City noise as a potential stressor on the urban bird: an analysis of

heterophil/lymphocyte ratios in European Starling nestlings

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ABSTRACT

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Anthropogenic disturbances are known to induce stress in a variety of urban
species. This study aims to determine if one particular disturbance, ambient urban
noise, influences the stress levels of nestling European Starlings (*Sturnus vulgaris*).
Nest boxes were randomly assigned to either an experimental (increased urban
noise level) or a control treatment (normal ambient noise). Heterophil/lymphocyte
(H/L) ratios were determine and used to estimate stress levels of nestlings at 11 and
15 days of age (day 0 being day of hatch). Higher H/L ratios are associated with
increased stress levels. I predicted that nestlings in the experimental treatment
would have higher H/L ratios than those in the control group. I also predicted that
habituation or sensitization to the noise would occur; therefore nestlings would
have either lower or higher H/L ratios on day 15 than on day 11. No significant
difference in H/L ratios was detected between the two treatments at day 11.
However, at day 15, control nestlings had significantly higher H/L ratios than did
experimental nestlings. Experimental nestlings tended to have a lower H/L ratio at
day 15 than at day 11, but no such pattern was detected in control nestlings. These
findings suggest that, contrary to my prediction, constant loud noise may not be as
important to inducing stress in urban-thriving European starling nestlings as was
previously thought. A future avenue of research would be to examine how the
unpredictability of noise affects H/L ratios in this species.

ACKNOLEDGEMENTS

INTRODUCTION

Urban populations of wild animals are becoming increasingly common due to the continuous expansion of human populations into formerly wild areas. Venter (2006) showed that 44% of natural habitat loss was due to urbanization. Urban environments pose unique challenges to animals compared to natural environments. The altered landscape associated with urban areas causes immediately obvious effects such as habitat loss. However, for those species that are able to survive in the urban environment, the presence of humans and their associated activities continue to cause strain on the species. For example, due to the majority of human activity taking place during the day, some species are forced to become nocturnal to reduce their interaction with people (Ditchkoff, 2006). Something that may seem initially advantageous to urban adaptors is the reduced abundance of natural predators. However, human pets and vehicles are uniquely urban threats that cause mortality (Ditchkoff, 2006; Forman, 1998). An increased rate of disease transmission is also common between animals as a result of increased population density, the close proximity of food resources, and decreased species richness which reduces the number of possible hosts (Ditchkoff, 2006; Bradley, 2007). The sum of such pressures creates a very different living environment for urban species than that in their traditional wild habitats.

Due to the unique stimuli presented in urban environments, differences are often seen in urban populations of one species compared to their rural counterparts. Since the urban populations are experiencing unnatural stimuli, after repeated exposure, these animals may have responses that cause them to habituate or

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sensitize, thus leading to the observable differences. Sensitization describes an increased response to the stimuli, while habituation refers to a decreased response over time (Blumstein, 2014). Many studies on birds use flight initiation distance (FID) to determine whether a bird has become habituated to the presence of people (Blumstein, 2014; Lowry et al., 2011; Rodriguez-Prieto et al., 2009). Birds in urban settings tend to have a shorter FID, arguably because they have habituated to the presence of humans (Blumstein, 2014; Lowry et al., 2011; Rodriguez-Prieto et al., 2009). Habituation may stem from a learned behavior. In the case of FID, birds learn that spending a great deal of energy to avoid people or initiating alarm calls is not energetically favorable since they pose no real threat. While some urban stimuli are well understood from behavioural observations, the responses to multiple types of human disturbances need to be understood in order to truly understand the effects of urbanization (Blumstein, 2014).

The effect of noise pollution on avian species is one aspect of urbanization that has received a lot of interest. In urban environments, sound produced by vehicles and machinery creates a very different acoustic environment than that found in natural environments (Francis et al., 2009). Ambient urban noise is a sensory disturbance that may be harmful to urban animals by causing direct stress, reducing predator detection, and masking vocalizations (Slabbekoorn, 2007; Campo et al., 2005; Lowry et al., 2011). For example, a study conducted on laying hens showed that raising hens in 90dB versus 65dB conditions significantly increased their stress levels (Campo, 2005).

There is a wide range of avian species in urban areas, with the most common being Rock Doves (*Columba livia*), European Starlings (*Sturnus vulgaris*), and House Sparrows (*Passer domesticus*) (Clergeau, 1998). They have varying levels of success in these urban regions. City centres have the highest concentration of urbanization, and avian species richness decreases with proximity to these centres (Clergeau, 1998). Species that are able to live successfully in these areas are urban exploiters and are typically generalists (McKinney, 2006). They are likely to be cavity nesters, and are attracted to the food resources that exist within these areas (McKinney, 2002).

