

University of Nevada, Reno

**Measuring Noise-Induced Stress in Rural and Urban Songbirds**

A thesis submitted in partial fulfillment  
of the requirements for the degree of

Bachelor of Science in Biology and the Honors Program

by

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## Abstract

With landscapes becoming increasingly urban worldwide, it is important for wildlife conservation efforts to monitor the effects that such urbanization has on wildlife and figure out why some individuals become urban adapters while others are displaced. Anthropogenic disturbances, such as traffic noise, can cause an increase in stress in vertebrates and could be the cause of displacement for many individuals. However, it is unclear if individuals accustomed to urban noise have a different physiological makeup compared to their rural counterparts. We examined baseline and stress-induced corticosterone levels in House Wrens, *Troglodytes aedon*, before and after an experimental noise treatment using a repeated measures design. We found that before treatment, the urban birds had higher baseline corticosterone levels in comparison to their rural conspecifics. Rural birds that had experimental traffic noise played to them significantly increased their baseline corticosterone levels as compared to rural birds in the control group. Urban birds in both the control and noise experimental groups had corticosterone levels that did not differ pre- and post-treatment. These results show that the stress response mechanisms between urban and rural House Wrens differ, possibly due to the urban birds habituating to the anthropogenic disturbances in their environment.

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## Chapter 1: Introduction

Urbanization refers to the physical growth of urban areas, and defines regions of the world where both human population and built structure densities are high (Pickett et al. 2011). Roughly 66% of land cover will be considered urban by 2050, as compared to 54% three years ago, and 30% sixty-five years ago (United Nations 2014). This increase in urbanization not only affects the growing cities, but also affects the ecology of the declining rural areas. In some cases, as an environment becomes increasingly urban, the diversity and abundance of species decreases (McKinney 2002). The relationship between urbanization and wildlife populations raises concerns for overall wildlife conservation. Understanding if, how, and at what rate species adapt to the disturbances of new urban environments is vital to both wildlife conservation and evolutionary ecology.

Anthropogenic disturbance in terms of changing shelter, food, light and noise throughout a habitat can affect particular organisms. Recently, in the field of urban ecology, there has been a focus on the challenges urban noise presents to wildlife (Ware et al. 2015, Barber et al. 2010). Specifically, studies on avian species report that anthropogenic noise such as traffic noise is challenging for anti-predator behavior (Meillere et al. 2015), reproduction (Halfwerk et al. 2010), alarm calls (Templeton et al. 2016, Halfwerk et al. 2009) and overall habitat degradation (Ware et al. 2015). While determining these challenges is important in the understanding of the impact of urbanization on wildlife, the next step is to pinpoint the physiological responses to these challenges. There is a growing body of literature focused on how the challenges presented by urbanization create stress for wildlife. Many studies have focused on the



effects of urbanization on physiological stress responses in certain species (Fokidis et al. 2011, Fokidis et al. 2009, Partecke et al. 2006).

When organisms are faced with novel experiences or challenges, they respond physiologically via stress responses mediated by the endocrine system. Several studies have already shown differences in endocrine function and response when a variety of vertebrates are observed in urban versus nonurban environments (e.g., Bonier 2012, Fokidis et al. 2009, French et al. 2008). However, the direction of the response may vary by sex, year and species. Avian species are commonly used in studies measuring the effect of urbanization on stress responses because of the information already available regarding their endocrine systems, as well as their presence in urban ecology (Bonier 2012, Hau et al. 2016).

To study stress in birds, one must examine the activation levels of the bird's hypothalamic pituitary adrenal (HPA) axis, because the HPA axis responds to external stressors in the environment. The HPA axis is responsible for the release of steroid hormones at different concentrations, depending on the level of activation. One class of the steroid hormones called glucocorticoids (GCs) has elevated concentrations when organisms are under stress-induced conditions (Hau et al. 2016). Specifically, corticosterone, hereafter referred to as cort, is the main GC in birds. Circulating cort concentrations in the blood are recorded in order to quantitatively measure stress, with typically higher cort concentrations being recorded when a bird is stressed (Hau et al. 2016). Studies have simulated stressful conditions in order to record and compare a bird's baseline cort and stress-induced cort, with results showing that rural birds showed more variability in stress responses than urban birds

(Fokidis et al. 2009, Fokidis et al. 2011, Partecke et al. 2006). For example, urban birds show more consistent stress responses, while rural birds have stress responses that are more susceptible to change. The differing stress responses between urban and rural birds suggest that there is some alteration of the HPA axes between the two groups of birds. However, it is also unclear if the differences between rural and urban birds are due to genetics and microevolution or to phenotypic plasticity. A study by Miranda et al. proposes that behavioral differences between rural and urban European blackbirds (*Turdus merula*) are due to microevolutionary changes between populations, since urban and rural born nestlings portrayed different behaviors even after being raised in the same, controlled lab setting (2013). In contrast, hormone repeatability and urbanization gradient studies suggest that behavioral stress responses are plastic traits (Bókony et al. 2012, Ouyang et al. 2011).

Elevated anthropogenic noise in urban environments can cause changes in the overall mechanism of stress responses, because organisms exposed to more noise would in turn experience more episodes of stress. Specifically, traffic noise from roads can increase physiological stress in birds, as reflected in increased cort levels. For example, male sage grouse that remained on breeding grounds where traffic noise was played had higher cort levels than males in control breeding grounds (Blickley et al. 2012). Similarly, another study showed that chicks exposed to traffic noise also responded with elevated cort levels (Crino et al. 2013). In contrast, a study by Angelier et al. showed that traffic noise did not result in differences in cort levels between urban and rural house sparrow chicks (2016). The variance between studies regarding the relationship between cort levels and traffic noise could be due to other

factors such as species-specific traits, life-history stage, age, and year—all of which also influence HPA axis activation (Crespi et al. 2012, Foltz et al. 2015, Johnstone et al. 2012). Furthermore, these studies only experimentally tested noise in one environment, whereas response to noise may be context-dependent. For example, stress responses of an individual may be dependent on the individual's past and current exposure to noise, which would differ depending on what type of environment they live in.

