that they are not affected by it as much as we expected. While urban areas have novel structures and food sources, there is a great reduction in biodiversity of both plant and animal species (Chace & Walsh, 2006; McKinney, 2008). Thus, birds from natural areas may generally be more exposed to a range of bird species (i.e. novel forms to them as they develop), resulting in reduced wariness to approach another species, especially if that species is not a threat.

Taken together, these mixed results generally mirror those of prior studies on response to novelty in animal populations, where there is some evidence for both neophobia and neotolerance in urban wildlife. For example, in a review of response to novelty, Griffin et al. (2017) found urban birds showed higher levels of neophobia in five studies, lower levels in four, and no difference in three. Our results most closely resemble those of Bokony et al. (2012), which found no difference in latency to approach novel food or objects between urban and rural house sparrow (*Passer domesticus*) populations. Though house finches are a species native to the desert southwestern USA, they now inhabit human-developed areas through most of the country following an introduction in the east in the 1940s (Hill, 1993). Their ability to thrive across a range of environments/climates may be a result of reduced fear of novelty, especially given their need for opportunism (e.g., breeding, feeding) in their native desert environment where resources are dispersed in both space and time. We have shown elsewhere that house finches do not show an increase in plasma corticosterone after introduction to a novel environment (Weaver et al., 2018); similarly, European starlings (*Sturnus vulgaris*) showed no increase in heart rate following an introduction to novel food and objects (Fischer et al., 2016). Thus, for certain environmental stimuli, especially those perceived as new opportunities or added resources, these birds (whether urban, suburban, or rural) may not respond to them as novel stressors but instead as commodities worth investigating, as opposed to cues like sound that carry perceived threat or greater risk than reward.

Ultimately, as urban areas expand worldwide and present animal populations with unique abiotic and biotic challenges, we must continue to improve our understanding of which anthropogenic stimuli are most supportive of or disruptive to animal populations and biodiversity. Although we found little evidence for urban-rural differences in neophobia/neotolerance in house finches, this may have occurred because house finches are an adaptive species that have demonstrated an ability to thrive in both native desert and urban environments. Conducting these robust novelty studies on species that do not acclimate to urban environments or only thrive around humans could elucidate more patterns in different levels of neotolerance/neophobia. We did find that rural birds took longer to approach a feeder and spent less time feeding in response to novel noises than urban and suburban birds. Given that studies on response to novelty across an urban gradient have been mixed, but many studies have demonstrated that anthropogenic noise can negatively impact biodiversity, we suggest that more researchers incorporate response to novel noises into future urban behavioral-ecological studies. Our experimental results suggest that environmental noise is a driving force shaping avian acclimation or adjustment to urban areas.

Figures

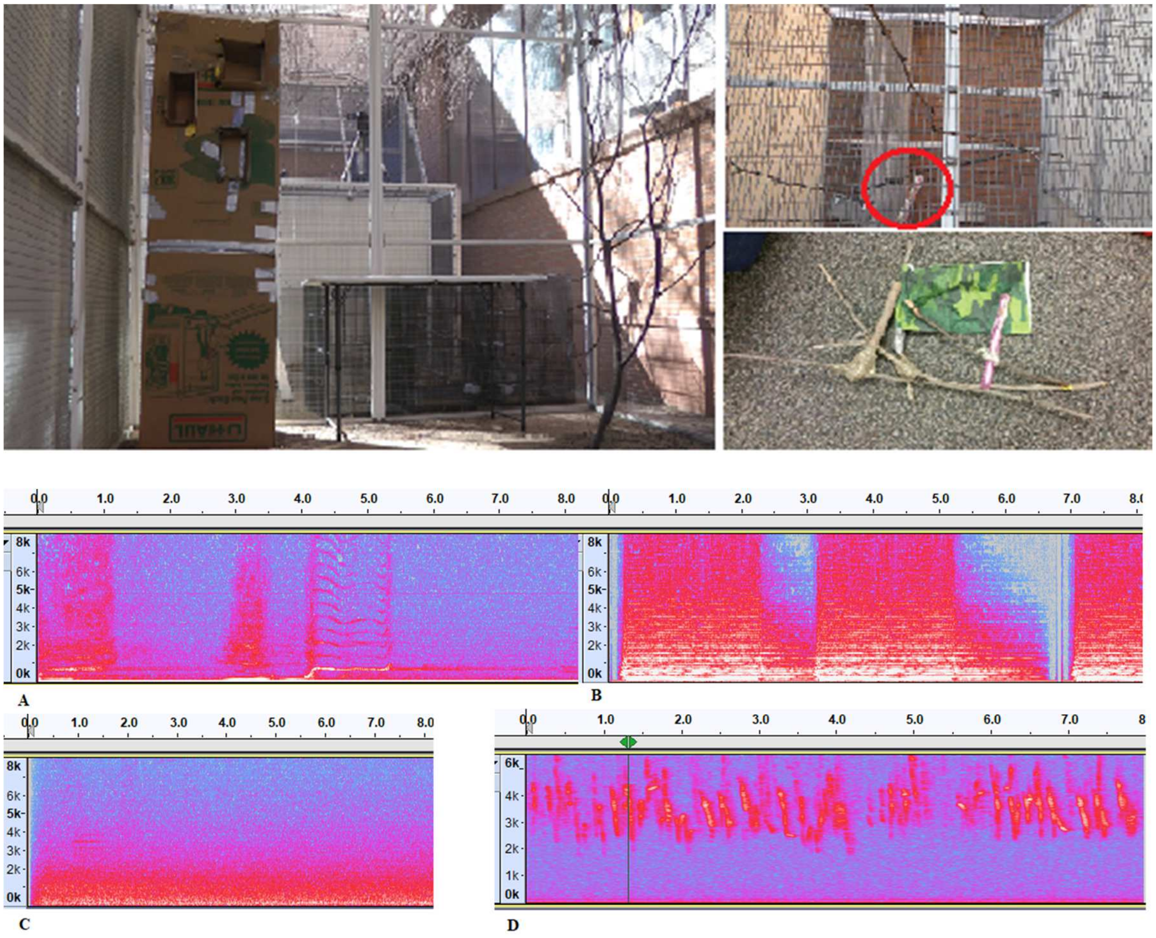

Figure 1.1. Photo at top left illustrates the large novel environment, containing a taped cardboard storage box (left), white and black table (center), and branch (right). The pair of photos at top right illustrate the glowstick (top) and all objects (bottom) used as novel objects. Spectrograms illustrate whale sound (A), ship sound (B), urban noise (C), and house finch song (D).

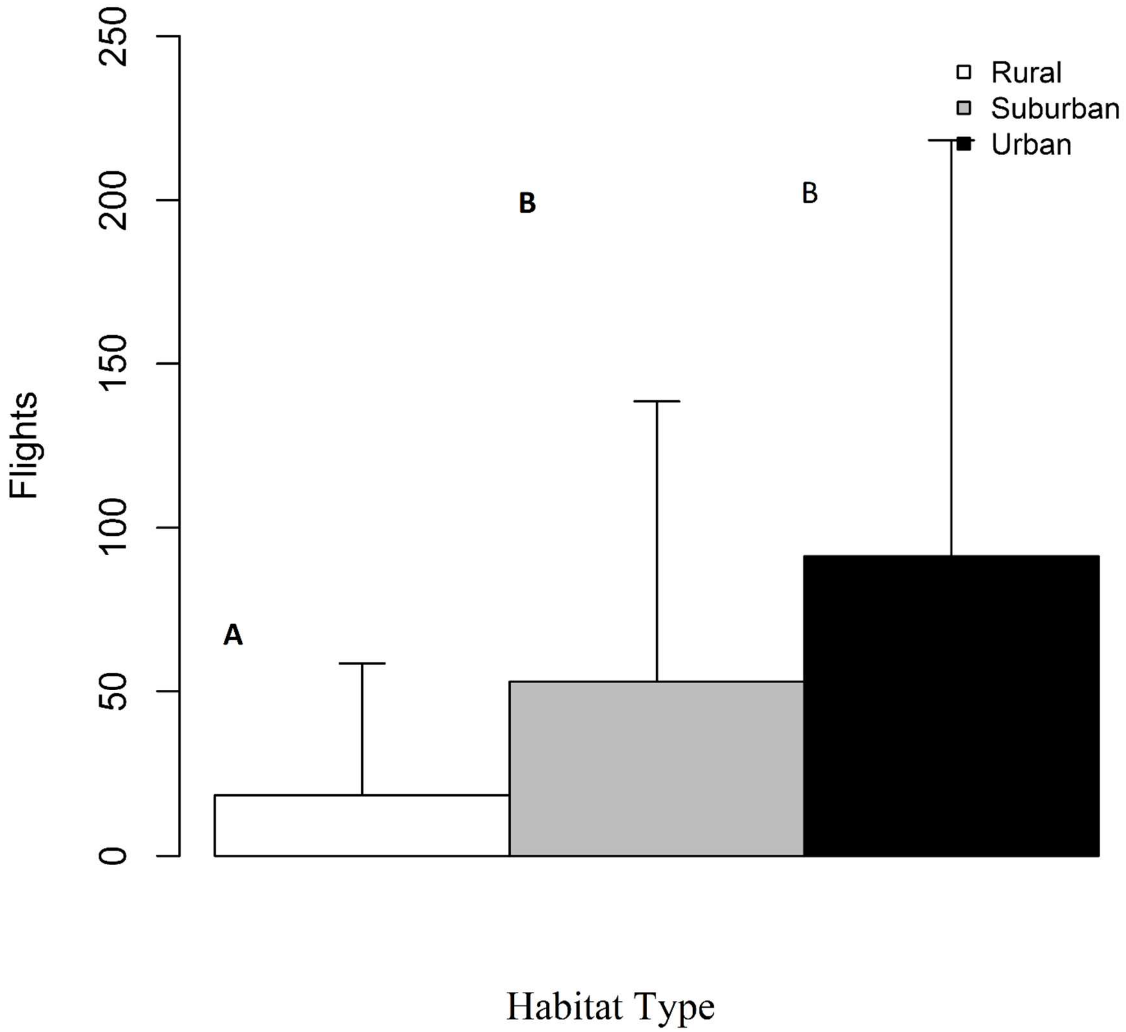

Figure 1.2. When birds were video-recorded in their home cages to quantify baseline behavior, we found that urban and suburban birds were significantly more active (i.e. had a greater number of distinct flights within the cage in the 30-minute timeframe; mean + SD depicted) than rural birds. Unshared letters denote groups that were significantly different after post-hoc testing.

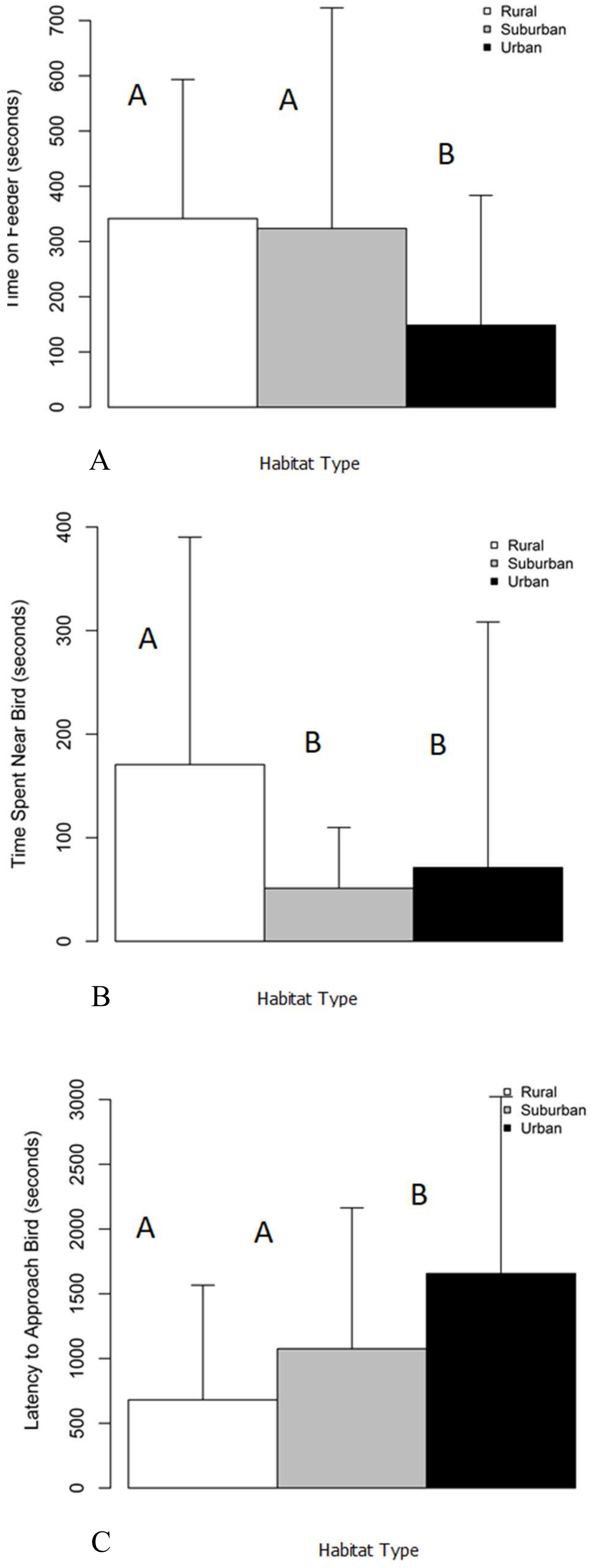

Figure 1.3. In our novel-environment trials, urban birds spent significantly less time on the feeder than did rural and suburban birds (A), urban and suburban birds spent less time near the novel birds than did rural birds (B), and urban birds took longer to approach the novel birds than rural birds (C). Again data are represented as means + SD and unshared letters denote groups that were significantly different.

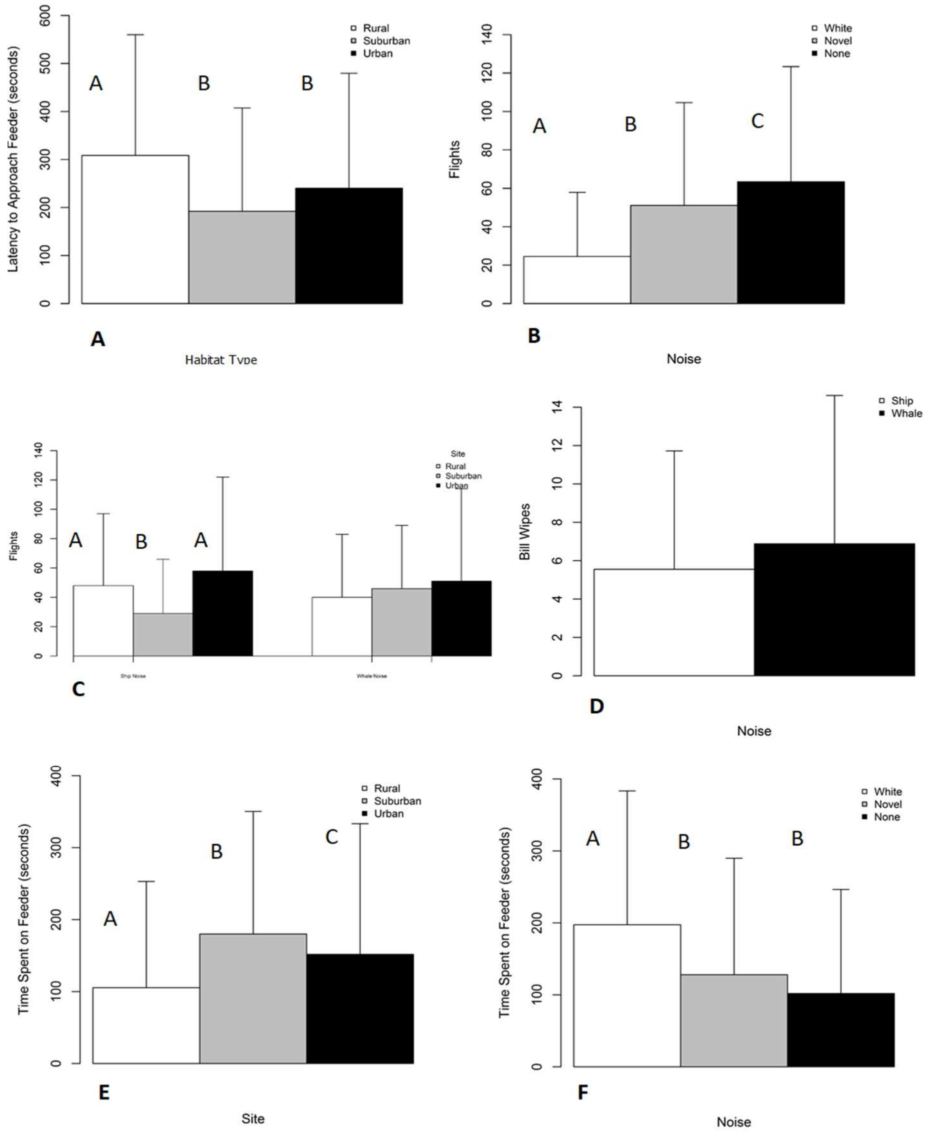

Figure 1.4. In the novel-noise trials, rural birds took significantly longer to approach the feeder than did urban and suburban birds (a), birds flew least before the novel noise and most after it (b), urban and rural birds were more active than suburban birds during the ship, but not whale, noise (c), birds bill-wiped more during the whale noise than ship noise (d), rural birds spent the least amount of time on the feeder and suburban birds spent the most, regardless of the noise (e), and birds spent more time on the feeder before and during the noise than after the noise (f).

Tables

Table 1.1. Results of ANOVAs for the activity and novel-environment experiments, including effects of each of the variables measured and their interactions on different finch behaviors. Because we applied sequential Bonferroni corrections, $p < 0.01$ denotes statistical significance.

Experiment	**Behavior**	**Term**	**F**	**df**	**p**
Activity	Flights	Site	9.03	2, 127	**<0.01**
		Sex	1.11	1, 128	0.29
		Site*Sex	3.93	2, 127	0.02
	Bill Wipes	Site	3.11	2, 127	0.04
		Sex	1.38	1, 128	0.24
		Site*Sex	0.28	2, 127	0.76
	Time on Feeder	Site	0.84	2, 127	0.44
		Sex	0.01	1, 128	0.92
		Site*Sex	1.36	2, 127	0.26
Environment	Flights	Site	0.07	2, 144	0.93
		Sex	0.40	1, 145	0.53
		Site*Sex	1.36	2, 144	0.26
	Bill Wipes	Site	3.61	2, 144	0.03
		Sex	0.01	1, 145	0.93
		Site*Sex	0.18	2, 144	0.91
	Time on Feeder	Site	9.26	2, 144	**<0.01**
		Sex	0.25	1, 145	0.62
		Site*Sex	0.04	2, 144	0.97
	Time on Branch	Site	3.04	2, 144	0.05
		Sex	0.07	1, 145	0.80
		Site*Sex	0.32	2, 144	0.73
	Time on Table	Site	2.33	2, 144	0.10
		Sex	0.01	1, 145	0.94
		Site*Sex	0.52	2, 144	0.59
	Time on Box	Site	0.53	2, 144	0.59
		Sex	0.80	1, 145	0.37
		Site*Sex	0.08	2, 144	0.93
	Time Spent Near Bird	Site	17.73	2, 144	**<0.01**
		Sex	0.41	1, 145	0.53
		Site*Sex	1.25	2, 144	0.29

Time to Feeder	Site	3.56	2, 144	0.04
	Sex	2.00	1, 145	0.17
	Site*Sex	0.86	2, 144	0.43
Time to Table	Site	1.67	2, 144	0.19
	Sex	0.46	1, 145	0.50
	Site*Sex	0.05	2, 144	0.96
Time to Box	Site	0.66	2, 144	0.52
	Sex	0.01	1, 145	0.99
	Site*Sex	2.17	2, 144	0.12
Time to Bird	Site	7.87	2, 144	**<0.01**
	Sex	1.66	1, 145	0.20
	Site*Sex	1.14	2, 144	0.32

Table 1.2. Results of ANOVAs (repeated measures for object and noise, with band number used as a random effect) for the novel-object, novel-noise, and novel-food experiments, including effects of each of the variables measured and their interactions on different finch behaviors. Because we applied sequential Bonferroni corrections, $p < 0.01$ denotes statistical significance.