Several methods exist to measure the effects anthropogenic disturbance on urban species including behavioural responses to human presence, relative reproductive success, and physiological effects (Tarlow & Blumstein, 2007). Measuring FID is one common method, but can be impacted by many variables that exist within a flock, between individual animals, or in the surrounding environment (Tarlow & Blumstein, 2007). Reproductive success is easily measured in avian populations, as nests can be monitored from the egg laying to fledging stages (Tarlow & Blumstein, 2007). Physiological effects may include changes in heart rate, ratios of white blood cells, or glucocorticoid levels (Cyr et al., 2009; L. Romero & R. Romero, 2002; Gross & Siegel, 1983). White blood cell (WBC or leukocyte) analysis, particularly that of heterophil to lymphocyte (H/L) ratios, is the most widely used method of quantifying stress in birds. Originally developed to analyze the stress levels of chickens being transported, this method is now commonly used for many bird species in both the food industry and ecological studies (Gross & Siegel, 1983).

Avian blood has five white blood cell types, which include both non-granular and granular leukocytes. Lymphocytes and monocytes are considered non-granular leukocytes as they lack stainable cytoplasmic granules. Lymphocytes are smaller than monocytes, and have a round shape and large central nucleus. They are easily distinguished from monocytes which are considerably larger, containing a large amount of cytoplasm and a typically curved nucleus (Lucas & Jamroz, 1961).

The granulocytes include basophils, eosinophils, and heterophils, so named because they contain granules that are easily visible with staining. Basophils have granules that stain darkly with basic dyes, often masking the nucleus and colourless cytoplasm. Granule shapes are a useful feature to distinguish eosinophils from heterophils. Normal, mature eosinophils have round granules while normal mature heterophils have rod-shaped granules. Variations in the granule shape occurs based on the maturity of the leukocyte, so observing other characteristics such as cytoplasm, nuclear lobes, and nuclear staining are important as well (Lucas & Jamroz, 1961).

Heterophils and lymphocytes are the two most abundant WBC types in birds, playing different roles in immunity (Davis et al., 2008). Heterophils are responsible for phagocytosing pathogens in the first line of immune defence, while lymphocytes provide acquired immunity thorough T-cell mediated and antibody-mediated immunity, as well as non-specific natural killer responses (Mallory, 2015; Kogut et al., 2005; Grasman, 2002). Several avian studies have shown that in response to physiological stress, heterophil levels increase and lymphocyte levels decrease

making H/L ratios a reliable stress indicator (e.g. Cirule et al., 2012; Davis, 2005; Gross & Siegel, 1983; Mallory 2015).

Another well-known indicator of stress is that of relative corticosterone level. The hypothalamic-pituitary-adrenal (HPA) axes control the release of glucocorticoids, such as corticosterone, which increases during stressful situations (Sapolsky et al., 2000; L. Romero & R. Romero, 2002). However, this stress response is difficult to measure in avian species because corticosterone levels can change within just two to three minutes and are affected by catching and handling (L. Romero & R. Romero, 2002). Conversely, H/L ratios change at a significantly slower rate in a variety of avian species including chickens (*Gallus gallus*), arctic marine birds such as Common eiders (*Somateria mollissima*) and Arctic terns (*Sterna paradisaea*), and numerous passerine species such as Rufus-collared sparrows (*Zonotrichia capensis*), and the Tufted Titmouse (*Baeolophus bicolor)* (Gross & Siegel, 1983; Mallory et al., 2015; Ruiz et al., 2002; Cirule et al., 2012; Carlo, 2013). Cirule et al. (2012) found that the change in H/L ratio of Great Tits (*Parus major)* stressed by handling occurred between 60-120 min after initial contact. Therefore, considering the time taken to capture and sample blood from any bird species, H/L ratios are more likely to yield accurate results of the stress level at time of capture than coritcosterone

Two common methods of conducting avian WBC counts are using a haemocytometer, and using blood smears. Haemocytometers allow for rapid and accurate counts. However, red blood cells are easily over-stained in this method thus making it difficult to identify WBCs (Walberg, 2001). Blood smears are inexpensive

to prepare, and can provide a good estimate of overall WBC counts. They are also easily stained to provide clear distinction between different WBC types. A definite advantage of this method is that the slides can be preserved, whereas haemocytometer samples must be discarded after analysis (Walberg, 2001; Gross & Siegel, 1983). Blood smears are the preferred method of field biologists, as a large number of samples can be preserved and stained, and viewed at a later date. Haemocytometer analysis is more practical in a clinical setting.

Although the use of H/L ratios is prevalent in avian research (e.g. Cirule et al., 2012; Clark, 2014; Davis, 2005), like any other method, it has its limitations. While H/L ratios are a good indicator of stress, little to no baseline values exist. The levels among individuals of different populations have to be compared to determine relative stress, and for most populations, it is not understood what "normal" levels are. As more studies report their findings, baseline values will become more common. A recent study on Arctic marine birds aimed to establish baseline values for nine species (Mallory et al., 2015). Such studies contribute to H/L analysis being even more useful to accurately assessing stress levels in avian species.

