Effects of Anthropogenic Noise on Communication in Dark-eyed Juncos (*Junco hyemalis*)

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Abstract

Noise pollution has numerous consequences for wildlife, including the disruption of acoustic communication through its impacts on signal production, signal transmission, and signal perception. In this thesis, I demonstrated, using complementary correlational and experimental approaches, that dark-eyed juncos (*Junco hyemalis*) increase the frequency of their songs in response to noise. Next, I broadcasted noise-altered and unaltered songs in noisy and quiet environments and re-recorded them along a 40-m transect, with microphones set up at 1, 5, 15, and 40 m. I measured song degradation along the transect and found that songs degraded predictably with increasing distance, but that recording environment (noisy or quiet), playback environment (noisy or quiet), and the interaction between the two had no effect on song degradation. This indicates that, although juncos increase the minimum frequency of their songs in response to noise, the shift does not, by itself, increase the transmission of the song through noise.

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Chapter 1: A Review of the Effects of Noise on Animal Ecology and Acoustic

Communication

Growing cities have led to increased temperature within cities (the urban heat

island effect), a reduction in natural habitat and associated food for many species, large-

scale chemical pollution, and, more recently, noise pollution (Slabbekoorn &

Ripmeester, 2008). Anthropogenic noise is concentrated near busy roadways, trainways,

industrial sites, and airports (Ripmeester et al., 2010), and is known to have numerous

negative effects on animals, including: (1) a reduction in species richness in anurans

(Eigenbrod et al., 2008), birds (McClure et al., 2013), and arthropods (Bunkley et al.,

2017); (2) reduced reproductive success in birds (Halfwerk et al., 2011b); and (3)

physiological stress in human and non-human animals (Wright et al., 2007). Additionally,

the effects of anthropogenic noise on the production, transmission, and perception of

animal signals have been studied in many species (Rabin et al., 2006; Iorio & Clark, 2009;

Luther & Baptista, 2009; Ripmeester et al., 2010; Cardoso & Atwell, 2011; Lampe et al.,

2012; Vargas-Salinas et al., 2014; Putland et al., 2017), particularly since anthropogenic

noise is concentrated at low frequencies that often overlap the lower frequencies

contained in animal signals (Francis et al., 2009).

Animals using acoustic communication in noisy conditions can increase their

signal-to-noise ratio (the ratio of energy in the signal to energy in the environment)

through at least five mechanisms: (1) avoiding noisy areas (Wright et al., 2007; Francis et

al., 2011; McClure et al., 2013); (2) shifting signal production to quieter times of the day

(Wright et al., 2007; Bermúdez-Cuamatzin et al., 2010); (3) increasing the minimum

frequency of signals above those contained in noise (Ripmeester et al., 2010; Cardoso &

Atwell, 2011; Luther & Magnotti, 2014); (4) increasing signal amplitude (Brumm, 2004;

Brumm & Zollinger, 2011); or (5) increasing signal tonality (i.e. concentrating energy in a

narrower range of frequencies; Hanna et al., 2011). These alterations are not mutually

exclusive; for instance, the frequency and amplitude of song covaries in some birds

(Cardoso & Atwell, 2011).

Effects of Noise on Avian Communication

Birds rely on acoustic communication for many purposes, including contacting

conspecifics, signalling the presence of danger, indicating the discovery of food,

attracting mates, and defending territories (Brenowitz et al., 1997; Brumm, 2013). In

temperate breeding birds, it is mostly the males that sing (Brenowitz et al., 1997); male

songbirds produce songs that advertise the direct (e.g. available food and territory) and

indirect (e.g. size and stamina) benefits they can provide to females (Brumm &

Ritschard, 2011; Ripmeester et al., 2010; Derryberry, 2007), as well as their ability and

readiness to defend their territories against rivals (Wood & Yezerinac, 2006). Noise may

thus disrupt avian communication and reproductive behaviour by interfering with song

production and/or song detection.

Birds may deal with anthropogenic noise in multiple ways, such as simply

avoiding noisy locations or noisy times (Wright et al., 2007; Arroyo-Solis et al., 2013;

McClure et al., 2013), or altering the structure of their songs in the presence of noise to improve the signal-to-noise ratio of their acoustic signals. For example, species such as red-winged blackbirds (*Agelaius phoeniceus*) counter noisy conditions by increasing the tonality of their song. Concentrating the energy of a signal in fewer frequencies can increase signal detectability in the presence of low-frequency background noise (Hanna et al., 2011). Additionally, species such as nightingales (*Luscinia megarhynchos*) increase the distance over which their songs can be heard by increasing song amplitude (Brumm, 2004; Brumm & Zollinger, 2011). Species such as European blackbirds (*Turdus merula*) and dark-eyed juncos (*Junco hyemalis*) sing with increased minimum frequency, which can reduce masking by low-frequency noise (Ripmeester et al., 2010; Cardoso & Atwell, 2011; Luther & Magnotti, 2014). With respect to signal alteration, these are three of the most common vocal adjustments documented in birds to increase the signal-to-noise ratio of their signals, but other vocal adjustments, such as increased duration (Luo et al., 2015) or redundancy (i.e. repetition of song elements; Brumm et al., 2004) have been documented in other groups in response to noisy conditions.

Most previous studies focusing on the alteration of birdsong in the presence of noise assume that the observed alterations increase song transmission (Brumm, 2004). Signal transmission is influenced by the local environment, including vegetation, temperature, wind, and ambient noise (Morton, 1975). Cities have created a novel acoustic environment for birds, with less natural habitat and more urban infrastructure, including large amounts of concrete and buildings (Ripmeester et al., 2010). These novel

characteristics increase the number of reverberant surfaces and can alter the transmission properties of birdsong (Derryberry, 2009). Previous studies have focused on the effects of these physical properties of the environment on the transmission of birdsong (Slabbekoorn et al. 2007; Sandoval et al., 2015; Graham et al., 2017), yet no study that I am aware of compares transmission properties of noise-altered and unaltered songs when only the noise environment is changed, as would be the case for birds living in a forest next to a loud highway.

Some song adjustments, such as frequency shifts, have been investigated in the context of female perception of male quality (Halfwerk et al., 2011a; Halfwerk et al., 2011b; Luther et al., 2016). Increasing the minimum frequency of songs, without adjustment to maximum frequency, can lead to a narrowing of frequency range, which reduces perceived male quality in some species, including great tits (*Parus major*) and white-crowned sparrows (*Zonotrichia leucophrys*; Halfwerk et al., 2011a; Luther et al., 2016). Halfwerk et al. (2011b) studied a nest-box population of great tits and found that females preferred low-frequency songs, and that males producing low-frequency songs were rewarded by their mates seeking fewer extra pair copulations. The presence of low-frequency anthropogenic noise could therefore disrupt female selection in systems such as this by masking low-frequency songs.

Anthropogenic noise can also affect songs used in contest competition. For example, male great tits react more strongly to lower-frequency songs produced by birds living in rural areas, as compared to higher-frequency songs produced by birds

living in urban areas (Mockford & Marshall, 2009). Similarly, northern cardinals

(*Cardinalis cardinalis*) react more strongly to songs that have been digitally altered to

have lower frequencies (Luther & Magnotti, 2014), and white-crowned sparrows react

more strongly to unaltered songs than to songs that have had their lower 500 Hz filtered

out (Luther et al., 2016). In northern cardinals and great tits, however, the stronger

responses towards lower-frequency songs decreased and, in the case of great tits,

reversed as low-frequency ambient noise increased. If males must use higher-frequency

songs of lower perceived quality to defend territories in noisy areas, then this may alter

interactions with neighbouring males, and affect contest competition.

In addition to reducing signal efficacy, modification of song characteristics in

response to local noise environments may cause song divergence between populations

living in noisy versus quiet conditions. Song divergence may, in turn, influence mate

choice and, in extreme cases, lead to reproductive isolation (Slabbekoorn & Smith,

2002a; Ripmeester et al., 2010). Such local changes in song production can occur due to

natural and anthropogenic sources of noise. For instance, compared to populations of

white-crowned sparrows (*Zonotrichia leucophrys*) living under quiet conditions, those

living next to loud surf, wind, or anthropogenic noise sing songs of higher frequency due

to cultural transmission (Derryberry et al., 2016). Differing noise environments have also

been implicated in song divergence and morphological divergence in parapatric little

greenbuls (*Eurillas virens*) in the ecotone forest and rainforest in Africa. In this case, song

diversification and female mate selection for habitat-dependent dialects may be a

mechanism driving reproductive isolation (Slabbekoorn & Smith, 2002b). Adjustment of birdsong may therefore affect the evolution of bird species, and not just an individual's survival and reproduction.

To understand how noise affects acoustic communication in animals, it is necessary to address the issue from multiple perspectives. For example, do animals adjust their acoustic signals in the presence of noise by altering signal structure or other aspects of signals, such as when and where they are produced? If the signaller does alter aspects of their signal or signal production, do these changes affect the transmission of the signal through noise? Research to determine the transmission properties of altered and unaltered signals could help to determine the function of these alterations. This thesis will focus on the production and transmission of territorial songs of a northern temperate-breeding songbird.