Experiment	**Behavior**	**Term**	**F**	**df**	**p**
Object	Flight	Site	3.85	2, 404	0.15
		Trial Object	3.29	2, 404	0.19
		Sex	2.11	1, 405	0.15
		Site*Trial Object	9.27	4, 402	0.05
		Site*Sex	2.67	2, 404	0.26
		Sex*Trial Object	1.06	2, 404	0.59
	Bill Wipe	Site	5.76	2, 404	0.06
		Trial Object	2.51	2, 404	0.28
		Sex	0.10	1, 405	0.76
		Site*Trial Object	2.48	4, 402	0.65
		Site*Sex	0.39	2, 404	0.82
		Sex*Trial Object	2.39	2, 404	0.30
	Time on Feeder	Site	1.99	2, 404	0.37
		Trial Object	5.79	2, 404	0.06
		Sex	3.10	1, 405	0.08
		Site*Trial Object	3.83	4, 402	0.43
		Site*Sex	2.74	2, 404	0.25
		Sex*Trial Object	4.77	2, 404	0.09
	Time to Feeder	Site	1.74	2, 404	0.42
		Trial Object	2.37	2, 404	0.31
		Sex	0.11	1, 405	0.74
		Site*Trial Object	2.03	4, 402	0.73
		Site*Sex	0.62	2, 404	0.73
		Sex*Trial Object	1.56	2, 404	0.46
	Breath Rate	Site	1.16	2, 810	0.56
		Trial Object	5.80	2, 810	0.06
		Measurement Period	0.37	1, 811	0.54
		Site*Trial Object	4.11	2, 808	0.39
		Site*Measurement Period	0.83	2, 810	0.66

Noise	Flight	Site	8.41	2, 838	**0.01**
		Trial Type	3.35	1, 839	0.07
		Noise Type	24.13	2, 838	**<0.01**
		Site*Trial Type	16.88	2, 838	**<0.01**
		Site*Noise Type	8.05	4, 836	0.09
	Bill Wipe	Site	1.40	2, 838	0.50
		Trial Type	5.70	1, 839	**0.01**
		Noise Type	0.80	2, 838	0.67
		Site*Trial Type	0.92	2, 838	0.63
		Site*Noise Type	2.03	4, 836	0.73
	Time on Feeder	Site	13.57	2, 838	**<0.01**
		Trial Type	0.30	1, 839	0.58
		Noise Type	11.44	2, 838	**<0.01**
		Site*Trial Type	2.42	2, 838	0.30
		Site*Noise Type	2.80	4, 836	0.59
	Time to Feeder	Site	11.21	2, 838	**<0.01**
		Trial Type	0.32	1, 839	0.57
		Noise Type	0.50	2, 838	0.78
		Site*Trial Type	6.61	2, 838	0.04
		Site*Noise Type	11.11	4, 836	0.03
	Breath Rate	Site	1.61	2, 538	0.45
		Trial Type	0.26	1, 539	0.61
		Measurement Period	0.12	1, 539	0.72
		Site*Trial Type	0.09	2, 538	0.95
		Site*Measurement Period	5.29	2, 538	0.07
Food	Time Feeding	Site	0.99	2, 141	0.37
		Sex	0.09	1, 142	0.77
		Site*Sex	0.25	2, 141	0.78
	Time to Feed	Site	0.45	2, 141	0.64
		Sex	0.76	1, 142	0.39
		Site*Sex	0.33	2, 141	0.72

Table 1.3. Repeatability of finch measurements for birds from each site during the novel-object trials.

Behavior	**Site**	**Repeatability**
Pre-Trial Breath Rate	Rural	0.458
	Suburban	0.477
	Urban	0.509
Post-Trial Breath Rate	Rural	0.508
	Suburban	0.136
	Urban	0.505
Flights	Rural	0.326
	Suburban	0.392
	Urban	0.430
Bill Wipes	Rural	0.394
	Suburban	0.329
	Urban	0.354
Time on Feeder	Rural	0.408
	Suburban	0.541
	Urban	0.172
Latency to Approach Feeder	Rural	0.403
	Suburban	0.298
	Urban	0.294

Table 1.4. Repeatability measurements between two observers that watched every video from the human-approach trials.

Experiment	**Behavior**	**Repeatability**
Winter 2012	Hop	0.96
	Fly	0.93
	Ruffle	0.74
	Bill wipe	0.90
	Time on feeder	0.93
	Time in hide	0.83
Summer 2012	Hop	0.87
	Fly	0.86
	Ruffle	0.66
	Bill wipe	0.71
	Time on feeder	0.92
	Time in hide	0.87

CHAPTER 2

REARING ENVIRONMENT HAS STRONGER EFFECTS THAN URBAN/RURAL HABITAT OF ORIGIN ON BEHAVIORAL RESPONSES OF SONGBIRDS TO HUMANS AND NOVELTY

Abstract

Urban areas continue to expand worldwide and reduce animal biodiversity. Some species thrive in cities, but in most populations it is unclear if they are adapted or acclimated to urban environments. Also, for species that appear to acclimate to urban environmental stimuli, it is largely unknown at what point during development acclimation occurs. Here we used a common-garden experiment with juvenile birds to assess developmental differences in behavior between urban and rural birds. We captured fledgling house finches (*Haemorhous mexicanus*) at urban and rural sites in Phoenix, AZ, USA and randomly assigned them to either a low- or high-human-exposure treatment. We then assessed their behavior in response to an approaching human and a novel object at the beginning, mid-point, and conclusion (~70 days) of the study. At the mid-point and conclusion of the trial, we also ran similar behavioral tests on wild-caught birds from both habitat types for ecological comparison. We found that, at all time points and regardless of habitat of origin, birds that were less exposed to humans during development had higher activity levels when approach by a human than birds frequently exposed to humans. Wild-caught birds showed higher activity levels when approached than captive birds. We also found that, during the mid-point trials only, urban birds and wild-caught birds spent more time on the novel object than rural birds, suggesting that there is a critical learning period where exploratory

behavior may be stronger in juveniles in a complex environment. Taken together, these results provide experimental support for the notion that juvenile acclimation is a key driver of animal tolerance of humans in urban settings.

Introduction

The world is currently experiencing a massive, rapid land use change as more people move from rural areas to cities. Urbanization can negatively impact animals in many ways (reviewed in Marzluff, 2001; Shochat et al., 2006), including through loss of native habitat, exposure to nonnative competitors and predators, and urban noise and light pollution. Many species are unable to tolerate these changes, resulting in reduced biodiversity in cities (McKinney, 2006). Animals that do persist in cities must co-exist with humans and utilize anthropogenic food sources and structures for nesting and shelter. This often requires behavioral modifications (reviewed in Miranda et al. 2013), such as altered responses to predators (McCleery, 2009; Kitchen et al., 2010), competitors (Hasegawa et al., 2014), or mates (Lane et al., 2011), or adjusting choice of habitat (Miller et al., 1998, Yeh et al., 2007), vocalizations (Brumm, 2004; Barber et al., 2009), or feeding sources (Møller, 2008; Liker & Bokony, 2009). Often, studies on urban animal populations measure difference in 'boldness,' response to a known threat, and 'exploration,' response to an unknown stimulus (first defined in Reale et al., 2007), as animals that are successful in urban environments should better tolerate or utilize novelty, including built structures, food sources, the presence of people, and urban noise.

A large body of literature supports the idea that urban animals acclimate to human presence, demonstrating that urban populations of a variety of bird and mammal species flee at decreased distances from an approaching human than their rural counterparts (Arroyo et al., 2017; Carrete & Tella, 2017; Cavalli et al., 2016; Clucas & Marzluff, 2012; McCleery, 2009; Møller, 2008; but see Valcarcel & Fernandez-Juricic, 2009). Studies on the response of urban animals to other novel stimuli are not as clear-cut. Griffin et al. (2017) reviewed studies on bird exploration across an urban gradient and found that in all but two studies, urban and rural populations differed, indicating that animals are responding to humans through behavioral changes, though the directionality of the studies is mixed. In half of the studies, urban birds show greater levels of neophobia than their urban counterparts, and in the other half, they show decreased neophobia. This reveals more questions than answers in determining how exactly animals acclimate to life with humans.

Phenotypic plasticity, the ability of animals to adjust behavior, morphology, or physiology through time or across environments (first proposed by Baldwin, 1896), may be the driver behind responses to urban change, which must happen rapidly and effectively to keep up with urban growth (Charmantier et al., 2008). In fact, a meta-analysis showed that the rate of phenotypic change is greater in urban areas than natural areas or other anthropogenic systems (Alberti et al., 2017), suggesting that urban animals are acclimating to cities rather than being predisposed to survive in those areas. However, less is known about when during an animal's lifetime this occurs. A handful of behavioral studies are now recognizing the importance of developmental plasticity (Snell-Rood, 2013), and specifically the ontogeny of personality (Ruploh et al., 2013), in understanding how animals might change their phenotype in response to environmental changes, such as human-induced changes. Differences in early-life environment can alter short- and/or long-term behavioral responses in many species, including birds (*Taeniopygia guttata*, Ruploh et al., 2015; *Parus major*, Naguib et al., 2011; van Oers et al., 2015; *Cyanistes caeruleus*, Arnold et al., 2007; *Aphelocoma californica*, Pravosudov & Kitaysky, 2006; *Anas platyrhynchos*, Butler et al., 2011), which are commonly used in studies of acclimation to urban ecosystems. However, many of these studies test birds at just one time point during development, potentially missing differences in behavior that may appear during unknown critical developmental periods. In addition, most of these studies are conducted in a lab environment, without comparison to animals going through similar phases of their development under natural conditions, and few study urban-rural differences between populations.

To our knowledge, no study has utilized a common-garden experimental approach to investigate developmental differences in behavior between urban and rural populations. Therefore, we conducted a multi-faceted study that incorporated both field and lab trials and multiple time points to elucidate differences in response to human presence on juvenile house finches (*Haemorhous mexicanus*) from urban and rural sites in and around Phoenix, Arizona. House finches from their native range in the southwestern USA are prevalent in both human-dominated and natural (desert) areas, making them an excellent system for studying population-level behavioral variation. In a previous study on adult house finches at these sites, we found that, when approached by a human, rural birds had increased activity and stress behaviors compared to urban birds (Weaver et al., 2018b), indicating that urban birds are more tolerant of the presence of humans/novelty. Here, we experimentally tested the effects of human presence on development of these behaviors under controlled captive conditions. We captured house finches just after fledging and randomly assigned them to either a low- or a high-human-exposure treatment and ran behavioral trials at three different times during their juvenile life stage, which included the first 90 days of their development. At the mid-point and end-point of the trials, we also performed behavioral tests on wild-caught hatch-year finches at the same urban and rural sites to compare behavior with those developing in captivity. If animals are acclimating to urban environments, we predict that birds in the high human exposure treatment would show modified behavioral responses (less activity, fewer stress behaviors, more time spent on novel object) than low exposure birds, regardless of capture site. Alternatively, if some populations are genetically adapted to (i.e. innately attuned to behaving in) urban environments, we expect that urban birds would already exhibit differences in behavior at an early age (i.e. our initial testing) and be more neotolerant than rural birds, regardless of treatment type. We also predict that captive birds would be more tolerant of humans/novelty than wild-caught birds captured at roughly the same time-points in their development, since they are not being housed in captive conditions in close proximity to humans.

Methods

Field Methods

From 21-24 May 2014, using basket traps baited with sunflower seeds, we trapped ~20 juvenile house finches just after fledging at each of four sites: two considered urban (n = 44) and two considered rural (n = 44), based on urban land use parameters measured by the Central Arizona-Phoenix Long-Term Ecological Research program (Giraudeau et al., 2014). The two urban sites were the Arizona State University campus and a neighborhood near downtown Phoenix. The two rural sites were natural park areas at South

Mountain and Estrella Mountain Regional Parks (see Appendix C for map). Juveniles were identified based on unique plumage characteristics (Pyle, 1987). Based on previous research on house finch breeding in Arizona (Badyaev et al., 2012), we estimated the age range of the birds at capture to be 28-60 days (assuming first-egg-dates starting in late February). Since house finches do not disperse from their natal site until end of the summer (Veit & Lewis, 1996), we believe that we captured birds very near their nest during the selected time periods. At capture, we fitted each bird with a numbered United States Geological Survey metal identification band, measured body mass (using a digital scale, to the nearest 0.01 g), and inspected them for signs of avian pox. Birds of atypically low body mass (less than 15g) or with visible pox lesions were released without being tested. We transported the rest of these birds back to Arizona State University in a paper bag for the follow-up testing and experiment.

Behavioral Trials with Captive Birds

Birds were individually housed in small wire cages (60cm x 40cm x 30cm) in a climate-controlled vivarium and given a diet of black sunflower seeds and tap water *ad libitum*. The rooms were kept at a temperature of 25° C on a natural outdoor light cycle. In the week following capture, we ran the initial bout of behavioral tests from 26-28 May 2014. We placed each bird individually in a large cage (0.77 m tall x 0.59 m long x 0.50 m wide; Figure 1) in our outdoor courtyard aviary between 0600-1000 hrs. (only during mornings because of the extreme Phoenix summer heat, when daily maximum temperatures can exceed 45° C). We tested four birds simultaneously (in different visually separated aviary compartments) and thus ran 16 trials per day. We used trial date and time of day as covariates to statistically account for this temporal variation, but removed them from the final model because they had no significant effect. The cage was equipped with a shaded area and feeders with sunflower seeds and water. We added a novel object (a perch made of a child's sword that flashes colorful light) to measure latency to approach an unfamiliar perch. The perch was placed at the highest (i.e. preferred) level of the cage, while the typical stick perches were placed lower in the cage. We selected this object because it was the size of perch but glowed with flashing colors that would be novel to birds from all sites.

Each bird was given 30 minutes to acclimate in the new cage while we recorded all behavior with a handheld video camera mounted on a tripod placed 5 m from the cage. After the acclimation period, one person (MW) walked toward the cage starting from a distance of 10 m at a pace of one step per second until touching the cage and walked away at the same pace. We recorded the bird's behavior during this approach as well as for 15 minutes afterward. From each video, two independent observers scored three behaviors using the software program Cowlog (Hanninen & Pastell, 2009): frequency of flights (used as a measure of activity level), frequency of bill wipes (proposed as indicators of stress; Tinbergen 1940), and time spent on the novel perch. Data collected by each observer were tested for repeatability (Lessells & Boag, 1987), and behavioral scores were averaged for final analyses. Before averaging scores, observers were expected to reach 70% (0.70) interobserver repeatability for each behavior (final repeatability for flights = 0.82, bill wipes = 0.70, and time on novel object = 0.90). If observers had not reached 0.70 repeatability, a third observer would watch the videos until obtaining interobserver repeatability of 0.70. We would then use data from the observer with which the third observer was repeatable.

After administering the initial “human-approach test,” we randomly assigned 10 birds from each site to a “low human exposure” group and 10 to a “high human exposure” group (*sensu* Cook et al. 2017). Birds from the “low human exposure” treatment were exposed to a human in their housing room for up to 15 minutes per day, which occurs during normal husbandry (e.g. feeding, watering, sweeping), except on one day every two weeks when cages were changed out (which took about 60 minutes). In the “high human exposure” housing room (in addition to the baseline human activities outlined above for the “low human exposure” treatment), a research assistant slowly paced throughout the room for one randomly selected daylight hour every other day, at a rate of one step per second, which is meant to mimic human activity that might occur around birds living in urban areas.

Birds were kept in three different rooms, two that held 20 and one larger room that held 40 finches, housed only with birds belonging to the same treatment group. Birds were rotated on rolling cage racks among rooms every two weeks to remove any room effects on the experiment. We kept the birds in captivity for 70 days, which we selected in order to keep the birds in captivity during the first 90 days (or longer) of their development. This duration is just longer than the critical learning period for song development (reviewed in Brainard & Doupe, 2002) recorded in zebra finches (*Taeniopygia guttata*; George et al., 1995), song sparrows (*Melospiza melodia*; Marler & Peters, 1987), and white-crowned sparrows (*Zonotrichia leucophrys;* Marler, 1970). We retested each bird at the halfway point of the study (30 June-4 July) and again just before we released all birds back into the wild (10-13 Aug.), the same periods during which we tested birds in the wild (see more below). In the weeks following these human-approach trials, finches were also tested for problem-solving ability as part of another study (Cook et al., 2017), but all birds were tested in the same fashion as to not affect the outcome of the present experiment in any way. Because of an unexpected avian pox outbreak during the study, not every captive bird participated in all three trials. Birds that showed signs of pox were euthanized to prevent further spread and thus not included in subsequent trials. Thus, 73 birds (38 high exposure consisting of 17 urban and 21 rural birds, 35 low exposure consisting of 14 urban and 21 rural birds) participated in trial 2 and 57 in all three trials (30 high exposure consisting of 12 urban and 18 rural birds, 27 low exposure consisting of 12 urban and 15 rural birds).

Behavioral Trials with Wild-Caught Birds

We returned to each field site twice more (once at the mid-point of the study, 5-12 July, and once at the completion of the study, 15-23 Aug.) to capture and perform the above-described trials on wild-caught juvenile house finches. We ran tests at the field site from 0600-1000 hrs. in the same type of shaded outdoor microhabitat that captive birds were experiencing. Based on what we know about house finch ecology and behavior, we believe that this reduced stress levels that would have been exacerbated by transporting birds back to campus and keeping them in captivity overnight and that, by conducting these trials in the field in a similar microhabitat, we were able to gain better insight into their natural behavior. We tested up to 12 birds per day (three simultaneously, at locations at a site separated by 10 m), so we visited each site twice during a week-long period. Overall, we tested 160 wild-caught juveniles (39 urban and 40 rural in July; 37 urban and 44 rural in August) at these time points.

Statistical Methods

We ran all statistical analyses in the R computing environment (Ver. 2.15.1). In our prior work, none of our three behavioral measures (flights, bill wipes, and time spent on novel object) were significantly intercorrelated (Weaver et al., 2018), so we analyzed each behavior in a separate analysis of variance (ANOVA) rather than combining them using principal components analysis or running a multivariate ANOVA (MANOVA; Huberty & Morris, 1989). To determine whether the behavioral responses of house finches to an approaching human and to a novel object differed based on degree of urbanization or human exposure condition, we ran a series of repeated-measures ANOVAs using capture site (urban v. rural), condition (high-exposure captive v. low-exposure captive v. wild caught), trial number in captive birds only (first, second, third), time of trial (before approach, after approach) and their interactions as the predictors/covariates and flights, bill wipes, and time spent on novel object as the separate response variables. In addition, we entered individual as a random effect to account for the fact that birds were tested in multiple trials. Because all behaviors met assumptions of normality and equal variance, we used parametric statistics for each ANOVA. When necessary, we used Tukey's honest-significant-difference tests for post-hoc comparisons.

Results

First, we found a significant main effect of the human approach on flight frequency (Figure 2; Table 1), such that birds flew more after the human approach than before it; thus, finches were clearly responding to the approaching human stimulus. We found a significant effect of the captive human-exposure treatment on frequency of flights during the human approach trial (Figure 2). Finches experimentally exposed less to humans flew more than those birds who were frequently exposed to humans (Figure 2, Table 1). We also found an overall effect of trial period on frequency of flights (Table 1). Birds flew significantly less during the third trial than in the first and second trials (Figure 2). Last, we found a significant effect of captivity on flight frequency (Table 3). Captive birds flew significantly less than wild birds during their final trial but did not differ in flights during the mid-point trials (Figure 4). We found no other significant effects on flight frequency (Table 1).

We found no significant effects of any variable or the interactions on bill-wipe frequency (Table 1). However, we found a significant effect of the habitat type on time spent on the novel object (Table 2). Urban birds spent more time on the object than rural birds, but only during the mid-point trial (Figure 3). In addition, we found a significant effect of captivity on time spent on the novel object (Table 2). Also, during this mid-point trial only, wild-caught birds spent more time on the object than captive birds, and high exposure birds spent more time on the object than low exposure birds (Figure 3). We found no other significant effects on time spent on the novel object (Tables 1, 2, 3).

Discussion

To better understand development of behavioral differences in urban and rural house finches, we captured birds just after fledging at urban and rural sites and exposed them to either a low human approach treatment or a high human approach treatment. We then conducted behavioral trials pre-, mid-, and post-treatment, and compared the last two trials to trials conducted on wild-caught birds in the field. We found that, regardless of capture site and treatment, birds were less active in response to human approach in the post-treatment trial than the pre-treatment and mid-treatment trials. In addition, we found that, during the post-treatment trial, wild-caught birds flew more frequently in response to a human than captive birds, regardless of site. Finally, we found that, during the mid-point trial only, urban birds spent more time on the novel object than rural birds, and wild-caught birds spent more time on it than captive birds. Among captive finches, high-human-exposure birds spent more time on the novel object than the low exposure birds. These results suggest that house finches, regardless of site, have the ability to acclimate to the presence of humans and change their response to humans accordingly when exposed to them frequently as juveniles, as they would be in urban environments.

A number of studies have explored behavioral differences between urban and rural populations and discovered that urban birds are indeed capable of modifying behavior in response to anthropogenic conditions (reviewed in Miranda et al., 2013). However, little attention has been paid to the age of the birds, and tests on juvenile animals are comparatively rare. In our earlier study on adults from this population of house finches, we found that urban birds flew less and showed fewer stress behaviors in response to human approach than their rural counterparts (Weaver et al., 2018b). As adults may have already established their habitat and habitat-specific behavior, we replicated this study with juveniles here and added an experimental approach, by exposing them to low or high levels of human exposure. We did not find any clear and consistent urban/rural differences in behaviors of young birds, but we did show that, regardless of habitat of origin, high-human-exposure birds flew less than low-exposure birds (with no difference in stress behaviors or time spent on novel object). These results suggest that young birds from the different habitats did not inherently differ in behavior (i.e. due to genetic predisposition or to very-early-life conditions) and did not differentially respond to levels of human exposure during development. Instead, because all captive groups seemed to become accustomed to repeated exposure to humans for weeks, it appears that acclimation during the post-fledging phase accounts for observed urban finch tolerance of humans (i.e. in cities). However, it is worth noting that there was a small pre-study difference in flight behavior between the two treatment groups and no interaction between treatment and trial period, indicating that, although birds were randomly assigned to groups, finches from the low-human-exposure group were also more active before treatment onset. As a post hoc analysis, we ran a repeated measures ANOVA on the mid-treatment and post-treatment trials using the pre-treatment behavior as a covariate to further elucidate treatment differences and found no differences in flights between low-exposure and high-exposure treatments. Thus, while we can say that all birds acclimated to human exposure, there was no different in treatments ($F_{2, 227} = 1.55$, $p = 0.43$).

