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James Madison University

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Is immediate flexibility present in a vocal mimic, the gray catbird (*Dumetella carolinensis*), across an urban gradient?

Shannon K. Eppert

A thesis submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

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FACULTY COMMITTEE:

Committee Chair: Dr. Dana Moseley

Committee Members/ Readers:

Dr. Heather Griscom

Dr. Patrice Ludwig

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Abstract

Increasing urbanization has increased anthropogenic noise levels near developed areas. Urban noise is high amplitude and low-frequency, and these frequencies can overlap with the signals animals use to communicate, including bird songs. Many urban birds sing higher minimum frequencies in urban areas, which avoids some masking by noise, but the mechanism behind this difference is not well understood. Immediate flexibility is the ability to alter song in real-time in the presence of sudden noise, allowing for avoidance of masking and better signal transmission. I investigated if male catbirds increased signal transmission in the presence of anthropogenic versus high-frequency noise playback compared to pre-playback. I conducted this experiment along an urban gradient from Virginia to the Washington D.C. metro region with 17 male gray catbirds (*Dumetella carolinensis*). I then measured song minimum frequencies using two sampling methods; one using the peak frequency contour (PFC) tool in Raven Pro to measure all elements, and second, the peak amplitude threshold (PAT) method in Signal 5 to measure the very lowest minimum frequency. The PFC analysis showed significantly greater average minimum frequencies during urban low-frequency noise playback relative to pre-playback minimum frequencies, but the effect size was small at only 73.3 Hz, opening the question of whether this shift would allow songs to avoid noise masking. Catbirds also showed flexibility of their maximum frequencies during masking high-frequency noise, decreasing roughly 260.9 Hz. In contrast, there was no significant difference between pre-noise and during low-frequency noise in the PAT method, which measured the very lowest frequency of each 10-second song clip. The results provide no clear evidence of immediate flexibility in catbirds, because the effect size of the

minimum frequency shift of all catbird notes was not greater than the frequency resolution of Raven Pro. Moreover, this small shift of minimum frequencies did not reach the effect size observed in other species of birds such as Parids and may be suggestive of a by-product from the Lombard effect rather than an overall shift of minimum frequency.

Introduction

Acoustic Communication

Acoustic communication is used across several taxa and functions in contexts of both survival and reproduction such as species recognition, alarm and/or distress calls, mate attraction, competition, as well as other functions (Bradbury & Vehrencamp, 1998). Birds, for example, use songs and calls for a variety of functions. Alarm calls can be used as an anti-predator measure, both to warn conspecifics or to chase away a predator (mobbing calls). Calls are also used in parent-offspring communication, with an example being begging behavior in chicks (Catchpole & Slater, 1995; Warren et. al, 2006). Songs are a type of vocal signal that are learned, complex, and function in attracting mates and defending territories (Borror, 1961; Catchpole, 1980). Therefore, songs and calls are a crucial aspect of birds' natural history and are essential in mediating interactions among individuals that contribute to the survival and reproductive success of individuals. For communication to be successful, a signal must transmit through the environment and reach its intended receiver, otherwise this signal would cease to function in a communication system.

Species do not exist in a quiet world, however, and in order to communicate, they must compete with the noise in their environment. Noise is defined as interference such as sounds, or other environmental factors that impede signal transmission, i.e., a signal reaching its receiver. Examples of auditory noise include abiotically generated sounds such as by streams and wind, or biotically generated ones such as by animal movement or heterospecific calls, and, increasingly in modern history, noise generated by anthropogenic sources, such as traffic, construction, and industry. There is thus selective

pressure on the sender of a signal to produce a signal that can be detected by a receiver after transmitting through the environment of both biotic and abiotic noise.

Urbanization

The process of urbanization has drastically altered the acoustic landscape by contributing additional noise pollution, and thus has created new selection pressures for animals that communicate acoustically (reviewed in Patricelli and Blickey, 2006; Warren, 2006). The evolutionary responses to sensory pollutants like anthropogenic noise, run the gamut from behavioral to physiological changes (Swaddle et al, 2015). Noise pollution is often overlooked as a transient byproduct of human activity, but chronic exposure to noise has serious implications for organisms' ability to communicate within their environment (Slabbekoorn, 2013). Urban noise differs from naturally existing biotic/abiotic noise as it is typically both loud and low-frequency, with the loudest range falling in a frequency range peaking between 1 - 2 kHz (Slabbekoorn and Peet 2003; reviewed in Patricelli and Blickey, 2006). To explain the impact of anthropogenic noise on wildlife, I will first give a brief background on the measurements of sound as follows. Frequency, measured in Hertz, reflects the pitch of the sound and is measured by the length of the sound wave – high pitches have short, fast wavelengths, and low pitches have long wavelengths. While amplitude, measured in decibels, describes the loudness and is literally the amplitude of the wave. Anthropogenic noise, which is both high amplitude and low-frequency, often overlaps with the frequencies that some animals use to communicate. Essentially, this overlap of frequencies which either partially or entirely masks acoustic signals, results in the inability of animals to communicate effectively (Slabberkoorn and Peet, 2003; Halfwerk et al, 2011).

Three examples of noise interfering with either effective communication or other aspects of natural history include the masking of chicks' begging calls, reduced response to territory intruders, and increases in vigilance to compensate for predator detection difficulty. In tree swallows (*Tachycineta bicolor*), parents missed detections of nestlings' begging calls when nests were exposed to white noise (Leonard & Horn, 2012). Environmental noise also impacts responsiveness to territory intruders. European robins (*Erithacus rubecula*) increase low-frequency notes during aggressive encounters with rival males, but the strength of this response is reduced in noise. This reduced aggressive reaction could have cascading effects in territory defense and reproduction (Zwart et al, 2016). Urban noise also impacts anti-predator behavior, for example, chaffinch (*Fringilla coelebs*) display increased scanning behavior in the presence of noise, and this heightened vigilance may compensate for reduced auditory cue detection (L. Quinn et al, 2006). House sparrows (*Passer domesticus*) exposed to chronic traffic noise flushed more readily than those under quieter conditions, which is another example of compensatory vigilance (Meillère et al, 2015).

Given the importance of communication, it is not surprising that acoustic signals that avoid masking by noises or other features historically found in their environment, such as noises from insects or impedance from dense forests, appear to have been favored evolutionarily due to their greater efficiency and increased chance of signal transmission (Wiley, 1983). Richards and Wiley (1980) describe how species living in open fields sing with more broad frequency trills, while species living in dense forests sing with more tonality – each singing style optimizes transmission in the particular habitat. A decrease in the effectiveness of communication within the environment can have severe

consequences for maintaining associated functions (Warren et al, 2006). With the pressure to communicate effectively in the presence of any masking noise, birds may change aspects of their song or behavior.

In response to urban noise, which is relatively recent on an evolutionary time scale, birds also can employ behavioral changes in the short-term, such as shifts in location, timing, amplitude (loudness), or, in some cases frequency (pitch). Differences in bird song between populations has been documented since 2003. Several studies now have shown that bird populations within noisy, urban environments sing at higher minimum frequencies (lowest pitches) than populations within quieter rural environments (Slabbekoorn and Peet 2003, reviewed by Slabbekoorn 2013, Shannon et al., 2016). Singing at higher frequencies in noisy environments avoids masking by urban noise and may be an adaptive behavior. Low-frequency signals are more masked, and likely less effective in a noisy environment, shifts to higher frequency of the minimum frequencies, the lower pitched components of songs, would allow birds to communicate more effectively (Halfwerk et al, 2011). Interestingly, there are inconsistencies for what species show differences their song frequency in response to urban noise and by how much, suggesting these differences are not necessarily taxon specific (Hu and Cardoso, 2010). Additionally, few studies have investigated how individuals within the same species vary in their ability to alter their song.

While there is a benefit of signal transmission for birds in urban populations to sing at higher frequencies, there also may be a cost of losing their lowest frequencies. In addition to being selected based on the acoustic landscape, song is also a sexually selected trait (Catchpole, 1980). Song is a sexually dimorphic trait in most North

American birds; during breeding season male birds sing vocalizations defined as songs whereas most females do not sing song. Certain aspects of song quality have been correlated with male physical qualities such as body size, physical condition, age, or brain region size (Catchpole and Slater, 2003). Aspects of song can serve as an honest indicator of genetic quality or physiological condition. Thus, a high-quality signal may be defined as a signal that maximizes these sexually attractive components, whereas an effective signal would be one that is more easily transmitted through the environment. Song parameters that have been shown to function in sexual selection contexts include the lowest minimum frequency (Halfwerk et al, 2011) because lower minimums indicate larger body sizes (Searcy and Nowicki 2006), and related to minimum frequency, broad frequency bandwidths (Ballentine et al, 2006). As birds sing higher minimum frequencies, the use of low-frequency notes is lost. This loss may be a hidden cost to avoid masking by singing higher, because females of some bird species tend to prefer low-frequency notes. For example, female great tits (*Parus major*) show greater sexual fidelity to males that use a larger proportion of low notes in their songs, however, in noisier contexts, female great tits responded to higher frequency songs more strongly (Halfwerk et al, 2011). This contrast sets up a trade-off between sexual selection and natural selections because a higher frequency song may transmit better but be evaluated as a lower-quality song by females. Additionally, as birds raise their minimum frequencies, they may reach a theoretical maximum frequency, a point after which they are physically unable to sing higher. This reduced range of usable frequencies may be a problem if range (frequency bandwidth) or frequency jumps are an attractive feature to females. In species where the attractive qualities have not been identified, it is unknown

if shifts in minimum frequency will result in a tradeoff between avoiding masking and the use of attractive song parameters.

Three main mechanisms could be responsible for the findings that populations of birds in noisy habitats sing higher minimum frequencies than populations in quieter habitats, and they are not mutually exclusive. First, changes may be accrued through generations via natural selection, if a change in the frequency of alleles in a population for singing higher minimum frequencies was favored over time. Second, songs may have diverged between populations through cultural selection. Instead of acting on genes, cultural selection can proceed quickly as it acts on learned behaviors or memes, such as learning higher-frequency song types over others (e.g., Moseley et al. 2018). Third, individual birds may be immediately flexible by shifting the frequency of their songs when faced with anthropogenic noise – a topic I will discuss at length below.

Immediate Flexibility

Immediate flexibility is one proposed mechanism to explain the observed shifts in birdsong frequency. It refers to a signaler's ability to immediately alter song in response to urban noise exposure, and then to shift frequencies back when noise is reduced (Brumm and Todt, 2002; Derryberry et al, 2017; Gentry et al, 2017; Ríos-Chelén et al, 2018; Verzijden et al. 2010). There are two main ways that birds can immediately alter their song to compensate for urban noise; they may increase the amplitude (loudness) of their song, or they may shift the frequency of their songs (Brumm and Todt, 2002; Warren et al, 2006).

For changes in song frequency, the shift could be upward, raising the minimum frequencies of song in order to avoid masking (overlap) by low-frequency noise or downward of the highest (maximum) frequencies, which would focus song energy within a smaller bandwidth (Warren et al, 2006). For the first type of shift, the most common is to shift specifically the lowest, minimum frequencies higher. For example, great tits (*Parus major*) were recorded shifting their song when presented with low-frequency noise, and immediately shifted back when the noise ceased (Halfwerk & Slabbekoorn, 2009). Similarly, chiffchaffs (*Phylloscopus collybita*) exposed to simulated highway noise sang at higher minimum frequencies and returned to pre-exposure frequencies after the cessation of the noise broadcast (Verzijden et al., 2010). House finches (*Carpodacus mexicanus*) were recorded immediately shifting minimum song frequency when played loud noise (Bermúdez-Cuamatzin et al., 2011). One study conducted by Derryberry et al. (2017) found that white-crowned sparrows (*Zonotrichia leucophrys*) change amplitude in noisy environments. Another study on white-crowned sparrows found that urban males shift their maximum frequencies down (narrowing frequency bandwidth) which would concentrate more energy in a narrower range in response to noise, whereas rural birds did not (Gentry et al, 2017).