Study Species

The dark-eyed junco is a sparrow (in Passerellidae) found commonly throughout North America. They are ground nesters, and both parents take care of the chicks. They prefer habitat transition zones between forest and open areas, are common in urban environments, and feed predominantly on seeds and arthropods (Nolan et al., 2002). There are five subspecies of juncos, differentiated due to differing morphology and behaviour. These include *Junco hyemalis hyemalis*, *Junco hyemalis oreganus*, *Junco hyemalis aikeni*, *Junco hyemalis mearnsi*, and *Junco hyemalis caniceps* (Pieplow, 2019).

Dark-eyed juncos are a convenient model system to explore the effects of anthropogenic noise on avian communication. They are common in both quiet undeveloped environments, and noisy developed environments (Slabbekoorn et al., 2007). During the breeding season, breeding pairs defend territories, and males sing loud, simple songs that repel rivals and attract prospective mates (Ferreira et al., 2016). Although dark-eyed juncos tolerate anthropogenic disturbance (Rottenborn, 1999), it is still possible that anthropogenic noise negatively impacts urban populations. Ferreira et al. (2016) found that urban populations of juncos sing with more mistakes (identified as departures from typical song design) when compared to rural populations, and that this may be due to anthropogenic noise impeding either song learning or song production. A similar study showed that the frequencies contained within junco songs were higher in urban populations than in rural populations (Cardoso & Atwell 2010). The authors attribute the change in frequency to two mechanisms, including increasing the frequencies contained in certain song elements, and replacing low-frequency elements with higher-frequency ones (Cardoso & Atwell, 2010). Slabbekoorn et al. (2007) also showed that juncos in urban environments sing higher-frequency songs than juncos in rural populations, but do not alter signal duration or trill rate. All three of these studies were conducted in California and therefore likely sampled dark-eyed juncos in the sub-species *oreganus*. The current study investigates song structure in the slate-coloured dark-eyed junco (*Junco hyemalis hyemalis*).

Research Objectives

I investigated two aspects of vocal communication that can be influenced or disrupted by anthropogenic noise. In chapter 2, I determined if singing dark-eyed juncos alter the structure of their song in the presence of noise. I predicted that juncos would increase the frequency and decrease the frequency range of their songs in noisy conditions, as previous correlational studies on dark-eyed juncos have found (Slabbekoorn et al., 2007; Cardoso & Atwell, 2010). I used correlative and experimental approaches to test this; I compared songs recorded in a forested area next to the trans-Canada highway to those recorded in quieter, more secluded forested sites. This was complemented by an experiment in which traffic noise was broadcasted in an otherwise undisturbed forest. In this case, songs from territorial males were recorded before and during the playback of noise.

In chapter 3, I tested whether song alterations made in the presence of noise affect song transmission. If noise-induced changes in song structure are adaptive, then songs produced in noisy conditions should transmit better (i.e. degrade less over distance) than unaltered songs in noisy environments. To determine if this occurs, I broadcasted junco songs recorded in noisy and quiet conditions at both noisy and quiet locations (i.e., a 2X2 factorial design). During each playback, I measured song degradation along a transect extending in front of the playback speaker.

Co-authorship Statement

Although this research was conducted independently, my supervisor, David Wilson, contributed to all aspects of this project, including experimental design, statistical analyses, and writing. Additionally, chapter 1 made use of a dataset collected by Bronwen Hennigar in partial fulfillment of her Master of Science degree. I was able to use recordings taken by Bronwen Hennigar and Jeffrey Ethier during the field season of 2016 and 2017. Bronwen broadcasted traffic noise in the forest of Labrador to determine how birds reacted to different anthropogenic stimuli. Her project also included the presence of light in combination with noise. I used a subset of her recordings and analysed the song structure of dark-eyed junco songs produced in the presence and absence of noise. Therefore, should chapters 2 and 3 proceed to publishing, Bronwen Hennigar and David Wilson will be co-authors on chapter 2, and David Wilson will be a co-author in chapter 3.

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Chapter 2: Dark-eyed Juncos Adjust Song Structure in Response to Traffic Noise

ABSTRACT

Anthropogenic noise has been linked to reduced biodiversity and reproductive success in animals, possibly because noise disrupts acoustic signaling that is critical for survival and reproduction. Previous correlational studies suggest that some animals mitigate this disruption by altering the structure of their signals in ways that minimize acoustic masking, but there are far fewer experimental studies testing the causal effects of noise on communication. In this study, I used both correlational and experimental approaches. For the correlational component, dark-eyed juncos (*Junco hyemalis*) were recorded near a busy highway as well as in more secluded areas. I found that juncos in noisy habitats sang songs with higher minimum frequencies and smaller frequency ranges. However, correlational studies may be confounded by other variables, such as habitat alteration, that often accompany sources of anthropogenic noise. Therefore, I used data from a previous experiment that broadcasted traffic noise through a loudspeaker in an otherwise undisturbed boreal forest during the breeding season, and that recorded the vocal responses of nearby resident dark-eyed juncos (*Junco hyemalis*) using a microphone array. Using this experimental approach, I confirmed that traffic noise caused juncos to increase the minimum frequency and decrease the frequency range of their songs. These changes may facilitate communication in noisy environments by reducing acoustic masking, and may thus help explain why juncos, unlike many species, succeed in noisy environments.

INTRODUCTION

Noise pollution is a widespread problem with consequences for many kinds of animals (Brumm, 2013). For example, it can induce physiological stress (Wright et al., 2007; Slabbekoorn et al., 2018), reduce reproductive success (Halfwerk et al., 2011), contribute to reduced species richness, and alter spatial distributions of animals within communities (Eigenbrod et al., 2008; McClure et al., 2013; Bunkley et al., 2017). These effects have been documented in terrestrial and aquatic systems and across multiple taxa, including some arthropods, such as vocal insects (Lampe et al., 2012; Bunkley et al., 2017), anurans (Eigenbrod et al., 2008), fish (Putland et al., 2017), birds (McClure et al., 2013), marine mammals (Putland et al., 2017), and terrestrial mammals (Luo et al., 2015; Slabbekoorn et al., 2018).

The disruption of acoustic communication is one mechanism by which anthropogenic noise can affect components of an animal's fitness (Rabin et al., 2006; Iorio and Clark, 2009; Cardoso & Atwell, 2011). Noise can mask alarm calls that prey species rely on for avoiding predators (e.g. ground squirrels, *Spermophilus beecheyi*; Rabin et al., 2006, and great tits, *Parus major*; Templeton et al., 2016), disrupt echolocation systems used by foraging bats (Gomez et al., 2016), and cause changes to acoustic signals used to attract mates and repel rivals (e.g. male great tits; Halfwerk et al., 2011). Anthropogenic noise has rapidly altered the acoustic environment in which these communication systems evolved, and it is not clear whether the communication systems can adapt to, or tolerate, the change (Brumm, 2013).

Many species living near sources of low-frequency anthropogenic noise use acoustic signals that differ from rural populations; however, some species, such as the anuran *Hyla arborea*, do not adjust vocal signalling in noise (Lengagne, 2008). A hypothesis that is commonly invoked is that signals are altered in noisy conditions to preserve the functionality of the signal (Parks et al., 2010; Bunkley et al. 2015; Luther et al., 2016a). Therefore, species that are unable to alter their signals may be less able to tolerate urban conditions. Signal alteration is thought to be adaptive and to increase transmission distance, but this increased transmission may come at a price. For instance, structural changes may reduce the perceived quality of males during mate choice (Luther et al., 2016b), impair reliability of intrasexual signals important for competition (Luther & Magnotti, 2014; Luther et al., 2016a), or reduce detectability of alarm calls (Potvin et al., 2014; Templeton et al., 2016).

Animals may adjust their signals in different ways. For instance, animals signalling in noisy areas may signal mainly during pauses in background noise (Arroyo-Solís et al., 2013). Animals may also use structurally altered signals, which presumably transmit better through a noisy environment. Increased minimum frequency is a common feature found in animal populations exposed to low-frequency noise (Patricelli & Blickley, 2006; Ripmeester et al., 2010; Hotchkin et al., 2013). Increased tonality (Wood & Yezerinac, 2006; Hanna et al., 2011), increased amplitude (Brumm et al., 2004; Scheifele et al., 2005; Egnor & Hauser, 2006), increased duration (Luo et al., 2015), or redundancy (i.e. repetition of song elements; Brumm et al., 2004) of the signal have also

been documented in noisy habitats. These changes are thought to improve the signal-to-noise ratio in a noisy environment, though tests of how these changes affect signal transmission and detection are lacking.

Most studies investigating the effects of noise on signal structure are correlational (Brumm et al., 2004; Wood & Yezerinac, 2006; Parks et al., 2010; Luther & Magnotti, 2014; Potvin et al., 2014; Bunkley et al. 2015). Such correlational studies benefit from the use of realistic noise environments, such as those found along highways, adjacent to airports, or inside cities. However, they are unable to determine why individuals in noisy environments sound different than those living in quiet environments (Stangor, 2011). It is possible that individuals flexibly adjust signal structure when noise is present, or that individuals living in noisy environments have permanent structural differences in their signals as a result of vocal learning or local adaptation (Dereryberry et al., 2016; Zolinger et al., 2017). Alternatively, poor-quality individuals that sound different than high-quality individuals may be relegated to poor-quality, noisy environments (see also Verzijden et al., 2010; Zollinger et al., 2017). Experimental studies can potentially ascertain the mechanisms underlying song differences, but can be criticized for a lack of realism (Stangor, 2011). For example, playing back traffic noise at a peak sound pressure level of 85 dB results in noise that attenuates far more quickly than the traffic noise produced from a highway (see attenuation distance on page 28). Noise playbacks are also usually short-term and may not capture longer-term responses to the chronic noise, such as habituation or

avoidance when selecting breeding sites. Combining correlational and experimental

approaches could thus provide the most comprehensive understanding of the effects of

noise on vocal production.