In the post-treatment trials, all captive birds, regardless of treatment, flew less in response to an approaching human than the previous two trials and flew significantly less than wild-caught birds tested during the same time period. This makes sense, as captive birds were exposed to humans on a daily basis during the study period, allowing enough exposure with reinforcement (i.e. no negative consequences to nearby/approaching humans, except being captured for cage relocation once every two weeks) to acclimate more to human presence than wild-caught birds. Our results resemble those found in an experiment on adult house sparrows (*Passer domesticus*), in which Vincze et al. (2013) found that all birds acclimated to human presence after several trials, but urban birds acclimated more quickly than rural sparrows. Although we detected no site differences in behavioral responsiveness to humans, our low-human-exposure treatment was meant to be comparable to rare human encounters in rural areas, whereas high exposure was intended to simulate urban-typical, frequent experiences with humans. Interestingly, our results conflict with those of Miranda et al. (2013), who found that urban European blackbirds (*Turdus merula*) still differed behaviorally from rural counterparts when raised in a common-garden study, though these birds did not undergo different treatments in captivity and thus are not comparable to ours *per se*.

The developmental time during or over which juvenile behavioral investigations are conducted may also impact findings across studies. During the mid-point trials only, urban house finches spent more time on the novel object than did rural birds, regardless of treatment, and wild-caught birds spent more time on it than captive birds. Also, at this same testing time-point, within captive birds we found that high-human-exposure birds spent more time on the object than low-exposure birds. In our previous study of adult house finches, we found no urban/rural difference in time spent near novel objects (albeit different ones than the sword used here; Weaver et al., in press), which suggests that there is a critical juvenile developmental period in which exploratory behavior in house finches is most responsive to environmental variation (and does not persist into adulthood). This difference vanished by the post-treatment trials one month later. Though we have no way of knowing the exact age of the birds, based on timing of the typical breeding period for finches (Badayev et al., 2012) we estimate that they were between 60-90 days old during this time, which appears to be a sensitive window in behavioral development. Most of what is known about behavioral development in passerines comes from studies on song learning (reviewed in Brainard and Doupe, 2002), where birds have an early sensory learning period and a later sensorimotor period in which experiences during 60-90 days of life shape the final stages of learning (Ruploh et al., 2015). Adolescence is a period in which dramatic changes happen in most animal's lives, when they begin to rely less on their parents and often disperse from natal sites (Sachser et al., 2010). During this time, for example, mice increase risk-taking behaviors and exposure to novelty (Macri et al., 2002; Laviola et al., 2003). This has not been studied in songbirds, but Butler et al. (2011) found that mallard ducks (*Anas platyrhynchos*) exposed to an immune challenge in later adolescence showed behavioral changes, whereas ducks that were immunochallenged earlier in development did not. Our results suggest that understanding urban acclimation requires a greater understanding of critical periods in animal behavioral development.

In conclusion, we found stronger evidence for urban house finches acclimating to the presence of humans than for an early-life genetic/pre-fledging predisposition for human tolerance, and this was true regardless of whether birds were captured from urban or rural habitats. Birds exposed less to humans in this study behaved similarly to adult rural birds in our prior work, being more active in the presence of humans than high-human-exposure fledglings, regardless of capture site. However, because we only tested responsiveness to and effects of human exposure and a novel object under captive conditions, this does not rule out a combination of acclimation and predisposition to other urban-dominated stimuli (that we did not investigate) in nature. In addition, we found interesting temporal differences in juvenile behavior during development, during which birds in a more complex environment (urban vs rural; wild caught vs captive) spent more time near a novel object but only during the mid-point trials. This result opens the door for further studies on if or precisely when critical periods for personality development occur, which is not widely studied in wild animals.

Figures

Figure 2.1. Experimental setup for the human approach trials, equipped with a cardboard box for a shaded area, a novel object (glowing green and purple toy sword), tree branches for natural perches, and white food and water dishes.

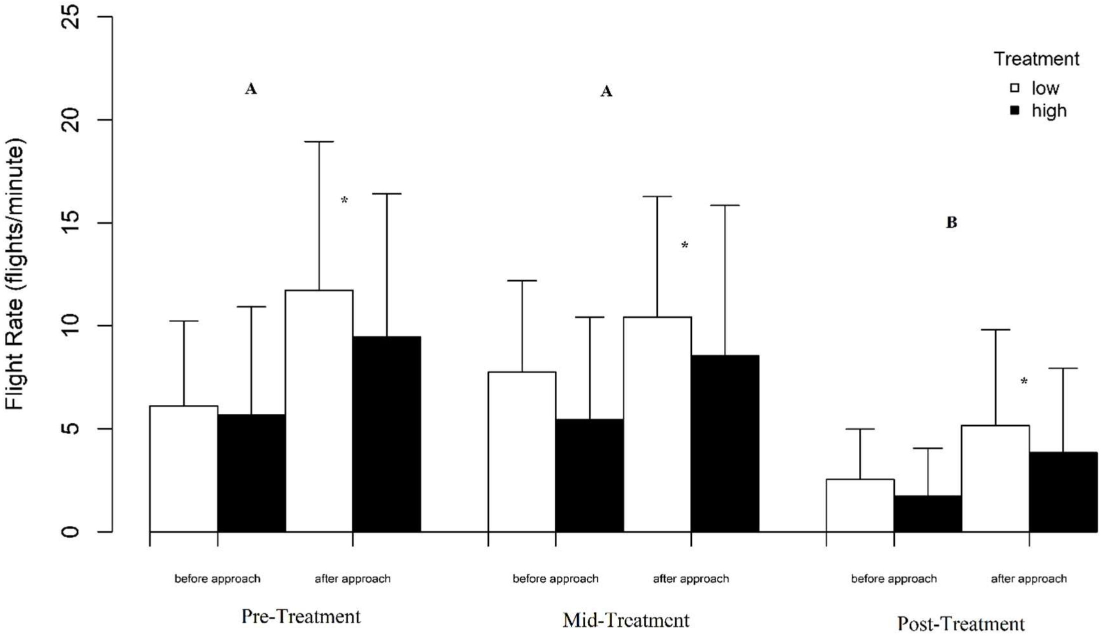

Figure 2.2. Differences in flight frequency as a function of human-exposure treatment (high or low), habitat type (urban or rural), trial period (pre-, mid-, or post-experiment), and time of testing (before or after the human approach). In captive trials, low-human-exposure birds flew significantly more than high-exposure birds, regardless of habitat type, trial period, and time of testing. Also, birds flew significantly more after the human approach than before, and flew more in the first two trials than the final trial. Graphs depict mean and standard deviation. Unshared letters represent significant differences in treatment time-points, and asterisks indicated differences in flight frequency before and after approach.

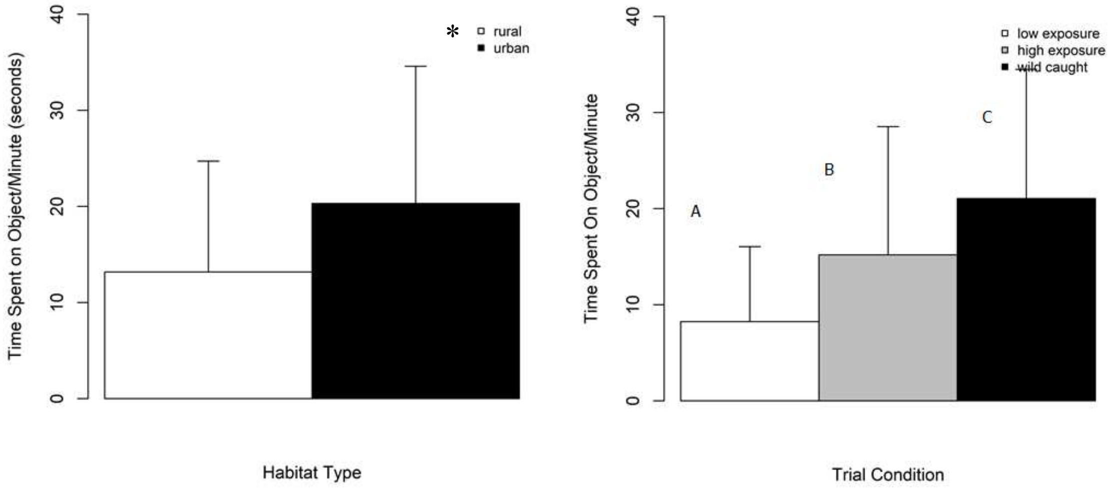

Figure 2.3. Differences in time spent on novel object as a function of urbanization (left) and treatment (right) during the mid-point trial in July. Urban birds spent more time on the novel object than rural birds, and wild caught birds spent more time on the novel object than captive birds while high exposure birds spent more time on the object than low exposure birds. Graphs depict mean and standard deviation, and unshared letters denote statistically significant differences between groups.

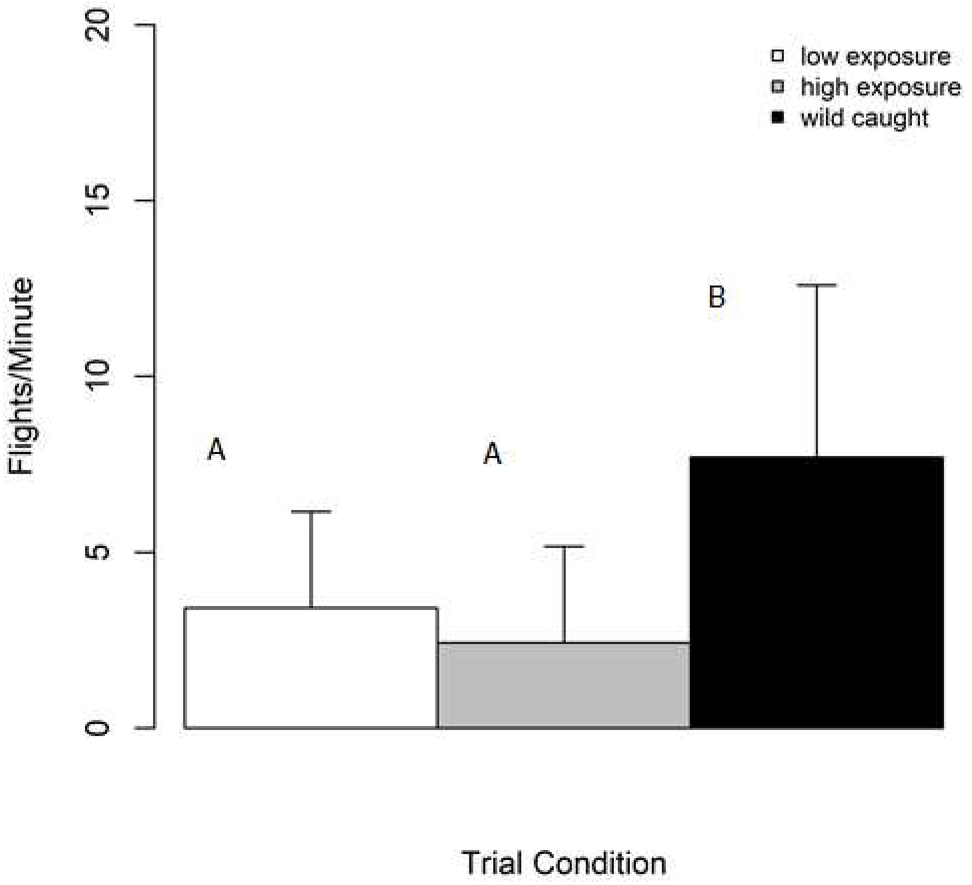

Figure 2.4. In the post-treatment trial, conducted in August, wild-caught birds flew more than captive birds, regardless of capture site or experimental treatment. Graph depicts mean and standard deviation.

Tables

Table 2.1. Results of repeated-measures ANOVAs testing the effects of habitat type, human-exposure treatment, trial period, time of testing, and their interactions on flight frequency. Statistically significant p-values are in bold.

Behavior	Term	F	df	p
Flights	Habitat Type	0.82	1, 527	0.36
	Treatment (low v high exposure)	3.69	1, 527	**0.05**
	Trial Time (before v after)	5.99	1, 527	**0.01**
	Trial Number (initial, mid, final)	9.48	2, 526	**0.01**
	Type*Condition	0.13	1, 527	0.72
	Type*Trial Time	0.05	1, 527	0.82
	Treatment*Trial Time	2.31	1, 527	0.13
	Type*Trial Number	2.47	2, 526	0.29
	Treatment*Trial Number	2.02	2, 526	0.36
	Trial Time*Trial Number	0.15	2, 526	0.93
	Type*Treatment*Trial Time	0.01	1, 527	0.95
	Type*Treatment*Trial Number	0.31	2, 526	0.85
	Type*Trial Time*Trial Number	0.13	2, 526	0.94
	Treatment*Trial Time*Trial #	2.09	2, 526	0.35
	Type*Treatment*Trial x*Trial #	0.12	2, 526	0.94
Bill Wipes	Habitat Type	0.18	1, 527	0.67
	Treatment (low v high exposure)	0.37	1, 527	0.54
	Trial Time (before v after)	0.02	1, 527	0.88
	Trial Number (initial, mid, final)	1.19	2, 526	0.55
	Type*Treatment	1.35	1, 527	0.25
	Type*Trial Time	0.04	1, 527	0.83
	Treatment*Trial Time	1.52	1, 527	0.22
	Type*Trial Number	0.73	2, 526	0.69
	Treatment*Trial Number	0.66	2, 526	0.72
	Trial Time*Trial Number	0.61	2, 526	0.74
	Type*Treatment*Trial Time	0.42	1, 527	0.52
	Type*Treatment*Trial Number	0.12	2, 526	0.94
	Type*Trial Time*Trial Number	2.01	2, 526	0.37
	Treatment*Trial Time*Trial #	1.23	2, 526	0.54
	Type*Condition*Trial x*Trial #	2.04	2, 526	0.36
Time on Object	Habitat Type	0.47	1, 527	0.49
	Treatment (low v high exposure)	0.30	1, 527	0.59
	Trial Time (before v after)	0.78	1, 527	0.38
	Trial Number (initial, mid, final)	2.63	2, 526	0.27

Type*Treatment	0.19	1, 527	0.67
Type*Trial Time	0.10	1, 527	0.76
Treatment*Trial Time	0.01	1, 527	0.98
Type*Trial Number	5.10	2, 526	0.08
Treatment*Trial Number	0.89	2, 526	0.64
Trial Time*Trial Number	0.29	2, 526	0.86
Type*Treatment*Trial Time	0.15	1, 527	0.70
Type*Treatment*Trial Number	3.50	2, 526	0.17
Type*Trial Time*Trial Number	0.57	2, 526	0.75
Treatment*Trial Time*Trial #	0.40	2, 526	0.82
Type*Treatment*Trial x*Trial #	1.90	2, 526	0.39

Table 2. Results of ANOVAs for midpoint trials, conducted in July, comparing wild caught and captive birds, including each of the variables measured and their interactions. Statistically significant p-values are in bold.

Behavior	**Term**	**F**	**df**	**p**
Flights	Habitat Type	0.01	1, 298	0.96
	Treatment (low vs high vs wild)	1.34	2, 297	0.51
	Trial Time (before vs after)	0.79	1, 298	0.37
	Type*Treatment	0.12	2, 297	0.94
	Type*Trial Time	0.02	1, 298	0.88
	Treatment*Trial Time	0.07	2, 297	0.97
	Type*Treatment*Trial Time	0.21	2, 297	0.90
Bill Wipes	Habitat Type	0.70	1, 298	0.40
	Treatment (low vs high vs wild)	1.48	2, 297	0.48
	Trial Time (before vs after)	0.10	1, 298	0.76
	Type*Treatment	3.07	2, 297	0.22
	Type*Trial Time	3.39	1, 298	0.07
	Treatment*Trial Time	1.00	2, 297	0.61
	Type*Treatment*Trial Time	2.93	2, 297	0.23
Time on Object	Habitat Type	4.12	1, 298	**0.04**
	Treatment (low vs high vs wild)	7.28	2, 297	**0.03**
	Trial Time (before vs after)	0.08	1, 298	0.77
	Type*Treatment	1.04	2, 297	0.59
	Type*Trial Time	2.35	1, 298	0.13
	Treatment*Trial Time	0.70	2, 297	0.71
	Type*Treatment*Trial Time	5.22	2, 297	0.07

Table 3. Results of ANOVAs for final trials, conducted in August, comparing wild-caught and captive birds, including each of the variables measured and their interactions. Statistically significant p-values are in bold.

Behavior	**Term**	**F**	**df**	**p**
Flights	Habitat Type	0.80	1, 269	0.37
	Treatment (low vs high vs wild)	37.9	2, 268	**<0.01**
	Trial Time (before vs after)	7.57	1, 269	**<0.01**
	Type*Treatment	2.83	2, 268	0.24
	Type*Trial Time	0.98	1, 269	0.32
	Treatment*Trial Time	4.56	2, 268	0.10
	Type*Treatment*Trial Time	1.57	2, 268	0.46
Bill Wipes	Habitat Type	2.20	1, 269	0.14
	Treatment (low vs high vs wild)	0.45	2, 268	0.80
	Trial Time (before vs after)	1.13	1, 269	0.29
	Type*Treatment	0.92	2, 268	0.63
	Type*Trial Time	2.44	1, 269	0.12
	Treatment*Trial Time	3.37	2, 268	0.19
	Type*Treatment*Trial Time	1.03	2, 268	0.60
Time on Object	Habitat Type	2.31	1, 269	0.13
	Treatment (low vs high vs wild)	4.65	2, 268	0.10
	Trial Time (before vs after)	0.08	1, 269	0.78
	Type*Treatment	3.94	2, 268	0.14
	Type*Trial Time	0.01	1, 269	0.90
	Treatment*Trial Time	1.59	2, 268	0.45
	Type*Treatment*Trial Time	0.25	2, 268	0.88

CHAPTER 3

CONTEXT-DEPENDENT VARIATION IN PROBLEM SOLVING ABILITY AS A FUNCTION OF URBANIZATION IN A SONGBIRD

Abstract

Recent human-induced transformations to the environment are significantly impacting wild animal populations. While some animals thrive due to these changes, others are being extinguished. Many studies have attempted to identify behavioral traits (e.g. personality, diet versatility, cognition) that allow some animals to succeed in human-dominated landscapes, but few have studied multiple traits or environmental contexts concurrently, despite the fact that different environments may require different types of behavioral performance. We presented house finches (*Haemorhous mexicanus*) captured from urban, suburban, and rural sites with two different environmental problems to solve (escaping from a novel environment and finding food in novel feeding structures) and measured success and speed of problem solving as well as activity levels and stress behaviors of the birds. We found that urban birds were better at solving the escape challenge, but rural birds were better at finding hidden food. In addition, we found that birds who solved the escape challenge were more active than those who did not solve this problem, although we observed no such behavioral difference in the food challenge. These results indicate that, because cognitive tasks can vary across environments, certain problem-solving abilities may not be favored for all urban-dwelling species.

Introduction

As human-dominated landscapes continue to expand across the globe, determining which traits enhance survival of animal species inhabiting urban environments has become an important challenge for ecologists. Cities typically have reduced biodiversity compared to surrounding natural areas, due to the novel, intense forms of anthropogenic environmental disturbance, which include destruction of native habitat, introduction of non-native predators and competitors, various types of pollution, and the presence of humans themselves (Marzluff, 2001; Shochat et al., 2006). However, many species successfully adapt to and thrive in cities, taking advantage of artificial food and water sources (Fokidis et al., 2009) and additional cover and nesting sites (Møller, 2008, Tryjanowski et al., 2015). Several behavioral traits have been implicated as potential drivers of exploitation of and adaptation to urban areas, such as boldness and plasticity (Lowry et al., 2013), but studies on behavioral variation along an urban-rural gradient have produced mixed results (Griffin et al., 2017), demonstrating that urban species may differ in the strategies that allow them to survive in their surroundings.

Because animals inhabiting urban areas face a number of behavioral problems they must solve, such as finding food in novel areas and searching novel structures for nesting sites and protection, many researchers have proposed that problem-solving ability should play a key role in determining which species can survive in human-impacted areas. Superior feeding innovation (or problem-solving ability; Griffin et al., 2017) is often found in urban birds (Audet et al., 2016) and mammals (Mangalam & Singh, 2013). It has been argued that this pressure to innovate stems from inhabiting harsher, more complex, unique environments (Roth et al., 2010), but few such studies have explored problem-solving in multiple contexts. Griffin et al. (2017) reviewed the literature on innovation in urban birds and found in six of eight studies that urban birds showed a higher level of innovation (the term used to describe ability to solve a problem) than their rural counterparts. However, in all but one of the studies, only the ability of animals to acquire food through a novel feeder was tested. Birds also face key navigational challenges in urban environments, such as escaping unique threats (e.g. feral cats) and locating novel nesting locations (e.g. buildings), which may require different abilities. Only Preiszner et al. (2017) has tested avian innovation in multiple contexts, finding that urban great tits (*Parus major*) were more successful both in clearing an obstacle to their nests and acquiring food through a novel feeder. To our knowledge, no ecological study has tested how animals solve a problem that presents an aversive challenge, such as escaping from confinement.