Overall, the variance among studies shows that there is still much disagreement in the field of urban ecology about the relationship between cort levels and stress responses. We currently need studies that compare stress responses for individuals of the same species inhabiting different environments, since previous studies focused on only one study site (Blickley et al. 2012, Crino et al. 2013, Goutte et al. 2010). No studies to date have focused on the relationship between stress responses and traffic noise in both rural and urban adult birds of the same species in an experimental design that focuses on two separate sites.

In order to determine a relationship between traffic noise and stress responses, we asked whether birds from different environments would have differing stress responses when exposed to experimental traffic noise. To answer this question, we measured how urban and rural House Wrens, *Troglodytes aedon*, physiologically responded to traffic noise disturbance, by comparing baseline and stress-induced cort levels from pre-noise treatment to post-noise treatment. We used two separate experimental sites, one rural and one urban, and proposed a different prediction for each site. We expected that the relationships between traffic noise and each

experimental group (urban and rural) would differ, as reflected in cort levels. Based on previous evidence, we expected that initial stress responses (measured by cort levels) recorded before the traffic noise treatment would be different between rural and urban birds, with urban birds showing elevated baseline and stress-induced cort levels. Secondly, we expected that after the experimental treatment of traffic noise playback, both baseline and stress-induced cort levels of urban birds would remain the same as the initial levels, whereas the rural birds would have elevated baseline and stress-induced cort levels when compared to their initial responses. We used a within and among-individual experimental setup to disentangle the mechanism behind adaptation to urban environment.

## **Chapter 2: Literature Review**

### **2.1 Urbanization**

Urbanization is referred to as human-induced changes of the earth, and is increasing globally (Ellis 2011). Urban landscapes have high built structure and human population densities, and are steadily increasing across the world (Pickett et al. 2011, United Nations 2014). The increase in urbanization changes the natural environment by introduction of pollution in forms such as material, light and noise. The introduction of anthropogenic pollution in previously undisturbed environments poses challenges for wildlife. Material pollution from construction and industrial waste results in wildlife habitat degradation from marine environments to montane forests (Cheevaporn et al. 2003, O’Dea et al. 2007). Anthropogenic light pollution from buildings, signs, and streetlights has been shown to negatively alter wildlife behavior and interactions, as many species rely on natural light cues and patterns for biological responses throughout a

lifetime (Longcore et al. 2006). For example, natural light is essential in birds' comprehension of time and seasons for migratory purposes. Therefore, artificial lighting from anthropogenic sources could disrupt the birds' natural timing cues and migratory behavior. Lastly, anthropogenic noise pollution has also shown to have negative effects on a variety of wildlife, primarily by causing stress, and has been an increasingly popular area of study (Francis et al. 2015, Luo et al. 2015, Meillere et al. 2015, Templeton et al. 2016). Specifically in avian species, noise pollution can disrupt communication between individuals, as birds struggle with adjusting the frequency of their acoustic signals to be heard over the noise (Templeton et al. 2016, Slabbekoorn et al. 2012). One type of anthropogenic noise that research has focused on recently is traffic noise, since the presence of traffic is typically abundant in urban areas. However, results among studies regarding the effects of traffic noise on wildlife are variable. As urbanization is predicted to continually rise in the near future (United Nations 2014), an increase in traffic noise is sure to follow. Therefore, it is important to further investigate the effects of traffic noise on birds, because understanding the relationships between urbanization and wildlife will improve the conservation and management of such organisms (Gianotti et. al 2016).

## **2.2 Quantifying Urbanization**

In order to measure effects of urbanization within an experiment, researchers must quantify urbanization of particular areas by establishing rural-urban gradients. The gradients take into account a combination of factors, such as physical densities of the following in a particular area: vegetation, buildings, roads and human population (Pickett et al. 2011). There is the need for a means to quantify urbanization by gradients in order to compare different sites within a single study, and, on a larger scale, compare different

studies across the field of research. However, there are currently a variety of methods used to create rural-urban gradients, which creates inconsistency among studies in regards to quantifying urbanization (Bókony et al. 2012, Gianotti et. al 2016, Seress et al. 2014, Zhang et al. 2011). Without a standardized system of urbanization quantification, the power of comparability between studies is poor, and further understandings of urban ecology, wildlife management, and wildlife conservation cannot be successfully reached (Gianotti et. al 2016).

Geoinformatics systems software (GIS) is a widely accepted tool to score urbanization and has been used in a similar urban ecology study examining urban and rural birds (Crino et al. 2013); however, GIS is not cost or time effective for our project. Manual urbanization scoring by individual observers in the field is also an option for scoring urbanization, as it has been used in similar avian studies in rural and urban environments (Bókony et al. 2012, Zhang et al. 2011). However, manual scoring is very time consuming as well. Alternatively, a newer, semi-automated software was developed to be cost and time effective, and readily available to download online (Seress et al. 2014). The software scores areas based on the densities of buildings, roads and vegetation in a 1 km x 1 km image from Google Maps. This method of urbanization scoring was shown to provide reliable results when compared to the more expensive GIS software, as well as the manual approach (Seress et al. 2014). This software will provide consistency within our research in order to be compared to research by others.

### **2.3 Corticosterone**

Glucocorticoids (GCs) are steroid hormones in the endocrine systems of vertebrates that are produced by the hypothalamic pituitary adrenal (HPA) axis. GCs are important

in maintaining an organism's responses to both predictable and unpredictable biotic and abiotic environmental factors (Hau et al. 2016). Corticosterone (cort) is the main GC in birds, reptiles and fish. In avian studies, cort is most commonly measured by circulating concentrations in blood (Angelier et al. 2016, Crino et al. 2013, Fokidis et al. 2011, Goutte et al. 2010, Johnstone et al. 2012). Cort concentrations are measured at baseline levels and stress-induced levels, and the recorded concentrations are then used to reflect the level of HPA axis activation.