Birds are a good model system for this research because they inhabit noisy and

quiet environments and rely heavily on acoustic signalling to communicate during social

interactions, mate choice, contest competition, and alarm signaling (Brumm, 2013). I

studied dark-eyed juncos (*Junco hyemalis*), an abundant species in noisy urban and quiet

rural locations. Male juncos use a simple trilled song to defend territories and attract

females (Ferreira et al. 2016). Juncos living in urban habitats sing songs with a higher

minimum frequency than those in quieter habitats (Slabbekoorn, et al., 2007; Cardoso &

Atwell, 2010). Urban juncos also make more mistakes (identified as departure from

typical song design) than rural juncos, suggesting that noise may hinder song learning or

song production (Ferreira et al., 2016). However, because these findings were from

correlational studies, it is unclear whether these differences were due to noise or to

other confounding variables, and whether differences reflect a plastic response to noise

or permanent differences among individuals. Furthermore, all previous research

regarding song structure alteration and the noise environment in juncos has been

conducted in California, USA (Slabbekoorn, et al., 2007; Cardoso & Atwell, 2010), and

therefore likely involved the sub-species *oreganus* (Pieplow, 2019).

I compared the structure of songs produced by dark-eyed juncos (*hyemalis*

subspecies) living in quiet forests versus along a noisy roadway to document vocal

differences between populations with different but realistic noise environments, and to

confirm previous reports based on a different location and the *oreganus* subspecies

(Pieplow, 2019). To supplement this correlational study, traffic noise was also

broadcasted in an otherwise undisturbed boreal forest to determine the causal effects

of noise, independent of confounding factors, such as the presence of a highway, that

often accompany noise. Any change in song structure in response to transient noise

would indicate that juncos can flexibly adjust song structure, and that such vocal

flexibility could contribute to structural differences observed between birds living in

chronically noisy versus quiet environments. I predicted that, in the presence of both

transient and chronic noise, junco songs would have higher minimum frequencies, since

this should facilitate signal transmission in a noisy environment (Slabbekoorn, et al.,

2007; Cardoso & Atwell, 2010). I also expected a decreased frequency range as a result

of increased minimum frequency without a concomitant increase to maximum

frequency, as seen in other studies (Wood & Yezerinac, 2006; Mockford & Marshall,

2009; Montague et al., 2012).

METHODS

Study Species

The dark-eyed junco is a sparrow (in Passerellidae) found commonly throughout

North America. There are five subspecies of juncos, differentiated due to differing

morphology and behaviour: *Junco hyemalis hyemalis*, *Junco hyemalis oreganus*, *Junco*

hyemalis aikeni, *Junco hyemalis mearnsi*, and *Junco hyemalis caniceps* (Pieplow, 2019). I

studied a non-migratory population of the slate-coloured subspecies (*Junco hyemalis*

hyemalis) in Newfoundland and Labrador, Canada. Males are territorial, sing loud,

simple songs for territory defence and mate attraction (Ferreira et al., 2016), and are

common in both undeveloped (i.e. quiet) and developed (i.e. noisy) environments

(Slabbekoorn et al., 2007).

Study 1: Song Structure During Chronic Traffic Noise

      I recorded birds living in a noisy forest in Pippy Park, St. John's, Newfoundland

and Labrador (47.5776° N, 52.7481° W), within 300 m of the busy 4-lane Trans-Canada

Highway. The highway has a calculated traffic volume on weekdays of 38 vehicles/min or

2259 vehicles/hour along the segment that transects my study site (calculated from

traffic data from 26 January 2016 between 07:00 and 10:00 h; Newfoundland

Government, Department of Transportation and Works). I also recorded juncos living in

the same forest, but at least 500 m away from the highway, where I could no longer

hear the traffic noise. Recordings at noisy locations were only taken during weekdays

during morning rush-hour traffic, whereas recordings at quiet locations were taken

during weekends and weekdays. All recordings were taken during the 2017 and 2018

breeding seasons between 15 May and 15 July.

      Subjects were unbanded (n = 14) and colour-banded (n = 4) male juncos. The

territory size of dark-eyed juncos in this population ranges from 25 m to 45 m in

diameter, as determined by following juncos and marking their singing perches over a 1 – 2 hour period (personal observation). Therefore, to ensure that each male was a new individual, I separated recording sites of unbanded males by at least 300 m. At each quiet and noisy site, singing juncos were located and recorded opportunistically between 06:00 and 12:00 h, when windspeed was less than 25 km/h and there was no precipitation. Subjects were recorded for a minimum of twenty songs from a single singing bout as they patrolled their territories. Sunrise occurred between 05:18 h and 05:24 h at the study site, so all recorded songs were diurnal songs and not part of the pre-dawn chorus. Most recordings were taken using a parabolic microphone (n = 16 males; MKH20-P48 omnidirectional microphone with a Telinga 22'' Parabola) connected to a digital audio recorder (Marantz professional PMD 661 MKII solid-state recorder; WAVE format, 16-bit amplitude encoding, 44.1 kHz sampling rate; Marantz professional, Cumberland, Maryland, USA), though some (n = 2, including 1 from a quiet habitat and 1 from a noisy habitat) were recorded with a shotgun microphone (Sennheiser ME66; 40 - 20000Hz frequency response; Sennheiser, Wedemark, Lower Saxony, Germany) connected to the same recorder.

I reviewed each recording as a spectrogram (Hamming window, 512-point FFT, 87.5% overlap) in Audacity software (Audacity recording and editing software, version 2.3.1; https://audacityteam.org/) and selected up to 10 high-quality songs from each male. Songs (± 0.5s) were exported as standalone clips (WAVE format, 16-bit amplitude encoding, 24 kHz sampling rate) using the tuneR and Seewave packages (Ligges et al.,

2018; Sueur et al., 2008) in R (R Core Team, 2018; version 3.4.2). I measured minimum

frequency (kHz) and frequency range (kHz) following Zollinger et al. (2012) and Podos

(1997). For each song, I constructed an averaged power spectrum (Hamming window,

512-point FFT, 87% overlap) of the entire song in Raven Pro software (version 1.5;

Bioacoustics Research Program, 2014). Minimum and maximum frequencies were taken

30dB below peak amplitude of the power spectrum to measure the frequency of the

songs, but to avoid inclusion of low-frequency background noise (Figure 2.2; the red

horizontal lines and dots indicate an example of minimum and maximum frequency

measurements). I calculated frequency range as the difference between minimum and

maximum frequency. After the initial song selection, I did not listen to recorded songs to

ensure I could remain blind to treatment while taking all song measurements.

To confirm that noisy and quiet locations differed in background noise level, I

measured sound pressure level (SPL) of the background noise at quiet (n = 8) and noisy

(n = 8) recording sites with a RadioShack sound level meter (RadioShack model 33-2055,

C-weighting, fast response; RadioShack Corporation, Fort Worth, USA). In many quiet

locations (n = 5), the background noise level was < 50 dB (the minimum sensitivity of the

sound level meter). In these cases, where the actual value could not be determined, a

value of 50 dB was assigned. Assigning a value of 50dB would artificially increase the

noise level of quiet sites, ensuring a conservative analysis.

Study 2: Song Structure During Transient Experimental Traffic Noise

Dark-eyed juncos were recorded in the presence and absence of experimentally broadcasted traffic noise in an otherwise undisturbed boreal forest during the 2016 and 2017 breeding seasons (16 May to 10 July 2016; 13 May to 27 June 2017). Each trial occurred over two days. The first day (beginning at 17:00 h) served as a quiet baseline. During the second day (also beginning at 17:00 h) researchers broadcasted a traffic noise stimulus through a loudspeaker from 17:00 h to 08:00 h the next day (see details below). This design allowed me to examine changes in song structure within each site (i.e. the change from the quiet baseline day to the treatment day), which controls for differences among sites, such as habitat and bird community composition.

Trials were conducted at 17 sites around Happy Valley-Goose Bay, Newfoundland and Labrador, Canada (53.5255° N, 60.1430° W), as part of a previous study (Ethier & Wilson, 2019; Hennigar et al., 2019). Trial locations from this previous study were determined by generating random UTM coordinates (RANDOM.org) within a 50-km radius of Happy Valley-Goose Bay. Inclusion criteria were that the locations were separated by at least 500 m to minimize the risk of the same birds being recorded at multiple sites, were within 1 km of a road or trail to facilitate access, and were not located in a swamp, bog, or water body. The UTM coordinates were plotted on topographic maps to determine if they met the above requirements.