Here we explored the extent to which problem-solving ability varies as a function of urbanization and task type (aversive vs rewarding). We administered two types of problem-solving challenges (locating food, considered rewarding, and escaping confinement, considered aversive) to house finches (*Haemorhous mexicanus*) captured across an urban gradient in Phoenix, Arizona, USA and recorded the behavior of all individuals to examine problem-solving success and its potential correlates (activity level and stress-related behaviors; Weaver et al., 2018). House finches are seed-eating passerines (Family Fringillidae) found in both urban and natural (desert) areas in their native range, and in previous work several behavioral (Valcarcel & Fernandez-Juricic, 2009; Weaver et al., 2018; Cook et al., 2017) and physiological differences (Giraudeau et al., 2014) have been identified between urban and rural birds. We initially predicted, based on prior work in other species (Preiszner et al., 2017) and because, in a pilot study, urban finches were found to escape from live traps better than rural birds (pers. obs.), that urban birds would be better problem-solvers regardless of task type. In addition, because previous studies had shown urban-rural variation in behavioral responses to stress in house finches (Weaver et al., 2018), we predicted that urban birds would have lower activity scores and display fewer stress behaviors during these trials, as reduced stress would give the birds a greater ability to solve the problem.

Methods

Field Methods

We trapped after-hatch-year house finches during two pre-breeding seasons (January-March 2012 for the cage-escape tests and January-February 2015 for the food-location tests), which we selected because, in our previous work (Weaver et al., 2018), we saw greater urban-rural behavioral differences at this time of year than during the summer molt season. We used basket traps baited with sunflower seeds to capture finches at two urban, two suburban, and two rural sites across the greater Phoenix area; these sites were categorized based on human population density and urban land-use parameters measured by the Central Arizona-Phoenix Long-Term Ecological Research program (Cook et al., 2017; see map in Appendix C). The two urban sites were the Arizona State University-Tempe campus and a neighborhood near downtown Phoenix. The two suburban sites were a landscaped park in Gilbert, AZ and a residential neighborhood in southeast Tempe, AZ. The two rural sites were natural, desert-foothill areas at South Mountain and Estrella Mountain Regional Parks. At capture, we fitted each bird with a numbered United States Geological Survey metal band for individual identification. We studied 50 urban (24 females, 26 males), 46 suburban (22 females, 24 males), and 50 rural (26 females, 24 males) finches in 2012 (81 males, 71 females), but because we found no consistent differences in suburban birds in early work (Weaver et al., 2018), we tested only urban (n = 56; 31 females, 25 males) and rural (n = 57; 25 females, 22 males) birds in 2015.

Birds studied in the escape challenge were behaviorally tested in the field and then released immediately at their capture site (see below). Birds given the food-finding challenge were transported to the Arizona State University-Tempe campus in a paper bag and kept in captivity for roughly two weeks before testing (see below); after conducting two additional, unrelated behavioral studies with them, this group of birds was also released at their capture site. For both types of challenges, we ran five behavioral trials per day, between 0700-1100 hrs. We used time of day as a covariate in our original model but removed it because it was not significant.

Field Cage-Escape Trials (2012)

We placed each bird individually in a large cage (0.77 m tall x 0.59 m long x 0.50 m wide; Figure 3.1) on the ground at its capture site, in similar light conditions to those where they were captured in the trap and adjacent to a human path (sidewalk at the urban areas and parking lot at rural areas, which we guarded to ensure trials were not interrupted). The cage was equipped with a hide area and feeders with sunflower seeds and water, as well as a small tunnel through which the finch could escape the cage. Each bird was given a 20-minute time period to escape the cage on its own. If it did not escape by the 20-minute mark, a human (MW) walked toward the cage (Weaver et al., 2018) to putatively stress the bird and assess if this stimulus would trigger it to escape the cage during the subsequent

10 minutes. All trials were video-recorded with a camera (JVC Everio, Long Beach, CA) mounted on a tripod about one meter from the cage. Three birds were tested at a time, each in its own cage that was separated by at least 20 meters from the other cages. Videos were scored for the time it took for a bird to escape the cage, the number of flights (used as a measure of activity level) and bill wipes (proposed as a behavioral indicator of stress; Tinbergen, 1940; Clark Jr., 1970), and the amount of time spent on the feeders and in the hide area. Behavioral data from this experiment were also previously utilized to understand response to humans across an urban gradient (Weaver et al., 2018), and in this paper, we only analyze the behaviors related to solving the escape challenge. From each video, two independent observers scored the behaviors using the software program Cowlog (Hänninen & Pastell, 2009). Data collected by each observer were tested for repeatability (Lessells & Boag, 1987), and behavioral scores were averaged for final analyses. Observers were pre-screened on training videos and expected to reach 0.75 repeatability with each behavior before participating in official data collection. If this did not occur, a third observer watched a subset of videos, and we selected the data from the observer with which the third person's repeatability was > 0.75.

Captive Food-Finding Trials (2015)

For two weeks (acclimation phase), birds were individually housed in small wire cages (60cm x 40cm x 30cm) in a climate-controlled vivarium on the Arizona State University-Tempe campus and given a diet of black oil sunflower seeds and tap water *ad libitum*. The rooms were kept at a temperature of 25º C on a natural outdoor light cycle. We chose not to food-deprive all birds prior to food-finding trials because we did not want hunger to be the driving factor in locating food sources, which could potentially measure another metric, such as nutritional/body condition, rather than problem-solving ability. In addition, we found no differences in time spent on feeder across sites in multiple experiments conducted on these populations (Weaver et al., 2018; Weaver et al., in press), so we do not believe their motivation to feed is different between sites. After the two-week adjustment period to captivity, each bird was placed a large flight cage (9' L X 5' W X 9' H) within ASU's School of Life Sciences courtyard aviary. Each flight cage was equipped with a black plastic trashcan, a small metal food dish hidden behind a dish towel, and a child's plastic cup with an attached plastic straw (Figure 3.1), all containing sunflower seeds hidden inside where the bird must enter the item to find the seeds. We chose common objects that even birds at rural parks might have some exposure to, so as not to test novelty per se. Each bird spent an hour in the flight cage, and we recorded all behavior with a video camera mounted on a tripod and performed video analyses as described above. Videos were scored for each bird's ability to find each of the three hidden food sources (yes/no), the speed with which they located the hidden food source(s), as well as the number of flights and bill wipes (as above).

Statistical Methods

We ran all statistical analyses in the R computing environment (Ver. 2.15.1). For the field cage-escape trials in 2012, we calculated the proportions of escapes per site and compared them using chi-squared tests. We then ran multiple analyses of variance (ANOVAs) to determine if birds who did and did not escape the cage significantly differed in their numbers of flights and bill wipes or in the time spent on the feeder/hide and if this varied by site. For the hidden-food trials in 2015, we also ran chi-squared tests on proportion of birds that located each of the three different food sources per site. We measured these variables separately since they were spatially-distinct and object-specific challenges (Papp et al., 2015). We then ran multiple ANOVAs using flights and bill wipes as independent variables to determine if finding hidden food sources was linked to these behaviors and differed by site. We included sex in our analysis but found no differences in ability to solve problems ($\chi^2 = 0.35$, $p = 0.55$ in 2012 and $\chi^2 = 2.31$, $p = 0.13$ in 2015). When necessary, values were log-transformed to normalize them, and we used Tukey's honest significant difference tests for post-hoc comparisons.

Results

Field cage-escape trials

Urban birds were significantly more likely to escape the cage than rural birds ($\chi^2 = 4.36$, $p = 0.037$; Figure 3.2), though suburban birds did not differ from either. Of the 51 urban birds, 11 escaped (22%), whereas eight of 49 suburban birds escaped (16%) and three of 54 rural birds escaped (5%). Of the 19 escapes, 14 came before the human approached and five came after; there was no significant difference in time to escape among sites ($F_{1,\,153} = 1.50$, $p = 0.25$). Regardless of site, birds that escaped the cage had higher activity levels (i.e. more flights/minute) than those who did not escape (Figure 3.2), but there were no other behavioral differences between birds that escaped and those that did not or among birds from different sites (Table 3.1). There also were no significant interactive effects of capture site and escape likelihood on frequency of flights or bill wipes (Table 3.1).

Captive food-finding trials

Rural birds were more likely than urban birds to find the food in all three structures: behind the towel ($\chi^2 = 9.48$, $p < 0.01$), in the cup ($\chi^2 = 13.10$, $p < 0.01$), and in the trash can ($\chi^2 = 3.99$, $p = 0.05$; Figure 3), but for those who solved the challenges urban and rural birds did not differ in time to find any of the food sources (Figure 3.3). We found no significant effects of capture site, solving success, or their interaction on trial behaviors (e.g. flights, bill wipes; Table 3.2).

Discussion

Urban environments often introduce novel structures (e.g. feeders, nesting areas, physical obstacles) that require innovation - either to avoid danger or exploit a resource - from city-dwelling species. Though several single-challenge studies support this claim (Griffin et al., 2017), here we aimed to test if urban animals are better problem-solvers at multiple tasks (food finding and escaping confinement), including a solving a problem that presents an aversive challenge (escaping confinement) rather than just finding a reward (food finding). Based on previous studies, most of which that found urban birds to be better problem solvers (reviewed in Griffin et al., 2017), we predicted that urban house finches would be better at solving both types of challenges. Instead, we found task-dependent differences in problem solving ability between urban and rural birds. House finches that we captured from urban sites were more successful at escaping from a holding cage, but rural birds were more likely to locate hidden food sources in a free-flight aviary. These results are consistent with the notion that urban environments do not favor superior innovation ability in all situations and that particular life-history traits, environmental limitations/needs, and types of tasks should be considered when understanding pressures favoring problem-solving ability in human-modified and natural environments.

The fact that urban birds were more successful at escaping from a novel environment than rural birds is consistent with previous studies on problem-solving and urbanization, in which six of eight studies found that urban birds were more like to solve a problem than rural counterparts (Griffin et al., 2017), including Preiszner et al. (2017) which found that urban great tits solved both an obstacle and food-innovation problem better than their rural counterparts. Because space-confinement, like exposure to humans, is an environmental stressor, we might expect urban birds (exposed more to both physical obstacles and humans) to show decreased stress response and increased performance under both conditions. Although studies testing responses to humans have produced conflicting results, as some urban populations of house finches show a greater behavioral-stress response to human approach (Valcarcel & Fernandez-Juricic, 2009), our previous study using these finch populations demonstrated that urban birds showed decreased response to human approach than rural birds (Weaver et al., 2018; also see Møller, 2008; Carrete & Tella, 2011 for similar findings in other species). Thus, this may give urban birds an advantage in reacting to anthropogenic stimuli and solving threatening, human-related problems.

We sought to isolate particular behavioral correlates of problem-solving during the cage-confinement tests, and, interestingly, regardless of site, birds that solved the problem were more active (i.e. more flights/min) than those that did not. Thus, in this case, movement in and exploration of the novel environment may be the key contributor to problem-solving success. This is consistent with results from previous studies in songbirds that have linked exploratory behavior and ability to solve a problem in a novel environment (Guillette et al., 2009; Webster & Lefebvre, 2001; Sol et al., 2011), including a study on our finch population that demonstrated that the best predictor of solving a novel task was visual assessment of the novel task (Cook et al., 2017).

In contrast to our findings from the cage-escape tests, however, we found that, when we tested ability to find hidden food, rural birds were more successful than urban birds at locating each of three different food sources. This opposing result may arise from the increased difficulty of finding food in a natural, desert environment as compared with urban environments, which are now increasingly supplemented with human food sources, such as bird feeders and trash dumps (Tryjanowski et al., 2015). Thus, although cities may require some species to become more innovative in seeking out rare or novel food sources (Griffin et al., 2017), house finches are granivores and often found at backyard bird feeders. Increased food accessibility in the urban environment may allow some species like house finches to reallocate attention/resources to other challenges, such as exposure to humans, non-native predators, and novel structures (e.g. for nesting, navigating around). In contrast to studies that have proposed the idea that novel environments are catalysts for behavioral innovation, Kotraschal and Taborsky (2010) proposed that, in fact, *changing* environments (i.e. like natural deserts but unlike more stable/predictable cities) select for greater food-related problem-solving abilities, finding that cichlid fish (*Simochromis pleurospilus*) raised with changes in food rations performed better at food innovation tasks later in life (see Laland & Reader, 1999 for similar results with guppies; *Poecilia reticulata*). In addition, Federspiel et al. (2017) found that rural common mynas (*Acridotheres tristis*) were also better at a learning challenge than urban birds, which have also been well-established in human-modified areas and have been exploiting human food sources for generations.

Also in contrast to the cage-escape study, in the food-finding trials we found no differences in activity level or bill wipes for birds that did and did not discover the hidden food sources. Few avian studies have investigated the link between food-related problem-solving and neophobia, and some have found a positive association between the two (Guillette et al., 2009; Webster & Lefebvre, 2001; Sol et al., 2011). However, this is not true in all cases. Audet et al. (2016) found that Barbados bullfinches (*Loxigilla barbadensis*) who were more likely to solve a food-related challenge were also more neophobic, and Lermite et al. (2017) found that common mynas (*Acridotheres tristis*) who were more likely to solve a novel foraging problem showed no difference in neophobia from those that could not solve it. Lermite et al. (2017) attributed differences between their results and those of other studies on neophobia and cognition partly to giving birds free access to food before the challenge, as we did, therefore measuring problem-solving ability rather than motivation to feed. Because rural birds are thought to have to search greater distances for more sparse resources (Tryjanowski et al., 2015), they may be better adapted to solve this challenge, even if not more likely to be habituated to novel feeding structures (Griffin et al., 2017). Thus, these challenges we employed in this study may have highlighted differences in problem-solving abilities necessary to survive in each environment, where rural birds are more skilled at finding food but urban birds faced with spatially navigating in more structurally complex (compared to sparsely vegetated deserts) or novel physical environments.

In summary, we exposed birds captured along an urban-rural gradient to two different problem-solving tasks, an escape challenge and a food-finding challenge, and, although we predicted that urban birds would be more successful in both, we found that urban birds were more effective in the escape challenge, but rural birds were more successful in locating the food. In addition, whereas activity level was found to positively predict solving success in the cage-escape trials, no such behavioral predictor of solving success was found in the food challenge. Because urban birds receive supplementary food from bird feeders and trash cans but face other spatial/navigational challenges in their environment (e.g. novel structures and predators), they may need to prioritize certain types of problems to solve over others. Our findings call for additional work on trade-offs in innovation, specifically as a function of the importance of different behavioral tasks and the presence and strength of different environmental selection pressures.

Figures

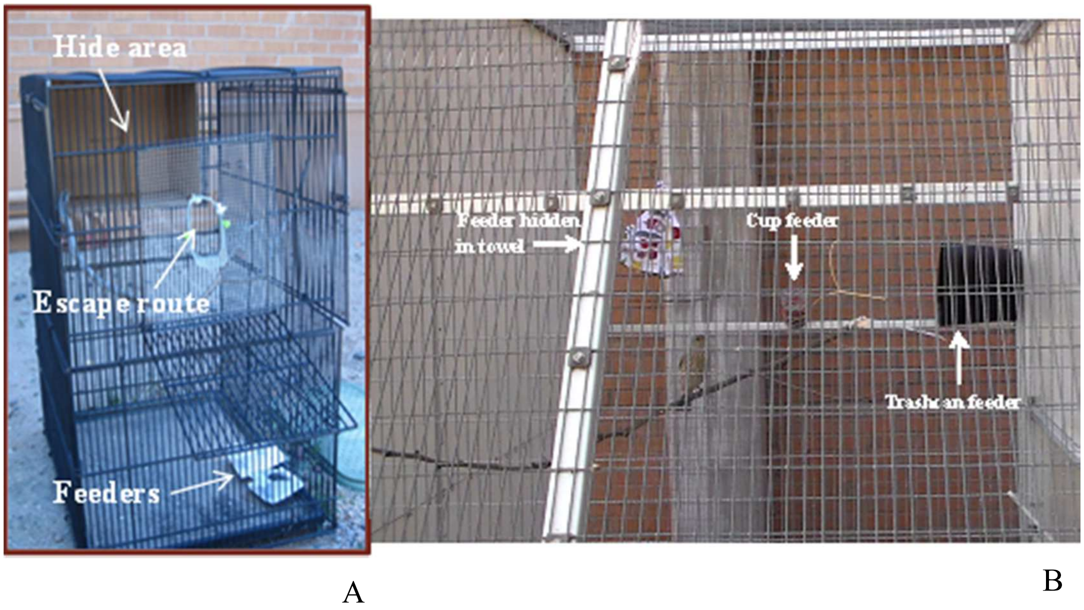

Figure 3.1. (A) Cage used in the escape trials. (B) Aviary used in the food-finding trials, in which we hid food in novel feeding structures.

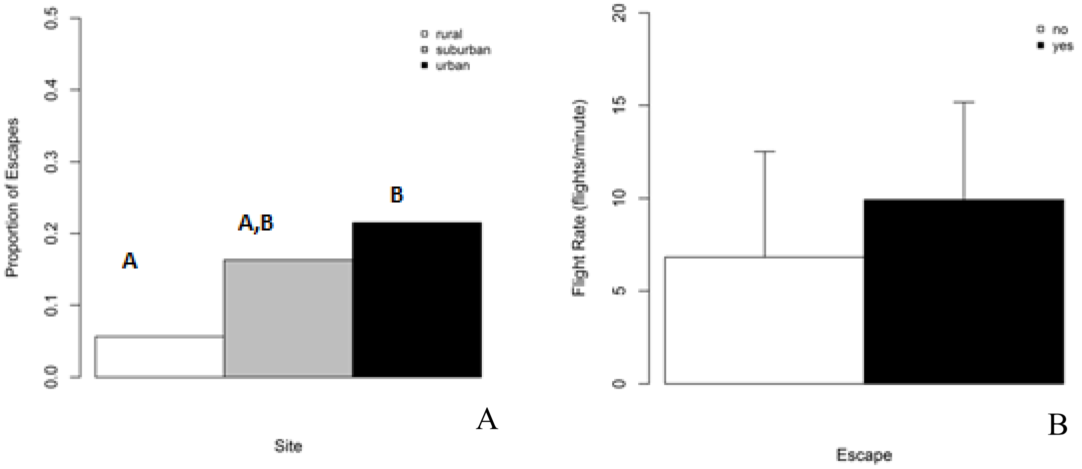

Figure 3.2. Urban birds were more successful at escaping the cage than rural birds (left panel). (B) Birds that escaped the cage were more active (as measured by number of flights per minute) than birds who did not, regardless of site. Histogram boxes denote means, with lines representing standard deviation. Unshared letters denote statistically significant differences between groups.

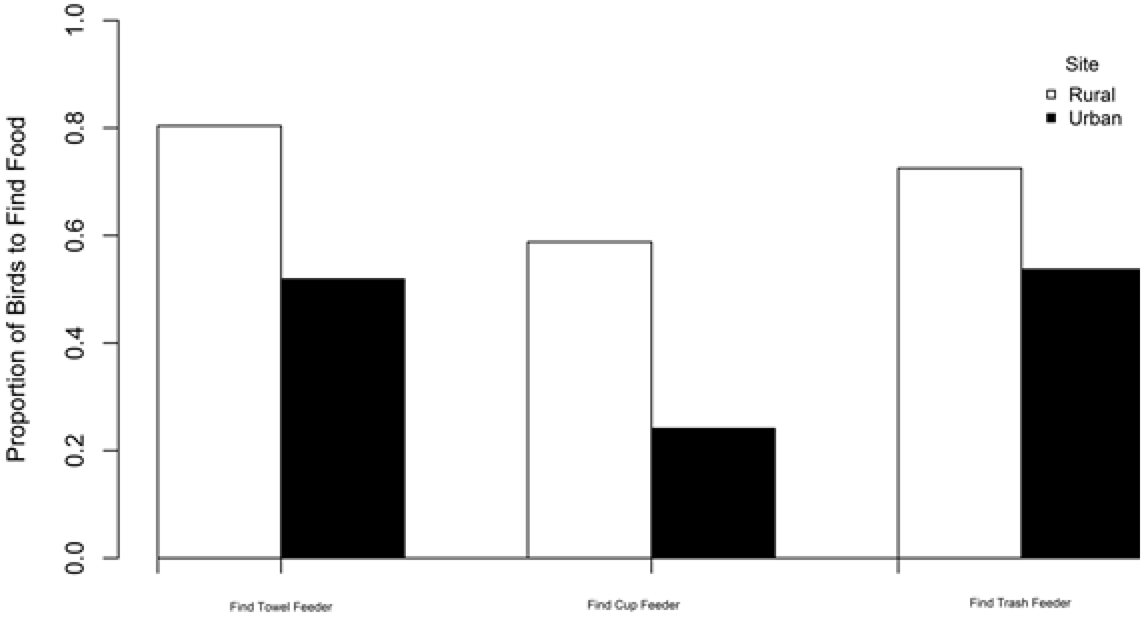

Figure 3.3. Rural birds were more successful in finding food in all three novel areas during the food-finding challenge.