Baseline cort levels represent activation of the HPA axis simply due to background stressors (Johnstone et al. 2012). Variation in baseline levels can be found among individuals depending on life-history events, such as during reproduction cycles and migration periods (Crespi et al. 2012), as well as during periods of increased workload, thermoregulation and immune responses (Hau et al. 2016). Baseline levels often reflect metabolic rates with higher levels, indicating increased energy expenditure, e.g., breeding, as opposed to other life history stages (Romero 2002). Therefore, life-history stages and assessment of previously mentioned factors of individuals are considered when researchers choose a population to study.

Stress-induced levels of cort represent the level of HPA axis activation when individuals are exposed to unpredictable changes or challenges in the environment. Cort concentrations are typically lower at baseline conditions, and higher at stress-induced conditions (Hau et al. 2016). In birds, elevation of cort concentrations begins to occur within three minutes after exposure to a challenge or unpredictable experience, with concentrations peaking between fifteen minutes to an hour (Hau et al. 2016). Therefore, blood collection should be completed during the appropriate period of time in order to

accurately record the stress-induced cort concentrations. The difference between baseline cort concentrations and the elevated stress-induced cort concentrations is a phenotypic endocrine trait used to determine stress in before mentioned vertebrates.

Furthermore, researchers determined that baseline cort and stress-induced cort concentrations are phenotypically plastic traits (physical traits that change based on the environment), but plasticity among species and even individuals may be variable (Ouyang et al. 2011). Stronger, more specific data can be recorded when studies focus on stress responses in within-individual paradigms, as well as within species-specific populations. The purpose of a within-individual paradigm is to show the variance in responses, measured by cort concentrations, of individuals over a period of time, which will reflect the flexibility of each individual's phenotype.

#### **2.4 HPA Axis**

The hypothalamic pituitary adrenal (HPA) axis is the central stress response system in vertebrates. Activation levels of the HPA axis are used to measure physiological stress. The HPA releases steroid hormones called glucocorticoids (GCs), such as the stress hormone cort, into the bloodstream in response to stress (Johnstone et al. 2012). In order for cort to be released into the bloodstream, the HPA axis has to release a series of hormones (Figure 1). First, in the presence of a stressor, the hypothalamus is stimulated by the central nervous system and releases the corticotropin releasing hormone (CRH). Next, CRH signals the pituitary gland to release the adrenocorticotropic hormone (ACTH). Finally, ACTH signals the adrenal gland to release corticosterone (CORT).



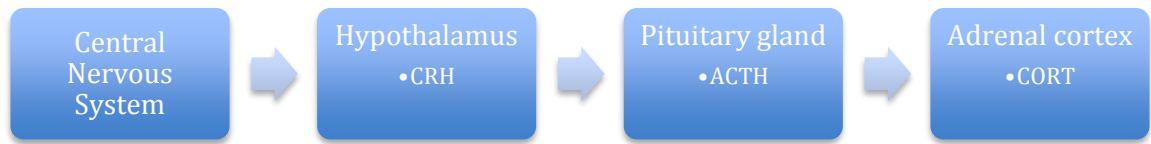


FIGURE 1. The HPA axis. When a vertebrate is exposed to a stressful stimulus, the central nervous system signals the HPA axis to release a series of hormones. The final hormone released is cort, and cort concentrations in a vertebrate's circulating blood are measured to quantitatively define stress.

Quantifying physiological stress is based on measuring the amount of cort released by the HPA axis into the bloodstream. The cort concentrations measured are used as primary data, so it is important that the data collection is precise. Stress hormone levels distributed by the HPA axis can change within three to five minutes of capture, handling, or other unpredictable disturbances, so appropriate timing is vital to accurate data (Johnstone et al. 2012).

It is suggested that vertebrates have adapted to lower their stress responses by the HPA axis down-regulating cort responses after long-term exposure to stressors from urban environments (e.g. French et al. 2008, Partecke et al. 2006). Alterations of the HPA axis were shown in Curve-billed Thrashers (*Toxostoma curvirostre*), and the researchers suggests that the HPA axis is up-regulated so urban birds can more easily adjust to urban stressors (Fokidis et al. 2011). Changes in the HPA axis are supported by evidence of a cort concentration repeatability study, which proposed cort concentrations are plastic traits (Ouyang et al. 2011). Also, Bókony et al. showed that baseline cort concentrations in the Tree Sparrow (*Passer montanus*) are positively correlated with the degree of urbanization of the sparrow's residence (2012). The correlation also shows the plasticity of cort and the HPA axis.

With evidence that changes in the HPA axis do occur, it is then important to determine at what point in a vertebrate's life that physiological changes are occurring in order to further understand the impact of urbanization on individuals. A study on House Sparrow (*Passer domesticus*) chicks showed that an urban stressor (traffic noise) did not change the cort levels of the chicks, suggesting the chicks were not stressed by the noise. Therefore, the researchers concluded that the HPA axis is not affected during chick development (Angelier et al. 2016.) Another study by the same primary researcher reported that in Snow-Petrels (*Pagodroma nive*) there was no correlation between age and stress-induced cort concentrations (Angelier et al. 2007).

Alternatively, there is evidence that in Snow-Petrels (*Pagodroma nive*) stress responses decrease with age, and then begin elevating again with older age (Goutte et al. 2010). Researchers propose that the changes of stress responses with age could be due to individuals reducing their energy input in stress responses as they age in order to focus on reproduction, and then at a certain, older age the HPA axis itself experiences senescence and the stress responses elevate (Wilcoxon et al. 2011). The variance in results between Angelier et al. 2007, Angelier et al. 2016 and Goutte et al. 2010 shows the inconsistencies in the understanding of the mechanism controlling the HPA axis responses.