For shifts in amplitude, a greater singing amplitude would theoretically compensate for the increased environmental noise and achieve a favorable signal to noise ratio as advantageous, and then reverting when the noise ceases. This concept was first explored in Japanese quail; individuals exposed to white noise called at increased amplitude and increased the length of calling bouts (Potash, 1972). Nightingales (*Luscinia megarhynchos*) have also been recorded altering their vocal amplitude higher in

response to the immediate noise level in the environment, and then returning to a lower amplitude when the environment quiets again (Brumm & Todt, 2002).

Changes in minimum frequency and amplitude are not mutually exclusive. Many instances of raised amplitude may be due to the Lombard effect—the tendency to increase signal volume in the presence of noise. When in a noisy environment, one way to overcome masking is to overpower the volume of the background noise, increasing the strength of the birdsong signal relative to the background noise (S:N). This effect has been observed not only in birds, but also within amphibians and primates, including humans (Lombard, 1911; Love & Bee, 2010; Sinnott et al., 1975; Brumm & Zollinger, 2011). Due to the physics of sound, this tendency to increase signal amplitude also comes with an involuntary increase in minimum frequency. It is possible that the increase in minimum frequency is a byproduct of an increase in amplitude (Brumm et Zollinger, 2011).

In order to synthesize the literature on immediate flexibility and resolve various hypotheses about shifting minimum frequencies and the methods by which the data was analyzed, I summarized all publications with immediate flexibility and report aspects of their methodology and results here.

Literature Review Methods

The intent of this review was to gather all extant papers that conduct a manipulative immediate flexibility experiment. I conducted searches for literature over the course of May 2019- June 2021. Preliminary paper collection was completed by Morgan Rhodes in 2019. Multiple databases and search engines were used, including:

Google Scholar, Scopus, and Web of Science, as well as forward and backwards citing.

Terms and keywords searched include: Acoustic adaptation, immediate flexibility, urban noise, song plasticity, vocal plasticity and other similar iterations.

Table 1. Table of known papers testing immediate flexibility in song spectral parameters.

In the columns below each abbreviation indicates, NP not provided by authors, no shift: no change in either min frequency, max frequency, or amplitude, N/A refers to details that were not applicable either based on the species or the design of the study.

Authors	Species	Order	Oscine or Sub-oscine?	N	number of stimuli	control	minimum adjusted?	maximum adjusted?	Other	Stimuli given to all individuals?	Distance from birds	dB of noise	N of songs
(Dorado-Correa et al, 2018)	Mallard (<i>Anas platyrhynchos</i>)	Anseriformes	N/A	15	Two white noise stimuli (60 and 70 dB)	No noise stimulus	Peak frequency increase (+227 Hz at 70 dB)	Not provided	Increased call amplitude	yes	N/A	60 and 70 dB(A) at test distance	15 call notes per treatment
(Pytte et al, 2003)	Blue-throated hummingbird (Lampornis clemenciae)	Apodiformes	N/A	2	Two creek noise stimuli	No noise stimulus	Not provided	Not provided	Increase call dB	yes	Not provided, but monitored	35 and 40 dB at 8 m	22-28 per condition
(Brumm et al, 2009)	Domestic Chicken (Gallus gallus)	Galliformes	N/A	19	Varied broadband noise (60, 67, 75, 80 dB)	No noise	No frequency shift	No frequency shift	Increased call dB	yes	Minimum of 30 cm	60, 67, 75, 80 dB	>10 calls per male
(Potash, 1972)	Japanese Quail (Coturnix coturnix japonica)	Galliformes	N/A	24 (12 pairs)	Low amplitude white noise (45 dB), and high amplitude white noise (60 dB)	No Noise Stimuli	Not provided	Not provided	Increased amplitude, call rate	yes	N/A	45 or 60 dB at test distance	N/A
(LaZerte et al, 2016)	Black-capped chickadee (Poecile atricapillus)	Passeriformes	oscine	28	Traffic noise simulation	Pre-noise	Proportion of low notes decrease	No frequency shift	Not provided	yes	Not provided but monitored	65 dB at test distance	Varied
(Goodwin & Podos, 2013)	Black-capped Chickadee (Poecile carolinensis)	Passeriformes	oscine	64	two; masking tone or control tone (5000 Hz)	pre-broadcast song	Shift to a higher song type	Not provided	Not provided	yes	<10 m	80 dB at 0.5 m	Not provided
(Verzijden et al, 2010)	Chiffchaffs (Phylloscopus collybita)	Passeriformes	oscine	22	highway noise	pre-noise	Increased minimum frequency (296±34 Hz)	Not provided	Song length increase in noise	highway noise played to all birds	8-16 m	60–66dB(A) at test distance	First 10 songs
(Gross et al, 2010)	Common Reed Bunting (Emberiza schoeniclus)	Passeriformes	oscine	23	Traffic noise, biotic noise, control (chiffchaff song)	biotic noise control and a pre-broadcast recording	Increased minimum frequency (190 Hz)	Not provided	Song length decreased, song rate decreased	yes	15-20 m	90 dB at 1 m	Not provided
(Montague et al, 2013)	European Robin (Eritacus rubecula)	Passeriformes	oscine	19	Low-frequency noise stimuli	pre-noise recording	Increased minimum frequency (550 Hz)	No frequency shift	Reduced song complexity, shorter song, slower song rate	N/A	7- 15 m	90 dB at 1 m	10 songs per condition (10 pre, during and post)
(Hallwerk & Slabbekoorn, 2009)	Great Tit (Parus major)	Passeriformes	oscine	19	City noise (low), inverse city noise (high) and white noise	high frequency and white noise	Shift to a higher song type	Lower maximum frequencies in high-frequency noise	Not provided	no	8-16 m	60-66 dB at birds distance	Randomly selected 6 strophes for each category of analysis

(Bermúdez-Cuamatzin et al. 2011)	House Finch (Carpodacus mexicanus)	Passeriformes	oscine	10	low amplitude noise and high amplitude noise (56-65 dB)	low volume noise (44-57 dB)	increased minimum frequency during increased noise dB (~150 Hz +)	No frequency shift	No frequency shift	Songs with shared syllables were shorter during noise	yes	3 m	low amp (44-57 dB); high amp (56-65 dB)	Not provided
(Brumm & Todt, 2002)	Nightingale (Luscinia megarhynchos)	Passeriformes	oscine	10	Varied white noise between 55 and 75 dB in 5 dB increments)	no noise	No frequency shift	No frequency shift	Increased song dB	either given multiple noise levels or the control		Minimum of 40 cm	50-75 dB	27 songs
(Hanna et al. 2011)	Red-winged Blackbird (Agelaius phoeniceus)	Passeriformes	oscine	20	Experimental noise (low frequency white noise) and quiet control (silence)	quiet control recording	Increased tonality with an increased focus on lower frequency	No frequency shift	Increased tonality of trills	yes	yes	5-30 m	89 dB at 1 m	Treatments played until 3 to 7 songs were collected
(Potvin & Mulder, 2013)	Silvereye (Zosterops lateralis)	Passeriformes	oscine	45	Low-frequency (1-4kHz), high frequency (5-8kHz)	No Noise Stimuli	Lower minimums during high frequency noise (112.4 Hz)	Not provided	Increased duration, increased call dB	all three noise conditions played to each bird		N/A	65 dB at test distance	Not provided
(Ríos-Chelén et al. 2018)	Vermillion Flycatcher (Pyrocephalus obscurus)	Passeriformes	suboscine	12	One noise stimuli	Pre-noise and post-noise	No frequency shift	No frequency shift	N/A	yes	yes	N/A	Varied	Randomly selected about 4 bouts per treatment per male
(Gentry et al. 2017)	White-crowned Sparrow	Passeriformes	oscine	107	City noise (low), Inverse noise (high), and white noise	Pre-broadcast song recording	No frequency shift	Urban males decreased maximums	N/A	No, one per individual		5 - 17 m	78 Laeq at 1 m	2- 23 songs per male
(Derryberry et al. 2017)	White-crowned Sparrow (Zonotrichia leucophrys)	Passeriformes	oscine	16	Changes in background noise level at time of recording	Pre-noise and post-noise	No frequency shift	No frequency shift	Amplitude increase with noise above 48 dB	No	Varied but corrected for in model	Variable	Variable	28 songs (males n = 16)
(Cynx et al. 1998)	Zebra Finch (Taeniopygia guttata)	Passeriformes	oscine	6	6 Noise stimuli	No Noise Stimuli	Not provided	Not provided	Increase amplitude	Yes	N/A	N/A	60 to 90 dB in 5dB steps	>5
(Potvin & MacDougall-Shackleton, 2015)	Zebra Finch (Taeniopygia guttata)	Passeriformes	oscine	20	Traffic Noise	No Noise Stimuli	Lowered minimum frequency	No frequency shift	No	Yes	N/A	N/A	65 dB	Long term collection

(Schuster et al. 2012)	Tinamou (Eudromia elegans)	Tinamiformes	N/A	5	low amplitude noise, high amplitude noise (65 dB)	low amplitude noise (45 dB)	Increase peak frequency (161 Hz)	Not provided	Increased amplitude of calls (14 dB)	yes	Minimum of 30 cm	65 dB	12 calls per male per treatment
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Lack of obvious pattern of flexibility across phylogeny

The majority of immediate flexibility studies have focused on Passeriformes, the order of songbirds, however there are other bird orders that have shown evidence of immediate flexibility. In addition to the first study on Japanese quail described above, immediate flexibility has also been observed in domestic chickens (*Gallus gallus*, Order: Galliformes) and Mallards (*Anas platyrhynchos*, Order: Anseriformes) (Dorado-Correa, 2018), indicating these landfowl and waterfowl are immediately flexible in that they raise their amplitude and slightly raise their minimum frequencies in response to noise. Immediate flexibility has also been documented in Elegant crested tinamous (*Eudromia elegans*, Order: Tinamiformes). Tinamiformes is a sister group to the ratites, an early diverging lineage of large flightless birds like ostriches and rhea. The presence of amplitude alteration by species of these older lineages may suggest that immediate flexibility is not a newly evolved trait and may have been present as early as 119 MYA (Schuster et al, 2012). The presence of immediate flexibility in these basal bird species also makes the inability in other more recent lineages with more complex song surprising.

However, evidence of immediate flexibility within an order doesn't mean that all species within that order are capable. Within both suborders of Passeriformes (Passeri and Tyranni), there are species that are capable and those that appear incapable of this vocal plasticity. Oscines are a suborder of Passeriformes (Passeri), commonly referred to as songbirds. This taxon displays high vocal control and acquires song through imitative learning. Within this group, there are birds that display immediate flexibility such as white-crowned Sparrows (Derryberry et al, 2017; Gentry et al, 2017), European robins (Montague et al, 2013), black-capped chickadees (Goodwin & Podos, 2013).

On the other hand, suboscines, the other suborder of Passeriformes (Tyranni), do not learn their song and are thought to have less vocal control compared to oscines. Despite this, eastern wood pewees (*Contopus virens*), a suboscine species, were recorded increasing the minimum frequency in songs per site, by the immediate traffic noise level (Gentry et al, 2018). While this study did not directly test immediate flexibility, as it did not confirm the shift within individual, this site-based vocal plasticity may infer that suboscines could be immediate flexible. Vermillion flycatchers (*Pyrocephalus rubinus*) another suboscine, were not found to be immediately flexible (Ríos-Chelén et al, 2018), which follows more closely with what we may predict based on their lack of song learning. These variations in which species, populations, and in what way immediate flexibility is demonstrated emphasize the need for further study of this mechanism.