Since their initial release in New York in 1890, European Starlings have flourished throughout North America (Kessel, 1953). These cavity nesters are known for their adaptability, particularly to urban environments (Feare, 1984; Clergeau, 2007). Their mating season in eastern Canada is from April-July, and they produce two broods. Adults tend to form monogamous pairs, though polygyny is also present. Females typically lay 3-7 eggs in each brood, and upon hatch, both parents help provision the young (Feare, 1984). Vocal communication between

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parents and nestlings is an important part of ensuring nestlings are being adequately nourished (Kacelnik et al., 1995).

Like many other avian species, Starlings increase their survival and reproductive success by selecting the best nest sites (Smith & Bruun, 1998). Optimal locations are usually near large grassy environments that have many invertebrates—the Starling's primary food source (Bruun & Smith, 2003; Latham & Latham, 2011). Selecting locations that offer protection from predators is another important factor (Nilsson, 1984; Mitchell, 1996).

The main objective of this study is to experimentally analyze the effects of an isolated anthropogenic disturbance on European Starling nestlings. Since there are many possible disturbances that could be experienced, this study aims to control as many variables as possible so as to analyze the effects of city noise as a potential stressor on nestlings. The results will reveal to what extent European Starlings are urban adapters by analyzing the physiological response of nestlings to urban noise played into experimental nest boxes for seven hours a day. While the effects of other urban stimuli have been thoroughly researched through behavioural observations, few studies have analyzed whether habituation occurs at the physiological level. Additionally, though sound is a known stressor in birds, little is understood about its effects on the nestling. If nestlings from the experimental treatment have higher overall H/L ratios it can be assumed that urban noise is a significant stressor on nestlings. Non-significant results would further support research that shows the resilience of this species in urban environments. I predict that although European Starlings are known to be good urban adaptors, persistent and loud levels of urban

noise will be a significant stressor at the more vulnerable nestling stage of life. It is also important to understand how animals habituate to an urban distraction over time (Chan & Blumstein, 2011). Therefore, H/L ratios over two different nestling ages (Day 11 and 15) will be analyzed to determine whether or not nestlings habituate to urban noise over time. I predict that H/L levels will indicate either habituation (decreased stress response) or sensitization (increased stress response) to the sound stimulus.

METHODS

Field Methods

Nestlings from ten first broods and eight second broods were used in this study; they hatched in some of the 38 nest boxes located on the campus of Saint Mary's University, Halifax Nova Scotia, Canada (44.6317° N, 63.5797° W). Nests were checked daily and then monitored from the time the first egg was laid until nestlings fledged. The first clutch was laid in April and early May 2015 while the second clutch was laid in June 2015. A general survey of daytime sound readings across campus showed much higher sound levels by the nest boxes on the streets than those within campus. An experiment with controlled sound levels was conducted to determine whether variance in sound had a negative effect on developing nestlings. Nests were paired in the quieter areas of campus so that experimentally controlled sound could be effectively introduced. Pairs were selected (Figure 1) such that one was the control and the other the experimental treatment based on the following similar criteria: 1) their proximity to each other (same

environment, same levels of noise and human activity), 2) number of nestlings, and 3) number of parents (both male and female present or both have a single parent) for the nest. The type of treatment for each pair was determined by flipping a coin. Control and experimental nest boxes were visited the same number of times.
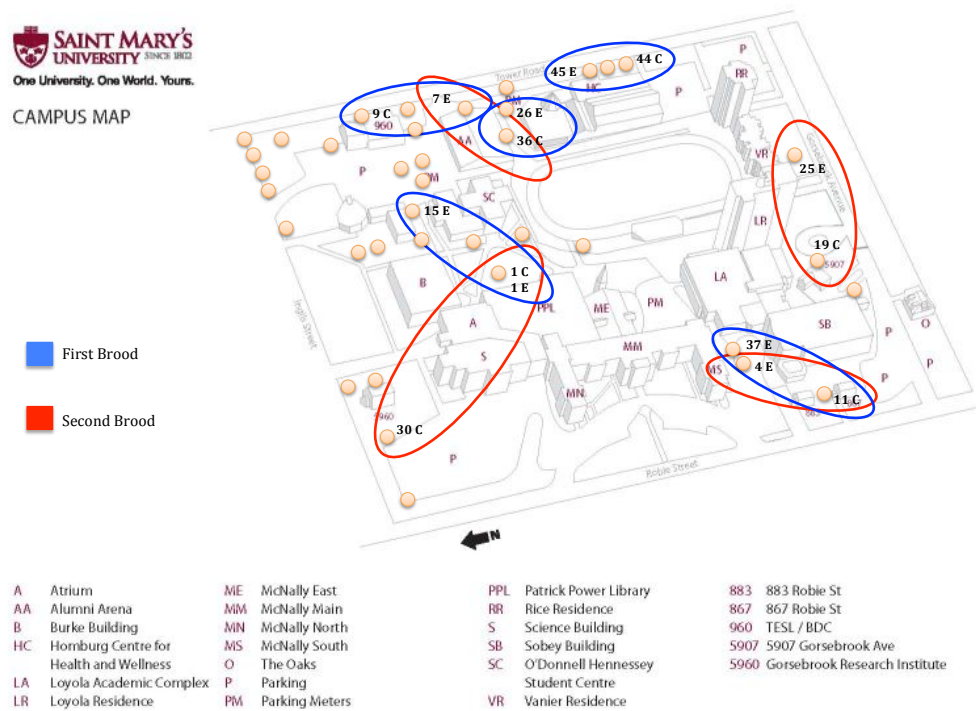

Figure 1: First and second brood nest box pairings located at Saint Mary's University. Nest boxes are labeled by number followed by treatment (E = experimental, C = control). Note that nest box 1 was used as a control in the first brood and an experimental in the second brood.