Noise was broadcasted for 15 h from an MP3 player (Hipstreet HS-636-4GBBK MP3 player) connected to an amplified loudspeaker (Sharper Image SBT1009BK with a

woofer diameter of 20.32 cm and tweeter diameter of 7.62 cm) that was placed face-up

inside a plastic bag on the ground. The noise was one of three recordings of traffic noise

selected at random for each trial: a low rate of traffic noise that contained

approximately 7 vehicles passing per minute (the recording was 1 hour long and looped

for the duration of the trial); a medium-level noise stimulus that contained

approximately 10 vehicles passing per minute (6 hours long and looped); and a high-

level noise stimulus that contained approximately 40 vehicles passing per minute (8

hours long and looped). All noise recordings were normalized to a peak amplitude of -1

dB and played back at 85 dB peak sound pressure level, as measured with a digital sound

level meter held 1 m from the centre of the speaker. Noise amplitude was measured at

1, 5, 10, 15, 20 and 25 m from the speaker to determine the effective range of noise.

According to these measurements, the traffic noise attenuated to less than 50 dB at a

distance of 25 m from the speaker (Hennigar et al., 2019).

Songs from juncos living near the playback site were recorded with four

autonomous audio recorders (Wildlife Acoustic SM3s; Wildlife Acoustics, Concord, MA,

USA; stereo WAVE format, 24 kHz sampling rate, 16-bit amplitude encoding, 10 dB gain)

arranged as a 40 m X 40 m microphone array that was centred on the playback speaker.

Each recorder was attached to a tree, approximately 1.5 m above the ground. A built-in

omnidirectional microphone (50–20000 Hz frequency response) was pointed towards

the centre of the array, and a second, external microphone was hung over a branch

approximately 2.5 m directly above the recorder. This configuration allowed me to

record vocalizations produced within the array, and to ensure that each vocalization was recorded with a high signal-to-noise ratio by at least one microphone. Because juncos are territorial, it is likely that only one male was recorded at each array; however, I cannot rule out the possibility that more than one individual was recorded at each array.

I reviewed each 8-channel recording (i.e. 4 stereo files derived from the 4 recorders during a given 2-h period of the trial) as a multichannel spectrogram (Hamming window, 512-point FFT, 87.5% overlap) in Audacity. I selected up to 10 high-quality songs from the baseline day (8.1 ± 2.6SD, range: 4-10) and up to 10 additional songs from the treatment day (8.4 ± 2.4SD, range: 4-10) of each trial, though there were often fewer songs available. I targeted songs produced after 18:00 h to ensure that birds had already been exposed to the noise treatment for a minimum of one hour on the treatment day. It was my intention that one hour would allow birds to adjust to the sudden onset of noise and begin producing sounds appropriate for a noisy environment, as opposed to songs whose structure reflected a sudden disturbance. I did not select songs after 22:00 h because juncos did not sing after that time. I also did not select songs from the following morning because the battery in the speaker was depleted, and the playback amplitude reduced. Thus, all songs collected for analysis were produced between 18:00 h and 22:00 h on either the baseline or treatment day. I exported the songs (± 0.5 s) as standalone clips (WAVE format, 16-bit amplitude encoding, 24 kHz sampling rate) from the channel in which they had the highest signal-to-noise ratio using the tuneR and Seewave packages in R.

Previous studies have included length and rate of note delivery when considering song alteration in response to noise (Slabbekoorn et al., 2007). They suggest that increased reflective surfaces associated with an urban environment would lead to increased reverberation, and that birds might be expected to decrease song length and trill rate to reduce these effects and increase signal detectability (Slabbekoorn et al., 2007). My study did not include such reflective surfaces because it was conducted in a forest. I therefore did not expect duration and trill rate to change, and without a strong rationale for including them, they were not considered in my study.

Before measurement, songs were filtered using a 2 kHz high-pass filter and normalized to a peak amplitude of 0dB. The structure of each song was measured using Raven Pro software. I measured minimum frequency (kHz) and frequency range (kHz) using an averaged power spectrum of the entire song. I then measured the minimum and maximum frequency that occurred 20 dB, rather than 30 dB (used for the chronic noise study), below the peak amplitude. Due to the poorer quality of recordings taken with the Song Meter SM3s (versus the parabolic microphone used in the chronic noise study), I was only able to move down 20dB to exclude background noise without clipping the lower frequencies of the song. During song measurement, I was blind to the treatment of each recording.

Statistical Analysis

In the chronic noise experiment I compared background noise measurements between noisy and quiet sites using a linear model, where the response variable was the sound level recorded and the explanatory variable was the treatment (within 300 m of the highway or beyond 500 m from the highway). This model was executed in R.

In both the chronic and transient noise studies, I compared the minimum frequency and frequency range of songs recorded in the presence and absence of noise with linear mixed effects models. The explanatory variable was treatment (noisy or quiet), with male identity (in the chronic noise experiment) or array location (in the transient noise experiment) as a random effect to account for nonindependence among songs derived from the same male or from the same site. Separate models were conducted for minimum frequency and frequency range, and alpha was adjusted to 0.025 (i.e., 0.05/2) to control experiment-wise type I error. Analyses were conducted in R using the lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) packages. All p-values below 0.025 (the corrected alpha value) were considered statistically significant and all p-values between 0.025 and 0.05 were considered statistical trends.

In a study conducted by Cardoso and Atwell (2010), a population of 151 male juncos sang 262 distinct song types; therefore, juncos may increase the minimum frequency of their songs either by shifting the frequencies within a given song type upward, or by switching to a different song type that has higher frequencies. Both mechanisms would reduce masking from low-frequency background noise. In this study,

without access to a similarly large song database for accurate classification of song types in my population, I did not include song type in my analysis.

Validation of Acoustic Measurements

In both studies, I sampled locations with differing levels of background noise, but this was low-frequency noise, largely concentrated below the frequencies contained in junco songs. If juncos adjust the minimum frequency of their song to avoid masking by low-frequency background noise, the amplitude of background noise present within the frequency range of the junco song will not be different between noisy and quiet environments, as juncos would adjust the frequencies they use in their songs to avoid masking by noise. However, it was still possible that when measuring song frequency, some background noise overlapped the frequencies contained in songs and could not be removed by filtering. This overlap would confound subsequent frequency measurements because the two treatments inherently contain different levels of background noise. For the presence of differing levels of background noise to be a confounding variable, there would need to be a significant difference in background noise levels between noisy and quiet treatments within the frequency range of junco songs. To test for this confound, I selected background noise clips (5 s of background noise preceding the songs) from recordings made under noisy and quiet conditions in the chronic noise experiment (n = 8 noisy clips, n = 8 quiet clips) and the transient noise experiment (n = 10 noisy clips, n = 10 quiet clips). I also included a sample of songs from each quiet location (n = 8 during

the chronic noise experiment and n = 10 for the transient noise experiment). For each

clip, I generated an averaged power spectrum (Hamming window, 512-point FFT, 87%

overlap) using Raven Pro. R (pavo package; Maia et al., 2018) was then used to graph the

mean and standard error of the averaged power spectra from each group (noisy

background noise samples, quiet background noise samples, and songs). If the standard

errors of the two types of background noise overlapped in the frequency range of songs,

then noise from the playback could not confound my measurements of minimum

frequency and frequency range.

RESULTS

Study 1: Song Structure During Chronic Traffic Noise

Background noise levels were significantly louder at 'noisy' sites (i.e. within 300

m of the highway) than at 'quiet' sites (i.e. beyond 500 m from the highway; general

linear model: $F_{1,14}$ = 61.28, p < 0.001; background noise levels were 10.5 ± 1.3dB higher

at noisy sites). Furthermore, songs recorded in noisy sites had higher minimum

frequencies (linear mixed effects model, effect of noise condition: t = -7.44, p < 0.001)

and narrower frequency ranges (linear mixed effects model, effect of noise condition: t =

19.80, p < 0.001; Figure 2.1) than songs recorded at quiet sites (Table 2.1).

Study 2: Song Structure During Transient Experimental Traffic Noise

Junco songs recorded during the noise playback had higher minimum frequencies than songs recorded during the quiet baseline period (t = -2.33, p = 0.022; Figure 2.1), and tended to have narrower frequency ranges than songs recorded during the quiet baseline period (t = 2.24, p=0.027; Figure 2.1).

Validation of Acoustic Measurements

Background noise in the frequency range of junco songs did not differ significantly between noisy and quiet conditions, as evidenced by the overlapping error bands across the frequency range of the junco song (Figure 2.2). The presence of low-frequency background noise within the frequency range of junco song was therefore not a confounding factor in this analysis.

DISCUSSION

I compared the structure of dark-eyed junco songs recorded in noisy and quiet conditions. Recordings were taken when birds were exposed to both chronic and transient, experimentally broadcast noise, for comparison with quiet locations. I found that juncos respond to traffic noise by increasing the minimum frequency and decreasing the frequency range of their songs. They make these changes almost instantaneously even in an undisturbed forest, where traffic noise is unusual. The

analysis was not confounded because background noise within the frequency range of junco songs did not differ between noisy and quiet treatments.

My chronic noise study corroborates the correlational findings of Cardoso and Atwell (2010) and Slabbekoorn et al. (2007); juncos in noisy environments sing with an increased minimum frequency. These previous studies were conducted in California, and likely sampled juncos of the subspecies o*reganus*. My study shows that juncos of the *hyemalis* subspecies*,* singing in chronically noisy habitats in Newfoundland and Labrador, Canada, also sing with an increased minimum frequency. Furthermore, my transient noise experiment shows that traffic noise causes juncos to increase the minimum frequency of their song, which likely explains the correlations between the presence of traffic noise and increased minimum frequency that have been observed in previous studies. Increasing the minimum frequency of song likely increases the song's signal-to-noise ratio and detectability by preventing frequency overlap with low-frequency background noise (Cardoso & Atwell, 2010; Ripmeester et al. 2010).