Differences along the urban gradient

There can also be differences in flexibility between populations of the same species. White-crowned sparrows appear to be capable of immediate flexibility within urban populations but not within rural populations (Derryberry et al, 2017). These differences in ability along an urban gradient opens more questions about what variation in ability we see within the same species. Black-capped chickadees were found to be more flexible with previous urban noise exposure (LaZerte et al, 2016). This finding suggests that flexibility could have a learned aspect to it and could explain the differences between urban and rural capabilities. This could mean that open-ended learners have a greater potential to be flexible or to learn to be flexible.

Differences in Response Type and Magnitude

Seven of the papers described studies that showed significant increases in minimum frequency, on average the effect size was 296.5 Hz. (Birds that shifted to higher frequency song types in their repertoire were excluded from this average). There was variation in how birds achieved this shift in frequency. Some birds exploited the natural variation in their song. In the presence of masking noise, these birds could switch to a higher song type rather than shifting a crystalized song upwards. One example in an early paper on immediate flexibility, great tits (*Parus major*) when exposed to city noise switched to higher frequency syllable types (Halfwerk & Slabbekoorn, 2009). This response was also observed in black-capped chickadees, where masking noise increased the speed that the birds switched to a higher non-masked song (Goodwin & Podos, 2013). Others species directly shifted their song minimum higher in frequency, such as the common reed bunting (+ 190 Hz shift) or the European robin (500+ Hz shift). But there is still the question of how much is enough of a frequency shift to be ecologically relevant, and why we see such a variation between species.

Other species lower their maximum frequencies rather than raise their minimum frequencies, which can concentrate their vocal energy into a narrower bandwidth and thus increase transmission. In anthropogenic noise, red-wing blackbirds (*Agelaius phoeniceus*) do not change their minimum frequency, however they concentrate more energy into their low-frequency notes (Hanna et al, 2011).

Differences in Methodology

While a few studies could conduct a natural experiment using active fluctuations in noise on site, while still measuring the same individual (ex: white crowned sparrows (Derryberry, et al, 2017)), most used noise playback, conducted on either wild singing birds or captive birds. Captive studies included the tinamou, domestic chicken, nightingale, vermilion flycatcher studies and more. The difference between in lab and on-site conditions may influence some of the differences measured, either by the proximity which can be achieved in captivity or by potential differences in bird stress level.

There are also noticeable differences in the noise stimuli and controls used in these studies. Most use silence or ‘no noise’ as their control, but not all report the average ambient sound pressure level of that silence. Relatively few go on to incorporate different types of noise (ex. high or low-frequency concentrated noise), to use a negative control. Instead, especially in the amplitude-focused papers, the stimuli used are white noise (broad bandwidth) at difference volumes. Even at high volumes (dB), white noise is not the best simulation of urban noise, which is low-frequency concentrated. This net of noise would not provide a “masking-free” acoustic space to use.

Debate over method of sampling

There is debate among researchers who study immediate flexibility as to whether observed shifts in frequency are due to a true shift or if it is merely a byproduct of increasing amplitude due to the Lombard effect. The Lombard effect describes how singing louder also results in a slight increase in frequency (pitch). In some cases,

however, it is also possible that these differences are due to the method in which the data was sampled. This is what is argued by Zollinger (2012) about the minimum frequency shifts seen in dark eyed juncos (*Junco hyemalis*) reported by Cardoso & Atwell (2011), should not have been accepted as evidence against the Lombard effect, due to the flaws with using the “by-eye” method. This methodology was also discussed by Brumm and Zollinger (2017), where they argue that false positives in frequency shifts can readily occur with inappropriate sampling technique. Relying on “eye-balling” minimum or maximum frequencies from a spectrogram introduced human error and bias based on the study hypothesis. This same technique was criticized by A. Ríos-Chelén et al (2016). However, despite recent criticism and community acceptance of the unreliability of frequency measurements taken visually from a spectrogram, many studies within the literature review (Table 1) either use this method or fail to state what method they used to measure frequency.

Testing Immediate Flexibility within Gray Catbirds

Given the background information in the previous section’s literature review above, my research question asks if the gray catbird (*Dumetella carolinensis*) is immediately flexible in their songs in response to noise, and if this ability varies across an urban gradient. The gray catbird provides a unique study species to investigate how individuals vary in their ability to communicate effectively in an urban landscape for multiple reasons. First, catbirds can be found breeding within urban areas as well as suburban backyards and rural forest edges, and the fact that they occupy this wide breadth of the urban gradient sets up a natural experiment. Second, while most species studied for their ability to be immediately flexible have short, stereotyped songs (e.g. Parids and

Passerellids) (Halfwerk & Slabbekoorn, 2009; Goodwin & Podos, 2013; Gentry et al., 2017), catbirds sing long song bouts with a large variable repertoire. Catbirds are members of the family Mimidae, meaning they mimic the songs of other birds or animals. This imitative ability, in addition to their ability to improvise and invent their own notes, makes gray catbird song highly variable between individuals and is used as part of a large repertoire of song elements (Fletcher and Smith, 1978). Third, gray catbirds are also open-ended learners, meaning they continue to learn throughout their lives (Catchpole & Slater, 2003). This learning program may enhance their ability to respond to the masking effects of low-frequency noise and allow them to be more flexible in their songs. Closed-ended learners like many species in the Passerellidae family, crystalize anywhere from a small repertoire to only one song type and sing this song type in a stereotyped manner for their lifetime (Catchpole & Slater, 2003), limiting their songs' potential flexibility. Catbirds' large song repertoire, use of mimicry, and continuous learning may give catbirds a greater potential for behavioral adaptation and flexibility to maximize signal effectiveness and minimize loss of quality.

To my knowledge, there are no other studies testing this mechanism – immediate flexibility in response to noise – in a vocal mimic. Few studies have even assessed if mimics sing differently in noisy versus quieter habitats. In a previous study conducted by the Moseley lab comparing the songs of catbirds along an urban gradient, Rhodes et al. (*unpublished data*, 2020) found that populations in noisier habitats sing with higher minimum frequencies than catbirds in rural populations. Another study compared male northern mockingbirds (*Mimus polyglottos*) by high-traffic roads to males within residential areas and found that an increase in noise had a positive correlation with

increased average peak frequency and an increase in peak frequency of the lowest syllable types. Though supporting the idea that vocal mimics have song differences between more and less urban locations, with their lowest notes being more influenced by anthropogenic noise, this prior study did not track changes within an individual's song during changes in noise amplitude, i.e., it did not test for immediate flexibility in this species.

Experiment and Hypothesis

I conducted a manipulative study to test whether catbirds can immediately alter their singing behavior in response to experimentally broadcast low- or high-frequency noise in real time along an urban-noise gradient. This type of noise broadcast experiment has been described and used in similar studies, such as Halfwerk & Slabbekoorn, (2009), Gentry et al (2017), and Verzijden et al. (2010), and is a modified version of a traditional playback experiment in which a noise stimulus is played at a targeted individual to observe their response. The low-frequency noise stimulus was used to simulate the frequency profile of anthropogenic noise, and the high-frequency noise stimulus was used as a negative control to account for a potential Lombard effect, as the catbird would not be likely to raise their minimum frequencies in response to high-frequency noise except as a byproduct of singing with higher intensity (Brumm & Zollinger, 2011).

I hypothesized that the frequencies of male catbird song are influenced by the immediate noise level in their environment, and that male gray catbirds can immediately alter their minimum frequencies in response to anthropogenic masking noise. I predicted that in response to broadcasted low-frequency noise, catbirds would shift their minimum

frequency higher compared to pre-noise exposure singing. Additionally, I predicted that catbirds would either maintain their minimum frequencies during high-frequency stimuli, and potentially lower their maximum frequencies away from the high-frequency noise. Finally, I predicted that this ability may vary across an urban gradient as has been found in previous studies (Gentry et al, 2017). To measure frequencies accurately, I used two sampling methods from two different sound analysis programs; one using the peak frequency contour (PFC) tool in Raven Pro to measure all elements, and second, the peak amplitude threshold (PAT) method in Signal 5 to measure the very lowest minimum frequency of a ten-second bout of song. Importantly, I'm not evaluating these two methods for accuracy or precision but rather using them to efficiently measure different aspects of catbird song.

Methods

Study Species

The gray catbird, *Dumetella carolinensis*, is a common migratory bird that ranges across eastern North America. Gray catbirds employ a complex song using mimicked, improvised, and invented sounds (Fletcher and Smith, 1978). Male catbirds are the most vocally active during their breeding season, which spans from April to July. During this time, males sing to attract females to establish territories against rival males (Borror, 1961; Catchpole, 1980). Catbirds can be found from more rural forest edges to urban environments and as a result are often exposed to human-generated, low-frequency noise that can overlap with the frequency of their song.

Study Sites

I sampled catbird ambient song and conducted a noise-playback experiment at 3 sites in DC (Wheat Regional Park, 39.05971, -77.0392; -77.0607; Smithsonian National Zoo, 38.92957, -77.0498; Opal Daniels Park, 38.98164, -77.0048;) and from 6 sites in Northwestern Virginia. (Bells Lane, 38.166, -79.035; Crusher Farm, 38.332744, -78.8312; Westover Park, 38.44939, -78.8826; James Madison University Arboretum, 38.429, -78.8629; Longview Oaks Apartments, 38.429, -78.848; and Purcell Park, 38.430, -78.881).

Sites were selected first based on the presence of breeding catbirds and then for their representation of the urban gradient. Most critically for this study, we measured the average noise level of a site to represent its level of urbanization. In a previous study in the Moseley Lab, the appropriate category was determined using a PCA of noise level,

percent of impervious surfaces and the percentage of tree cover surrounding the site (Rhodes thesis, 2020), but for this study I am only using the noise level. To ease identifying individuals, birds were marked with 3 color bands and one USGS aluminum band. However, banded and unbanded males were both used in this study. When recording unbanded birds, the males were first observed in order to identify an approximate territory with singing perches and nest sites, when possible. Additionally, I selected focal males from non-adjacent territories to avoid recording the same male twice if the neighboring males were both unbanded. No trials were performed on males on days that they were handled or banded.

Table 2. Table of sites, state, urbanization category, average noise level and number of males per site.