When nestlings were five days old (day 0 is day of first hatch), a pair of headphone ear buds was attached to the inside of each focal nest box (control and experimental). SONY MDR-E820LP stereo headphones were used to play back sound

in the experimental nests, and less expensive headphones were placed in the control nest boxes where sound was not played (both types of headphones were similar in appearance and size). The headphones were attached to the back corners of each nest box with black tape such that the earbuds were located 27cm into the box. A Nextech omnidirectional tie clip microphone was placed 12cm into the front right corner of each nest box, for use in another study. A medium-sized ziplock bag was stapled to the base of each nest box, and housed the cables from the headphones and the microphone.

In the experimental nest boxes, a 4GB Hipstreet Prism MP3 playing a City Ambience sound effect (Finnolia Sound Effects, 2013) at maximum volume was connected to the headphones starting between 07:30-08:30 on Day 5 of the nestling period. This sound played continuously for 3.5 hours, and was then stopped so that the MP3 player could be recharged for an hour. Sound was then played continuously for another 3.5 hours. On days 8, 12, and 14 of the nestling period, recorders were set up during this one-hour quiet period for another study. At the end of the second play period each day, the MP3s were taken down. Each time an experimental nest box was visited, so too was the control nest box and its ziplock bag was rustled to simulate the disturbance of the MP3 set up or take down.

Sound level measurements were also taken at each nest box, to ensure that there was a distinct difference in noise level between the experimental (when noise was being played) and control nest boxes. Sound levels were measured in decibels, which indicate the loudness of noise (Stevens, 1972). So as to take these measurements without disrupting the nestlings, an empty nest box filled with nest-

like material, with headphones attached to the same specifications as previously

described, was placed upright on the ground directly below the actual nest box.

Using a Check Mate SPL meter, a maximum dBA reading was taken every 30 seconds

over a 3 minute period to give an average dB reading for that nest box. The meter

was oriented within the nest box opening, with the lid closed, in order to incorporate

noise from the surrounding environment and within the nest box. The average dB

level ± SD for the control treatment was 65dB ± 2.45 and for the experimental

treatment was 87dB ± 1.50 and the treatments were found to be significantly

different (W=45.00, n=9, P=0.0039) showing that the added MP3 sound effectively

increased the noise level.

The nestlings were handled on days 5, 11, and 15 of the nestling period. On

day 5, wing chord length was measured using a wing chord ruler to the nearest 0.5

mm. A series of tarsus length measurements were taken using digital callipers to the

nearest 0.01 mm to determine average tarsus length. Mass was taken by placing the

nestling into a nylon stocking and weighing it with a zeroed 30 g or 100g Pesola

spring scale to the nearest 0.5 g. Each nestling was banded with a different colour

plastic band on their left leg to individually identify them from their siblings. A small

blood sample (20 – 50 μl) was taken by pricking the right brachial vein with a 25G

needle, and collecting the blood with a 50 μl capillary tube. Most of this blood was

then placed in an eppendorf tube containing 1 mL of 95% ethanol, for another study.

Two drops of this blood (one for each slide) was deposited near the base of a clean

slide and smeared along the length of the slide with the edge of another clean slide

as outlined by Bennett (1970). Two slides were made for each individual.

On Day 11, the nestlings were handled mid-day in the one-hour period where no sound was playing. Before mass, tarsus and wing chord measurements were taken, a small blood sample was taken to make two blood smears. This was done within 0.5-2 minutes of taking the nestling out of the nest to eliminate the possibility of handling stress affecting WBC counts. A Canadian Wildlife Services (CWS) band was placed on the right leg of each nestling.

On Day 15, two more blood smears were made for each nestling after the morning round of MP3 play. The nest box hole was plugged with a cloth bag to eliminate the risk of premature fledging while handling the nestlings. After this blood sample was taken, the study was considered complete. The nest boxes were left untouched until the nestlings fledged, typically six days later. Fledging success was determined by re-visiting the nest boxes a few days after estimated fledge occurred.

Lab Methods

All blood smears were fixed in 100% methanol within 48 hours of being made. They were then stained with Hema III staining solutions and left to dry overnight. Cover slips were then added to the dry slides using 3-4 drops of Permount and were left to dry for a minimum of 24 hours before being examined under a microscope.