Besides age, additional factors contribute to the HPA axis responses. For example, multiple studies have shown that HPA axis responses can vary depending on life history stage (Crespi et al. 2012, Fokidis et al. 2009, Fokidis et al. 2011, Partecke et al. 2006). Furthermore, an individual's current conditions, such as workload or time of day and season can influence HPA activity (Hau et al. 2016, Johnstone et al. 2012).

Because the HPA axis responses are not constant throughout an organism's lifetime, controlling for age, life history stages, and individual conditions, ideally by use of a within-individual paradigm, is an important factor to ensure precision, and more specific results in future studies.

## **2.5 Traffic Noise**

Traffic noise is one type of anthropogenic disturbance that causes stress in vertebrates. For species that have not adapted to traffic noise, the disturbances cause stress not only because the noise is novel and unpredictable, but also because the noise interferes with communication and behavior, which is problematic for avoiding predation (Meillère et al. 2015, Templeton et al. 2016). It was shown that traffic noise drowns out great tit (*Parus major*) alarm calls, and the tits are not able to adjust their calls to be heard over the noise (Templeton et al. 2016). Alarm calls are used to warn other birds of danger, so inability to adjust the alarm calls can increase predation. One study theorized that urban birds must adapt to increase both the amplitude and frequency of their songs and calls in order for their calls to be heard better and over longer distances in the presence of anthropogenic noise (Slabbekoorn et al. 2012). Halfwerk et al. showed that the great tit (*Parus major*) adjusted the frequency of their songs, based on surrounding noise (2009). The inability of a bird to adapt to the noise challenges would cause increased stress and an overall decrease in fitness. In addition to communication challenges for birds, rural female house sparrows (*Passer domesticus*), negatively altered their anti-predation behavior in response to traffic noise exposure (Meillère et al. 2015). The inability of alarm call communication between the tits and the alteration of anti-

predator behavior for the sparrow, both due to traffic noise, results in increased predation and stress levels, and, ultimately, lowered fitness for the organism.

While these studies focus on *why* traffic noise stresses birds, it is also important to understand *how* the stress from traffic noise impacts birds physiologically. In order to actually measure stress in vertebrates, researchers monitor circulating cort concentrations in the bloodstream of birds. Cort levels due to stress caused by traffic noise early in a bird's life could alter the development of the individual. One study examined the effect of traffic noise on overall physiological development in mountain white-crowned sparrow nestlings by comparing cort levels in nestlings exposed to traffic noise to control nestlings (Crino et al. 2013). The traffic-noise exposed nestlings showed elevated cort levels in comparison to control nestlings, which suggests that traffic noise does affect the developing young bird. In contrast, a study by Angelier et al. showed that traffic noise did not result in differences in cort levels between urban and rural house sparrow chicks (2016). The researchers concluded that traffic noise does not affect the developing chick, possibly because the chicks are insensitive to urban stressors at a young age (Angelier et al. 2016). The discrepancy between these studies could be due to species-specific physiological responses, which are determined by genetics and/or environment. Therefore, it is important to control for species type when performing similar traffic noise studies.

In order to use traffic noise as a variable in studies on birds and other vertebrates, some studies have used playback of recorded traffic noise at nest sites (Brischoux et al. 2017, Meillere et al. 2015), while another study categorized individuals by how close the birds were to roads (Crino et al. 2013). In order to create the most accurate and controlled

recorded playbacks of traffic noise, experimenters surveyed the areas of study to determine average noise levels (Meillere et al. 2015). In the study where the effects of traffic noise on study individuals were determined by proximity to a road, GPS and GIS data were used to measure the distance of study sites to a highway (Crino et al. 2013). The playback method is appropriate when experimenting in areas where traffic noise is not present, such as in rural areas, while the proximity method is useful when the experimental site is an urban area where traffic noise and roads are already present.

## **2.6 Summary**

Overall, as urban areas are expanding, interactions between wildlife and anthropogenic challenges are growing increasingly inevitable. In order to improve conservation efforts during a rapidly growing urban time, it is important to determine the impacts of urbanization on wildlife, as well as the ways in which they respond. However, to date, the responses of stress levels due to traffic noise across urbanized landscapes are far from conclusive within the literature. There is a lack of control for context-dependent factors in the environment and among individuals, such as life history state and anthropogenic noise exposure, which results in the variation seen among previous study results. In order to improve upon previous studies and obtain clearer conclusions, a within and among-individual paradigm should be used. The within and among-individual paradigm would mitigate variation by controlling for environmental and individual context. The within and among-individual paradigms will show variance of an individual's phenotype over time, which will give more specific insight into the mechanisms behind the phenotypic flexibility, according to the conditions it is in.

In order to determine a relationship between traffic noise and stress response, we asked whether birds from different environments would have differing stress responses when exposed to experimental traffic noise. To answer this question, we measured how urban and rural House Wrens, *Troglodytes aedon*, physiologically responded to traffic noise disturbance. First, we will use a within and among-individual paradigm to improve the understanding of stress levels over an urban-rural gradient, by exposing different populations of House Wrens (*Troglodytes aedon*) to experimental noise, and measuring their physiological stress responses. All data were collected from House Wrens during the same season (post-egg hatch in early summer), in order to control for species and life-history stage. Unlike previous studies, two separate environmental sites (one urban and one rural) were included, in order to compare responses among individuals across environments within one study. To control for context-dependent responses, based on different levels of noise exposure, all study individuals at both environmental sites experienced experimental noise exposure (traffic noise or control noise). This method also singles out traffic noise in particular as being the source of the stressor rather than noise in general. By using within and among individual paradigm to control for species, life-history stage, noise exposure levels, and separate sites, this study helped to disentangle the mechanisms behind stress responses to urban traffic noise with less error from variation than previous studies have, and is important to the growth of the field of urban ecology, as it will fill the gaps present in current literature.