Tables

Table 3.1. ANOVA tables depicting effects of site, escape likelihood, and their interactions on finch behaviors (flights and bill wipes) during the cage-escape challenge. Statistically significant p-values are in bold.

Behavior	**Term**	**F**	**df**	**p**
Flight Rate	Site	0.50	2, 152	0.61
	Escape	6.45	1, 153	**0.01**
	Site*Escape	2.09	2, 152	0.13
Bill-Wipe Rate	Site	0.68	2, 152	0.51
	Escape	0.47	1, 153	0.49
	Site*Escape	0.52	2, 152	0.59
Time Spent on Feeder	Site	5.09	2, 152	**<0.01**
	Escape	2.32	1, 153	0.12
	Site*Escape	0.34	2, 152	0.71
Time Spent in Hide	Site	1.19	2, 152	0.31
	Escape	0.02	1, 153	0.88
	Site*Escape	0.11	2, 152	0.90

Table 3.2. ANOVA tables depicting effects of site, task type, and their interactions on finch behaviors (flights and bill wipes) during the food-finding challenge.

Behavior	**Term**	**F**	**df**	**p**
Flight Rate	Site	0.11	2, 103	0.74
	Discover Towel	0.07	1, 104	0.79
	Discover Cup	0.32	1, 104	0.57
	Discover Trash	0.09	1, 104	0.76
	Site*Discover Towel	0.05	2, 103	0.83
	Site*Discover Cup	0.30	2, 103	0.59
	Site*Discover Trash	2.40	2, 103	0.13
Bill-Wipe Rate	Site	1.92	2, 103	0.17
	Discover Towel	0.27	1, 104	0.61
	Discover Cup	0.04	1, 104	0.84
	Discover Trash	0.61	1, 104	0.44
	Site*Discover Towel	0.22	2, 103	0.64
	Site*Discover Cup	3.97	2, 103	0.05
	Site*Discover Trash	1.91	2, 103	0.17

CHAPTER 4

CONCLUDING REMARKS

Although urban ecologists have learned much to date, using a range of methods on several taxa, about the behavioral responses of animals to urbanization, many questions still remain regarding the specific features of urban development/areas that are having the greatest impact on the ability of animals to acclimate or adapt to cities. A review by Sol et al. (2014), for example, found that life-history traits (e.g. fecundity, body size, relative brain size) were actually poor predictors of which bird species succeed in urban areas, but did not explicitly assess behavioral traits such as exploration of novelty and problem-solving ability. In this dissertation, I developed a comprehensive, multi-year study with both field and lab components to investigate the effects of major novel urban environmental stimuli on the behavior of an avian species (the house finch; *Haemorhous mexicanus*), a songbird that is commonly found in both urban and natural areas throughout their native southwestern USA range. I chose the top four stimuli that I predicted would drive behavioral differences in urban and rural populations: human presence (Møller, 2008; Valcarcel & Fernandez-Juricic, 2009), novel structures (Drent et al., 2003), novel food sources (Robb et al., 2008), and novel noise (Barber et al., 2010). Then, I observed behavioral and hormonal responses to these stimuli in adults and juveniles from urban and rural sites.

As predicted, urban and rural finches behaviorally differed more in response to the presence of humans (urban-associated stimuli) than to a native raptor predator (which is found in both developed and natural areas). When replicating the human-approach experiment in juveniles, I found that, unlike in adults, there were no site differences, but that all birds acclimated to humans by the post-treatment trials. However, the only other differences we found in response to other novel urban stimuli was that urban birds had decreased response to novel noise than rural counterparts and context-dependent problem-solving abilities within those novel environments. Finally, we found very few differences between urban and rural birds in hormonal response to humans or to novelty. Taken together, our findings (1) reveal that different novel or anthropogenic environmental conditions can vary in their salience to acclimating/adapting urban wildlife and (2) provide consistent support for the notion that urban animals are more behaviorally resilient to putatively aversive novel anthropogenic stimuli.

One of the biggest contemporary questions in urban behavioral ecology is whether phenotype differences between urban and rural animals occur because of a genetic predisposition or phenotypic plasticity (Wong & Candolin, 2014). When I initially proposed the experiments for this dissertation, I expected to discover traits that formed an urban ‘personality,’ but as I began my experiments, I realized that I was examining phenotypic changes in animals from urban environments rather than consistency in traits through time and across contexts, as the definition of personality denotes. While genetic adaptation may take generations, plasticity happens more quickly, making it the more likely driver of urban acclimation (Chevin & Lande, 2010), especially for longer-lived vertebrates like birds. A recent meta-analysis of phenotypic plasticity indicated in fact that phenotypic changes happen on a much greater scale in urban areas vs natural conditions (Alberti et al., 2017), and results from my dissertation generally support this notion.

Perhaps the most compelling evidence of acclimation is that, despite finding site differences in response to humans in adult house finches (Appendix B), there were no capture-site effects in juveniles from urban and rural sites in Chapter 2. If urban and rural birds had inherited tendencies to respond differently to humans, we expected to see that they would show these differences even at their initial testing at 28-60 days of age. However, urban and rural birds did not differ in behavioral responses to humans at the initial time point, or at any time point throughout the experiment (i.e. being exposed to the same common-garden experimental conditions). Instead, we saw experimental effects of the human-exposure treatment on both urban and rural finches, such that all birds acclimated to the benign presence of humans. In addition, compared to wild-caught birds, regardless of site, at that same time period, all captive birds showed decreased activity levels in response to human approach. The importance of this finding is magnified because there are few studies on personality development in non-human juvenile animals (Petelle et al., 2013), and most of those are conducted on domesticated mammal species (Farrell et al., 2015), even though this is a critical time period when animals experience dramatic changes, such as dispersing from their natal environment (Sachser et al., 2010). Since events that modify juvenile behavior can have lasting effects into adulthood (Ruploh et al., 2015), a compelling line of future research would be determining critical learning periods at which point juveniles are more malleable to environmental influences on their behavior patterns.

Although Chapter 1 revealed few populations differences, urban birds demonstrated reduced behavioral response to urban noise, indicating another avenue through which they might be acclimating to city life. Given the key role of vocal/auditory communication (e.g. contact calls, mating songs) in this and other songbird species (Hill 1993), response to novel noise may be a primary driver in urban acclimation. While studies have shown that birds tend to avoid urban noise (Rheindt, 2003; Bayne et al., 2008) or modify song to compensate for any communication disturbances it may cause (Potvin & MacDougall-Shackleton, 2015; LaZerte et al., 2017), only one study has incorporated noise into a novelty study (Von Donselaar, 2018), and, in that study, urban black-capped chickadees (*Poecile atricapillus*) were exposed to urban noise rather than novel noise. That I found significant results, and found that rural birds responded more strongly to one novel sound (whale noise) than the other (ship noise), suggests the need for a greater understanding of how novel noise drives behavioral changes in urban areas. As urban areas rapidly shift, changes in human-created noises could also shift in frequency and decibels. Understanding how animals respond to novel noise could help us predict how this will affect biodiversity in human-modified areas.

Overall, the first three studies in my dissertation revealed that humans and urban noise were the key drivers of behavioral changes in urban animals. In a meta-analysis conducted on urban phenotypic change, Alberti et al. (2017) argued that biotic interactions (e.g. introduction of predators or competition) and habitat modification were the most salient environmental regulators of urban animal acclimation. While my dissertation did not suggest that humans are perceived as predators, my initial study (Appendix B), which found that rural birds showed a higher stress response to humans, provided evidence for the first claim, indicating that human presence is a biotic interaction that may dictate which species fail to persist in urban environments. While noise may not directly fall into the habitat modification category, I would argue that

Chapter 3 does address habitat modification, as ability to escape confinement may be an acclimation that results from evading predators, searching for shelter, and navigating through novel structures, most of which are a result of habitat modification. While urban animals may face introduction of novel predators (e.g., cats; Loss et al., 2013) and structures (Isaac et al., 2014; Møller, 2009), they have year-round, supplemental food sources (Tryjanowski et al., 2015) that reduce the need to shift foraging strategies as the food sources change, as they do in natural environments (Kotraschal & Taborsky, 2010). Though earlier studies proposed that urban areas may select for larger brains (Reader & McDonald, 2003; Sol et al., 2005), later studies (Evans et al., 2011; Sol et al., 2014) found weak correlations between brain size and likelihood to colonize urban areas in bird species. My findings that urban birds are better at solving one type of problem but not another call for additional investigations into potential differences in brain structure and/or tradeoffs associated with problem-solving ability in urban and rural areas.

Finally, Appendix A revealed no differences in plasma corticosterone (a blood stress hormone) between urban and rural populations, despite finding multiple behavioral differences. This is an interesting finding because it suggests that, although urban animals may modify their behavior in response to humans (as seen in Appendix B), they may still be experiencing stress in response to humans. However, since a meta-analysis on urban-rural differences in corticosterone levels (Bonier, 2012) also revealed few differences, our results may also be another in a series of studies that reveal plasma corticosterone to be a poor indicator of urban stress response. Additional work is thus needed on other mechanisms that may underlie these behavioral differences. Is there another hormone (e.g. epinephrine, Koolhaas et al., 1999; vasopressin, Englemann et al., 1996) or physiological measure, such as metabolism (Holtmann et al., 2017), hematology (Fokidis et al., 2008), or body condition (e.g. oxidative stress), that is a better measure of the effects of acclimating to life in the city? These are questions I hope to tackle in future work as I delve further into the overall question that drove this dissertation – what makes some species adaptable and others intolerant to human presence?

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APPENDIX A

CIRCULATING CORTICOSTERONE LEVELS VARY DURING EXPOSURE TO ANTHROPOGENIC STIMULI AND SHOW WEAK CORRELATION WITH BEHAVIOR ACROSS AN URBAN GRADIENT IN HOUSE FINCHES (*HAEMORHOUS MEXICANUS*)

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Circulating corticosterone levels vary during exposure to anthropogenic stimuli and show weak correlation with behavior across an urban gradient in house finches (*Haemorhous mexicanus*)

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ABSTRACT

Urban environments are rapidly expanding and presenting animal populations with novel challenges, many of which are thought to be stressors that contribute to low biodiversity. However, studies on stress responses in urban vs rural populations have produced mixed results, and many of these studies use a standard stressor that cannot be replicated in the wild (e.g. restraining an animal in a bag). Pairing physiological and behavioral measurements in response to urban-related stressors improves our understanding of the mechanism underlying animal success in human-dominated landscapes. Here, we examined the physiological stress (plasma corticosterone, CORT) responses of a songbird species (the house finch, *Haemorhous mexicanus*) to two different anthropogenic stimuli – (1) the presence of a human and (2) a captive environment containing man-made objects. During three field seasons (summer 2012, winter 2014, and winter 2015), we captured birds at six sites along an urban gradient in Phoenix, Arizona, USA and measured plasma CORT levels both before and after each trial. Though CORT levels did increase post-human exposure, though not during exposure to novel environment, indicating only one of the treatments caused a physiological response, baseline or post-trial plasma CORT levels did not differ between finches between urban and rural birds in 2012 or 2014. However, rural birds demonstrated relatively low pre- and post-trial plasma CORT levels during the human-exposure trials in 2015. Furthermore, we found few correlations between behavioral and physiological responses. A significant positive correlation was only detected between activity behavior after human approach and post-trial plasma CORT levels in 2012. Taken together, our results reveal a weak, conditional relationship between stress physiology, behavioral responses, and urbanization in house finches.

1. Introduction

As human populations grow, urban areas expand and thus dramatically shift global land-use. Animal populations are negatively affected by urbanization in a multitude of ways (Marzluff, 2001; Shochat et al., 2006), including loss of native habitat, exposure to nonnative competitors and predators (Vitousek et al., 1997), and pollution (Partecke et al., 2006). These factors can lower survival, health (Liker et al., 2008), and reproduction (Partecke et al., 2006) of wild animals and ultimately deplete biodiversity (McKinney, 2006). Some species, however, benefit from living in proximity to human development, by gaining access to anthropogenic resources (e.g. human refuse, bird feeders, agricultural sources), water (Fokidis et al., 2009), cover and nesting sites (e.g. buildings, shade trees; Møller, 2009), as well as more moderate temperatures (Shochat et al., 2006). Studies that have examined the behavioral and adaptive response of animals to anthropogenic environmental challenges show that animals with relatively higher levels of cognition or more plastic behavior are more likely to benefit from living in proximity to humans (reviewed in Sol et al., 2013). By comparison, we understand less about the physiological mechanisms underlying behavioral acclimation/adaptation in the city.

The acute stress response, characterized by a transient and rapid elevation of plasma glucocorticoids (Wingfield and Ramenofsky, 1999), is one of the best-studied physiological mechanisms for coping with city stressors. The release of CORT is controlled by the hypothalamic-pituitary-adrenal (HPA) axis and contributes to mobilizing energy stores and modifying behavior when an animal is faced with a stressful situation (Sapolsky et al., 2000). In the short-term, this is generally considered to be an adaptive response that can aid an animal in surviving a dangerous event, such as escaping a predator (Adamo et al., 2013) or navigating a novel environment (Martins et al., 2007).

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However, long-term elevation of glucocorticoid levels, as experienced during chronic stress, can impair reproductive, immune, and brain functions (Sapolsky, 1992). Thus, if animals are experiencing greater daily stress in human-developed areas, increased levels of glucocorticoids may reduce fitness and, over generations, lead to adaptive suppression of physiological stress responses.

However, results from studies linking animal stress responses to human-caused environmental change have been mixed, with no consistent, directional relationship between urbanization and CORT levels. In fact, Bonier (2012) reviewed the urban physiological-ecology literature and found that urban birds had higher baseline CORT levels in five studies, that rural birds had higher levels in five studies, and that urban and rural birds did not differ in nine studies. The results were virtually identical for stress-induced CORT levels (higher in urban birds in three studies, higher in rural birds in three studies, no difference in seven studies). Also, in a study of several songbird species from the Sonoran desert, Fokidis et al. (2009) found that urban birds of some, but not all, species had higher plasma CORT than their rural counterparts. Results comparing hormone levels with behavioral differences (e.g. boldness, exploration; Reale et al., 2007) have also shown high levels of interspecific variation. Although studies on captive great tits (*Parus major*; Carere et al., 2003), zebra finches (*Taeniopygia guttata*; Martins et al., 2007), and house mice (*Mus musculus domesticus*; Veenema et al., 2003) have demonstrated that bold personality types have lower plasma CORT than those labeled as shy, this was not the case in common lizards (*Zootoca vivipara*; Mell et al., 2016), European starlings (*Sturnus vulgaris*; Apfelbeck and Raess, 2008), house sparrows (*Passer domesticus*; Lendvai et al., 2011), and rough-skinned newts (*Taricha granulosa*; Neuman-Lee et al., 2015). These diverse findings indicate a need for additional studies to understand the complex interaction between stress physiology, behavioral responses, and urbanization. Because many abiotic and biotic environmental factors impact animals in cities, identifying the specific urban stressors that affect animal behavior and physiology may be critical for understanding how species acclimate and adapt to human-inhabited settings.

To this goal, we measured changes in plasma CORT levels in a common North American bird species (the house finch, *Haemorhous mexicanus*) sampled across an urban gradient in Phoenix, Arizona, USA following exposure to potential anthropogenic environmental stressors. A predominant difference between urban and natural environments is the abundance of (i.e., exposure to) people. Previous work on house finches in an urban environment has shown that urban birds flock in greater numbers and take flight earlier when approached by humans than rural finches (Valcarcel and Fernandez-Juricic, 2009). To investigate the physiological mechanism underlying this behavioral difference, we tested the behavioral (boldness and exploration; Reale et al., 2007) and CORT responses of finches exposed to human presence. Furthermore, because urban animals encounter novel/artificial obstacles (e.g. buildings, electrical poles, windows), we also measured behavioral and plasma CORT responses in finches by exposing birds to man-made objects in a large aviary. We predicted that urban and suburban birds would have higher baseline plasma CORT levels than their rural counterparts because urban birds face repeated daily stressors from high levels of exposure to humans and human-related environmental changes. However, because urban and suburban birds experience more constant exposure to stressors, they may demonstrate lower plasma CORT levels when compared to their rural counterparts after treatment due to acclimation/adaptation to often benign anthropogenic threats like nearby humans. In addition, we predicted that urban and suburban birds would show lower activity levels and decreased stress-related behaviors than rural finches. Because environmental conditions/stressors (e.g. temperature, food, water availability) change seasonally in native (desert) and urban finch habitats, we captured birds during three different seasons (summer and in two consecutive winters) and conducted three different experiments to determine if finch behavioral and physiological responses to humans and man-made structures varied among sites or remained consistent through time.

2. Methods

2.1. *Ethics statement*

This work was approved by the Arizona State University Institutional Animal Care and Use Committee (protocol 12-1234R), the Arizona Game and Fish Department (collecting permit # SP654951), U.S. Fish and Wildlife Service (collecting permit MB088806), and the Federal Bird Banding Lab (permit 23862). Authors are not aware of any conflicts of interest.

2.2. *Field methods*

During three different field seasons (summer 2012, winter 2014, and winter 2015), we trapped finches using basket traps baited with sunflower seeds at six sites across the greater Phoenix area that vary in human population density and urban land-use parameters (as determined by the Central Arizona Phoenix Long Term Ecological Research program; Giraudeau et al., 2014). The two urban sites were the Arizona State University – Tempe campus and a neighborhood near downtown Phoenix, which contained high human population density, buildings, and artificial landscaping. The two suburban sites were a park in a Phoenix suburb and a residential yard in a suburban neighborhood, which had an intermediate amount of human residents, buildings, and artificial landscaping. The two rural sites were natural park areas at South Mountain and Estrella Mountain, which had very few nearby human inhabitants, man-made structures, or artificial landscaping.

In summer of 2012 (July-Oct), we trapped and collected blood samples from 99 birds (37 rural, 28 suburban, 34 urban). These birds were used in the human presence trials, which were conducted in the field (see Section 2.4 below). In winter 2013–2014 (Dec-Feb), we trapped and collected blood samples from 72 birds (39 rural, 10 suburban, 23 urban). These birds were used in the novel environment trials (described in Section 2.5), which were conducted in aviaries on the Arizona State University – Tempe campus. In winter 2015 (Jan-March), we trapped and collected 44 samples from urban and rural sites only (18 rural, 26 urban) since previous studies had revealed few behavioral differences between urban and suburban birds (Weaver et al., 2018a). These birds were used in the human presence trials that were conducted in our campus aviaries (described in Section 2.5). All blood samples were taken from the alar vein, within three minutes of capture (for the field samples) or of handling prior to captive trials (for baseline CORT measurement) and within three minutes of the conclusion of each trial. Blood was stored on ice until it could be centrifuged to separate the plasma, which was stored in a −80 °C freezer until the assay.

At capture, we fitted each bird with a numbered United States Geological Survey metal identification band. In addition, we measured body mass (using a digital scale, to the nearest 0.01 g) and tarsus length (with digital calipers, to the nearest 0.01 mm) of each bird so that we could calculate body condition as the residuals between mass and tarsus length (multiple regression: $F_{1,}$ [illegible] = 10.81, $r^2 = 0.038$, $p < 0.01$; Schulte-Hostedde et al., 2005). Birds with abnormally low body mass (< 15 g) or showing signs of avian pox were released at capture. Because there were no significant site differences in body condition in our studies (all $p > 0.29$), we did include this as a variable in our analyses. Captured birds were transferred immediately into captivity for roughly two weeks, when they underwent various novelty and problem-solving trials (see more below) and were then released back into the wild at their capture site.

2.3. *General details of captive birds and trials*

Captive finches were individually housed in small wire cages

Fig. 1. Experimental Design. Photo at left illustrates the cage in which birds were tested for the human-approach trials; it is equipped with a hide area, escape route and food/water dish. Photo at right illustrates the large novel environment, containing a storage box (left), table (center), and branch (right).

(60 cm × 40 cm × 30 cm) with visual access to each other in a climate-controlled vivarium and given a diet of black sunflower seeds and tap water *ad libitum*. The rooms were kept at a temperature of 25 °C on a natural outdoor light cycle. For all experiments, we ran five trials per day, between 0700 and 1100 h (the most active hours of the day for finches). Order of stimulus presentation (approaching human, sitting human, control, or novel environment) was randomized for each bird from each site.

2.4. *Human response trials*

We performed human response trials during two different seasons, summer 2012 (July-Oct, n = 99) and winter 2015 (Jan-Mar, n = 44), with some differences in experimental protocol between the seasons (see below). In summer 2012, just prior to beginning a trial, we placed each bird in a large cage (0.77 m tall × 0.59 m long × 0.50 m wide; Fig. 1) equipped with a hide area and feeders with sunflower seeds and water. Field trials were all conducted in similar lighting conditions between 0600 and 1100 h. Trials were conducted earlier in the day during summer than winter because of the earlier time of sunrise and high late-morning temperatures in summer. Each bird had 20 min to acclimate in the trial cage while we recorded behavior with a video camera (JVC Everio, Long Beach, CA) mounted on a tripod. After the acclimation period, one person (MW) walked toward the cage starting from a distance of 20 m at a pace of one step per second until touching the cage with a foot and walked away at the same pace. We then recorded the behavior of the birds for 10 min. Three birds were tested at a time, each in its own cage at least 20 m from the other two cages in similar lighting conditions. From each video, two independent observers scored two key measures of behavior using the software program Cowlog (Hänninen and Pastell, 2009): the number of flights in the cage (used as a measure of general activity level) and number of bill wipes (a presumed behavioral indicator of stress; Tinbergen, 1940). We used time (behavior before approach and behavior after approach) as a co-variate in the analysis to determine if behavior changed from the acclimation period to after stimulus presentation.