<i>Site</i>	<i>State</i>	<i>Urbanization Category</i>	Average Noise (dB)	N Males
Wheaton Regional Park	DC	Rural	52.5	2
Bells Lane	VA	Rural	35	4
Crusher Run	VA	Rural	48.7	3
Opal Daniels Park	DC	Suburban	49.7	1
Longview Oaks Apartments	VA	Suburban	46.3	1
Purcell Park	VA	Suburban	34	1
Smithsonian National Zoo	DC	Urban	56.9	1
JMU Arboretum	VA	Urban	58.7	3
Westover Park	VA	Urban	52.5	1

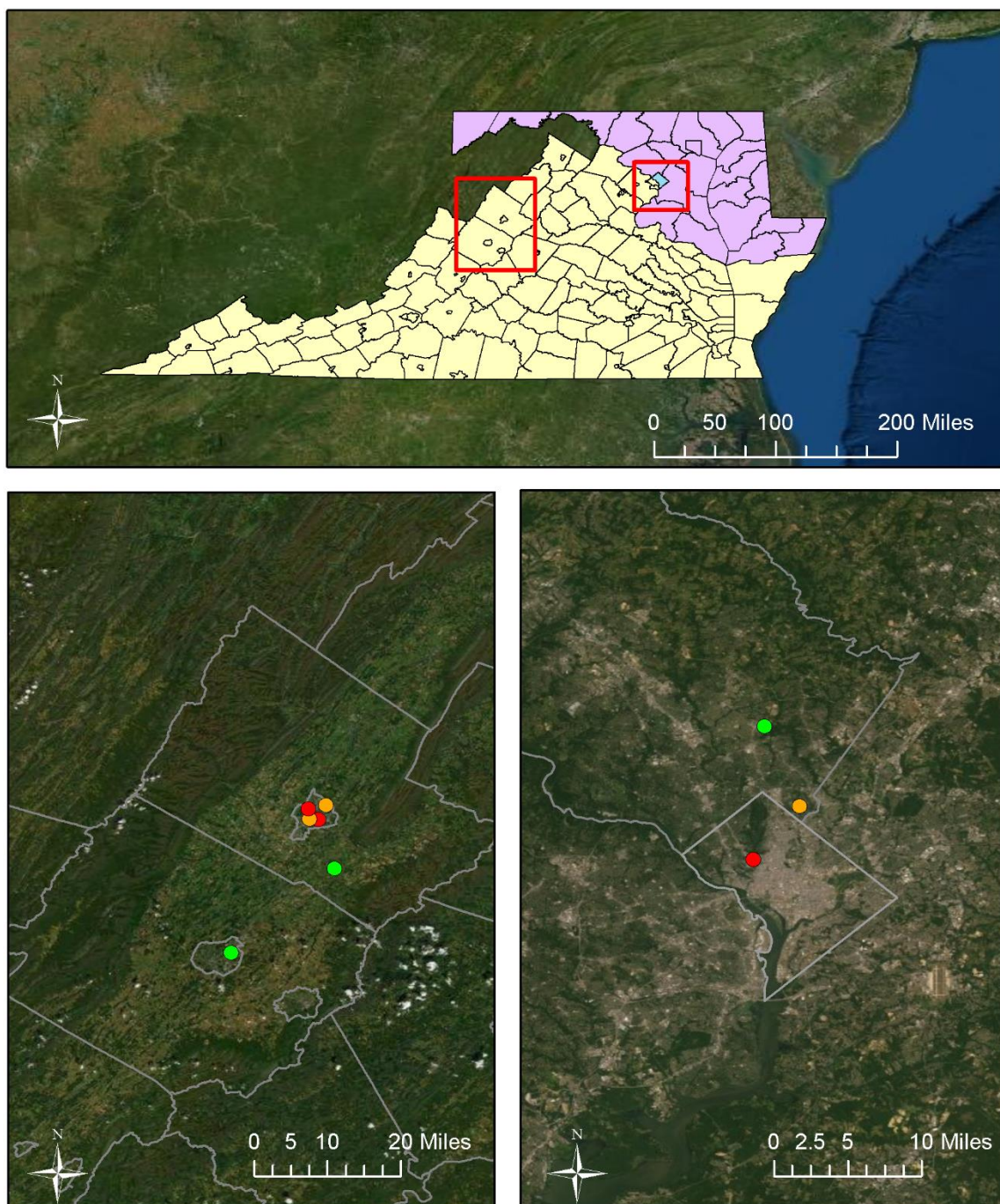
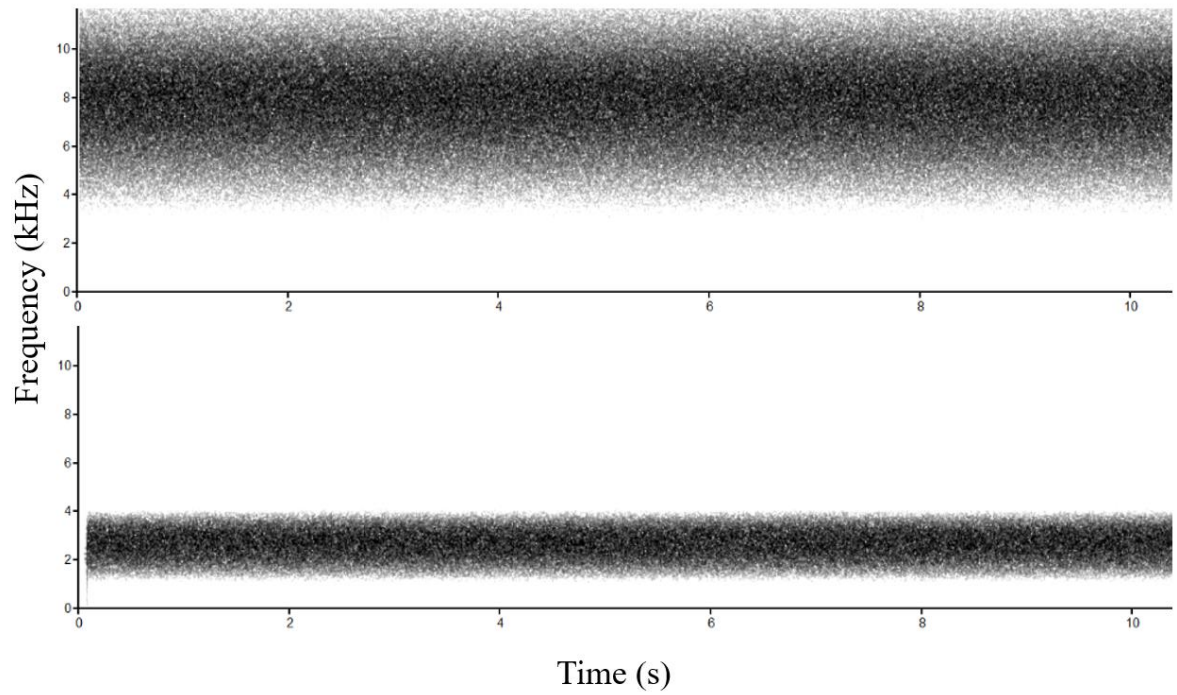


Figure 1. A map of the 9 sites used in this study categorized by their level of urbanization (Urban, red; suburban, orange; rural, green).

Noise Playback Stimuli

I created two noise stimuli using the white noise generator function in the program Audacity (v. 2.3.2). For the low-frequency stimulus, I generated noise between 1-3.5 kHz to use as the anthropogenic masking noise, and for the high-frequency stimulus, I generated noise between 6-10 kHz to use as a negative control as it was above the peak frequency of much of catbird song though it overlapped with elements between 6-9.5 kHz. Each playback stimulus included 3 minutes of noise stimulus preceded by 10 seconds of silence to prevent premature noise exposure due to false starts. The amplitude of each stimulus was set to achieve 85 dB measured at 1 m in the lab.

a)



b)

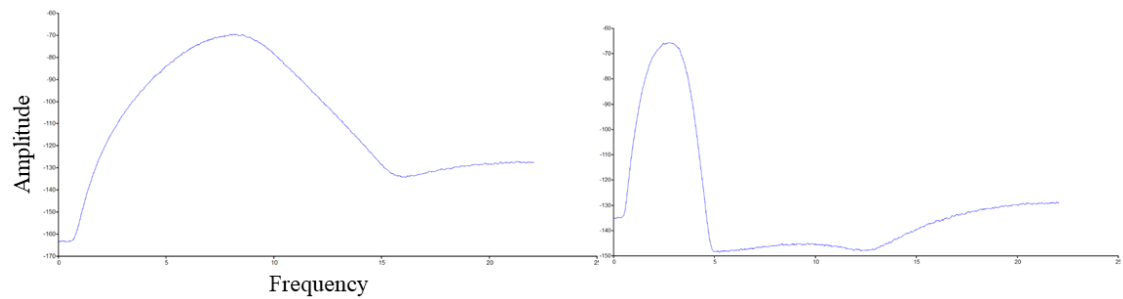


Figure 2. Visualization of noise stimuli used in noise playback: a) Spectrograms (frequency vs time) and b) amplitude spectra (amplitude versus frequency) of simulated and high-frequency control noise (top, left) and urban, low-frequency noise (bottom, right).

Playback Experimental Protocol

Trials were conducted between late May 2019 - July 5th in 2019 and June 20th, 2020- July 14th in 2020, during the gray catbird breeding seasons, between the hours of 5:30- 14:00. The majority of trials were completed prior to 10:00, but a liberal window was allowed for opportunistic recording. Before each trial I located an actively singing male catbird and placed our field speaker within 5 to 20 m of the target male.

I used two speakers, SME-AFS Amplified Field Speaker and a JBL Flip 5 for the SME-AFS Amplified Field Speaker, the volume was set level to achieve 85 dB at 1 m based on testing in the lab prior to site visits. Due to the specs of the JBL Flip 5 speaker, the max volume achieved a noise level of 80 dB at 1m, based on measurements in the lab. Given the open habitats and the varying distances to the focal males, we do not think this volume would have contributed to a substantial difference in exposure. The speaker was connected via a 10m 1/4 to 1/8 inch audio cable to an Apple iPod Nano (A1320) or Apple 32GB iPod touch (7th Generation, product number: PKHV2LL/A). Before playing the stimuli, an ambient noise measurement was taken in the field at the speaker placement using a SPL-meter (Galaxy Audio CM-170 IEC 61672-1 Type II). Due to equipment malfunction, 8 trials completed after July 8, 2020 were completed using the JBL Flip 5 (speaker which was connected to the iPod Touch) by Bluetooth wireless.

As a control I recorded males for a minimum of 3 mins prior to any experimental noise exposure (pre-noise). The experimental noise broadcast then commenced, and the target male was presented with one of the two noise broadcast tracks (low-frequency or high-frequency) for 3 minutes. After the initial broadcast, we allowed for a minimum of 2

minutes of silence to serve as a “cool down period” before presenting the same target male with the alternative noise stimulus. The order of the stimuli (High/Low, or Low/High) was first selected randomly and then alternated with subsequent noise broadcasts to achieve a balanced design. During the 2019 field season all noise broadcasts (high and low tracks) were completed within a 3-hour window to limit changes in song based on other factors such as nesting status. Due to the limited time available to obtain songs during 2020, this window was expanded to allow trials to be completed within a maximum of 48 hours from the first recording.

Two observers recorded catbird song during the trials and dictations prior to and after the trial – one observer used parabolic microphone (Stith Telinga Universal MK2 parabola with omni-directional Sennheiser ME62 microphone), and the other used a Sennheiser ME66 directional microphone, each of which were connected to Marantz digital recorders (PMD561) with XLR cables. A lapel microphone and smartphones (iPhone 6s) were used to capture the dictation from the observers while catbirds were singing. Song recordings were saved as uncompressed WAV files, at a 44.1 kHz sampling rate, additional dictation recordings were saved as MP3 files for distance references. In addition to recording song, observers dictated the changes in distance from the bird to the speaker, noting when the bird flew out of the 20m range.

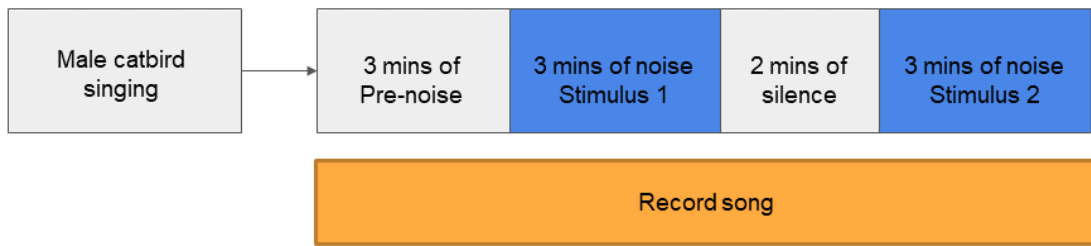


Figure 3. Timeline of noise broadcast. Actively singing males were recorded for an attempted three minutes, the one of the two noise stimuli (low- or high-frequency noise) would be played for three minutes. A “cool down” period of at least two minutes would be given before proceeding to the alternate stimuli.