One blood smear for each of two randomly selected nestlings from each nest box was examined under an Olympus CX41RF microscope for WBC analysis. Under 1000X magnification, the first 100 WBC observed were identified as either heterophils, eosinophils, basophils, lymphocytes, or monocytes. The number of

fields of view, as well as the estimated number of RBC in each field of view were recorded. From this information, the number of WBC per 10,000 RBC was calculated (not used in analysis), and the heterophil to lymphocyte ratios were determined. When all of the smears were analyzed, ten randomly selected smears were re-checked and correlated with the original counts to ensure that the results were repeatable. There was a significant correlation between the original H/L ratios and the rechecks (r=0.705, n=10, P=0.023). Figure 2 shows that there was a close match, and therefore the results were repeatable. The least repeatable measurements (nestling B and F) were made on poor quality slides compared to the other samples. This methodology does not provide exact H/L ratios, but instead provides an overall estimate of H/L.
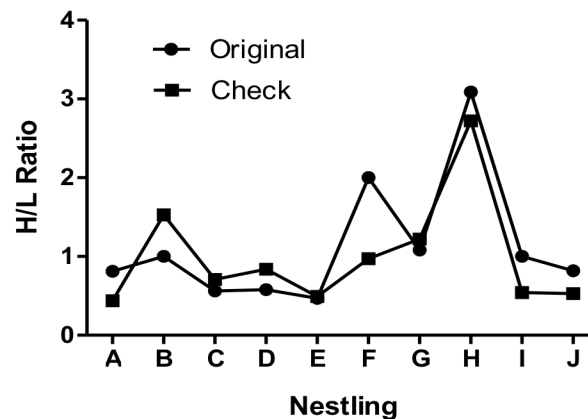

Figure 2: Correlation between first and second H/L counts from 10 randomly selected nestlings A-J.

All data were analyzed using GraphPad Prism 5. Before the predictions were tested, the repeatability of H/L ratios within a nest box was determined. Since resulting H/L ratios of the two nestlings selected from each nest box were averaged to give an overall H/L value for a given nest box, I wanted to know if there was a significant difference between nestlings of the same nest box. A nonparametric paired test (Wilcoxon test; data were not normal) showed no significant difference between the H/L of individual nestlings within each selected nest box (Day 11 W=23.00, n=18, P=0.60; Day 15 W=-1.000, n=17, P=1.00), so the averages were assumed to be a good indicator of the overall nestling stress level for that nest box.

To ensure that any significant results between the H/L ratios were not due to individual differences in nestling condition, residuals were determined by regressing mass against tarsus length for all nestlings in the breeding season, and correlating these condition residuals with H/L ratios. The higher the residual is above zero, the greater the condition. Conversely, the lower the residual is below zero, the poorer the condition. No significant correlation was found (see Figure 4), therefore the predictions were tested knowing nestling condition should not impact the results.

When testing the predictions, experimental data were first compared to the control data. The data sets were tested for normality using a D'Agostino-Pearson omnibus normality test, and found to be non-normally distributed. Non-parametric tests for paired data (Wilcoxon) were then conducted on H/L ratios for both Days 11 and 15, comparing experimental to control treatments. Non-parametric paired tests

were also conducted within each treatment, comparing Days 11 to 15 to determine changes over time within the same nest boxes. Results were considered significant when $P \leq 0.05$.

RESULTS

No significant difference in H/L ratios were detected between the two randomly selected nestlings within each nest box on either Day 11 (Wicoxon W=23, n=18, P=0.60) or Day 15 (W=-1.000, n=17, P=1.00; Figures 3 and 4).
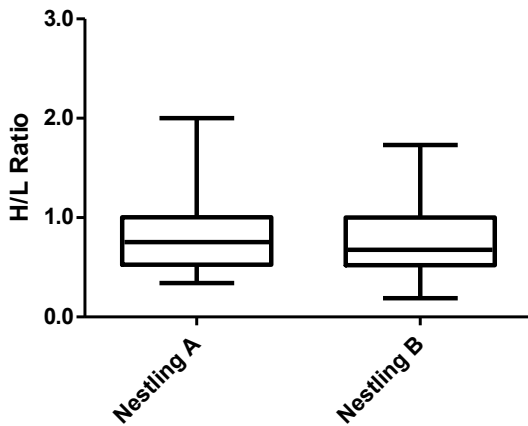
Figure 3: Comparison of H/L ratios of first versus second nestlings sampled in each nest box at Day 11 (n = 18).
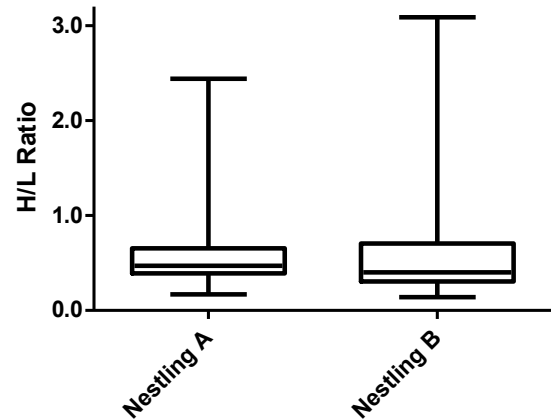
Figure 4: H/L ratio comparison for first versus second nestlings sampled in each nest box at Day 15 (n = 17).