In winter 2015, birds were placed in the same trial cage, but this cage was placed in a larger aviary back at the ASU campus, instead of in the field, in order to more carefully control environmental/human exposure. Two cages were placed five meters apart with a lawn chair in between them. In this lawn chair, we placed either a human or a bed sheet rolled up to the size of a human (as a control stimulus to differentiate presence of human from presence of a similarly sized object). The stimulus was hidden for a 20-minute acclimation period behind a cardboard divider, at which time the divider was removed and the stimulus was in view of the bird for 20 min. The divider was then brought back in place for another 20 min. Each bird was exposed to both the control and the human stimulus in randomized order on different days, and we collected blood samples before/after each trial. We were primarily interested in differences in plasma CORT between the two trials, so we did not record behaviors in these trials because they were similar to the human approach trials, which we had recorded in summer 2012.

2.5. *Novel environment trials*

In winter 2014, each bird was released into the large aviary cage (Fig. 1) mentioned above, but now equipped with materials comprising the 'novel environment': a large (6′ × 2′) storage box, a picnic table, a large branch, and two zebra finches (a male and female) for one hour. The large storage box was intended to create a novel feeding structure that birds had never experienced, the table represented a human object frequently present at both urban and rural sites, and the branch was to simulate a natural environment (i.e. a reference object). Each of these three different areas had the bird's normal captive food (sunflower seeds) placed in it or on it (Echeverria and Vassallo, 2008). The zebra finches served as novel biotic stimuli since birds in urban areas may be exposed to competitors introduced by humans (e.g. house sparrows, *Passer domesticus*; rock pigeons, *Columba livia*). As zebra finches are not native to this area, it is unlikely that a house finch would have encountered one. We recorded the same behaviors listed above as well as latency to approach novel structures (and the branch, for comparison) and time spent (i.e. within two bird lengths) near novel birds. In a previous study, we found differences in activity levels and stress behaviors in this cage vs their home cage for rural birds (Weaver et al., 2018b), which provides some evidence that this novel environment is stressful to finches.

2.6. *Corticosterone assay*

To measure plasma corticosterone, we used a commercial enzyme-linked immunoassay from Enzo Life Sciences, following instructions outlined by the manufacturer. We diluted the plasma 20x in assay buffer containing 2.5 μl displacement reagent:97.5 μl plasma. All samples were assayed in duplicate on a single plate, but samples were randomized on each plate so that samples from urban, suburban, and rural sites were on each plate. Each plate included a complete standard curve and an internal control. For the first human-approach trials, the assays were conducted in winter 2013. The assay sensitivity was 25.56 $pg\,ml^{-1}$ and the inter- and intra-assay coefficients of variation were 7.38% (N = 2 samples assayed on each plate) and 14.84% (N = 302 samples), respectively. We conducted the assays for the remaining experiments in winter 2016. The assay sensitivity was 32.24 $pg\,ml^{-1}$ and the inter- and intra-assay coefficients of variation

Table 1
This table shows correlations between baseline CORT levels and behavior.

Year	Behavior	Rho	p-value	Year	Behavior	Rho	p-value
2012	Fly (before)	0.19	0.06	2014	Fly	−0.07	0.17
	Bill wipe (before)	0.01	0.97		Bill wipe	0.21	0.08
	Fly (after)	0.19	0.06		Time to box	0.14	0.22
	Bill wipe (after)	−0.02	0.86		Time to table	0.06	0.60
	Time to calm (after)	0.03	0.71		Time to bird	−0.05	0.67
					Time to feed	0.18	0.40

were 8.18% (N = 3 samples assayed on each plate) and 4.89% (N = 315 samples), respectively.

2.7. *Statistical methods*

We ran all statistical analyses in the R computing environment (Ver. 2.15.3). We ran repeated-measures analyses of covariance, using the before and after CORT values as the repeated response variable. Data were log transformed when necessary to meet the assumption of parametric statistics. In the winter 2015 trial, the model included the values from both the human trials and control trials. Although order was randomly selected in the human presence trials, we added it as a co-variate along with time of day. When neither was significant, we also removed those terms. To account for multiple variables in our analysis, we ran a Bonferroni correction and reported corrected p-values as our results. We then ran a series of univariate correlations between pre- and post-trial CORT levels and our behavioral measures (frequency of flights and bill wipes, and latency to approach the objects (novel environment only)) in the human-approach and novel-environment trials (behavioral methods and results reported in detail in Weaver et al. (2018a,b); see Tables 1, 2). Thus, we ran a total of 20 correlations (pre-trial CORT levels and post-trial CORT levels with flights, bill wipes, time spent on each of the four objects, and latency to approach each of four objects in the novel environment). In addition, we conducted Bartlett's test of homogeneity of variance among the sites to see if the variability of CORT levels within populations varied significantly across an urban gradient. We tested both pre- and post-trial CORT levels and then conducted repeatability tests (Lessells and Boag, 1987) on the pre- and post-trial CORT levels of each bird during all three seasons to determine if the difference in variance between sites was caused by within-individual or between-individual differences.

3. Results

3.1. *Human response trials*

In the 2012 and 2015 human-response trials, we found a significant effect of presentation of human on plasma CORT levels, such that birds had higher post-trial than pre-trial plasma CORT (2012: $F_{1,98} = 34.31$, $p < 0.01$; 2015: $F_{1,42} = 7.67$, $p = 0.01$; Fig. 2). Although the treatment*site effect was not significant in 2012 (see Table 3 for full results), it was in 2015, such that rural birds had lower plasma CORT before and after the human, but not control, trials, when compared to urban birds ($F_{1,42} = 5.54$, $p = 0.01$; Fig. 3). There was no effect of site (2012: $F_{2,97} = 0.57$, $p = 0.75$; 2015: $F_{1,42} = 0.18$, $p = 0.67$) or the site*treatment interaction (2012: $F_{2,97} = 0.71$, $p = 0.70$; 2015: $F_{1,42} = 0.01$, $p = 0.93$) on CORT levels in both 2012 and 2015. Only one behavior was correlated with plasma CORT: in 2012, the frequency of flights after the human approached was positively correlated with post-exposure CORT levels (rho = 0.22, $p = 0.03$; see Tables 1, 2), but this was not significant after application of Bonferroni correction (Table 3), applied to account for running multiple tests, which changed alpha to 0.01. In addition, we found significant differences in variance in baseline CORT levels (Fig. 4) during both 2012 ($p = 0.02$) and 2015 ($p = 0.01$). However, we found that birds had low repeatability between pre-trial and post-trial CORT levels (rural = 0.11, suburban = 0.01, urban = 0.33), though urban birds did have the highest repeatability levels. These results include the 2014 birds, whose results are described in detail below as well.

Table 2
This table shows correlations between post-trial CORT levels and behavior.

Year	Behavior	Rho	p-value	Year	Behavior	Rho	p-value
2012	Fly (before)	0.22	0.03	2014	Fly	−0.10	0.39
	Bill wipe (before)	−0.03	0.74		Bill wipe	0.18	0.12
	Fly (after)	0.07	0.52		Time to box	−0.07	0.54
	Bill wipe (after)	−0.06	0.58		Time to table	0.03	0.78
	Time to calm (after)	−0.05	0.63		Time to bird	−0.15	0.18
					Time to feed	0.15	0.22

3.2. *Novel environment trials*

In contrast to the human response trials, we found no significant effects of exposure to novel environment on site ($F_{1,78} = 0.66$, $p = 0.42$), site ($F_{2,78} = 0.35$, $p = 0.84$), or the site*treatment interaction ($F_{2,78} = 0.50$, $p = 0.78$) on plasma CORT levels during the novel environment trials. There were no significant correlations between CORT levels and behaviors (flights, bill wipes, time spent on the feeder, time spent near novel birds, time spent on novel objects and latency to approach novel objects; Tables 1 and 2) during these trials. We did find significant site differences in variance in CORT levels ($p = 0.01$), such that urban birds were more variable.

4. Discussion

In this study, we tested physiological stress responses (as measured by plasma CORT) of house finches across an urbanization gradient to two different types of anthropogenic stressors (presence of a human and exploring a novel environment). As we conducted these studies during three different years with different birds (and with changes in study design), our aim was not to compare individual bird responses across the multiple experiments but investigate how different stressors may affect animal populations across an urban gradient. Though previous findings have been mixed in urban CORT studies of birds, we tested populations of house finches, which had previously shown urban-rural behavioral differences (Weaver et al., 2018a) and in contexts that more closely mimicked challenges that urban birds might face. We predicted that, because urban and suburban birds are more often exposed to anthropogenic stressors (including the presence of humans and novel environments), they would have higher baseline CORT but lower stress-induced CORT than rural birds because they are facing constant non-life-threatening urban pressures and therefore have decreased response to such stressors. We also predicted that urban and suburban birds would have lower activity levels and fewer stress behaviors than rural birds. However, these predictions were not supported by our results, which showed very few differences between urban and rural birds in both baseline and stress-induced CORT during multiple years/seasons and when exposing finches to various types of anthropogenic stimuli.

One pattern we did consistently observe across sites and seasons, in the human-response trials only, was an elevation in post-trial CORT levels compared to pre-trial levels. This repeated effect suggests that we were successful in administering 'stressful' treatments to the birds, such that they (regardless of site) regularly were perceiving humans as immediate threats. Alternatively, because we handled birds prior to the start of each relatively brief trial, the during-trial elevation in CORT

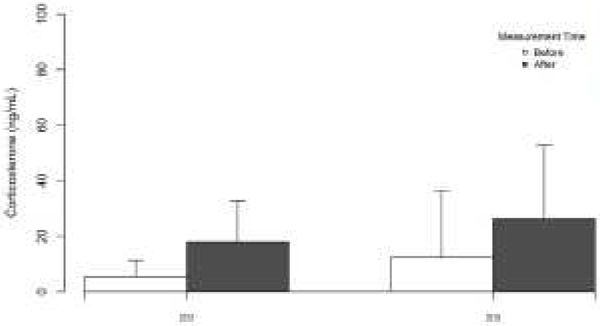

Fig. 2. Human Presence Results. In both the 2012 human approach trials (left) and 2015 human presence trials (right), house finches showed higher levels of blood CORT after than before the trial (F = 34.31, p < 0.01 in 2012; F = 7.67, p = 0.01), regardless of site of capture. This graph illustrates averages with lines representing standard deviation.

Table 3
This table shows all results from the analysis on pre-trial and post-trial plasma CORT levels in each of the three seasons.

Year	Term	F	df	p
2012	Site	0.57	2, 97	0.75
	Measurement time	34.3	1, 98	< 0.01
	Site*Measurement time	0.71	2, 97	0.70
2014	Site	0.35	2, 75	0.84
	Measurement time	0.66	1, 76	0.42
	Site*Measurement time	0.50	2, 75	0.78
2015	Site	0.18	2, 41	0.67
	Measurement time	7.67	1, 42	< 0.01
	Trial	1.53	1, 42	0.22
	Site*Measurement time*Trial	0.02	2, 41	0.88
	Site*Measurement time	0.01	2, 41	0.93
	Site*Trial	5.54	2, 41	0.02
	Measurement time*Trial	0.01	2, 41	0.98

may be due to that previous handling stress. Recent studies have demonstrated that introduction to captivity can increase plasma CORT for up to three months (de Assis et al., 2015; Love et al., 2017), much longer than we kept these birds in captivity. This may have affected our results if all birds were too stressed to reveal differences in responses. However, Partecke et al. (2006) studied European blackbirds (Turdus merula) collected across an urban gradient in captivity for a period of one year and found consistent between-site differences, thus setting a precedent for studying birds in captivity. In addition, in our trials, birds showed differences between some captive trials and not others, indicating that there were changes occurring in the plasma CORT levels in the time period that we kept them in captivity. This leads us to believe that not all our results can be linked to stress of captivity.

The only observed site difference in CORT was that, in the 2015 human-exposure trials, we found a significant site-by-treatment interaction, in which rural birds had lower pre- and post-trial CORT levels than urban birds, but only in the human-exposure (not the control) trial. This was contrary to our predictions that rural birds would have lower baseline, but not acute, CORT levels. The result most closely mirrors those from Bonier et al. (2007) and Zhang et al. (2011), who observed higher CORT levels in urban compared to rural white-crowned sparrows (*Zonotrichia leucophrys*) and tree sparrows (*Passer montanus*), though these studies measured baseline CORT only. Studies on European blackbirds and song sparrows (*Melospiza melodia*), which compared both baseline and stress-induced CORT across an urban gradient, showed no urban-rural difference in baseline CORT levels but found that urban birds had a lower stress response after exposure to handling stress (Partecke et al., 2006; Ouyang et al., 2014). Our results may have differed from these studies because we used a different type of stressor (human presence rather than confinement in a bag), as we tried to simulate a situation that may affect birds in urban areas and therefore mimic a stress response that they may experience in the wild. However, it is interesting to note that rural birds in our study were less stressed in these trials even before being exposed to the human (no site*time interaction), though they were not during the control trials (site*trial interaction). As birds were randomly assigned their trial

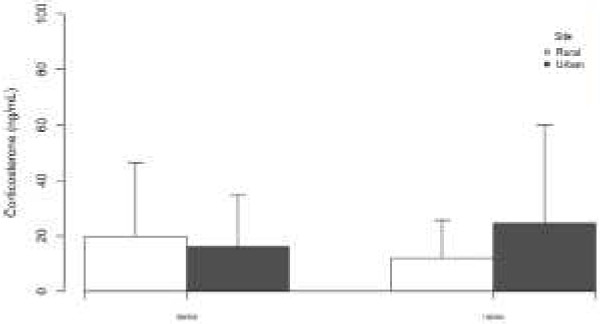

Fig. 3. Human Exposure Results. In the 2015 human presence trials, rural house finches showed a lower blood CORT response to human exposure than urban house finches (p = 0.02). This was not true when both groups were exposed to the human control (p = 0.90; a sheet rolled up to the size of a human). See Fig. 2 for additional details of the figure.

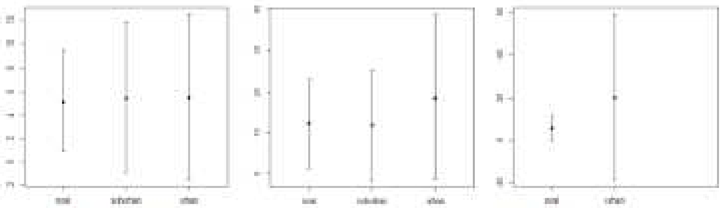

Fig. 4. Variance in CORT levels. From left to right, this graph shows the variance in the baseline CORT levels of the 2012 human approach trials ($p = 0.02$), the 2014 novel environment trials ($p = 0.01$), and the control trial from the 2015 human presence trials ($p = 0.01$), found to be significant in a Bartlett's test. There were no significant site differences in variance in the post-trial CORT measurements. The dots denote the mean for each site and the error bars denote standard deviation.

order, we do not believe this could be due to carryover effects, and we found no effect when we included trial order in our statistical model. It is interesting to note that we did not observe similar site differences in CORT levels in the 2012 human-approach trials. One reason that this may have occurred is difference in season. 2012 trials were conducted during the molt season, during which some species of passerines have been shown to suppress the stress response because CORT inhibits the production of proteins with which birds make their new feathers (Romero et al., 2005; Strochlic and Romero, 2008). We ran the trials in two different seasons because we wanted to see if there were seasonal effects on CORT levels. However, these results should also be approached with caution since captivity has been shown to affect CORT levels in animals (reviewed in Calisi and Bentley, 2009), and it is possible that rural birds had a lower response to captivity than urban birds, which is why differences appeared in 2015 but not in 2012. While many birds were in captivity longer than two weeks, not every bird was and may have had lingering captivity-acclimation effects. Since we did not see these differences in the 2014 trials, when none of the birds were in captivity longer than 10 days, this still suggests a significant effect of the human presence treatment on CORT levels.

In addition, we did not find any urban-rural differences in CORT levels in the 2014 novel-environment trials. Overall, our results suggest that hormone levels are a weak predictor of differences in behavior or stress response across an urban gradient. This is consistent with Bonier's (2012) equivocal interspecific findings in her urban-rural meta-analyses of baseline and stress-induced CORT levels, as well as with four additional, more recent studies on wild-caught birds (Meillère et al., 2015; Foltz et al., 2015; Strasser and Heath, 2013), in which only Grunst et al. (2014) reported consistent differences in CORT levels between urban and rural birds, finding a negative correlation between acute, but not baseline, CORT levels and degree of urbanization. However, the results of other studies were not as clear cut, as Foltz et al. (2015) found an urban-rural difference in CORT for some seasons but not others, and Heath (2013) found an urban-suburban difference in CORT of breeding females. Thus, one reason for the absent, or at least inconsistent, links between urbanization and CORT could be the complexity of factors that affect CORT levels, such as body condition and ambient temperature (Hau and Goymann, 2015), which are often unaccounted for and/or can vary among and within species/seasons. In addition, though urban birds may experience some additional/unique stressors, they also can have access to a more stable food supply, which could mitigate stress (Fokidis et al., 2009). Taken together, this indicates that urban environments do not strongly or consistently alter CORT levels of birds, and another physiological measure, such as metabolism (Holtmann et al., 2017), hematology (Fokidis et al., 2008), or body condition (e.g. oxidative stress), may be more likely to consistently respond to (i.e. serving as a coping mechanism for) life in the city.

Unlike site differences in CORT, we did observe significant urban-rural differences in behavior during the human-approach and novel-environment trials in our previous work (Weaver et al., 2018a,b). Rural birds showed higher levels of activity after human approach than did urban and suburban birds, which indicates potential habituation to humans and human-related environmental changes by urban populations. However, we did not find significant correlations between any behaviors performed during the trials and either pre- or post-trial CORT levels in these experiments, except that post-trial CORT levels were significantly positively correlated with activity behaviors after the human approached, though this was not significant after Bonferroni correction. Increased flight activity (i.e. escape interest) after a human approach is likely a sign of behavioral stress. If that is the case, this may indicate that this type of stress response may not be expressed through glucocorticoid regulation, but some other hormone (Koolhaas et al., 1999; Suarez and Gallup, 1981) and may provide evidence that the presence of humans is a source of stress for some city-dwelling species.

One reason why some animals better-adapt than others to human-impacted areas is that populations vary in plasticity (i.e. ability to rapidly respond and acclimate to environmental conditions; reviewed in Diamond and Martin, 2016). Studies that focus on plasticity generally test behavioral responses, but animals may be plastic in their physiological responses as well. As a way of better understanding CORT plasticity in our house finch study populations, we examined the variance in both the pre- and post-trial CORT levels in our experiments and found significant differences in the baseline (but not post-trial) plasma CORT between sites in all three seasons (Fig. 4). In 2012, both suburban and urban finches showed much greater variance in baseline CORT levels than rural birds, which suggests that rural birds came into the study more similar to each other, perhaps due to the unique conditions they endure in the wild. Because urban and suburban areas can vary in human density and natural vegetation (e.g. park vs strip mall vs downtown), urban and suburban birds may vary in their response to diverse human-inhabited areas, whereas rural birds may not share this experience, resulting in lower variance in responses from rural birds than urban. Site differences in CORT variance were also present in the winter 2014 trials, although here only between rural and urban populations, such that the urban populations again showed higher variance than rural populations. In 2015, we tested only urban and rural populations, but there were still significant site differences in variance for pre-trial CORT levels in both the control and human trials. We believe that these patterns reflect between-individual differences since results of the repeatability test indicate low repeatability between pre- and post-trial CORT levels regardless of site and season. Because site differences in CORT variance disappear after experimental testing, combined with the fact that we uncovered few/no mean site differences in plasma CORT, this provides the compelling suggestion that urban and rural birds responded similarly in all of our trials simply because they were being subjected to the same environmental/testing conditions. Although tracking stress events for individual birds in the wild is notoriously difficult, especially in a non-territorial study species like ours, we now must better understand the frequency and intensity of natural environmental stressors in the lives of finches along the urban gradient to better contextualize the behavioral and CORT-related findings from our lab experiments.

As urban areas continue to expand worldwide and more animal populations are affected by human activities, studies on the physiological and behavioral mechanisms underlying organismal acclimation and/or adaptation will continue to be important. This study highlights that circulating CORT may not reliably capture physiological or behavioral responses of house finches to anthropogenic stimuli (like human presence or navigating in a new environment), at least when tested under confined or lab conditions. Instead, in opportunistic species like house finches (as native desert and city inhabitants), it may be more likely that acclimation/responsiveness to local environmental conditions explains field differences in physiology and behavior, and that presenting them with the same environmental stimuli in the lab is unlikely to reveal strong, lasting, adaptive, genetic or developmentally rooted population differences in phenotype. Additionally, it may be valuable for urban ecologists to pursue additional physiological measurements, such as metabolic rate or body condition, in order to improve our resolution of how CORT and behavior are linked across an urban environmental gradient.