Song Processing

The program Raven (v. 1.5) was used for sound analysis. To limit low and high frequency environmental noise outside the range of catbird song, all audio files were filtered using the Band Pass Filter tool to isolate the range of 1000-9500 Hz. After filtering, if the files had a good quality signal:noise ratio (meaning that the bird song was sufficiently louder than the environmental noise) based on the waveform, then I proceeded to process the control and trial files within Raven. While multiple males were excluded if they did not successfully receive all 3 trial periods, only one male was excluded because of poor quality of recording.

First, the bouts or periods of song were identified. If the catbird paused for one or more seconds, then the bout was considered complete and a new bout would start when the bird resumed. Within these bouts, I then selected individual components, or

“elements”. The start and stop time were marked within each trial and each element was identified as being either “pre-noise”, during the low-frequency “Low”, during the high-frequency “High”, or “Post-stimulus”. For each element, minimum, maximum, and peak frequency measurements were generated. Minimum and maximum frequencies obtained for each element using the Peak Frequency Contour feature within Raven. The tool tracks the peak frequency (pitch) over the course of an element, then selects either the minimum or maximum frequency to report out.

Table 3. Definitions of sound parameters taken in the Raven Pro sound analysis program.

Sound Parameter	Definition	Units
Minimum PFC	The minimum frequency identified from the Peak Frequency Contour tool in Raven Pro. The Peak frequency contour tool breaks a spectrograph selection into time bin slices and determines the peak frequency per each slice but using a contour to predict the slope of the sounds. From this series of peaks, the lowest peak within a selection is determined.	(Hz)
Peak Frequency	The frequency with the most energy/highest amplitude.	(Hz)
Maximum PFC	The highest frequency identified from the Peak Frequency Contour tool. See description of PFC	(Hz)

The amount/duration of elements during the noise broadcast was used to determine if a trial was successful. For preliminary analysis, a male needed to have a minimum of 5 seconds of singing, not counting the silence between elements, as a “floor” (a minimum for inclusion in analysis). For some males with proliferate song, I stopped sampling after a “ceiling” of 100 elements. For the trials that reached 100 elements, I

obtained a 1:1 ratio of Pre:During stimulus song selections. For trials with fewer than the ceiling in the total bird song, I obtained a 2:1 ratio of Pre:During stimulus selections.

Signal 5

In order to process song using the amplitude threshold method using Signal 5, I selected song recordings from each trial - pre-noise, during high-frequency, and during low-frequency playbacks in ten-second intervals (± 2 seconds) based on natural breaks in song. Additionally, I also collected a background noise sample for each track, during a period in which the focal male was not singing. For each ten-second interval, I measured peak and minimum frequencies using the amplitude power spectrum threshold method (Brumm & Zollinger, 2017; Podos 1997). A power spectrum is a visualization of the amplitude across the frequency range, and displays where the energy (loudness/amplitude) is distributed. I set a threshold of 12 dB under the peak to account for background noise in the recordings, and measured where the line of the frequency graph intersected with this -12dB threshold line at the lowest end to accurately measure minimum frequency of the birdsong over noise. I determined the frequencies from three ten-second clips for each experimental condition (pre-stimulus, low-frequency stimulus, and high-frequency stimulus) for each male. When possible, for the songs selected during noise playback, I selected one clip early, one clip in the middle and one clip late in the trial. If the male was not singing consistently throughout the trial, I selected sections with the best signal to noise ratio, favoring clips in the latter half of the trial.

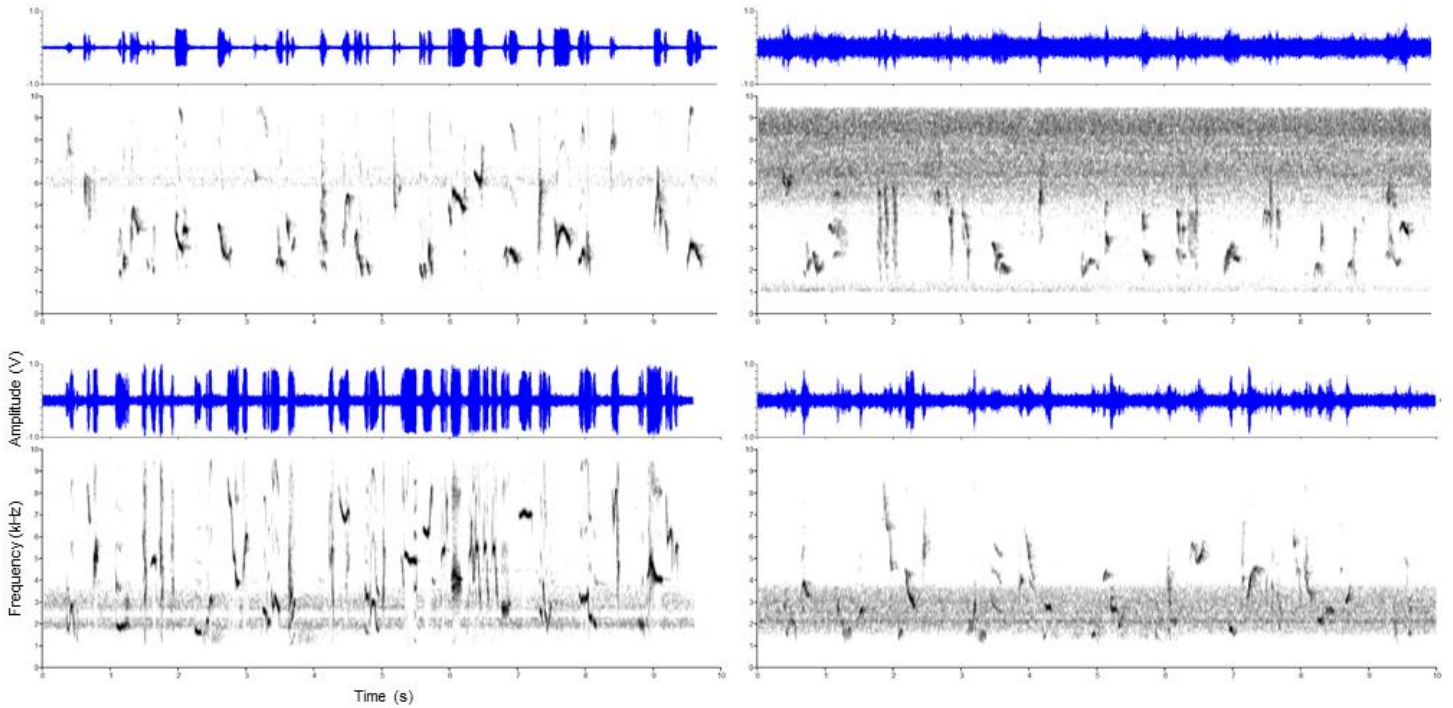


Figure 4. Four examples of catbird records used in analysis. The top two panels are song recordings taken during high-frequency stimulus, and the bottom two panels are examples from the low-frequency stimulus. The two images on the left are examples of high-quality clips, whereas the two on the right are lower quality but still deemed usable. Within each of the four quadrants, the blue waveform denotes the amplitude (V) throughout the recordings, and a spectrogram below depicting the frequencies (Hz).

Statistical Analysis

I conducted all statistical analyses using the program R version 3.6.2. Data from selections taken in Raven were imported into R, and song parameter averages (minimum frequency, peak frequency and maximum frequency) were obtained per male. I used one-

factor repeated-measures ANOVA. Male identity was incorporated as a the repeated-measures aspect in the ANOVA as each male received all three trials (pre, low, and high). For the post-hoc analysis of pair-wise tests of groups, in lieu of a Tukey's HSD test, I used paired t-tests because each male received all three treatments, which makes a Tukey's test difficult to run in R.

Catbirds were unlikely to shift notes with minimum frequencies already above 3,500 kHz as they would have already avoided the low-frequency stimulus (1000-3500 Hz), and alternatively they may not shift notes already below 6kHz which avoid interference from the high-frequency stimulus (6000-10000 Hz). Therefore, I filtered elements into the following groups: Elements with *minimum* PFC equal or less than 3500 Hz and elements with *maximum* PFC equal or above 6000 Hz.

Table 4. The song parameter, frequency range, and total number of elements per subsections.

Measurement	Frequency Range	Number of Elements
All Elements	0 to 9500	12444
Minimum Frequency	≤ 3500 Hz	9764
Maximum Frequency	≥ 6000 Hz	2711

Table 5. Table of the average elements measured per male per treatment group (mean \pm SEM = 191.5 \pm 2.4, min =17, max = 500)

Treatment	Min N of Elements	Max N of Elements	Mean N of Elements
Pre-Noise	65	295	192.4
Low-Frequency	39	465	195.1
High-Frequency	17	500	187.9

Linear Models

To test my hypothesis that catbird's ability to be immediately flexible would differ along the urban gradient, I ran three generalized linear mixed models (GLMM) on three outcome variables: 1) the average minimum frequency of elements under 3500 Hz in Raven, 2) the lowest minimum frequency data measured in Signal, and 3) maximum frequency over 6000 Hz in Raven. I used a random effects model using maximum likelihood testing the noise-level of the habitat and treatment groups as fixed effects with male identity as a random effect on each of the three outcome variables.

Results

Minimum Frequency Analyses: Peak Frequency Contour (PFC) Analysis in Raven

An analysis using the PFC tool in Raven Pro and analyzing song elements with minimum frequencies at or below 3,500Hz showed that males shifted their average minimum frequencies higher between pre-noise and during low-frequency noise stimuli (from here I will refer to these as pre, low, and high treatments). Average song minimum frequency was significantly different between treatment groups in a repeated-measures single-factor ANOVA (Fig. 5, $n = 17$, $df = 2$, $F = 6.654$, $p = 0.00383$), with songs recorded during the low-frequency noise trial and pre-noise differing significantly in a post-hoc paired t-test ($\beta_{\text{low-pre}} = 73.3$ $t = 1.9378$, $df = 30.122$, $p\text{-value} = 0.003923$). The low treatment mean minimum frequency was significantly greater than that in the high frequency treatment (Fig. 5, $t = 2.792$, $df = 16$, $p\text{-value} = 0.01305$). Post hoc analysis showed no significant difference between pre and high treatments (Fig. 5, $df = 16$, $t = 0.13186$, $p\text{-value} = 0.8967$). The overall pre versus low treatment effect size was 73.3 Hz, and low versus high was 76.17 Hz.

A GLMM random effects model comparing minimum frequency to treatment and to average noise level of each bird's habitat showed that minimum frequencies during low-frequency noise treatments were significantly higher than during pre-noise ($n = 17$, $df = 32.000$, $t = 3.098$, $p = 0.00404$). The effect size of low versus pre was 73.338 Hz. minimum frequencies were also significantly different between low and high treatments (Fig. 6, $n=17$, $df = 32$, $t = 3.218$, $p = 0.00296$), with an effect size of 76.175 Hz. The average noise level at the site was not a significant predictor of minimum frequency ($n=17$, $df = 15.000$, $t = 1.397$ $p = 0.18287$), but all lines showed positive slopes. While not

significant, birds in more urban, noisy habitats, however, shifted their average minimum frequencies the greatest amount between pre and low treatments, with minimum frequencies for the noisiest habitats higher than pre-noise by an average of 166.792 Hz (Fig. 6).