In the presence of anthropogenic noise, juncos sang with a reduced frequency range in both studies. A similar effect has been described in European robins (*Erithacus rubecula*) and song sparrows (*Melospiza melodia*), though not in great tits (Wood & Yezerinac, 2006; Mockford & Marshall, 2009; Montague et al., 2012). The decreased frequency range suggests that juncos increase the minimum frequency without a concomitant increase to maximum frequency, leading to a decrease in frequency range in response to noise.

The experimental approach used in this study, where traffic noise was broadcasted in an otherwise undisturbed forest, helps to explain why previous studies have found differences in the structure of songs produced in noisy and quiet environments. That juncos produced songs with higher minimum frequencies in response to transient experimental noise shows that juncos flexibly and rapidly alter their song structure in response to noise. This suggests that the increase in minimum frequency observed in previous correlational studies is due to the presence of noise, rather than to factors commonly associated with noise (e.g. habitat type, presence of other disturbances) or to population-level differences in song production (e.g. if poor-quality individuals are relegated to poor-quality habitats). I note, however, that the difference in minimum frequency between noisy and quiet conditions was greater in the chronic noise study compared to the transient noise study. This may reflect the fact that naturally produced traffic noise was louder and attenuated less quickly than the playback, but might also reflect geographic variation in song production or individual-level differences in song flexibility.

Templeton et al. (2016) have also used an experimental approach to test the effects of noise on great tit alarm call volume and detectability, and Verzijden et al. (2010) and Hanna et al. (2011) both used complementary correlational and experimental methods to demonstrate how noise influences the structure of songs produced by chiffchaffs (*Phylloscopus collybita*) and red-winged blackbirds, respectively. The experimental results from these papers, as well as from the current study, show that

birds are capable of making immediate alterations to their acoustic signals in the presence of noise. In such experimental work, researchers can determine that the observed increase in frequency in noisy habitats is due to the presence of noise, and that individuals are capable of adjusting their songs quite rapidly; additionally, correlational studies (Brumm, 2004; Slabbekoorn et al., 2007; Cardoso et al., 2010; Bunkley et al. 2015) can support that this shift in frequency is found in natural systems, and is a wide-spread phenomenon. However, experimental studies, due to the short-term exposure of noise and lack of long-term behavioural data, cannot rule out the potential role of other processes such as cultural evolution of birdsong, and local homogenization of birdsong in response to chronically noisy environments (Derryberry et al., 2016).

Previous studies have acknowledged the need to filter out background noise before measuring song parameters, as increased levels of background noise (associated only with noisy treatments) may alter both amplitude and frequency measurements (Zollinger et al., 2012, Mockford & Marshall, 2009). However, these studies do not consider the possibility that high-pass filtering may not fully account for differences in noise levels within the frequency range of the signal of interest. This is a potential confounding factor present in most studies comparing song structure between noisy and quiet conditions, and should be addressed. I therefore graphed the spectral profiles of junco songs and of the background noise profiles of noisy and quiet locations. I showed that background noise levels between noisy and quiet conditions did not differ

significantly within the frequency range of junco songs (Figure 2.2). I recommend that future studies follow this practice, particularly for species with low-frequency songs that overlap the range of frequencies where background noise levels likely diverge between noisy and quiet environments.

It is possible that the alteration of acoustic signals in response to noise does not affect the information conveyed within the signal; however, as calls and songs are often specific and identifiable at the individual level (Marler & Isaac, 1961; Nelson, 1989; Mathevon et al., 2008; Abe & Watanabe, 2011), it is possible that even seemingly minor changes to the structure of these signals have a large effect on how they are perceived. Konishi (1964) found that minimum frequency was the least variable part of junco songs, which may indicate that alterations to frequency could influence the song's ability to attract prospective mates (Luther et al., 2016b) or repel potential rivals (Luther & Magnotti, 2014; Luther et al., 2016a). It has also been suggested that increased minimum frequency may be a side-effect of singing louder (Nemeth & Brumm, 2010). Amplitude was not measured in this study, so it is unclear if amplitude also increased in response to noise. Future research into the transmission properties of adjusted songs should determine if frequency shifts, without a corresponding increase in amplitude, optimize transmission (Chapter 3) or influence the responses of signal receivers (Appendix A).

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Table 2.1: Comparison of song structure between noisy and quiet conditions during the chronic traffic noise experiment and the transient traffic noise experiment; (α=0.025); significant p-values are in bold and statistical trends are italicised (0.025 < p < 0.05).

Study	Song Parameter	Explanatory Variable	Estimate ± SE	t-value	p
Chronic	Minimum frequency[a]	Intercept	3.33 ± 0.11	30.73	**<0.001**
		Treatment	-0.58 ± 0.08	-7.44	**<0.001**
	Frequency range[b]	Intercept	3.13 ± 0.16	19.80	**<0.001**
		Treatment	0.34 ± 0.10	3.47	**<0.001**
Transient	Minimum frequency[c]	Intercept	3.51 ± 0.11	31.48	**<0.001**
		Treatment	-0.16 ± 0.07	-2.33	**0.022**
	Frequency range[d]	Intercept	2.22 ± 0.17	13.12	**<0.001**
		Treatment	0.27 ± 0.12	2.24	*0.027*

Estimates for treatment are for the quiet condition
[a]Random effect of male identity: variance= 0.10 ± 0.32; residual = 0.12 ± 0.35
[b]Random effect of male identity: variance=0.22 ± 0.46; residual = 0.41 ± 0.64
[c]Random effect of location: variance (variance ± SD) = 0.17 ± 0.41; residual = 0.05 ± 0.21
[d]Random effect of location: variance= 0.38 ± 0.62; residual = 0.07 ± 0.27
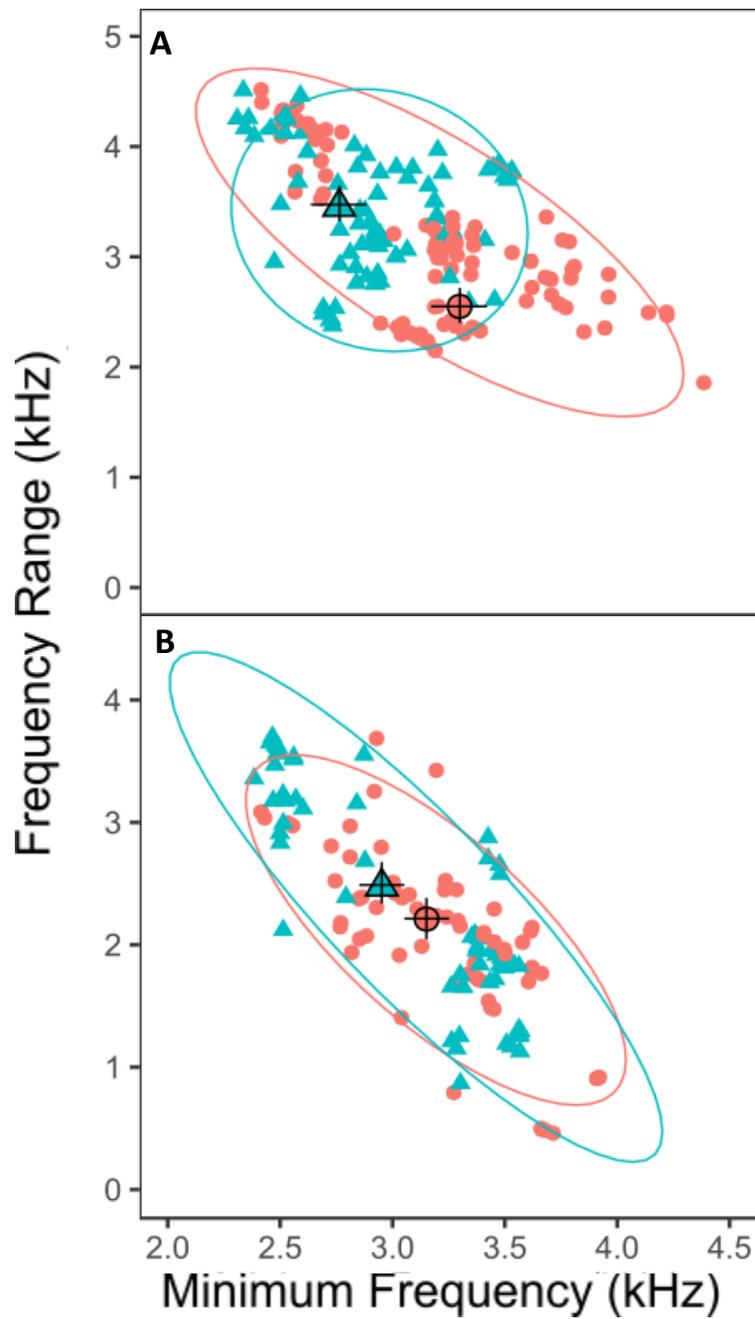
Figure 2.1: The relationship between minimum frequency and frequency range in noisy (light red circles) and quiet (light green triangles) conditions during the chronic noise experiment (A) and transient noise experiment (B), with centroids and standard error bars derived from their models.
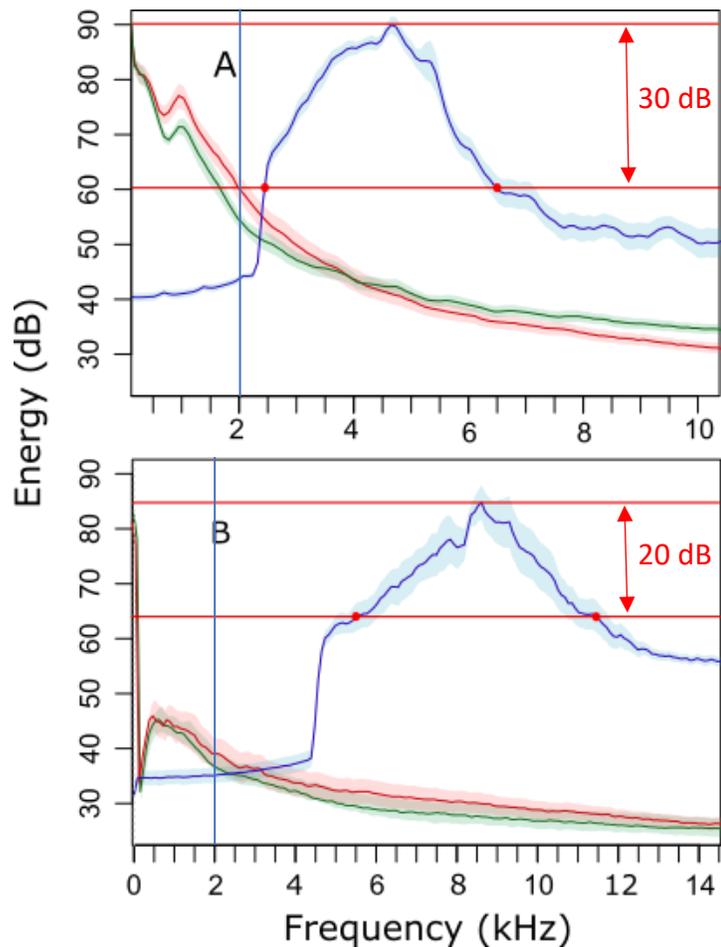
Figure 2.2: The spectral profiles of average background noise measurements from noisy