Nestling condition, as represented by residuals, was evenly distributed above and below 0 (average condition) (Figure 5). No significant correlation was found between nestling condition and H/L ratio (r= -0.1127, n=36, P=0.51, Fig. 6).
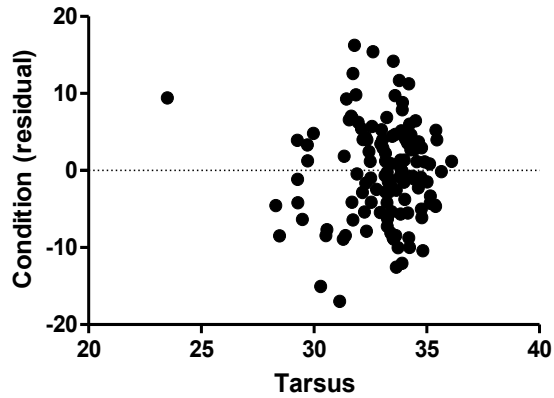
Figure 5: Day 11 residuals of mass vs. tarsus length in all nestling n =36 sampled in the 2015 breeding season. Indicates the relative condition of the nestlings.
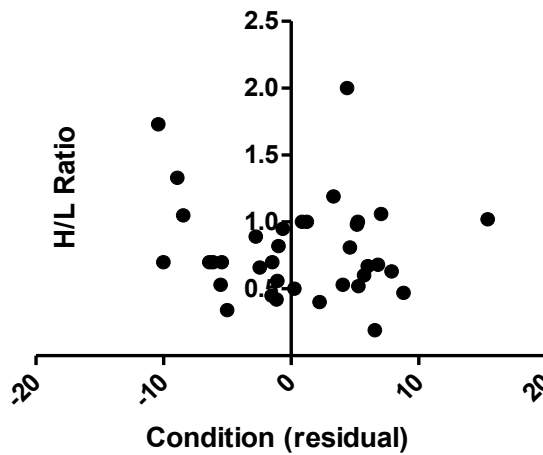

Figure 6: Day 11 residuals indicating the condition of the European Starling nestling compared to their H/L ratio.

Having determined the relative similarity of H/L ratios between the two randomly selected nestlings within a nest box, and ruling out condition as a factor affecting the findings, I tested my predictions. When comparing H/L ratios of

experimental and control treatments of Day 11 nestlings, no significant difference was found (one-tailed Wilcoxon W=17.00, n=9, P=0.18; Fig. 7).
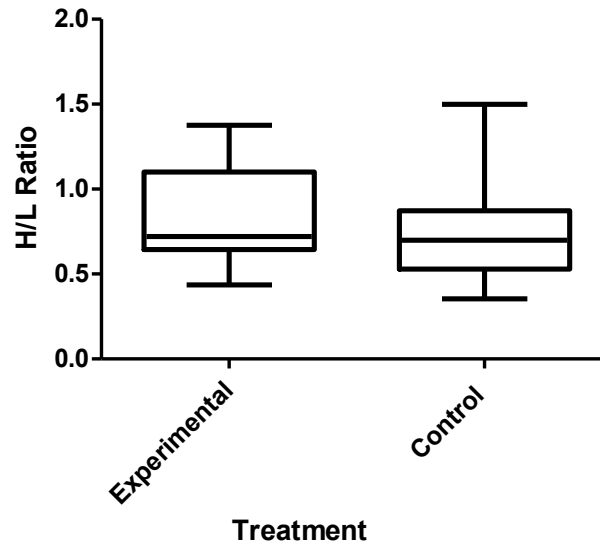

Figure 7: H/L ratios of Day 11 nestlings exposed to the experimental treatment (added urban noise) and the control treatment (no added urban noise).

However, H/L ratios were significantly higher for control than experimental nestlings at Day 15 (W=-26.00, n=8, P=0. 0.039). The test statistic (W) has a negative rank (meaning the control values are higher than the experimental values). It was predicted that the experimental nestlings would have higher H/L ratios (positive rank), so this is the opposite result than was predicted. Figure 8 illustrates the lower values in the experimental treatment compared to the control at Day 15.
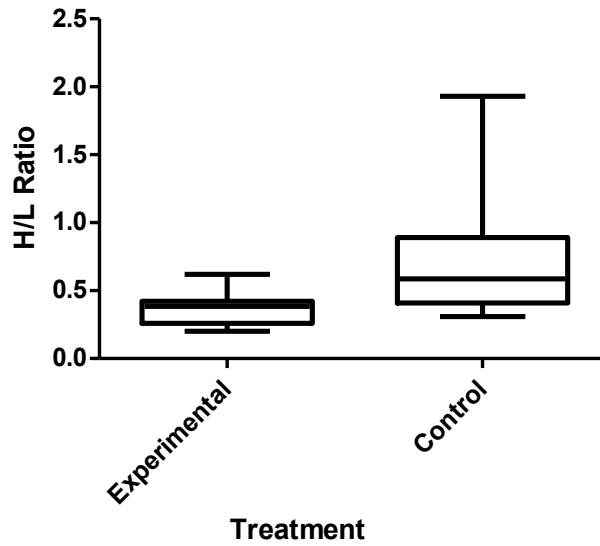
Figure 8: H/L ratios of Day 15 nestlings exposed to the experimental or the control treatment.