Analysis in Signal 5

When analyzing catbird songs in the sound analysis program Signal 5 using the threshold method, I measured the lowest frequency per each 10-sec bout of song – this method is different than averaging the minimum frequency of every element of a song in Raven Pro. For minimum frequencies measured with the threshold method, there were significant differences based on treatment, but in a direction against my prediction. (Fig. 7, $df = 2$, $t = 3.521$, $p = 0.0415$). The difference between low and pre-noise treatments, however, was not significant in a post-hoc test ($t = -1.3592$, $df = 16$, $p\text{-value} = 0.1929$), only the difference between low and high treatments was significant (Fig. 7, $t = 2.9398$, $df = 16$, $p\text{-value} = 0.009613$), with minimum frequencies lower during the low-frequency noise treatment as compared to high.

The generalized linear mixed model comparing minimum frequency measured by the threshold method to the average noise level at sites showed that the lowest minimum frequencies during low were not different than the pre-noise (Fig. 8, $df = 32.000$, $t = -1.342$, $p = 0.189$). Low and high treatments were significantly different with minimum frequencies higher with high treatment, while there were no significant differences between either the high or low treatments compared to pre-noise. (Fig. 8, $df = 32.000$, $t = -2.654$, $p = 0.0123$, $\beta_{\text{high-low}} = -95.374$ Hz). The average noise level at the sites was a

significant predictor of minimum frequency across treatments, with a positive slope of increasing minimum frequencies as noise level increased (Fig. 8 $df = 15.000$, $t = 2.248$, $p = 0.040$).

Maximum Frequency Analysis

Maximum frequency measured in Raven was significantly different between treatments in a repeated-measures single-factor ANOVA (Fig. 9. $n = 16$, $df = 2$, $F = 7.32$, $p = 0.00258$). Maximum frequencies were lower during high-frequency noise compared to pre-noise (Fig. 9, $df = 30.00$, $t = -3.586$, $p = 0.00117$, $\beta_{\text{High-pre}} = -260.95$), and compared to during low-frequency noise ($\beta_{\text{high-low}} = -214.55$). But there was not a significant difference in maximum frequencies low versus pre-noise ($\beta_{\text{low-pre}} = -46.40$ Hz). Average site noise level was also a reliable predictor of maximum frequency, with max frequency increasing with noise level (Fig 10, $\beta = 12.76$, $df = 14.00$, $t = 3.230$, $p = 0.00605$).

Table 6. Table of generalized linear mixed model outputs for minimum frequency (PFC and PAT) and maximum frequency data incorporating average noise level (dB).

1. Summary of GLMM random effects model using PFC minimum frequency measurements in Raven Pro.					
Fixed effects	Estimate	Standard Error	Degrees of freedom	t-value	p-value
Intercept	1908.244	130.367	15.334	14.637	2.01e-10 ***
Low vs Pre-noise	73.338	23.675	32.000	3.098	0.00404**
High vs Pre-noise	-2.837	23.675	32.000	0.120	0.90538
Average Noise	3.732	2.672	15.000	1.397	0.18287
2. Summary of GLMM random effects model using Signal 5 minimum frequency measurements.					
Fixed effects	Estimate	Standard Error	Degrees of freedom	t-value	p-value
Intercept	1486.138	102.594	16.293	14.486	9.94e-11***
Low vs Pre-noise	-48.241	35.942	32.000	-1.342	0.189
High vs Pre-Noise	47.133	35.942	32.000	1.311	0.199
Average Noise	4.656	2.071	15.000	2.248	0.040*
3. Summary of GLMM random effects model using PFC maximum frequency measurements.					
Fixed effects	Estimate	Standard Error	Degrees of freedom	t-value	p-value
Intercept	6773.16	199.02	15.32	34.033	7.43e-16 ***
Low vs Pre-Noise	-46.40	72.77	30.00	-0.638	0.52852
High vs Pre-Noise	-260.95	72.77	30.00	-3.586	0.00117 **
Average Noise	12.76	3.95	14.00	3.230	0.00605 **

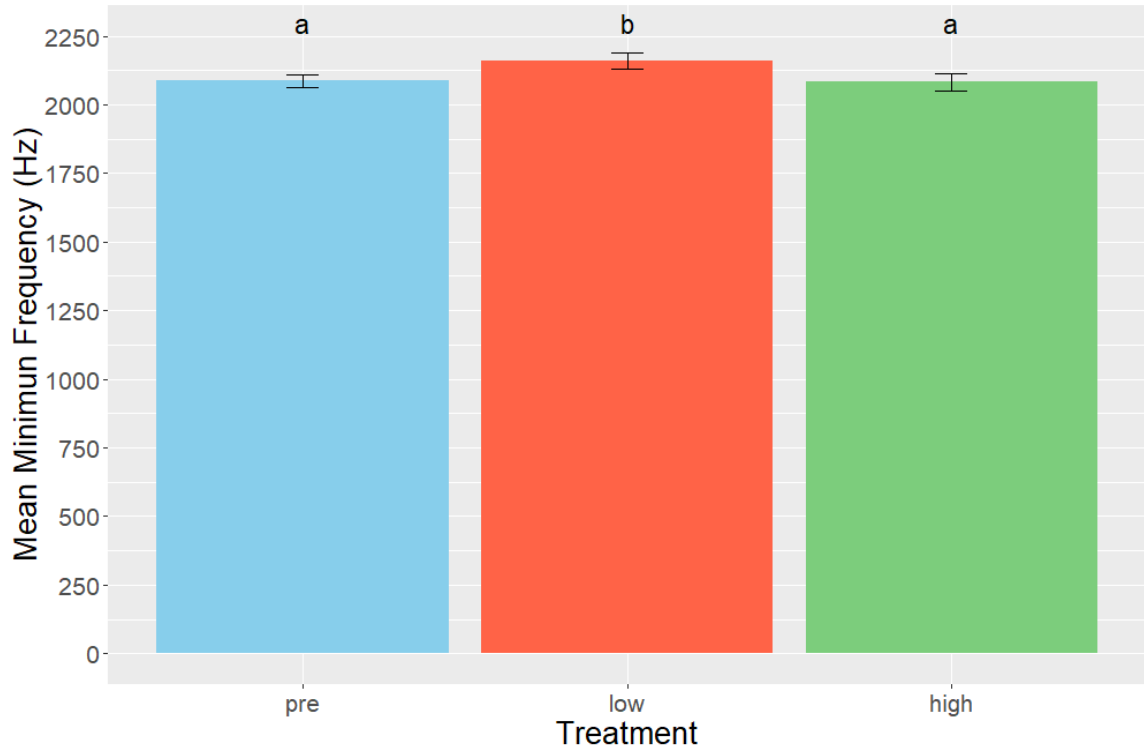


Figure 5. Minimum frequencies (Hz) measured with Raven Pro's peak frequency contour tool of song during the pre-noise playback, low-frequency noise, and high-frequency noise for elements with minimums under 3,500 Hz. Bars represent the mean \pm standard error of the mean (SEM) for $n = 17$ males. Treatment groups differed significantly (ANOVA, $df = 2$, $F = 6.654$, $p = 0.00383$), with minimum frequencies of low-pre ($\beta_{\text{low-pre}} = 73.3$, $t = 1.9378$, $df = 30.122$, $p\text{-value} = 0.003923$), low-high ($\beta_{\text{low-high}} = 76.17$, $t = 2.792$, $df = 16$, $p\text{-value} = 0.01305$) and high-pre ($p > 0.05$).

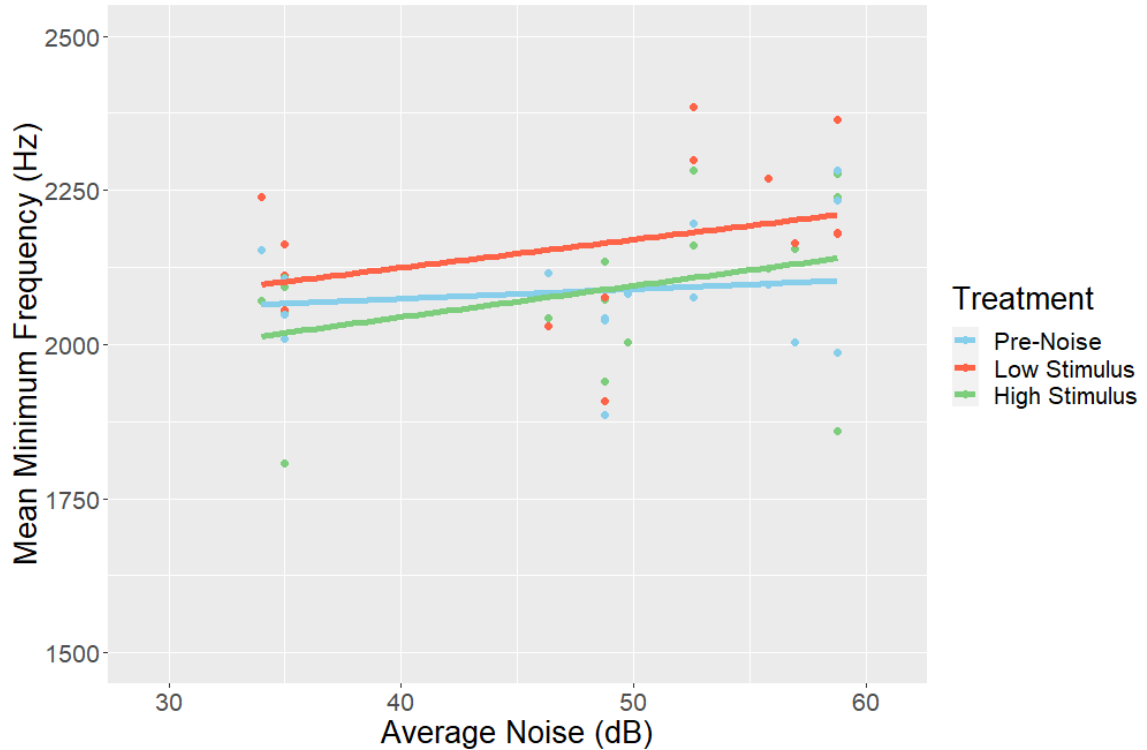


Figure 6. Plot of average minimum frequencies measured with the PFC tool in Raven during pre-noise, low-frequency noise, and high-frequency noise trials against the average noise level per site. Best-fit lines are shown per treatment. Treatment groups differed significantly (ANOVA, $n = 17$, $df = 2$, $F = 6.654$, $p = 0.00383$); low-pre (GLMM, $df = 32.000$, $t = 3.098$, $p = 0.00404$), low-high (GLMM, $df = 32$, $t = 3.218$, $p = 0.00296$), and high-pre (GLMM, $p > 0.05$). Noise (dB) was not a significant predictor of minimum frequency (GLMM, $p > 0.05$) but all slopes were positive.