(red) and quiet (green) conditions, as well as a typical junco song (blue), with standard

error depicted as shading around each line. The vertical blue line indicates the cut-off for

the high-pass filter, the top red horizontal line indicates the peak amplitude, from which

I came down 20 or 30 dB to measure minimum and maximum frequency (the left and

right red dots, respectively). A; created using recordings from the chronic noise

experiment (noisy sample of background noise, n = 8; quiet sample, n = 8; junco song, n

= 8) B; created using recordings from the transient traffic noise experiment (noisy

sample, n =10; quiet sample, n = 10; junco song, n = 10).

Chapter 3: Noise-induced Changes to Song Structure do not Improve Signal Transmission in Dark-eyed Juncos

ABSTRACT

Some animals adjust the fine structure of their acoustic signals in response to anthropogenic noise, and these adjustments are assumed to increase the distance over which the signal can be detected through noise. Increasing signal amplitude is one of the most common adjustments made in response to noise (the Lombard effect). Increasing minimum frequency is another common response to noise, but its function is less clear. It has been assumed to maximize signal detectability by reducing spectral overlap with low-frequency background noise, but transmission experiments comparing normal and frequency-adjusted signals are lacking. Here, I report on the transmission properties of dark-eyed junco (*Junco hyemalis*) songs that have had their minimum frequencies increased by the birds in response to noise. I recorded junco songs in noisy and quiet locations, confirmed that those recorded in noisy environments had higher minimum frequencies, and then broadcasted the altered and unaltered songs through a speaker at both noisy and quiet locations. I re-recorded the broadcasted songs along a 40-m transect at each location and quantified song degradation. Songs degraded predictably with distance, but recording site, transmission site, and the two-way interaction between them had no effect on degradation. This indicates that noise-induced changes to song do not improve signal detectability in noisy conditions, as they cannot be detected at a greater distance than unadjusted songs in noisy locations.

INTRODUCTION

Noise pollution has multiple negative impacts on wildlife. It can reduce species richness (Eigenbrod et al., 2008; McClure et al., 2013; Bunkley et al., 2017) and reproductive success (Halfwerk et al., 2011) and increase physiological stress (Wright et al., 2007). Noise can also interfere with acoustic communication; signals cannot be detected over as great a distance when produced in noisy environments (Rabin et al., 2006; Templeton et al., 2016; Grabarczyk & Gill 2019), which impairs communication critical for acquiring mates, repelling territorial intruders, and avoiding predators (Brumm & Ritschard, 2011; Halfwerk et al., 2011; Templeton et al., 2016).

In response to noise, many animals adjust their acoustic signals by shifting signal production to quieter times of the day, or by altering the acoustic structure of their signals (Brumm et al., 2004; Egnor & Hauser, 2006; Ripmeester et al., 2010; Arroyo-Solís et al., 2013; Hotchkin et al., 2013; Luther et al., 2016). Often alterations to signal structure include increasing the amplitude or frequency of their signal (Patricelli & Blickley, 2006; Wood & Yezerinac, 2006; Cardoso & Atwell, 2010; Montague et al., 2012; Hotchkin et al., 2013). There is considerable evidence that shifting signal production to quieter times of day and increasing signal amplitude improve signal detectability in noisy environments (Cynx et al., 1998; Nemeth & Brumm, 2010), but the function of increasing a signal's minimum frequency is less clear. Because anthropogenic noise is low in frequency, increasing the frequency of acoustic signals can reduce spectral overlap and

potentially increase the distance over which the signal can be detected (Francis et al., 2009; Luther & Baptista, 2009).

Few studies have investigated the effects of anthropogenic noise on the transmission and detectability of frequency-shifted signals. Some studies have compared signal transmission of low- and high-frequency signals between rural and urban environments, but these have focused on how frequency adjustments affect signal transmission when the amount of vegetation or human structures (buildings and roadways) differ (Slabbekoorn et al., 2007). Furthermore, the approach used by Slabbekoorn et al. (2007) to test signal transmission was to broadcast artificial tones of different frequencies through speakers in urban and rural environments. Nemeth et al. (2012) notes that such artificial stimuli may propagate differently than naturally produced birdsong. Pohl et al. (2011) tested the effects of frequency on signal detectability by broadcasting urban or rural noise and testing signal detectability when signal frequency is altered, but they used artificially manipulated signals rather than signals adjusted naturally by animals in response to noise. Nemeth et al. (2012) again argued that such stimuli are unrealistic because the resulting frequency shifts are unnaturally large. In support of their criticism, Mockford et al. (2011) showed that when they shifted the minimum frequency of great tit (*Parus major*) songs up 0.5kHz, signal detectability did not degrade in the same manner as naturally produced urban song. Grabarczyk and Gill (2019) subsequently avoided the issue by broadcasting naturally produced songs in their study. However, although their study focused on song

detectability in noisy and quiet sites, they did not compare signals with shifted and

unshifted minimum frequencies (Grabarczyk and Gill 2019). In the case of songs

produced in noisy areas, birds may re-allocate the energy contained in lower frequencies

to higher frequencies, which may increase the signal-to-noise ratio in the presence of

low-frequency anthropogenic noise and potentially increase the distance over which the

song is detectable. To date, no study has compared the independent effects of noise on

the detectability of naturally produced signals that have or have not been adjusted by

the animals in response to noise.

Dark-eyed juncos (*Junco hyemalis*) are a convenient study species for

investigating the effects of noise-induced structural changes on detectability. They are

common in both noisy and quiet environments, and males sing a simple trilled song

throughout the breeding season that functions to attract mates and advertise territorial

occupancy (Rottenborn, 1999; Slabbekoorn et al., 2007; Cardoso & Atwell, 2010;

Ferreira et al., 2016). Male dark-eyed juncos living in noisy environments, such as those

living alongside roadways, sing with increased minimum frequency, as compared to

those living in quiet rural environments (Slabbekoorne et al. 2007; Cardoso & Atwell,

2010; Chapter 2). Further experimental research involving the broadcast of traffic noise

or silence through a loudspeaker has confirmed that this difference in minimum

frequency is caused by noise (Chapter 2).

In this study, I tested how song adjustments made in response to traffic noise

affected song detectability as songs transmitted through the environment. I did this by

broadcasting songs that previously had been recorded under noisy or quiet conditions (Chapter 2) through a loudspeaker in both noisy and quiet conditions, and then re-recording them at fixed distances along a 40-m transect. I predicted that songs would degrade with increased distance, and that all songs would be less detectable (i.e. have poorer signal-to-noise ratios) when played back in noisy versus quiet conditions. Furthermore, if frequency shifting is adaptive, I predicted that frequency-shifted songs would be less detectable than unshifted songs when played under quiet conditions, and that unshifted songs would be less detectable than shifted songs when played in the presence of low-frequency traffic noise that would mask the lower frequencies of the unshifted songs.