## Chapter 3: Methodology

### 3.1 Species

We studied the House Wren, *Troglodytes aedon*, for this experiment. House Wrens are cavity nesters that prefer nest boxes or tree holes. They collect twigs to pile into their nest cavity, and then make a soft-lined cup in which they lay their eggs. Incubation lasts between 9 and 12 days, followed by the nestling period for around 15 to 17 days, with variation due to delayed incubation in some females. Breeding season runs from late spring through summer, and they can be found in most of North America (Cornell University 2015).

### 3.2 Urbanization Scores

The study included House Wren data from two separate sites, one urban (Idlewild Park) and one rural (Main Station Farm) in Reno, NV. In order to differentiate between urban and rural characteristics, each site was given an urbanization score by a semi-automated software. The software measures the urban-rural gradient based on evaluation of mean densities of buildings, roads and vegetation present in Google Maps images of the areas (Seress et al. 2014). The software captures a 1km x 1km image from Google Maps of each study area; in this study, one image for the rural location and one image for the urban location was used (Figure 2). Each image is divided into 100 squares, and each square is evaluated for the relative densities of buildings, paved roads and vegetation. Squares with very high densities of vegetation or buildings are denoted with the number 2. Squares with any paved roads present are denoted with the number 1. The software combines the data from the grids of each location and generates data for each location, which is used to ensure that the urban and rural sites are different in their compositions of

buildings, roads and vegetation (Table 1). The differences in building, vegetation and paved road densities distinguished the urban site (Idlewild Park) from the rural site (Main Station Farm).



FIGURE 2. Images of study sites. **A.** The 1km x 1km image of the urban site, Idlewild Park, from Google Maps that was used in the urbanization scoring software. **B.** The 1km x 1km image of the rural site, Main Station Farm, that was used in the urbanization scoring software.

TABLE 1. Urbanization index data. Data from the urbanization gradient software to compare the urban and rural sites by the densities of buildings, vegetation and paved roads. The numbers in the first three columns reflect the total number of individual squares in the 1 km x 1 km image where building density and vegetation was very high (denoted by “2”) and where there was a presence of paved roads (denoted by “1”).

	building density= 2	vegetation density= 2	paved roads= 1	average building	average vegetation
Main Station Farm (rural)	18	79	40	0.55	1.72
Idlewild Park (urban)	63	35	88	1.5	1.24



### 3.3 Locations and Experimental Setup

The urban location, Idlewild Park, is a local park in a suburban neighborhood in Northwest Reno, Nevada. The neighborhood park has residents visiting and cars passing throughout the day. The rural location is at the University of Nevada, Reno Main Station Farm in East Reno, Nevada. The farm has very little disturbance from humans and cars, as there is only one road accessed by the employees on the entire 900 acres. Wooden nest boxes were constructed (24cm x 10cm x 14cm) and attached to trees by two nails, one at the top of the box and one at the base of the box (Figure 3). The boxes were placed about 1.5m from the ground and at least 15m from one another, at both locations. In total, 150 nest boxes were set up at each site in order for the birds to choose from. Each nest box was designated a number for identification, in order to record which birds belonged to which box.



FIGURE 3. House Wren nest box. This is a photo of one of the 150 nest boxes that were set up at both the rural and urban sites. The boxes were nailed to trees 1.5m off the ground and were designated an identification number (see on right side of box).

### 3.4 Procedure

We caught adult House Wrens at the urban and rural sites from May 23, 2016 through July 4, 2016, between the hours of 8:00am and 11:00am, with an automatic spring trap on day 9 and day 11 of chick rearing (days 1 and 3 of the experiment). Within three minutes of capture we took the adults' blood from the brachial vein (<100 $\mu$ l) for baseline cort. The birds were held in an opaque bag for 30 minutes, and then we bled them again for stress-induced cort (Figure 4). On day 10 of chick rearing (day 2 of the experiment), experimental or control noise was played from 0800 to 1700 from an Arespark AS200 Speaker, which we placed 5 m in front of the nest box (Figure 5). An Audacity 2.0 (Carnegie Mellon Computer Music Group, Pittsburgh, PA, USA) was used to make a traffic noise tape by combining traffic noise from previously recorded clips of traffic noise in downtown Reno. A total of 200 sound clips from cars, trucks, ambulances, and sirens were recorded using a Sennheiser ME 67 shotgun microphone (Wedemark, Germany). For the control noise, we used a standard white noise tape. We normalized traffic and control noise to 0dB using Audacity, and calibrated both urban and control noise to have an amplitude of 65dB at 5m. We measured all sounds with SEW 2310 SL digital sound meter. All blood samples (N=116, 4 samples per individual bird) were stored on ice until taken back to the lab to be centrifuged. In the lab, we centrifuged each sample and the separated plasma was stored in a freezer at -80 °C until the hormone assays were run.

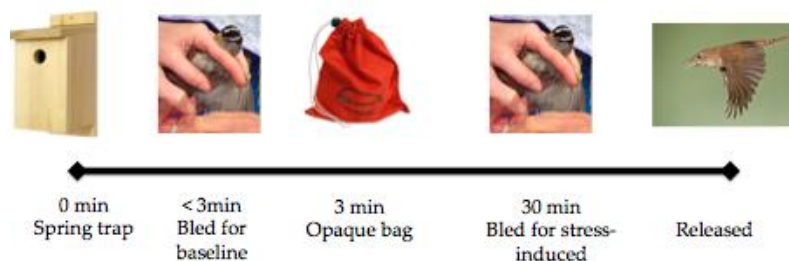


FIGURE 4. Capture and bleeding timeline. The timeline for experimental days 1 and 3 (equivalent to chick rearing days 9 and 11) where the House Wrens were captured and bled for pre-treatment and post-treatment data collection.