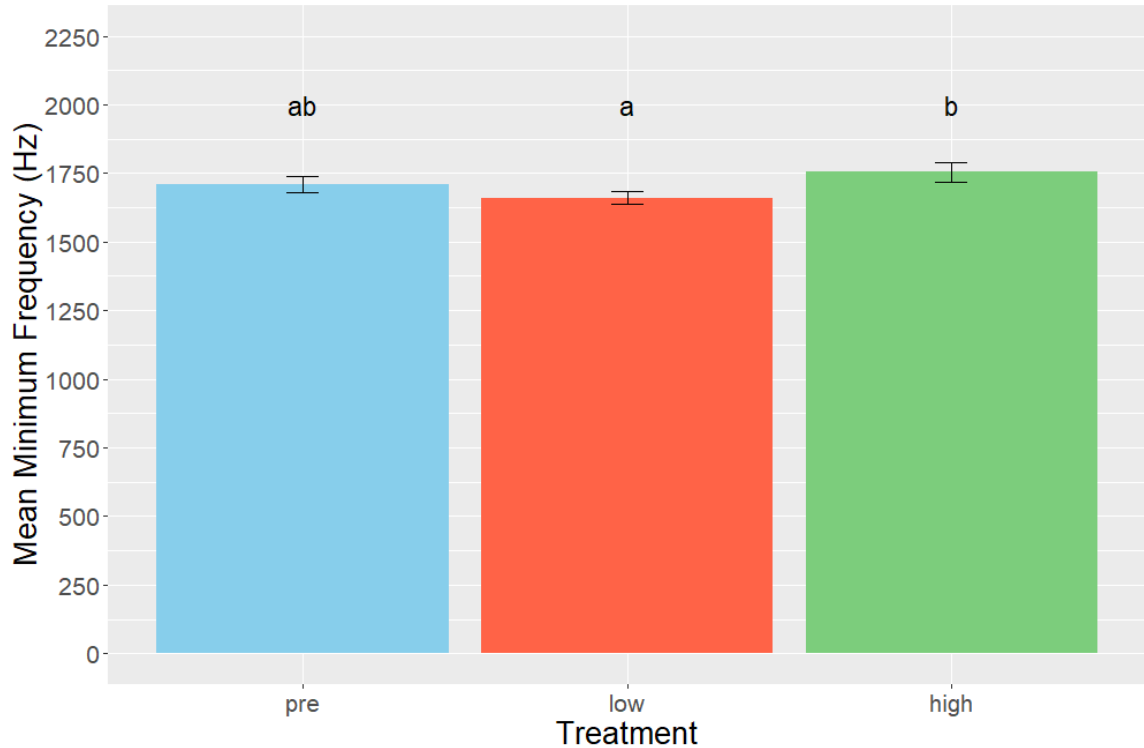


Figure 7. Minimum frequencies (Hz) measured using the amplitude threshold method in Signal 5, during the pre-noise playback, low-frequency noise, and high-frequency noise. Bars represent the mean \pm SEM of minimum frequency in Hz measured per three 10-sec song bouts averaged per male ($N = 17$). Treatment groups differed significantly (ANOVA, $df = 2$, $t = 3.521$, $p = 0.0415$) but only between low and high treatments ($t = 2.9398$, $df = 16$, $p\text{-value} = 0.009613$). Low-pre ($p > 0.05$), high-pre ($p > 0.05$).

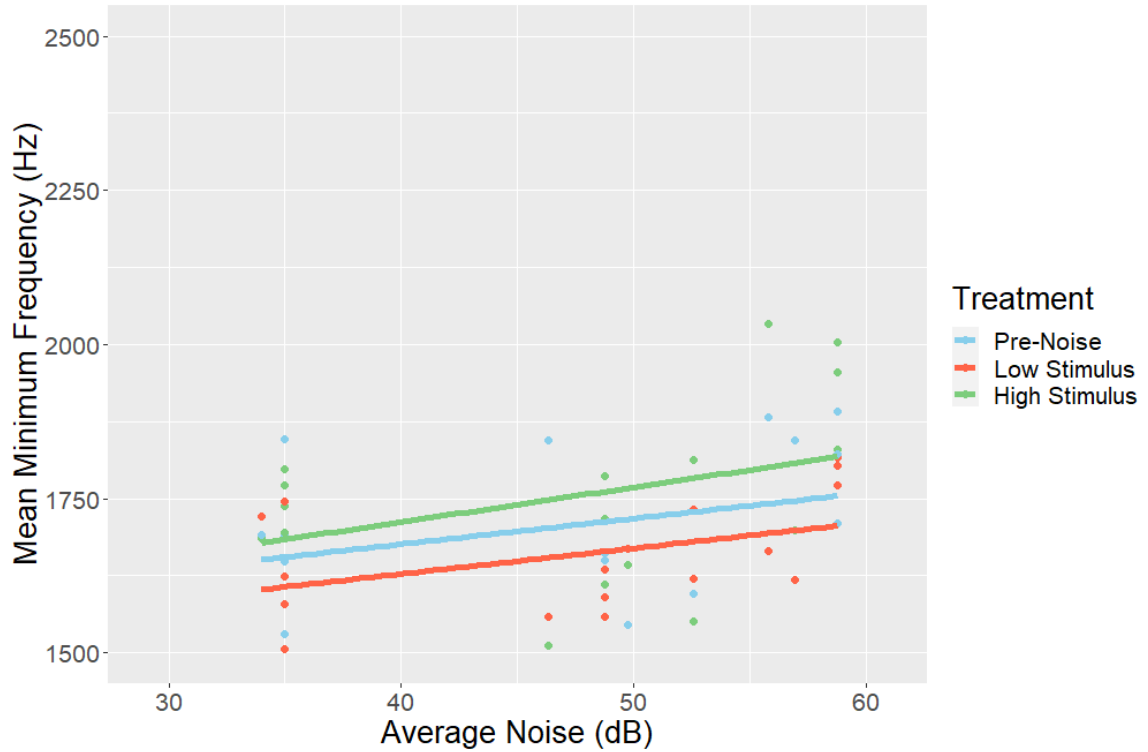


Figure 8. Plot of average minimum frequencies measured per three 10-sec song bouts averaged per male using the amplitude threshold technique in Signal during the pre-experiment song, high-frequency, and low-frequency noise playback stimuli against average noise level per site. Best-fit lines are shown per treatment. Treatments were significantly different per group (ANOVA, $n = 17$, $df = 2$, $t = 3.521$, $p = 0.0415$). GLMM showed high-low to be significant (GLMM, $n = 17$, $df = 32.000$, $t = -2.654$, $p = 0.0123$, $\beta_{\text{high-low}} = -95.374$ Hz), whereas low-pre and high-pre were not significant (GLMM, $p > 0.05$). Noise (dB) was a significant predictor of minimum frequency (GLMM, $p = 0.040$).

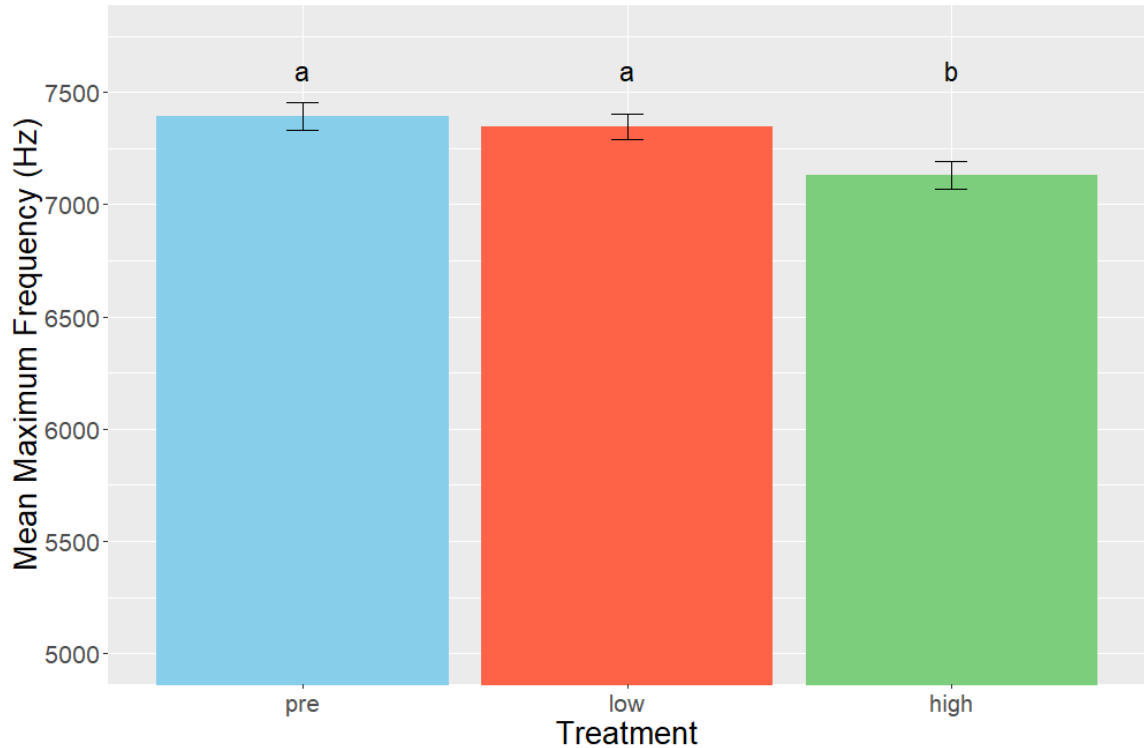


Figure 9. Maximum frequencies (Hz) measured with Raven Pro's peak frequency contour tool of song during the pre-playback, low-frequency noise, and high-frequency noise for elements with maximum PFC above 6,000 Hz. Bars represent the mean \pm SEM for $n = 16$ males. Treatment groups differed significantly (ANOVA, $n = 16$, $df = 2$, $F = 7.32$, $p = 0.00258$); mean maximum frequency was significantly lower during high-frequency noise compared to pre-noise ($df = 30.00$, $t = -3.586$, $p = 0.00117$, $\beta_{\text{High-pre}} = -260.95$), as well as in high-frequency compared to low-frequency (GLMM, $\beta_{\text{high-low}} = -214.55$, $n = 16$, $df = 30.00$, $t = 2.948$, $p = 0.00613$). High- pre ($p > 0.05$) and low-pre ($p > 0.05$) were not significantly different.

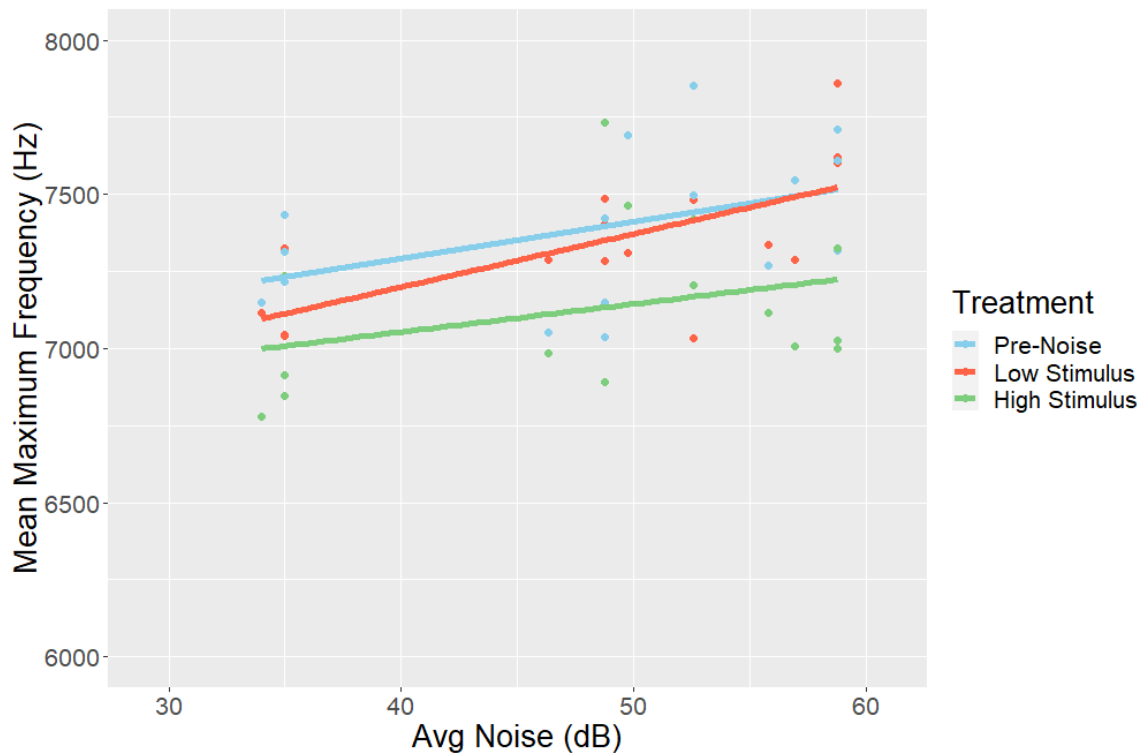


Figure 10. Plot of average maximum frequencies during pre-noise playback and playback of low- and high-frequency noise trials against the average noise level per site. Best-fit lines shown per treatment. In a GLMM, maximum frequencies were significantly low during high-frequency noise and pre-noise (GLMM, $n = 16$, $df = 30.00$, $t = -3.586$, $p = 0.00117$, $\beta_{\text{High-pre}} = -260.95$), as well as in high-frequency compared to low-frequency (GLMM, $\beta_{\text{high-low}} = -214.55$, $n = 16$, $df = 30.00$, $t = 2.948$, $p = 0.00613$). Average site noise level was also a reliable predictor of maximum frequency, with max frequency increasing with noise level (Fig 10, $\beta = 12.76$, $df = 14.00$, $t = 3.230$, $p = 0.00605$).