METHODS

Study Area

I conducted this study in Pippy Park (47.5776° N, 52.7481° W), a large (1,376 ha) suburban park with over 120 km of walking trails in the boreal forest adjacent to St. John's, Newfoundland and Labrador, Canada. The park is dominated by balsam fir (*Abies balsamea*) and black spruce (*Picea mariana*), and is bisected by the 4-lane Trans-Canada Highway. Traffic volume on a weekday was approximately 46 vehicles/min at the time of day (08:00 − 10:00 NDT) and location of my study, as measured in January 2016 by the Department of Transportation and Works (Government of NL). I considered locations

within 300 m of the highway as 'noisy', and locations beyond 500 m from the highway, where I could no longer hear the noise, as 'quiet'.

Stimulus Preparation

Songs used as stimuli in the transmission experiment were recorded opportunistically from 16 free-living male juncos as they patrolled their breeding territories from 15 May to 15 July in 2017 and 2018. They were recorded as a part of a previous study (Chapter 2) at the same noisy and quiet areas used in the current study. Four of the birds had been colour-banded in a previous study. I reduced the risk of recording the same unbanded males on multiple occasions by separating recordings of unbanded males by at least 300 m, which exceeds the $25 - 45$ m core territory diameter in this population (personal observation). Chandler et al. (1994) similarly showed that males spend approximately 95% of their time within 57 m^2. I recorded at least 20 songs from each male from a distance of $10 - 20$ m between 06:00 and 12:00 h NDT (all recordings were of diurnal songs), when wind velocity was < 25 km/h and there was no precipitation. Recordings were made with a parabolic microphone (MKH20-P48 omnidirectional microphone with a Telinga 22'' Parabola) and a digital audio recorder (WAVE format, 16-bit amplitude encoding, 44.1 kHz sampling rate; Marantz professional PMD 661 MKII solid-state recorder, Marantz professional, Cumberland, Maryland, USA).

I reviewed each recording as a spectrogram (Hamming window, 512-point fast Fourier transform, 87.5% overlap) with Audacity recording and editing software (version

2.3.1; Audacity® software is copyright © 1999–2019 Audacity Team; the name Audacity®

is a registered trademark of Dominic Mazzoni) and selected up to 20 songs (minimum =

10) with no overlapping background noise and high signal to noise ratio from each male.

Songs (± 0.5s) were filtered with a 2-kHz high-pass filter (24 dB cut-off), normalized to a

peak amplitude of 0 dB, and exported as standalone clips using the tuneR and Seewave

packages (Ligges et al., 2018; Sueur et al., 2008) in R (R Core Team, 2018; version 3.4.2).

The recordings I used in this study contained recordings used previously (Chapter

2) and included twelve recordings from quiet locations and twelve from noisy locations. I

compared minimum frequency of playback stimuli recorded from noisy and quiet sites

using the same methodology outlined in Chapter 2 and confirmed that the minimum

frequency of stimulus songs was higher for those derived from noisy versus quiet

conditions (mean ± SD: 3.18 ± 0.47 versus 2.97 ± 0.38 kHz, respectively; results of a

linear mixed effects model with noise condition as a fixed effect and male ID as a

random factor: t = -7.44, p < 0.001). Although increases in both amplitude and frequency

are thought to increase signal transmission and detectability in noisy environments, I

focused only on minimum frequency because my stimuli were not recorded with a

calibrated microphone system capable of determining signal amplitude. Since amplitude

data were not collected during stimulus recording, the effects of potential amplitude

adjustments on signal transmission and detectability could not be tested in the current

study. Potential amplitude differences were therefore removed by normalizing all

stimuli.

For each male, I used Audacity software to create a sequence containing his $10 -$ 20 songs, with 1 s of silence between songs. I used a digital audio player (Samsung mini S4) to broadcast the songs in the field.

Transmission Experiment

Transmission trials were conducted at six noisy locations (all noisy trials were conducted on weekdays) and six quiet locations during morning rush hour traffic (08:00 $-$ 10:00 h NDT) from 1 August to 9 October in 2017, 2018 and 2019. I conducted trials during this time of year because song production by local juncos had subsided and would not interfere with the transmission trials. Most trees at my sites are coniferous, so the vegetative cover changes little between the breeding season when birds are naturally singing and the early fall when my study was conducted. To minimize the effect of uneven topography on sound transmission, I selected flat locations for my trials. One trial was conducted per day, and noisy and quiet locations were tested alternately. Trials were only conducted when Environment Canada predicted wind velocities below 25km/h and no precipitation. Windspeed during the trial was later confirmed using Environment Canada data obtained from the St. John's International Airport, which is located approximately 3.4 km from my study site.

During each trial, I broadcasted two audio sequences that each contained $10 -$ 20 songs. One sequence was derived from a noisy environment and one from a quiet environment. Each sequence (n = 24) was therefore used in only one trial (n = 12), and

each trial included two different audio sequences. Before each trial, I set up a 40-m

transect parallel to the highway. At one end of the transect, I placed a portable amplified

loudspeaker (7-100 Pignose, 5 watts; Pignose Ind., Las Vegas, NV, USA) on a tripod 1 m

above the ground, facing the opposite end of the transect. I then set up four

microphones (Behringer studio condenser microphones; 20Hz – 20kHz frequency

response, cardioid polar pattern; Willich, Germany) along the transect to re-record the

songs being broadcast from the speaker. They were mounted on tripods 1 m above the

ground at 1, 5, 15, and 40 m from the speaker, oriented towards the speaker, and

connected with cables to a single multichannel audio recorder (TASCAM DR-70D linear

PCM recorder; TASCAM, Quebec, Canada). Using a sound level calibrator (Extech

instruments model 407744; Massachusetts, United States), I calibrated each recording

channel with a 1-kHz 94-dB reference tone. After calibration, the two sequences

selected for the trial were broadcasted three times each in a random order from the

audio player, which was connected to the loudspeaker. The volume was set so that

stimuli played at 80-dB sound pressure level, as measured with a sound level meter held

1 m in front of the loudspeaker (RadioShack; model 33-2055, C-weighting, fast response;

RadioShack Corporation, Fort Worth, USA). The songs were re-recorded (24-bit WAVE

files with a 44.1kHz sampling rate) by each of the four microphones.

Following each trial, I quantified vegetation at the site to test whether there

were any differences in vegetation between noisy and quiet test locations, since

vegetative structure can affect signal transmission. I mounted a 50 X 50cm checker-

board comprising 25 alternating black and white squares (10 x 10 cm) behind the

speaker and took photographs of the board from each microphone position. I scored

vegetation by counting the number of visible squares (squares were assessed visually as

0, 25, 50, 75, or 100% visible), which produced a total visibility score between 0 (where

no squares could be seen through the vegetation) and 25 (where I could see all squares).

I also recorded background noise at each trial site to ensure noisy and quiet sites

differed in noise level. I took an averaged measurement of background noise over a ten-

minute period at the location of the speaker using the sound level meter pointed

towards the highway.

My final data set included four treatments: songs recorded at a noisy location

and played back at a noisy location (noisy x noisy, n = 6), songs from a noisy location

played back at a quiet location (noisy x quiet, n = 6), songs from a quiet location played

back at a noisy location (quiet x noisy, n = 6), and songs from a quiet location played

back at a quiet location (n = 6, quiet x quiet).

Acoustic Analysis

I compared the structure of songs recorded at the 1-m microphone to the

structure of the same songs recorded at the 5-m, 15-m, and 40-m microphones. This

approach considers degradation that occurs exclusively during signal transmission, since

any degradation or artifacts introduced to the original recording by the playback system

would be present on the 1-m recording and thus excluded from measures of additional

degradation at subsequent microphones (Sandoval et al. 2015, Graham et al. 2017).

For each trial, I selected for analysis two of the $10 - 20$ songs from each of the

two audio sequences. I chose songs that were recorded with high signal-to-noise ratios

and no overlapping noise at any of the four microphones during at least two of the three

repetitions of that sequence. Thus, for a given trial, I analyzed four different songs (2

derived from a male in a noisy environment and 2 from a male in a quiet environment)

that were each broadcasted two times and recorded each time at four different

microphones, or 32 song recordings per trial. Songs were selected by viewing them as

spectrograms (Hamming window, 512 samples, 87.5% overlap) in Raven Pro (version

1.5).

For each song, I calculated : (1) attenuation, which is the change in energy in the

song over distance, (2) excess attenuation, which is the attenuation beyond what is

expected due to geometric spreading, and (3) blurring, which is the change in the

signal's overall temporal, frequency, and amplitude structure over distance (as in

Dabelsteen et al., 1993; Holland et al., 2001; Sandoval et al., 2015). Attenuation was

calculated as the energy of the song recorded at the 1-m microphone, minus the energy

of the same song recorded at each subsequent microphone. Energy was measured as in-

band power (dB), which measures the energy contained in the frequency range of a

selection (frequency limits were determined based on the spectrogram at the 1-m

recording), rather than the energy over all frequencies. This allowed me to measure the

energy in the song without measuring the energy in the ambient low-frequency

background noise. Excess attenuation was calculated by subtracting the expected

geometric spreading loss, as calculated in R using the seewave package, from the

observed attenuation. Blurring was derived using spectrogram cross-correlation. The

song, as recorded at the 1-m microphone, was correlated against the same song at each

subsequent microphone, and the resulting correlation coefficient was subtracted from

one to measure dissimilarity. The cross-correlation parameters were set so songs were

filtered with a 2-kHz high-pass filter and normalized prior to correlation. All

measurements were made in Raven, and the two measurements for each song at each

microphone were averaged to get one measurement per song per microphone.