No significant difference was detected in H/L ratios of control nestlings between Days 11 and 15 (two-tailed Wilcoxon W=0.00, n=8, P=1.000; Fig. 9).
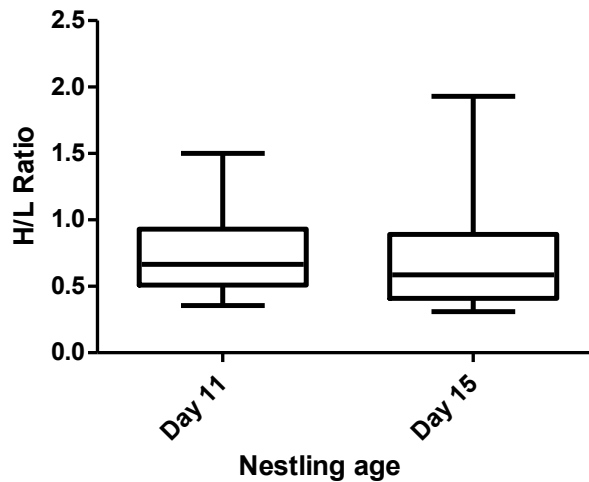

Figure 9: Comparison of H/L ratios of nestlings at Days 11 and 15 in the control treatment.

Similarly, no significant difference was found in nestling H/L ratios in the experimental treatment between Days 11 and 15 (two-tailed Wilcoxon W=29, n=9, P=0.098; Fig. 10). However, H/L ratio of nestlings in the experimental group tended to be generally lower at Day 15 than at Day 11.
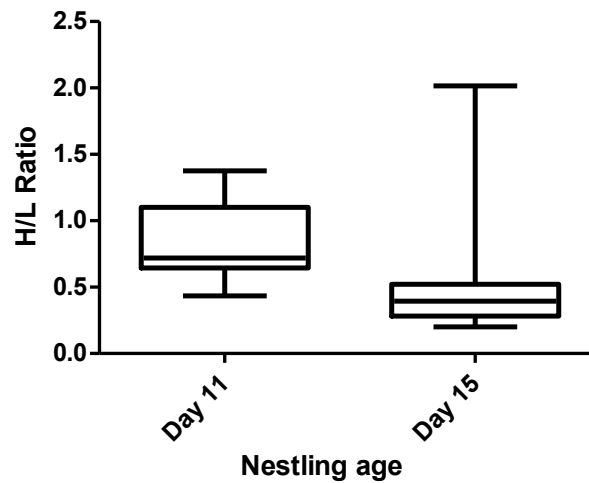
Figure 10: Comparison of H/L ratios of nestlings at Days 11 and 15 in the experimental treatment.

DISCUSSION

The initial analyses conducted before the predictions were tested were very important in validating the results of this study. Randomly selecting two nestlings from each nest box, to establish a mean H/L ratio per nest box was shown to be reliable since the selected nestlings had similar H/L ratios (Figs. 3 and 4). Confirming that nestling condition did not affect their H/L ratios aligns with previous findings (Copan, 2013; Yuill, 2014), and helps verify that the variation in

stress levels was mainly attributable to the treatments and not differences among individuals.

The results of this study appear to confirm that the European Starling is very well adapted to urban life. The lack of increased stress response (no significant increase in H/L ratio) to the added noise stimuli at Days 11 and 15 is contrary to my first prediction. Therefore, an increased volume of ambient city noise does not appear to be a significant stressor to European Starling nestlings.

My second prediction was that after prolonged exposure to the stimulus, nestlings would either become habituated or sensitized. The significantly lower stress response at Day 15 in the experimental treatment is evidence of habituation, which will be later discussed.

Stress levels in control nestlings did not change significantly. Therefore, unlike what was observed in the experimental treatment, no habituation occurred in control conditions. At Day 15, the control nestlings were significantly more stressed than the experimental nestlings. Taking a closer look at the methodology of this study and that of others, may shed light on why increased noise created a less stressful environment.