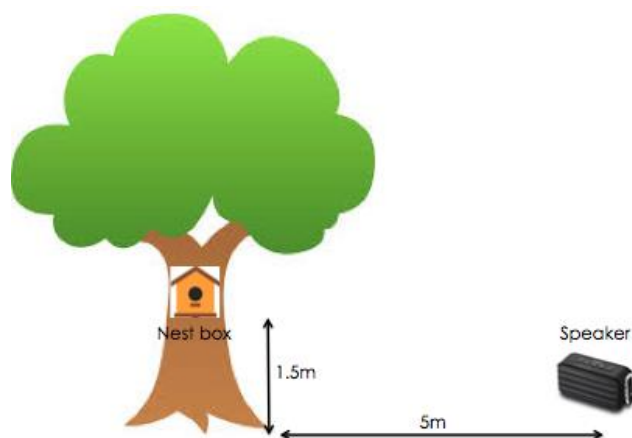


FIGURE 5. Experimental noise playback setup. On day 2 of the experiment (equivalent to day 2 of chick rearing) a speaker was placed 5 m in front of the nest box. The speaker played either traffic noise or control noise for nine hours during the day.

### 3.5 Hormone Analysis

To determine the cort levels in the House Wren plasma samples, we used an Enzo Life Sciences competitive enzyme-linked immunosorbent assay (ELISA) kit in the lab. To measure the cort in the plasma samples, 3.75 $\mu$ l of each sample was added to a single well and diluted 40x using the assay buffer, which contained the steroid displacement reagent (SDR) at 0.5% of the volume of the plasma samples. The samples were run in singles and randomly assigned across and within two microplates; however, the paired 0 minute and 30 minute samples from a single day for each individual were included on the

same plate. A standard curve was run on each plate, which ranged from 32 pg/ml-20,000 pg/ml. A cort antibody and a cort conjugate were added to each well and the plates were then placed on the plate shaker for 2 hours. After the plates were shaken, the wells were washed 3x with 400 $\mu$ l of wash solution. Next, 200 $\mu$ l of an enzyme substrate (p-nitrophenyl phosphate) was added to each well, then the plates were covered and incubated in a dark space for 1 hour. At the hour, 50 $\mu$ l of tri-sodium phosphate was added to each well to stop the enzymatic reaction. The plates were immediately read in a Fisher Scientific accuSkan FC microplate reader at 405nm. The assay sensitivity was 2.1pg/ml, with an intra-plate (2 plates) coefficient of variation of 10.3% and inter-plate coefficient of variation of 3.3%.

### **3.6 Statistical Analysis**

We performed statistical tests with R environment (version 2.12.2), to determine significance among the samples of cort concentrations. A within-individual paradigm was used to compare changes in cort levels within each individual. Linear mixed models (LMM) were run to test if baseline and stress-induced cort concentrations were changed by the interaction of experimental treatment (noise vs. control) and site (rural vs. urban), while controlling for both sex and body condition. Body condition was measured as the residuals from a regression of mass and tarsus Nest box identification number was included as a random effect in order to control for any common environmental effects, or interactions between a male and female pair at a particular nest box. To test whether or not baseline cort and stress-induced cort levels were different by experimental treatment and for an interaction between treatment and site, the Kenward-Roger approximation was used. All model assumptions were met and significance was taken at  $\alpha=0.05$ .

### 3.7 Ethical Note

Procedures were approved by all federal and state permit agencies, as well as the Institutional Animal Care and Use Committee (IACUC) of the University of Nevada, Reno (protocol #00677).

## Chapter 4: Results

Before experimental noise treatment, the baseline cort concentrations between the rural site and urban site were significantly different, with urban birds showing a higher baseline cort than rural birds ( $p < 0.0001$ , Figure 6, Table 2). In contrast, the stress induced cort concentrations between the rural site and the urban site were not significant before treatment (coef= -0.175, s.e= 4.31,  $t = -0.407$ ,  $p = 0.968$ ). Rural birds exposed to experimental traffic noise had a significantly larger increase in baseline levels from pre-treatment to post-treatment when compared to their rural counterparts exposed to control noise, and also the urban birds ( $p = 0.020$ , Figure 7, Table 2). The significant increase in baseline level from pre-treatment to post-treatment in rural birds shows an interaction between site and treatment for baseline levels. However, there was no significant interaction between site and treatment for stress-induced levels ( $p = 0.338$ , Table 3).

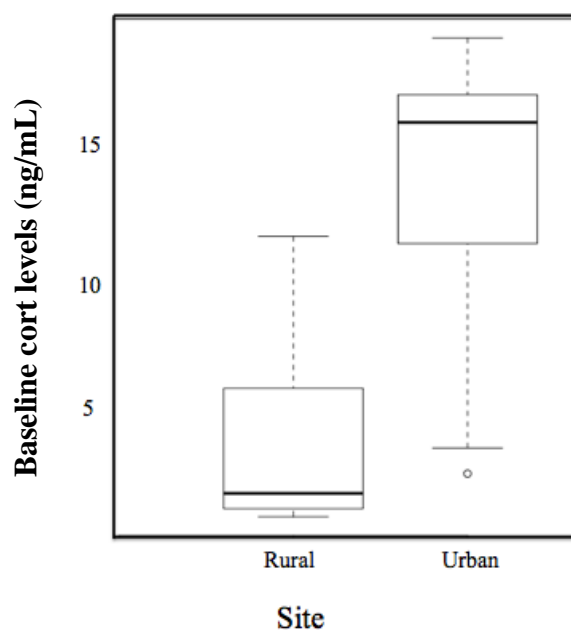


FIGURE 6. Boxplots for the initial baseline corticosterone levels of House Wrens at the rural and urban sites. Urban birds show higher mean baseline corticosterone levels than rural birds before the noise treatment. Shown are means, 25% and 75%, and outliers.