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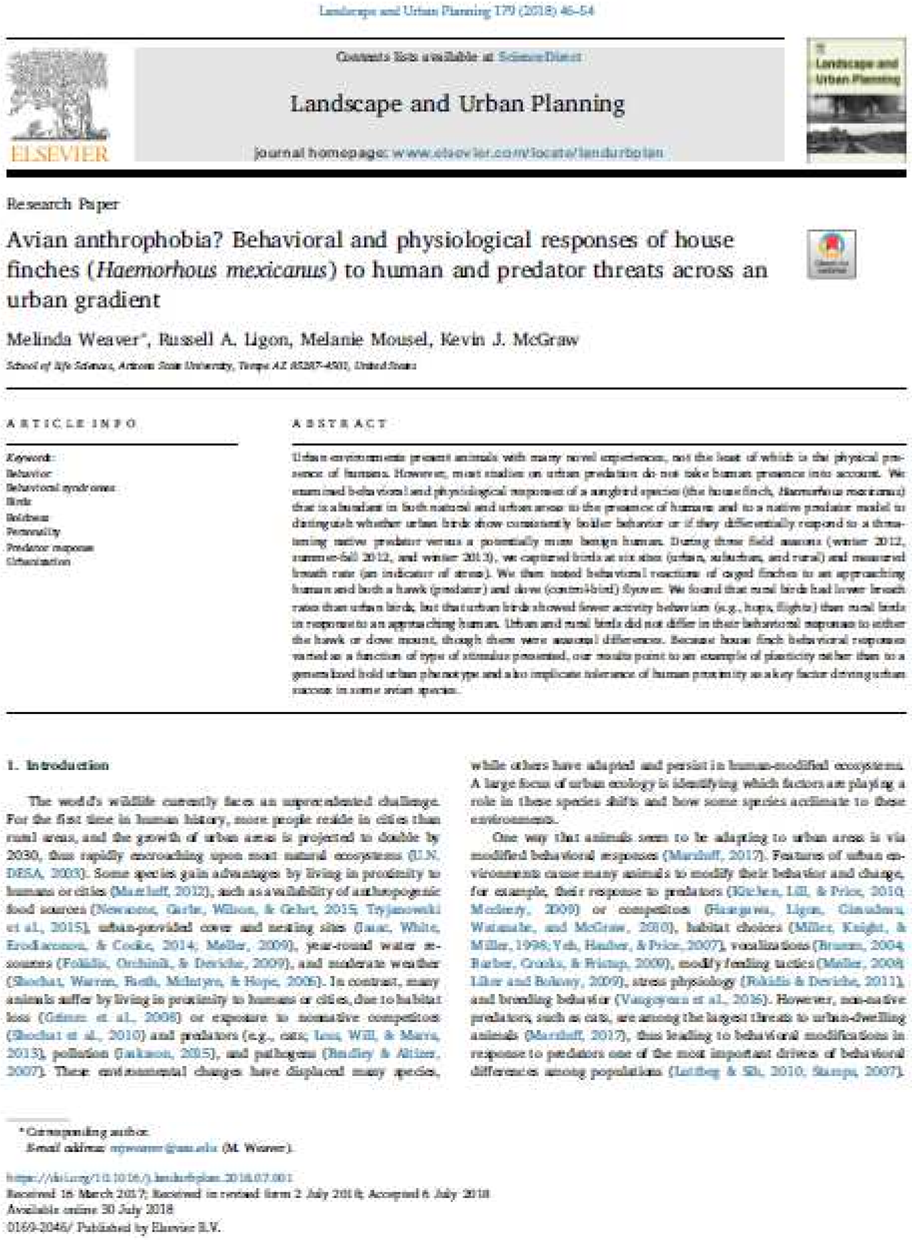
APPENDIX B

AVIAN ANTHROPHOBIA? BEHAVIORAL AND PHYSIOLOGICAL RESPONSES OF HOUSE FINCHES (*HAEMORHOUS MEXICANUS*) TO HUMAN AND PREDATOR THREATS ACROSS AN URBAN GRADIENT



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Research Paper

Avian anthrophobia? Behavioral and physiological responses of house finches (*Haemorhous mexicanus*) to human and predator threats across an urban gradient

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A B S T R A C T

Urban environments present animals with many novel experiences, not the least of which is the physical presence of humans. However, most studies on urban predation do not take human presence into account. We examined behavioral and physiological responses of a songbird species (the house finch, *Haemorhous mexicanus*) that is abundant in both natural and urban areas to the presence of humans and to a native predator model to distinguish whether urban birds show consistently bolder behavior or if they differentially respond to a threatening native predator versus a potentially more benign human. During three field seasons (winter 2012, summer-fall 2012, and winter 2013), we captured birds at six sites (urban, suburban, and rural) and measured breath rate (an indicator of stress). We then noted behavioral reactions of caged finches to an approaching human and both a hawk (predator) and dove (control-bird) flyover. We found that rural birds had lower breath rates than urban birds, but that urban birds showed fewer activity behaviors (e.g., hops, flights) than rural birds in response to an approaching human. Urban and rural birds did not differ in their behavioral responses to either the hawk or dove mount, though there were seasonal differences. Because house finch behavioral responses varied as a function of type of stimulus presented, our results point to an example of plasticity rather than to a generalized bold urban phenotype and also implicate tolerance of human proximity as a key factor driving urban success in some avian species.

1. Introduction

The world's wildlife currently faces an unprecedented challenge. For the first time in human history, more people reside in cities than rural areas, and the growth of urban areas is projected to double by 2030, thus rapidly encroaching upon most natural ecosystems (U.N. DESA, 2003). Some species gain advantages by living in proximity to humans or cities (Marzluff, 2012), such as availability of anthropogenic food sources (Newsome, Garbe, Wilson, & Gehrt, 2015; Tryjanowski et al., 2015), urban-provided cover and nesting sites (Isaac, White, Ierodiaconou, & Cooke, 2014; Møller, 2009), year-round water resources (Fokidis, Orchinik, & Deviche, 2009), and moderate weather (Shochat, Warren, Faeth, McIntyre, & Hope, 2006). In contrast, many animals suffer by living in proximity to humans or cities, due to habitat loss (Grimm et al., 2008) or exposure to nonnative competitors (Shochat et al., 2010) and predators (e.g., cats; Loss, Will, & Marra, 2013), pollution (Jackson, 2015), and pathogens (Bradley & Altizer, 2007). These environmental changes have displaced many species, while others have adapted and persist in human-modified ecosystems. A large focus of urban ecology is identifying which factors are playing a role in these species shifts and how some species acclimate to these environments.

One way that animals seem to be adapting to urban areas is via modified behavioral responses (Marzluff, 2017). Features of urban environments cause many animals to modify their behavior and change, for example, their response to predators (Kitchen, Lill, & Price, 2010; McCleery, 2009) or competitors (Hasegawa, Ligon, Giraudeau, Watanabe, and McGraw, 2010), habitat choices (Miller, Knight, & Miller, 1998; Yeh, Hauber, & Price, 2007), vocalizations (Brumm, 2004; Barber, Crooks, & Fristrup, 2009), modify feeding tactics (Møller, 2008; Liker and Bokony, 2009), stress physiology (Fokidis & Deviche, 2011), and breeding behavior (Vaugoyeau et al., 2016). However, non-native predators, such as cats, are among the largest threats to urban-dwelling animals (Marzluff, 2017), thus leading to behavioral modifications in response to predators one of the most important drivers of behavioral differences among populations (Luttbeg & Sih, 2010; Stamps, 2007).

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Shochat et al. (2006) suggested that predation could be one of the primary forces shaping the composition of urban animal communities as well. Initially, the safe-habitat hypothesis (Tomialojc, 1982) proposed that animals experience reduced predation risk in urban areas due to lack of native predators. However, researchers have since come to realize that, while predation rates may be lower in cities, vertebrate predator densities can be higher (i.e. the "predation paradox"; Fischer, Cleeton, Lyons, & Miller, 2012). In addition, many studies of urban predation have failed to consider the role of humans, which, through actual or perceived predation, can negatively affect animal foraging behavior (Ward & Low, 1997), patch use (Fernandez-Juricic and Telleria, 2000), population density (Fernandez-Juricic, 2000), and fecundity (Zanette, White, Allen, & Clinchy, 2011).

As humans abound and expand their range worldwide, how a species responds to human presence – and the ability to differentiate humans from predators and identify their presence as less threatening – may be a key predictor of urban acclimation and adaptation. Studies that have evaluated how urban populations of animals respond to human physical presence have produced mixed results. Although several studies found that birds show a relaxed response to humans in urban environments, taking flight later in response to their presence (Arroyo, Mougeot, & Bretagnolle, 2017; Carrete & Tella, 2011; Carrete & Tella, 2017; Møller, 2008), Valcarcel and Fernandez-Juricic (2009) found that urban house finches (*Haemorhous mexicanus*) took flight sooner in the presence of humans than rural counterparts. In addition, recent studies have found that the amount of time to take flight in response to human/predator presence can be affected by the presence of bird feeders (Møller, Díaz, et al., 2015; Møller, Tryjanowski, et al., 2015), the direction of the approach (direct vs tangential; Møller & Tryjanowski, 2014), and vehicle speed (DeVault, Blackwell, Seamans, Lima, & Fernández-Juricic, 2014). Therefore, it appears that animals use many cues to assess humans as potential threats in their environments, and thus behavioral responses, and their underlying physiological causes, could be more complex and require a more detailed understanding of their nuances.

While FID studies allow researchers to understand how animals respond to human presence in their environment, a more detailed understanding of behavioral responses, and the underlying physiological mechanisms, may identify additional behavioral differences that can better elucidate why some animals tolerate humans better than others. In addition, previous studies have not compared behavioral responses of animals to humans with those to native predators, to determine if urban and rural populations respond differently to different vertebrate threats, or perceive humans as threats at all (or as something novel). Here, we tested behavioral and physiological responses of house finches along an urbanization gradient to the presence of both an approaching human and a native (hawk) predator. By measuring finch responses to both a human, a hawk mount, and a control (dove mount) stimulus, we could (1) examine differences in behavioral and physiological stress responses to potential predators among rural, suburban, and urban populations, and (2) test whether urban birds show consistently bolder behavior toward potential vertebrate threats or differentially respond to a threatening native predator versus a potentially more benign human or dove. Because urban and suburban birds live in areas with higher human densities than rural birds but are not often directly or physically harmed by humans, we predicted that they would show lower levels of both behavioral and physiological stress when approached by a human, but that no such differences would be apparent when house finches were approached by a native predator (hawk), which can be found in both urban and rural environments. Though a previous study on house finches (Valcarcel & Fernandez-Juricic, 2009) found opposite results (i.e. that urban birds responded more strongly than did rural ones to the presence of humans), observations in our house finches, combined with several other published avian studies (Arroyo et al., 2017; Carrete & Tella, 2011; Carrete & Tella, 2017; Møller, 2008), led us to predict these results in our populations. We conducted our behavioral and physiological tests in two different seasons – winter and summer/fall – to examine if or how changes in climate or life-stage might influence responses of house finches to human and avian stimulus presentations.

2. Methods

2.1. *Study system*

To address our questions, we investigated house finches in the greater Phoenix, Arizona, USA area. House finches are native to the desert-southwestern USA and Mexico, but now also inhabit urban and suburban areas across most of the lower 48 United States (Badyaev, Belloni, & Hill, 2012). House finches also readily display behaviors like foraging and aggression in open environments (e.g., feeders, backyards), are abundant and easily captured, and are amenable to captive behavioral experimentation (Hill, 2002).

2.2. *Field methods*

Using basket traps baited with sunflower seeds, we trapped ca. 30 finches during each of three trapping seasons (see more below) at each of six sites (Supplementary Fig. 1): two urban (n = 185; 91 females, 94 males), two suburban (n = 183; 89 females, 94 males), and two rural (n = 184; 89 females, 95 males), based on urban land-use parameters measured by the Central Arizona Phoenix Long Term Ecological Research program (Giraudeau, Mousel, Earl, & McGraw, 2014). During the two winter seasons, we captured adult house finches, and during the summer season, we captured juveniles. We trapped finches during winter 2012 (January – March; human approach trials only: n = 153; 71 females, 82 males), summer-fall 2012 (July - September; both human approach and native predator trials: n = 206; 101 females, 105 males) and the subsequent winter (December 2012 – February 2013; native predator trials only: n = 192; 97 females, 95 males). We chose to study finches during winter and late summer/early fall since these should be contrasting times of environmental stress - summer molt (when it is extremely hot in the desert and birds are devoting energy to regrowing feathers; King, 1980) versus the winter non-breeding season (when the climate is mild and the only major resource investment is in self-maintenance; Bryant, 1997). In the first season, we focused on studying response to humans but decided in the second season that testing responses to both humans and a native predator would provide a more comprehensive test of behavioral variability. In our third season, we examined response to native predators only (since we already had two seasons of data on responsiveness to humans). At capture, we fitted each bird with a numbered United States Geological Survey metal identification band and measured breath rate, by counting number of breaths per minute for one minute just after capture as the bird was held in hand. This method of measuring stress is less invasive than taking a blood sample and previously was shown in great tits (*Parus major*) to increase following stressful situations and was correlated with anti-predator behavior, such that shyer birds had higher breath rates than those that were bolder (Carere & van Oers, 2004).

2.3. *Human approach trials*

After banding and measuring birds, we placed each individually in a large cage (0.77 m tall * 0.59 m long * 0.50 m wide; Fig. 1) in the field in similar light conditions (shaded in summer) to the trapping site between 0600 and 1100 h. The cage was equipped with a hide area and small dishes of sunflower seeds and water. Each bird had 20 min to acclimate to the cage while we recorded all behavior with a video camera (JVC Everio, Long Beach, CA, 24 Mbps recording) mounted on a tripod placed about three feet from the cage. After the acclimation period, one person (MW) walked toward the cage starting from a distance of 20 m at a pace of one step per second until reaching the cage and walked away at the same pace. We continued recording the

Fig. 1. Cage in which birds were housed during the human-approach trials, which included an escape route, food, water dish, and a cardboard box that served as a hide area.

Table 1
List of behaviors quantified for each trial and their definitions.

Behavior	Description
Hop	Bird relocates to a new place in the cage without using its wings
Fly	Bird relocates to a new place in the cage by flapping its wings
Ruffle	Bird puffs out feathers and shivers
Bill wipe	Bird wipes bill on cage/perch/feeder but not on self
Time on feeder	Time bird spends perched on or eating from feeder
Time in hide	Time bird spends in hide area
Latency approach feeder	How long it takes the bird to perch on feeder after the trial begins
Time to calm	Time bird first sits for five seconds without moving

behavior of the birds to measure direct response to the approaching/departing human and including the 10 min after the approach, to measure lasting effects of the human walk-by. We tested three birds at a time, each in its own cage at least 20 m from the nearest cage to prevent the birds from responding to an approach to a neighboring cage. We determined this distance by recording the distance at which the birds first reacted to human presence during a pilot study. Birds from winter 2012 were released immediately after the trial. Birds from summer-fall 2012 and winter 2013 were then placed in a paper bag and transported to campus for the native predator-presentation trials (see methods below). We tested a total of 12 birds per day and visited each site at least three times to obtain the required sample size of 28 birds per season, which we obtained from a power analysis with an effect size of 0.40. From each video, two independent observers scored several behaviors (see Table 1 for definitions of each behavior) using the software program Cowlog (Hänninen and Pastell, 2009): the number of hops and flights (used as measures of activity level), number of bill wipes and feather ruffles (proposed as indicators of stress; Clark, 1970; Tinbergen, 1940), and the amount of time spent on the feeders and in the hide area (Table 1). Data collected by each observer were tested for inter-person repeatability (Lessells & Boag, 1987), which measures the consistency between observers. Repeatability estimates for individual behaviors were > 0.75 (Supplementary Table 1), so the scores from the two observers were averaged for final analyses, except for two variables (Supplementary Table 1), where we had an impartial third party observe 20 videos and used the data of the person who was most closely repeatable to the third observer. Because videos were recorded in the field and sites were distinctive to those who had also participated in field work, observers are not blind to site when watching videos.

2.4. *Native predator trials*

Prior to participating in trials, we housed birds individually in small wire cages (60 cm * 40 cm * 30 cm) without visual access to one another within a climate-controlled vivarium and given an *ad libitum* diet of black sunflower seeds and tap water. The rooms were kept at a temperature of 25 °C on a natural outdoor light-dark cycle. The night before each trial, each bird's food was removed to ensure motivation to approach the feeder during the behavioral assays the following day. For the trial, we placed each bird individually in a large flight cage (2.75 m L * 1.5 m W * 2.75 m H) in a secluded courtyard on campus between 0600 and 1100 h, giving each bird 15 min to acclimate. Because there are four flight cages in the courtyard, we ran trials for four birds at a time, but birds did not have visual access to one another. After the acclimation period, either a stuffed sharp-shinned hawk (*Accipiter striatus*; treatment) or a stuffed mourning dove (*Zenaida macroura*; control) was flown over the aviary cages on a zipline with the appropriate species-specific bird vocalization being played via a portable speaker connected to an iPod. Each bird went through a hawk and a dove trial, thus testing each bird twice. Order of stimulus presentation was randomized to avoid order effects. The behavior of each finch was recorded following the flyover for 45 min; we chose this trial duration because pilot experiments showed that many birds did not approach the feeder for up to 30 min post-exposure. We conducted four sessions of trials/day (total of 16 trials/day) during the summer and five trial sessions/day (total of 20 trials/day) during the winter. We recorded trials on a video camera mounted to a tripod just outside of the aviary cages and scored the same behaviors listed above (see "Human Approach Trials") as well as latency to approach feeder (a common measure of boldness in predator studies (first defined by Greenberg & Mettke-Hofmann, 2001) and to 'calm' after stimulus presentation, which was the time it took for the bird to sit without moving for five seconds after the initial burst of flights that followed in every bird upon the model presentation (Table 1). As above, we tested data collected by each video observer for repeatability (Supplementary Table 2) and averaged the two values for use in statistical analyses. If a repeatability estimate fell below 0.75 (Supplementary Table 2), an impartial third party observed 20 videos, and we used the data of the person who was most closely repeatable to the third observer for averaging. To minimize observer bias, video watchers were blind to the origin site and bird identity.

2.5. *Statistical methods*

We ran all statistical analyses in the R computing environment (Ver. 2.15.1). To test for multicollinearity among finch behavioral variables, we ran initial correlations among the various finch behaviors and reduced number variables when two were significantly correlated (higher than 0.20 for Spearman's test). We found that the frequency of bill wiping and ruffling (displacement behaviors) were highly correlated (Supplementary Tables 3 and 4), so we randomly chose to analyze one

Table 2
Model-averaged estimates for life-history and ecological variables predicting behavioral responses of house finches during human-approach trials.

Behavioral response	Predictor	Estimate	SE	SE (adjusted)	z	p
Flight rate						
	(Intercept)	5.82	0.25	0.25	23.42	**< 0.0001**
	Pre-approach flight rate	0.00	0.00	0.00	5.49	**< 0.0001**
	Habitat (suburban)	− 0.45	0.27	0.27	1.66	0.097
	Habitat (urban)	− 0.42	0.26	0.26	1.62	0.106
	Season (winter)	− 0.69	0.17	0.17	3.96	**< 0.0001**
	Sex (male)	0.65	0.07	0.07	8.96	**< 0.0001**
Wipe rate						
	(Intercept)	0.10	0.42	0.43	0.24	0.812
	Pre-approach wipe rate	0.03	0.00	0.00	6.85	**< 0.0001**
	Habitat (suburban)	− 1.01	0.46	0.46	2.19	**0.029**
	Habitat (urban)	− 1.55	0.49	0.49	3.17	**0.002**
	Season (winter)	1.10	0.42	0.42	2.64	**0.008**
	Sex (male)	− 0.09	0.24	0.24	0.36	0.716
Feed rate						
	(Intercept)	− 2.49	1.46	1.46	1.71	0.088
	Pre-approach feeding rate	0.00	0.00	0.00	7.10	**< 0.0001**
	Habitat (suburban)	− 2.23	1.20	1.21	1.85	0.065
	Habitat (urban)	− 2.23	1.21	1.21	1.84	0.066
	Season (winter)	0.68	0.79	0.79	0.86	0.390
	Sex (male)	− 0.83	0.68	0.68	1.22	0.222
Hide rate	(Intercept)	1.54	0.50	0.50	3.08	**0.002**
	Pre-approach hiding rate	0.00	0.00	0.00	9.15	**< 0.0001**
	Habitat (suburban)	− 0.18	0.45	0.45	0.40	0.686
	Habitat (urban)	0.07	0.35	0.35	0.21	0.838
	Season (winter)	− 0.85	0.66	0.67	1.27	0.204
	Sex (male)	− 0.04	0.27	0.27	0.14	0.886
Breathing rate	(Intercept)	3.91	0.03	0.03	122.33	**< 0.0001**
	Pre-approach breath rate	0.01	0.00	0.00	31.75	**< 0.0001**
	Habitat (suburban)	0.00	0.00	0.00	0.03	0.977
	Habitat (urban)	0.00	0.00	0.00	0.06	0.949
	Season (winter)	0.00	0.01	0.01	0.05	0.959
	Sex (male)	0.00	0.00	0.00	0.02	0.987

The values are bolded if they have a significant p value.

of those behaviors (bill wipes) to reduce the number of behaviors examined. Interestingly, frequency of hopping and flying (activity behaviors) were also highly correlated in the human-approach trials, but in predator trials hopping frequency was correlated with frequency of bill wiping and ruffling (instead of with flying), so we analyzed flight frequency as the activity behavior for both experiments (Tables 2, 3). Since none of the remaining behaviors were highly correlated with one another, we analyzed each behavior rather than combining them using principal components analysis. We also calculated the repeatability (Lessells & Boag, 1987) of an individual's behavioral responses to hawk and dove since each bird participated in both trials (Table 4) and repeatability of behavioral responses to the birds who participated in both predator trials and the human approach trials in summer 2012 ($n = 124$; Table 4). Values higher than 0.20 were considered repeatable as $p < 0.01$ for those values.