Discussion

I hypothesized that gray catbirds would have the ability to be immediately flexible in response to noise and would shift their song higher in real time during the presence of anthropogenic noise in their environment. While my results show a significant increase in minimum frequency during low-frequency noise playback, the effect size was smaller than the size of measurable error in frequency differences. The direction of the shift in one analysis aligned with my prediction, but another analysis revealed that catbirds do not shift their very lowest frequencies – together, leading to the conclusion that catbirds are not immediately flexible in response to low-frequency anthropogenic noise. However, my analysis did reveal an unexpected result – gray catbirds lower maximum frequencies when exposed to high-frequency noise playback.

Measuring minimum frequencies of all elements with the peak frequency contour tool in Raven Pro, showed evidence that catbirds may be capable of immediate flexibility, but at a small effect size. Male catbirds had significantly higher minimum frequencies during low-frequency anthropogenic noise compared to pre-noise exposure. These shifts in minimum frequency during low-frequency anthropogenic noise were also significantly greater than the slight shift upwards during high-frequency noise. However, the effect size was incredibly small and at 73.3 Hz, the difference is technically smaller than the frequency-measurement error using the sampling rate and window size settings in Raven Pro's spectrograph settings. Moreover, the difference in effect sizes between low-pre (73 Hz) versus high-pre (-2 Hz), though significantly different are still too small to rule out raising minimum frequency as by-product of singing with greater amplitude as suggested by the Lombard effect. This pattern was confirmed in the GLMM

random-effect model. The average noise of each focal male's habitat (which increases with urbanization) had a positive relationship with the minimum frequencies in song, but this effect was not significant, nor did it appear to differ between treatment groups as all slopes showed a tendency to be positive. In other words, males in noisier habitats tended to sing with higher minimum frequencies but males did not differ in their responses to noise-treatment based on habitat. This contrasts with what we see in white-crowned sparrows, where males in urban areas significantly shifted their frequencies whereas their rural counterparts did not (Gentry et al, 2017). Likewise, in a study by LaZerte et al (2016) only black-capped chickadees at already noisy sites immediately shifted songs higher, but those in urban areas did not show the same flexibility. In contrast, this study testing the effects of both noise-playback and the noise level of the habitats showed a greater difference in minimum frequencies between pre-noise and low-frequency noise with increased urbanization. This difference was not statistically significant potentially because of the small sample of very urban males ($n = 2$). Which may have greater minimum frequency shifts with their previous exposure to noise, as has been observed in previous studies (e.g. Gentry et al., 2017; LaZerte et al., 2016).

Using the peak amplitude threshold method, however, I found that minimum frequencies during low-frequency noise were slightly but not significantly lower from pre-noise exposure, a finding which did not support my prediction. Minimum frequencies during low-frequency noise were significantly lower than during high-frequency noise treatments, a trend also opposing my prediction. The findings of the amplitude threshold analysis conflict with the findings of the PFC method. The different results observed between the Raven and Signal analyses may be attributed to inherent differences between

these two sampling techniques. Within Raven, minimum and maximum frequency values are obtained for each and every individual note within the song. Therefore, the average using the PFC minimum tool would generate a much higher value for minimum frequency than the absolute lowest frequency as measured with the threshold method in Signal. Specifically, when using the threshold method, I measured the overall lowest minimum frequency per approximately ten seconds of song. It may be that the catbirds sing a higher proportion of higher frequency notes, but did not eliminate their lowest frequency notes from their song. If that is the case, then a technique like the peak amplitude threshold method would still include these rarer lower notes in the average minimum frequency.

The retention of low notes may be due to the tradeoff between communicating with high signal transmission and high quality in a sexual selection context. Low notes are often a sexually selected trait, and males raising their minimum frequency to avoid noise may suffer a cost in mate attraction, as shown in great tits (Halfwerk et al. 2011), or male-male competition from diminishing their vocal range. Thus, it may be too costly to abandon the lowest notes entirely. Variation in low note use could also be influenced by the proximity of other birds. If a rival male or potential mate are close by then it may be more advantageous to sing low notes communicating quality than it would be to optimize singing across a larger distance. The social-context influence in song has been documented in chickadees overlapping (Mennill et al., 2002) making noise just one influence on song selection.

What we do see in both analyses is the tendency for the average minimum frequencies, for both the averages of all element minimums and the lowest of the low

notes, to increase in frequency with increasing average noise levels of the habitat. This agrees with another study (Rhodes et al., 2020), which found that urban catbird populations sing at a higher minimum frequency than rural males and places catbirds among the many species that display this phenomenon (Slabbekoorn & Peet, 2003).

Additionally, catbirds appeared to be vocally flexible in an unexpected way. During the high-frequency noise playbacks, gray catbirds significantly lowered their maximum frequencies in the presence of high-frequency noise. Unlike the small shifts in minimum frequency, the shifts in maximum frequency were substantial with effect sizes of $\beta_{\text{High-pre}} = -260.95$ was significantly lower in response to high-frequency noise compared to pre-noise and of $\beta_{\text{high-low}} = -214.55$ compared to during low-frequency noise. This result is considered an example of immediate flexibility, though on the higher end range of catbird song. The ability to lower maximum frequencies to avoid high-frequency noise, may be an ability to shift out of the range of other noise sources. Previous studies have found that birdsong is shaped by the environment including both abiotic noise and biotic noise including other organisms that communicate acoustically. A study on populations of green hylia (*Hylia prasina*) found that birds sang at lower frequencies appearing to avoid insect noise (Kirshcel et al, 2009). Some species showed behavioral flexibility to avoid insect noise, such as seen in Neotropical birds. Birds that sang song outside of the frequencies shared by the insect frequencies will continue to sing in the presence of insect noise, however birds with masked high frequency song wait to start singing until insect noise has stopped (Stanley et al, 2015; Hart et al, 2015). This is also similar to what we see in silvereyes (*Zosterops lateralis*) (Potvin & Mulder, 2013) (Table 1), birds lowered minimum frequencies during high-frequency noise. The authors

believed lowering minimums to be advantageous in the presence of other bird or insect noise. Silvereyes are another example of small to no change in minimum frequency to urban noise but a much more flexible response to noises that are less evolutionarily novel. Similarly, for catbirds the small shift upwards in the presence of low-frequency noise might be due to the novelty of anthropogenic noise compared to the time-scale of birds dealing with high-frequency insect noise.

The persistence of very low notes may also give insight into catbirds learning under noisy conditions. As open-ended learners, mimics are not constrained to a developmental learning period, and it appears the noise in the environment has not prevented high quality song learning of low-frequency notes. Fluctuation in noise, as well as the open-ended learning nature of catbirds appear to give catbirds sufficient opportunity to learn low-frequency song, and the threshold method measurements supports that catbirds have not dropped their lowest notes, but instead use higher notes from their repertoire on average during anthropogenic noise.

Overall, these results show that the gray catbirds in this study do not appear to be immediately flexible in response to low-frequency, anthropogenic noise. While the peak frequency contour analysis supports my hypothesis, the effect size of just 73.3 Hz between pre and low noise conditions is extremely small. This relatively low effect size may suggest that this is not a biologically significant shift in minimum frequency. This shift is noticeably smaller than the shifts observed in other species that raise their frequencies by roughly 300 Hz (see synthesis in Background section) as well as smaller than some shifts that have been attributed to the Lombard effect (e.g. >150 Hz). This small shift is especially surprising as previous studies suggest that species with variable

song types, such as great tits or black-capped chickadees, seem more readily able to shift their minimum frequency. Which begs the question as to why gray catbirds, regardless of their highly variable song, do not demonstrate a sizable shift in minimum frequency.

An effect size this small is also smaller than the frequency resolution possible using the settings in the Raven Pro software for this analysis (± 86.13 Hz when using a window size of 512 and a sample rate of 44,100 points). In other words, frequency differences below 86.13 Hz cannot be accurately measured using the settings in this study. Therefore, we must conclude that this effect size is not enough evidence to say that catbirds are immediately flexible. A larger effect size may have been achieved with a larger sample size, especially of the most urban birds ($n=2$), as we did see variation in effect size with urbanization, and it is possible that a much greater effect size would be observed with more urban birds sampled. It is also possible that the stimulus did not continue long enough for the birds to adjust. Studies on immediate flexibility vary greatly in their playback length with durations in noise playback range from 2 to about 30 minutes. If given a longer adjustment period, we may have seen a greater shift in song, and a larger effect size.

Another possibility that the low-frequency noise stimulus used in this experiment is too broad of a range (1-3.5 kHz). This same playback stimulus range was used by Halfwerk et al (2009), and by Gentry et al (2017), which yielded significant but different results for raising minimum frequencies in great tits and lowering frequency bandwidth in white-crowned sparrows. I made edits to remove roll-off noise but maintained the original bandwidth of the noise playback used in these prior studies. This larger range up to 3,500 Hz, was appropriately high to overlap Parid (tits) or Passerellid (sparrow) songs

and may have helped to elicit a response from great tits or white-crowned sparrows, which have on average higher frequency songs than catbirds. However, since most anthropogenic noise peaks at 2,000 Hz, a noise stimulus up to 3,500 Hz could be too high for the catbirds to avoid. We may have seen a clearer response with a more accurate recreation of urban noise (greatest amplitude from 1-2 kHz). This is the first study of immediate flexibility on a mimic and suggests that, despite their long song, open-ended learning, and large repertoire size, gray catbirds are at best limited in their ability to be immediately flexible in the minimum frequencies of their songs in response to anthropogenic noise. Further studies are needed to confirm if the small effect size seen in the peak frequency contour analysis is the result of a true shift in minimum frequency. Nevertheless, previous work in the Moseley Lab shows that urban birds sing at higher minimum frequencies, so my study suggests the mechanism for this finding may be due to natural or cultural selection instead of immediate flexibility.

In addition to a greater sampling effort of catbirds breeding in more urban and noisier habitats, further studies could investigate the influence of natural or cultural selection as the driving mechanism behind urban and rural populations frequency differences. Future studies could also investigate how noise may affect song qualities of gray catbirds like song rate, redundancy or repeats of syllables, or temporal changes.

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