TABLE 2. Model estimates to test the effect of site on initial baseline corticosterone concentrations. Site was significant in determining baseline corticosterone levels ( $p < 0.0001$ ).

variable	estimate	s.e.	<i>t</i>	<i>p</i>
site				
urban vs. rural	9.49	1.80	5.29	$p < 0.0001$
body condition	0.61	0.80	0.77	0.443
sex (male vs. female)	0.63	1.04	0.60	0.545

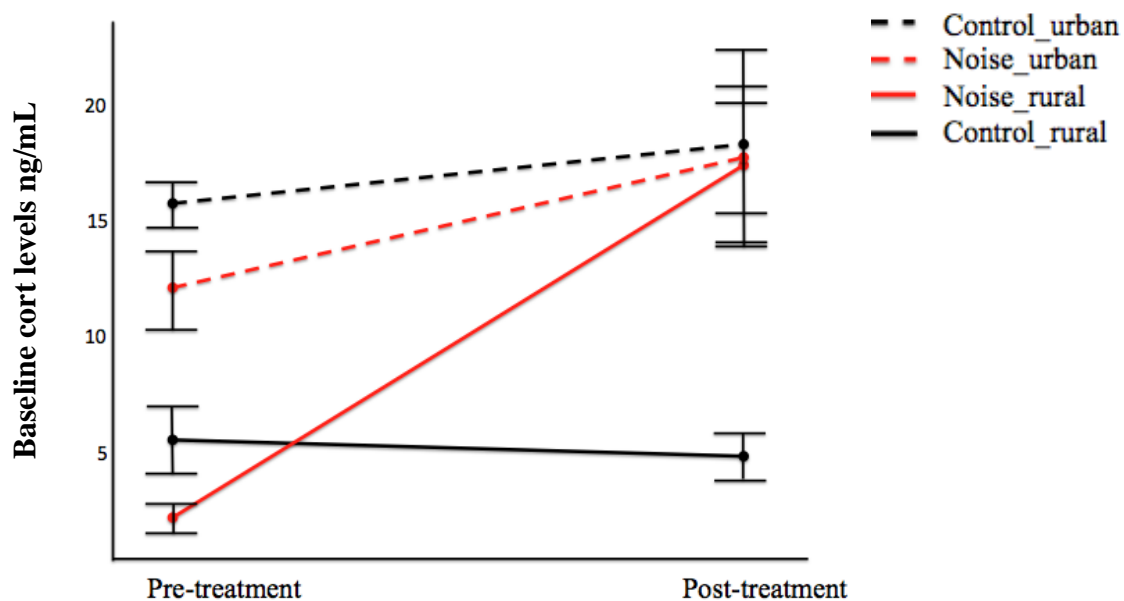


FIGURE 7. Concentrations of baseline corticosterone levels of the House Wrens before and after experimental noise treatment at the urban and rural sites. Rural birds exposed to traffic noise showed a significant increase in baseline corticosterone from pre-treatment to post-treatment, as represented by the red solid line. Shown are within individual changes and mean  $\pm$  1SE.

TABLE 3. Model estimates to test the effect of site and treatment on baseline corticosterone changes. The interaction between site and treatment was significant in the changing baseline corticosterone levels ( $p=0.0004$ ). The  $p$ -values for treatment and interaction were calculated according the Kenward-Roger approximation.

variable	d.f.	$F$	estimate	s.e.	$t$	$p$
treatment						
noise vs. control			15.90	4.49	3.54	0.0004
site : treatment	3, 13.7	4.57				0.020
urban vs. rural			1.86	4.55	0.41	0.683
body condition			0.87	1.80	0.48	0.630
sex (male vs. female)			-0.97	2.57	-0.38	0.706

TABLE 4. Model estimates to test the effect of site and treatment on stress-induced corticosterone changes. There was no significant interaction between site and treatment on stress-induced corticosterone changes ( $p=0.338$ ). The  $p$ -values for treatment and interaction were calculated according the Kenward-Roger approximation.

variable	d.f.	$F$	estimate	s.e.	$t$	$p$
treatment						
noise vs. control			4.87	3.60	1.35	0.175
site : treatment	3, 11.7	1.24				0.338
urban vs. rural			-2.44	3.81	-0.64	0.521
body condition			-0.99	1.69	-0.59	0.556
sex (male vs. female)			-2.23	2.53	-0.88	0.378

## Chapter 5: Discussion of Data

This study showed that the increase of traffic noise pollution due to urbanization creates stress for the House Wren, and can potentially change the mechanism in which wrens respond to stress. We found that urban birds have elevated baseline levels when compared to rural birds before noise treatment, and that baseline levels of the rural birds were elevated after noise treatment as compared to pre-treatment levels whereas urban birds' stress response remained the same. We also show that there were no significant differences in stress-induced cort levels with respect to treatment and site.

### 5.1 Differences in Baseline Corticosterone Levels

The differences in initial baseline levels of the rural and urban House Wrens, seen in Figure 6, show that the birds from the two sites differed physiologically before the experimental treatment. The initial difference in baseline cort levels between the two groups of birds suggests that the urban birds have already habituated to the disturbances



in their environment by maintaining elevated baseline cort levels in comparison to rural birds ( $p < 0.0001$ , Figure 6, Table 1). The adjustment of the baseline cort levels by the HPA axis of the urban birds shows some sort of adaptive difference between the urban and rural birds. The physiological adjustment would be due to higher volumes of traffic noise, as only the rural birds showed a significant elevation after experimental treatment, suggesting that urban birds may have already habituated to traffic noise ( $p = 0.020$ , Figure 7, Table 2).

Also, in our experiment, the treatment alone (traffic noise vs. control noise) was significant in baseline cort differences, regardless of site ( $p = 0.0004$ , Table 1). The significance of the treatment alone suggests that, in general, the presence of traffic noise provoked more stress in the birds, which is similar to previous findings reporting that traffic noise is fact a stressful disturbance for a variety of reasons (Halfwerk et al. 2010, Halfwerk et al. 2009, Meillere et al. 2015, Templeton et al. 2016, Ware et al. 2015).