We used generalized linear mixed models (with the *glmer* function in lme4 package; Bates, Mächler, Bolker, & Walker 2015) to evaluate the influence of several predictor variables on house finch behavior, including individual finch identity as a random effect in our models. Specifically, we evaluated the effects of habitat type (three categories: rural, suburban, urban), season (molt, winter), pre-stimulus behavior on finch response to the different stimuli. For example, when analyzing flights, we included an individual's pre-stimulus (either human approach or model flyover) flights, as well as the other predictor variables to control for any trial-specific behavioral tendencies unrelated to the specific stimulus (model or human). Additionally, we employed an information-theoretic model-averaging approach to incorporate parameter estimates from multiple models, in accordance with the relative strength of each model (Burnham & Anderson 2002; Burnham, Anderson, & Huyvaert 2010). Specifically, we limited our model-averaging to the 95% Confidence Set (the set of models that, collectively, have a summed Akaike weight of 0.95; Burnham & Anderson 2002). Within this framework, variables that only appear in poorly-supported models exhibit negligible parameter estimates, and variables that appear repeatedly in well-supported models will have larger parameter estimates (Supplementary Tables 5, 6). We used Cohen's d to calculate effect sizes, which were all ≥ 0.54.

3. Results

3.1. *Behavioral responses to an approaching human*

We observed higher rates of flying and bill-wiping after the human approached the cage than before the approach, indicating that birds were responding to the stimulus (Table 2). Capture site significantly predicted bird activity in the minute just after the human approached the cage (Table 2), such that rural birds flew more than both urban and suburban birds (Fig. 2). We found that urban and suburban birds had lower bill-wiping rates than rural birds during the trials, and overall birds flew and bill-wiped more in the 10 min after the human approached than during the 20-min acclimation period (Table 2, Fig. 2). We also found significant season and sex effects, such that birds had a higher flight rate during the molt season but higher bill-wiping rate during the winter season, and males had a higher flight rate overall than females (Table 2, Fig. 2). We found no significantly effects of site, season, sex, or their interactions on breath rate (Table 2).

3.2. *Behavioral responses to aerial avian presentations*

Though capture site did not predict any of the behavioral measures

Table 3
Model-averaged estimates for life-history and ecological variables predicting behavioral responses of house finches during avian model presentations. Habitat estimates are made relative to rural populations, model presentation estimates are made relative to dove models, season estimates are made relative to molt season, and sex estimates are made relative to female finches.

Behavioral response	Predictor	Estimate	SE	SE (adjusted)	z	p
Flight rate						
	(Intercept)	5.923	0.10	0.10	61.43	< 0.0001
	Pre-flyover flight rate	0.000	0.00	0.00	54.54	< 0.0001
	Habitat (suburban)	0.006	0.10	0.10	0.06	0.952
	Habitat (urban)	0.153	0.15	0.15	1.00	0.317
	Model (hawk)	0.018	0.00	0.00	4.60	< 0.0001
	Season (winter)	− 0.031	0.08	0.08	0.41	0.680
	Sex (male)	− 0.021	0.07	0.07	0.33	0.744
Wipe rate						
	(Intercept)	1.130	0.16	0.16	7.06	< 0.0001
	Pre-flyover wipe rate	0.003	0.00	0.00	6.28	< 0.0001
	Habitat (suburban)	0.274	0.21	0.22	1.27	0.203
	Habitat (urban)	− 0.056	0.15	0.15	0.38	0.704
	Model (hawk)	− 0.005	0.01	0.01	0.31	0.758
	Season (winter)	1.344	0.14	0.15	9.26	< 0.0001
	Sex (male)	0.304	0.17	0.17	1.77	0.076
Feed rate						
	(Intercept)	6.119	0.14	0.14	43.96	< 0.0001
	Pre-flyover feeding rate	0.000	0.00	0.00	33.15	< 0.0001
	Habitat (suburban)	− 0.051	0.13	0.13	0.41	0.682
	Habitat (urban)	− 0.044	0.11	0.11	0.39	0.697
	Model (hawk)	− 0.012	0.00	0.00	3.52	< 0.001
	Season (winter)	0.144	0.16	0.16	0.90	0.368
	Sex (male)	0.058	0.11	0.11	0.51	0.610
Latency to return to feeder						
	(Intercept)	4.880	0.09	0.09	53.65	< 0.0001
	Pre-flyover feeder latency	0.000	0.00	0.00	41.11	< 0.0001
	Habitat (suburban)	− 0.004	0.05	0.06	0.07	0.943
	Habitat (urban)	0.012	0.06	0.06	0.20	0.843
	Model (hawk)	0.054	0.00	0.00	12.16	< 0.0001
	Season (winter)	1.037	0.11	0.11	9.13	< 0.0001
	Sex (male)	− 0.003	0.06	0.06	0.05	0.961
Latency to calm						
	(Intercept)	4.573	0.14	0.14	33.62	< 0.0001
	Pre-flyover feeder latency	− 0.001	0.00	0.00	6.67	< 0.0001
	Habitat (suburban)	− 0.013	0.09	0.09	0.15	0.884
	Habitat (urban)	0.021	0.09	0.09	0.23	0.822
	Model (hawk)	− 0.253	0.01	0.01	29.00	< 0.0001
	Season (winter)	− 1.059	0.17	0.17	6.25	< 0.0001
	Sex (male)	0.010	0.09	0.09	0.12	0.909

The values are bolded if they have a significant p value.

during the avian-presentation trials, we did find significant effects of season and model presentation on finch behavior (Table 3). Birds had higher flight and bill-wiping rates, ate less, took longer to approach the feeder, and took longer to calm during the hawk flyover versus the dove flyover (Fig. 3, Table 3). We saw higher rates of flying and bill-wiping after the flyovers, regardless of model type, indicating that birds were responding to the stimulus (Table 3). Finches also had a higher bill-wiping rate and took longer to calm during the winter season than the summer-fall (Fig. 3, Table 3) Fig. 4.

3.3. *Repeatability of behavior across trials*

Flight rate was the most repeatable behavior between the two aerial avian trials (Table 4), and time spent on feeder was the least repeatable. Suburban birds showed the most consistency between trials (Table 4), whereas rural birds were consistent in flight rate and bill-wiping rate but not time spent on feeder or time to approach the feeder (Table 4). Urban birds were consistent in flight rate, latency to approach feeder, and in time spent on feeder, but not in latency to calm or bill-wiping rate (Table 4). We also tested repeatability of behaviors in birds who went through both the avian-flyover and human-approach trials (n = 124; Table 4). Birds from all three sites were repeatable in flight rate and time on feeder, as urban birds had the greatest repeatability in flight rate and suburban birds had the greatest repeatability in time spent on feeder (Table 4). None of the sites were repeatable on bill wiping rate, however (Table 4).

4. Discussion

In this study, we tested behavioral and physiological responses of house finches from urban, suburban, and rural sites to the approach of humans and native birds. As predicted, we observed greater behavioral differences across sites in the human-approach trials than the avian-flyover trials. We also found that rural birds were more active (i.e. flew around more in the cage) than both suburban and urban finches in the minute just after the human approach, and that this response was consistent among seasons. Many studies that evaluate the response of urban animals to humans utilize flight initiation distances (FID) to determine fear levels toward humans (Arroyo et al., 2017; Carrete & Tella, 2011; Carrete & Tella, 2017; Møller, 2008; Valcarcel & Fernandez-Juricic, 2009), predicting that animals that allow an approaching human to get close to them are less affected by human presence. While most of these studies have found that, in more than 25 species ranging from songbirds to raptors, urban birds had shorter FIDs than those from rural areas (Arroyo et al., 2017; Carrete & Tella, 2011; Carrete & Tella, 2017; Møller, 2008), which is consistent with our

Table 4
Repeatability between finch behavior in the aerial-flyover trials hawk vs dove and hawk vs dove vs human.

Trial	Behavior	Habitat Type	Repeatability
Hawk vs dove	Flight	Rural	0.57
		Suburban	0.64
		Urban	0.51
	Bill wipe	Rural	0.53
		Suburban	0.68
		Urban	0.19
	Time to calm	Rural	0.23
		Suburban	0.30
		Urban	0.09
	Time on feeder	Rural	0.09
		Suburban	0.23
		Urban	0.30
	Latency to feeder	Rural	0.09
		Suburban	0.25
		Urban	0.33
Hawk vs dove vs human	Flight	Rural	0.35
		Suburban	0.34
		Urban	0.58
	Bill wipe	Rural	0.03
		Suburban	0.14
		Urban	0.15
	Time on feeder	Rural	0.39
		Suburban	0.47
		Urban	0.28

findings, Valcarcel and Fernandez-Juricic (2009) found that house finches in urban areas actually took flight *earlier* than rural populations. Unfortunately, we could not calculate FID *per se* in our study because of the generally high levels of finch activity during confinement and acclimation, which makes it difficult to accurately compare our results to those of Valcarcel and Fernandez-Juricic (2009). However, we also found that rural house finches more frequently bill-wiped in response to the approaching human than urban and suburban birds, which is another indicator that rural birds were experiencing greater levels of stress in the presence of a human.

While we recognize that having only one mount of each stimulus type may be a limitation to our study, we still found that finches took longer to calm after the hawk flyover versus the dove, suggesting that the predator presentation may have served as a greater threat or stressor than the more benign dove. We found this same result in the human approach trials, as birds across sites showed differences in behavior before and after approach. We, of course, cannot ignore that these studies were conducted in captivity, which might have resulted in artificial differences across populations. To mitigate this, we conducted human approach trials in the field within five minutes of capture to get the most ecologically relevant results. Another limitation of this study is that we used different populations of birds across different seasons, which we discuss in more detail below.

In contrast to the results from the human-approach trials, we found no effect of capture site (*i.e.* urbanization) on any of the behaviors in response to an avian flyover (whether hawk or dove). This finding suggests that finches from urban, suburban, and rural sites show a consistent response to the threat of an aerial (*accipiter*) predator. Though a handful of studies have demonstrated that urban animals tend to be bolder in the presence of a predator than rural ones (Evans, Boudreau, & Hyman, 2010; McCleery, 2009; Myers and Hyman, 2016; Seress, Bokony, Heszberger, & Liker, 2011), Cote, Fogarty, Tymen, Sih, and Brodin (2013) showed that behavioral differences in populations of mosquitofish (*Gambusia affinis*) vanished in the face of predation, when all individuals tended to behave similarly when faced with a known threat. Since finches at all of our sites are exposed to aerial predators (pers. obs.), we would predict that birds from all sites would respond similarly to these predators. In addition, birds of all populations demonstrated a high level of repeatability in activity behavior between

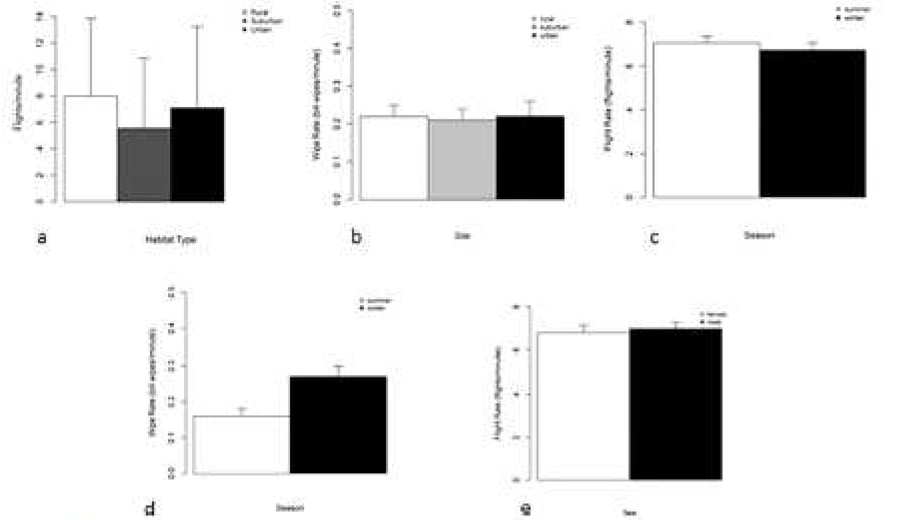

Fig. 2. During the human-approach trials, rural birds flew more than both urban and suburban birds in the minute just after approach (a), rural birds had a higher bill-wiping rate than both urban and suburban birds (b), birds had a higher flight rate in the summer than during winter (c), birds had a higher bill-wiping rate in the winter than during summer (d), and males had a higher flight rate than females (e). Mean + standard error shown in all panels.

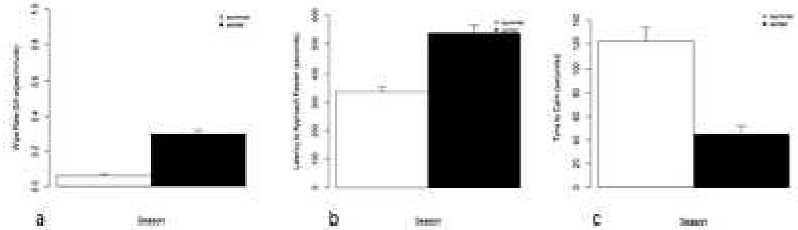

Fig. 3. During the bird-mount flyover trials, birds had a higher bill-wiping rate during the winter than during summer (a), took longer to approach the feeder after flyover in the winter than in summer (b), and took longer to calm during the winter than summer (c). Mean + standard error shown in all panels.

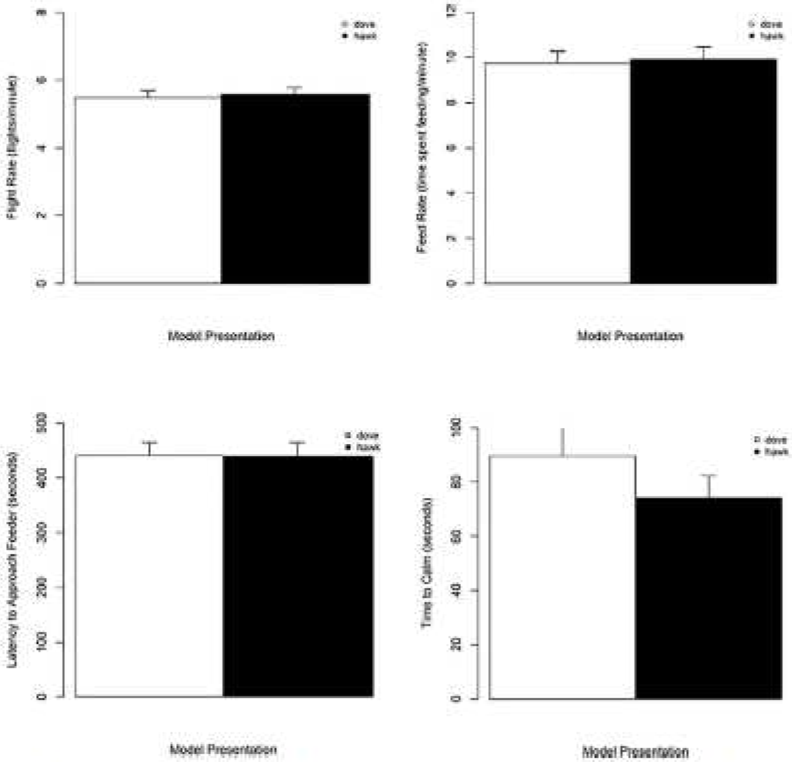

Fig. 4. Birds had a higher flight rate (a), ate less frequently (b), took longer to approach the feeder (c), and took longer to calm (d) after the hawk flyover compared to the dove flyover. Mean + standard error shown in all panels.

the three trials. However, while bill wiping was repeatable between hawk and dove trials, it was not repeatable between aerial trials and human trials, demonstrating that all populations are showing a great deal of plasticity in their response to larger vertebrates in their environments.

We did find several seasonal differences in both studies, as birds had a higher bill-wiping rate and took longer to approach the feeder during the winter avian-flyover trials than during the summer-fall. Also, in the human-approach trials, birds again bill-wiped more frequently in the winter than in summer but had a higher flight rate in summer compared to winter. However, it is also noteworthy that the majority of birds captured during the summer season were juveniles, whereas the majority captured during the winter were adults. Age can affect boldness, as juvenile perch (*Perca fluviatilis*) are less bold than adults when exposed to predators (Magnhagen & Borcherding, 2008), but boldness was found to decrease with age in domestic dogs (Starling, Branson, Thomson, & McGreevy, 2013). In addition, Frost, Winrow-Giffen, Ashley, and Sneddon (2007) found that previous experience altered boldness in rainbow trout (*Oncorhynchus mykiss*), such that individuals with previous experience with predators were less bold and exploratory. However, the majority of studies on juvenile behavioral traits have found that juvenile behavior, particularly boldness, does not predict adult behavior (Bell & Stamps, 2004; Petelle, McCoy, Alejandro, Martin, & Blumstein, 2013; Sinn, Gosling, and Moltschaniwskyj, 2008). Thus, difference in age (and experience with predators) may have contributed to our seasonal patterns. Future behavioral studies with juvenile finches have been planned to investigate this possibility.

In addition to measuring finch behavioral responses to humans and hawk/dove mounts, we also measured breath rate as an indicator of physiological stress (as in great tits; Carere & van Oers, 2004). Contrary to our prediction, we found no differences in breath rate among urban, suburban, and rural birds. This appears to conflict with our behavioral results, in which rural birds had higher activity levels and displayed more stress behaviors than urban and suburban birds in the human-approach tests. However, these tests were conducted at different time scales (immediately upon capture for breath rate versus monitoring behavior during a 30- or 60-min trial), which could capture different components of stress responsiveness, just as measuring corticosterone levels immediately after a stressor versus 3–5 min afterwards captures different stress measurements because corticosterone takes 3–5 min to increase in the blood post-stressor (Wingfield, Vleck, & Moore, 1992). Studies that have attempted to identify differences in breath rate across an urban gradient have been mixed as some studies have shown that urban great tits have higher breath rates (Charmantier, Demeyrier, Lambrechts, Perret, & Grégoire, 2017; Torné-Noguera, Pagani-Núñez, & Senar, 2014), while urban dark-eyed juncos had lower breath rates (Abolins-Abols, Hope, & Ketterson, 2016). However, another study in great tits found no difference in birds captured at urban and rural sites as breath rate can be affected by numerous seasonal and ecological variables (Senar et al., 2017). Future studies measuring breath rate in a variety of contexts, and pairing this information with both behavioral data and blood corticosterone levels, could lead to a better understanding of why rural birds in our study exhibited behavioral differences but no difference breath rate in response to humans.

5. Conclusions

Urban areas continue to grow at rapid rates. While cities are thought to be a safe haven from predators, studies have produced conflicting results about how animals perceive humans as threats. Although some studies have shown urban-rural differences in avian behavioral responses to humans, to our knowledge few studies have considered if such responses should be similar to or different from those when exposed to native predators or to benign heterospecific bird species. Here we showed that house finches display behavioral plasticity in response to presence of different vertebrates, such that differences in urban-rural responses to an approaching human did not also occur during native predator flyovers. Thus, when designing studies to test wildlife behavioral response to humans, it is important to also understand species responses to stimuli from both human-impacted and natural environments. Moreover, the fact that a bird species responded differently to humans versus aerial vertebrates as a function of urbanization indicates that human presence could indeed be an important reason behind why certain species avoid and are excluded from urban environments.

Ethics statement

This work was approved by the Arizona State University Institutional Animal Care and Use Committee (protocol 12-1234R), the Arizona Game and Fish Department (collecting permit # SP654951), U.S. Fish and Wildlife Service (collecting permit MB088806), and the Federal Bird Banding Lab (permit 23862). This work was funded by Arizona State University, Animal Behavior Society, Sigma Xi, and the Central Arizona Phoenix Long Term Ecological Research Project.

Conflict of interest

There were no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.landurbplan.2018.07.001.

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APPENDIX C

MAP OF FIELD SITES

Map of six capture sites, which were labeled urban, suburban, or rural based on human population density within 1 km of site as well as on land-use/land-cover metrics. A = Estrella Mountain (rural); B = Phoenix neighborhood (urban); C = ASU campus (urban); D = South Mountain (rural); E = Chandler neighborhood (suburban); F = Gilbert Crossroads Park (suburban). Map made on Google maps.

APPENDIX D

PERMISSION FOR INCLUSION OF PUBLISHED WORKS

All co-authors have granted permission for published work to be included.