Dooling and Popper (2007) stated that there was little literature describing the effects of road noise, which is a very key aspect of overall anthropogenic city noise, on avian species, and that poor methodology makes it difficult to draw definite conclusions. It is difficult to perfectly simulate conditions of the external world for an experimental treatment within a nest box. In the control treatment, the sound nestlings experienced followed typical patterns of a work day, but individual

noises would have been relatively unpredictable. Spontaneous noises such as sirens, garbage trucks, human voices, and dogs barking, might have been stressors that impacted the H/L ratios of control nestlings. In the experimental treatment, however, sounds came at predictable intervals because a three-minute track of ambient city noise was played on repeat for 7 hours daily. Though the volume was about 20dB higher, the treatment did not perfectly emulate what a nestling would typically experience in an urban setting. The sounds were predictable, unlike what would be found in the real world. Volume is therefore not the only difference between these treatments, and the predictability of the treatments needs to be addressed in order to gain a better understanding of the results.

In a study conducted on white-crowned sparrows (*Zonotrichia leucophrys oriantha*), nestlings exposed to five days of traffic noise were also found to have decreased stress levels (Crino et al., 2013). The experimental treatment in this study had five standardized recordings that were each 40 seconds in length. They played continuously, day and night, from Day 1 after hatch to Day 5 (Crino et al., 2013). This study, like my own, had a predictable set of sounds, as the treatment played continually for an extended period of time.

The lowered stress response over time shows that habituation occurred. Understanding acute and chronic stress, noise masking, and nestling learning may offer deeper insight into this phenomenon. Chronic stressors are those that exist repeatedly or for extended periods of time, while acute stressors occur for only a short time (Cyr & Romero, 2009). The treatment in both my study, and that of the white-crowned sparrows were chronic treatments. Studies have shown that

habituation is most likely to occur in response to chronic stressors than acute

stressors. (Evans et al., 2010; Rich & Romero, 2005; Cyr & Romero, 2009; Lynn et al.,

2010). This finding has also been shown in adult avian species. For example,

penguins (*Spheniscus magellanicus*) studied in tourist areas of Argentina were found

to have lower stress levels than those living in less disturbed areas. The constant

flow of tourists would be a chronic stressor, whereas penguins in remote study

areas only experience human disturbance occasionally (Fowler, 1999).

  Masking, the interference of one sound by another, may have contributed to

producing a chronic stress treatment (Dooling & Popper, 2007). It has been shown

that masking can negatively affect the ability of birds to perceive vocalizations or

approaching predators (Dooling & Popper, 2007; Leonard & Horn, 2012). The sound

in the experimental treatment was played from within the nest box at a higher

volume than the incoming background noise. The MP3 sound was therefore masking

incoming acute stressors, meaning the nestling were only experiencing a chronic

stressor.

  Habituation is often closely associated with learning. In fact, Cyr & Romero

(2009) state than learning to recognize a stimulus as non-threatening is necessary

for habituation to occur. In other words, the capacity to respond does not change,

but the perceived need to is reduced. Animals learn to re-assess risk after prolonged

exposure to a stimulus (Chan et al., 2010). In this experiment, the playback of urban

noise did not pose any real threat. It is possible that the nestlings recognized this,

resulting in habituation in the form of a decreased H/L ratio.

Very few studies have shown how nestling immunity develops as they age, specifically with regards to H/L ratios. Wilcoxen et al. (2011) showed that age did not affect corticosterone levels in adult Florida Scrub-Jays (*Aphelocoma coerulescens*) between the ages of 2-12 years, but they did not examine it in nestlings. The lack of change in H/L ratio in nestlings within the control treatment suggests that there may not be much difference in nestling WBC numbers between Days 11 and 15. The significant result in the experimental treatment can therefore more confidently be attributed to the experiment rather than changes occurring due to aging.

In order to confirm if the predictability, and not just the loudness of noise decreases H/L ratios, a different experimental design would be required. One possibility would be to amplify the external noise into the experimental nest boxes. This way, paired nest boxes would receive the same types of disturbances, but at different volumes. Nest boxes could also be analyzed with naturally occurring sound variation within the study sites. On Saint Mary's University campus, there are many nest boxes along Inglis street which are consequently exposed to frequent, and loud traffic sounds. The stress levels of nestlings along this street could be compared to nestlings in quieter areas to determine whether the results support those of this study.

Future work could also determine the precise time taken for a nestling to habituate. Habituation occurred somewhere between Day 11 and Day 15. However, the exact amount of exposure needed would not be known without more sampling.

Overall, there appears to be a need for physiological studies on nestlings. While adult European Starlings are frequently studied, little is known about their physiology in the early life stages. Several other researchers have also expressed this need (Crino et al., 2013; Dooling & Popper, 2007).

Many behavioural studies have been conducted on avian species, and conclusions about stress have been deduced from how birds respond behaviourally to a stimulus. For example, several studies attributed a short FID distance to birds habituating to human presence, though this has rarely been compared to a decreased physiological stress response. Since nestlings have been shown to reduce their stress response in the presence of increased noise volume, it would therefore be instructive to determine whether this reduced physiological response correlates with behavioural changes such as vocalizations.

CONCLUSION

To summarize, European Starling nestlings do not appear to be stressed by increased volume of ambient city noise. There is however, a decreased stress response over time in the experimental treatment, suggesting European Starling nestlings have the ability to habituate to a chronic stimulus.

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