When birds and other vertebrates are exposed to the same stimulus repeatedly, the responsiveness of the HPA axis can decrease (Fokidis et al. 2009, French et al. 2008, Partecke et al. 2006). Decreased responsiveness of the HPA axis would explain why the urban House Wrens, who have been exposed to traffic noise daily, did not have a significant change in baseline cort levels when compared to their rural conspecifics under the experimental traffic-noise treatment in our experiment (Figure 7), and also why the initial baseline levels between the two groups were different (Figure 6).

Alteration of responsiveness of the HPA axis can also be the result of reproduction, seasonality, or other life history stages (Boonstra 2004, Romero et al. 2008, Schoech et al. 2007). For example, one study showed that birds decreased their stress

responses to the harsh environment in order to expend the majority of their energy on reproduction instead of stress responses (Silverin et al. 1998). However, the data from our experiment were collected from birds during the same season and same life history stage, which suggests that the differences in baseline cort levels could in fact be due to the desensitization of the HPA axis to a chronic urban stressor.

An alternative explanation to the baseline cort differences between the urban and rural conspecifics in this study is resource availability. Research has been shown that birds with better food resources have the ability for higher stress response, reflected in elevated cort, since they have the energy to devote to physiologically responding to stress (Schoech et al. 2007, Schoech et al. 2004). In the study by Schoech et al., researchers supplemented a group of wild birds with additional food and found that the addition of supplements increased the birds' cort levels when compared to the group of wild birds that were not given food supplements (2004). Urban areas may have more opportunities for birds to obtain food from trash, birdfeeders, etc., which would result in elevated cort levels of an urban population. However, one study by Fokidis et al. showed that rural birds had elevated cort levels because the dairy farm they were living on provided abundant food resources (2008). The latter study suggests that the baseline cort differences between the urban and rural birds in our study are not due to stress desensitization of the urban birds, but to possible increased food availability of the rural birds. The rural site in our study is an agricultural farm where seeds and crops are much more abundant than at the urban site. For future studies, it would be beneficial to look at food availability and abundance between study sites.

## 5.2 Stress-Induced Corticosterone Levels

While baseline cort levels showed significant changes before and after treatment, the stress-induced levels did not (Table 4). The lack of significance in changes of stress-induced cort from pre-treatment to post-treatment could simply be a species-specific trait. However, we cannot conclude that unchanging stress-induced responses is a species-specific trait in House Wrens, because no previous studies have tested House Wrens. Therefore, we cannot yet compare our results to other studies. Additional studies on House Wrens are necessary in order to either support our findings of stress-induced cort responses, or oppose our findings.

Alternatively, unchanging stress-induced cort levels of the wrens could be due to the fact that the human handling of the birds before the stress-induced measurements was novel to both the urban and rural birds. That is to say, while urban birds may have habituated to the stress of traffic noise, it is likely that they have not been habituated to human handling since they are free-living birds. Previous studies have shown that handling results in stress responses in birds, which negatively effects both physiology and behavior (Balcombe et al. 2004). A study by Torné-Noguera et al. compared stress responses due to handling, measured by breath rate, in both urban and forest birds, with urban birds showing a higher stress response (2014). The study suggests that the higher stress response induced by handling of the urban birds could be due to urban wildlife having a higher sensitivity to stressors (Torné-Noguera et al. 2014). While that idea opposes the results of the baseline cort data collected in our experiment (which showed that urban birds may actually have down regulated stress responses), it is still important

to consider the stress that handling provokes and how it may have influenced our stress-induced cort data.

Another possible factor that could be influencing the unclear relationship between stress-induced cort levels in urban and rural birds is differences in personalities, still with respect to handling stress. A study on song sparrows has shown that stress responses to handling in urban and rural birds could potentially be due to individual personality differences (Evans et al. 2010). Specifically, studies have shown that in some avian species birds that have “slower” (less assertive and more shy) behavioral traits also have higher cort responses (Cockrem 2007, Stowe et al. 2010). Therefore, future studies could find clearer results if personality is considered and controlled for.

### **Chapter 6: Conclusion**

The increase of noise pollution due to urbanization can create stress for wildlife that inhabit urban areas, and can potentially provoke change in the mechanism in which wildlife respond to stress. Therefore, we asked whether House Wrens from two separate sites in Reno, NV, one urban site and one rural site, would have different stress responses in the presence of traffic noise. First, before the experimental noise treatments we expected the urban birds to have elevated baseline and stress-induced cort levels when compared to rural birds. Additionally, we expected that after the experimental noise treatment the baseline and stress-induced cort levels of the urban birds would match the initial levels, while the baseline and stress-induced cort levels of the rural birds would be elevated in comparison to initial levels.

This study showed that urban and rural wrens do have different physiological responses to stress, and that the environment (urban vs. rural) is an important predicting

factor of the differences. Specifically, wrens are experiencing adaptations or plasticity of the HPA axis in response to an increasingly urban landscape. We can conclude that urban birds are adapting to the urban environment because we found that the initial baseline cort concentrations were significantly different from the rural birds, but also because only the rural birds had elevated stress responses after being exposed to experimental traffic noise. However, further understanding of the physiological changes of the wrens' HPA axis due to environmental changes is necessary, as our study is the only in the field to study House Wrens.

While the role of the environment is a large part of this research, it is also important to increase the understanding of other factors that are influencing the behavioral differences between urban and rural birds. Factors such as food resources and abundance, handling protocol, and personality differences would be important to include and control for in similar, future studies.

Overall, additional research in the field of urban ecology is needed to clarify the relationship between the effect of urbanization on wrens, as well as other avian and wildlife species. Understanding if aspects of urbanization, like traffic noise, provokes stress in other species, and if those species can physiologically adapt as the House Wrens have shown to do, is important for the overall preservation and conservation practices of wildlife. Determining which species are unable to adapt or are less efficient at adapting to an increasingly urban environment can then be focused on in conservation efforts at local, national, and international levels.

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