Statistical Analyses

I tested for effects of microphone distance, noise environment at the recording

location (noisy versus quiet), noise environment at the transmission site (noisy versus

quiet), and the two-way interaction between the two noise environments on each of the

three measures. Effects were modeled using three separate linear mixed effects models,

with trial number $(1 - 12)$ included as a categorical variable with random effects to

account for possible dependencies among repeated measures from the same trial.

Distance (5, 15, or 40 m) was included as a covariate with fixed effects. Because I

conducted three separate tests of the same general hypothesis, I adjusted alpha using

the Bonferroni method ($\alpha = 0.017$). Songs degrade predictably over distance, and, as

distance increases, attenuation, excess attenuation, and blurring should all increase (Dabelsteen et al., 1993).

I tested if vegetative cover differed between noisy and quiet transmission sites using a linear mixed model in R. Distance and the interaction between distance and noise condition were included as fixed factors, and trial number was included as a categorical variable with random effects to account for non-independence in the data.

RESULTS

With increasing distance from the 1-m microphone, attenuation, excess attenuation, and blurring all increased (Table 3.1; Figure 3.1). There were no effects of the noise environment at the recording location, the noise environment at the transmission location, or their two-way interaction on any of the three measures (Table 3.1; Figure 3.1).

Mean vegetative cover ($\pm$ SD) was 10.7 $\pm$ 9.8 (out of a maximum possible score of 25) at noisy transmission sites and 11.7 $\pm$ 10.7 at quiet transmission sites. Vegetative cover did not differ significantly between transmission site noise environments (linear mixed model: $F_{1,15}$ = 0.44, p = 0.517), but did decline significantly with distance from the speaker ($F_{1,178}$ = 385.49, p < 0.001). There was no interaction between distance and transmission noise environment ($F_{1,178}$ = 0.18, p = 0.670).

DISCUSSION

I broadcasted noise-altered and unaltered junco songs in both noisy and quiet

environments and re-recorded them along a 40-m transect to measure signal

degradation and compare signal detectability. Although songs degraded predictably with

increasing distance from their source (Dabelsteen et al., 1993; Sandoval et al., 2015),

detectability was not affected by the noise environment at the recording site or

transmission test site, or by their two-way interaction. This indicates that frequency

adjustments made in the presence of noise are not effective at increasing song

detectability in noise.

With increasing distance from the speaker, attenuation, excess attenuation, and

blurring all increased, as expected; however, songs degraded so rapidly with distance

that they were nearly inaudible only 40 m from the speaker. Juncos commonly sing from

perches near the ground (Nolan et al., 2002), so the rapid attenuation of such songs

suggests that they are intended for mates or for neighbours from adjacent territories (a

junco's territory in this population is approximately 25 – 45 m in diameter). Juncos also

sing from the tops of trees at approximately the same amplitude that songs were played

in the current study (80 – 85 dB at 1 m; Nolan et al., 2002). In this context, songs remain

audible to human observers for 100 – 250 m (Nolan et al., 2002), suggesting that songs

produced from treetops are intended for a broad audience, and that they function as

advertisements to rival males or prospective mates from up to several territories away

(Titus, 1998). The idea of directing songs to different audiences by adjusting perch

height is further supported by Ketterson et al. (1992), who showed that unmated males

are more likely than mated males to sing from treetops, and that mated males are more

likely than unmated males to sing from near the ground.

Contrary to my prediction and to previous research (Grabarczyk & Gill 2019;

Templeton et al., 2016), song detectability did not degrade faster in the presence of loud

background noise. The discrepancy may reflect differences in the amplitudes of the

noise used in each study. In the current study, the background noise level at noisy sites

was 61.6 ± 3.3 dB sound pressure level (mean $\pm$ SD; range: $57 - 67$ dB), whereas

background noise in Templeton et al. (2016) was broadcasted at 76 db. However, the

background noise in Grabarczyk & Gill (2019), which was produced by nearby roadways,

was approximately 53 dB, which was even lower than the amplitude of the traffic noise

observed in the current study. Therefore, it remains unclear why these two previous

studies, but not this current study, found that the presence of noise decreased song

detectability.

Songs recorded in noisy locations had higher minimum frequencies than songs

recorded in quiet locations, yet their detectability did not degrade faster than unshifted

songs in quiet environments, or propagate farther than unshifted songs in noisy

environments, as I had predicted. These findings differed from those of Pohl et al.

(2011), who found that great tits were better able to detect higher-frequency songs in

noisier locations. The discrepancies among studies may be due to differences in the

degree of frequency shift. The average frequency shift of songs produced in a noisy

environment was 0.21 kHz in this study versus 1 kHz in Pohl et al. (2011). Nemeth et al. (2012) argue that a frequency shift of 1kHz is much larger than what has been reported in the field; therefore, more "natural" levels of frequency shift (0.21 kHz in this study) may be less effective at increasing signal transmission than artificial shifts. Alternatively, it is possible that our measures of degradation do not represent the signal characteristics that caused live birds to respond differently to shifted versus unshifted songs in noisy environments in previous studies (e.g. Pohl et al., 2011; Templeton et al., 2016).

It remains unclear whether birds are able to independently increase the frequency of their songs or whether an increased song frequency is an artifact created by the Lombard effect. Nemeth and Brumm (2010) modelled the masking effects of noise and concluded that signal transmission is increased more by increasing song amplitude than by increasing song frequency. My results are consistent with this argument, since increased frequency did not improve the detectability of songs through noise. Some authors suggest that there may be a biomechanical link between increased amplitude and increased frequency in birdsong, and that increased frequency may simply be a functionless by-product of the Lombard effect (Nemeth & Brumm, 2010; Verzijden et al., 2010). However, Cardoso & Atwell (2011) found that when juncos sang with a higher amplitude, they also sang with a decreased minimum frequency and increased frequency range. This makes it unclear whether increased minimum frequency in response to noise is linked to amplitude adjustments, or whether they can adjust

minimum frequency and amplitude independently. Further research into the

mechanisms of song production could help determine the relationship between

frequency and amplitude adjustments, and the possible function of frequency shifts in

noisy conditions. Even if increased frequency in the presence of noise has no effect on

song transmission, it may still be important to receivers trying to evaluate the signaler or

decode information from their signal (Slabbekoorn et al. 2012). Future research should

focus on how signal receivers react to songs that have or have not been frequency-

shifted in response to noise (see Appendix A).

Many studies have investigated the factors affecting song transmission, but most

have focused on the effects of vegetative structure (Dabelsteen et al., 1993; Derryberry,

2009; Graham et al., 2017), perch height (Dabelsteen et al., 1993; Sandoval et al., 2015),

song type (Sandoval et al., 2015), and the novel physical environments presented by

cities (Mockford et al., 2011; Slabbekoorn et al. 2007). Very few studies have

investigated the effects of noise, rather than the effects of the physical environment, on

signal detectability. In this study, I tested the effects of environmental noise and the

adjustments that birds make to their songs in response to noise on signal detectability.

Songs that were frequency-shifted in response to noise did not degrade less than

unshifted songs when played in a noisy environment, suggesting that frequency shifting

is not an adaptation for improving signal detectability through noise.

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Table 3.1: Factors affecting the detectability of dark-eyed junco song, including distance from the song's origin, noise level at the transmission test location (Tloc: noisy or quiet), noise level at the recording location (Recloc: noisy or quiet), and the two-way interaction between recording and transmission site noise levels (ReclocXTloc). Effects were modeled using a different linear mixed model for each parameter of song degradation, and trial number $(1 - 12)$ was included in each model as a categorical variable with random effects. Bold numbers indicate a significant relationship (Bonferroni adjusted $\alpha = 0.017$).

Song Parameter	Explanatory Variable	Estimate ± SE	t-value	P
Attenuation	Intercept	4.89 ± 1.20	4.11	**<0.001**
	Distance	0.98 ± 0.03	37.21	**<0.001**
	Recloc	-0.48 ± 1.13	-0.42	0.674
	Tloc	0.44 ± 1.58	0.28	0.780
	ReclocXTloc	0.86 ± 1.60	0.54	0.590
Excess Attenuation	Intercept	-1.83 ± 1.03	-1.79	0.098
	Distance	0.28 ± 0.01	21.69	**<0.001**
	Recloc	-0.48 ± 0.56	-0.85	0.397
	Tloc	0.45 ± 1.42	0.32	0.758
	ReclocXTloc	0.86 ± 0.79	1.09	0.278
Blurring	Intercept	0.96 ± 0.02	19.80	**<0.001**
	Distance	0.01 ± 0.00	15.31	**<0.001**

Recloc	-0.02 ± 0.02	-1.20	0.231
Tloc	-0.04 ± 0.03	-1.68	0.111
ReclocXTloc	0.03 ± 0.03	1.11	0.270

Distance is included as a continuous variable

Estimates for each factor are for the quiet level of the factor

Random effects for trial number (variance ± SD):

    Attenuation: 3.68 ± 1.92; residual = 30.85 ± 5.56
    Excess attenuation: 5.13 ± 2.27; residual = 7.58 ± 2.75
   Blurring: <0.01 ± 0.03; residual = <0.01 ± 0.09
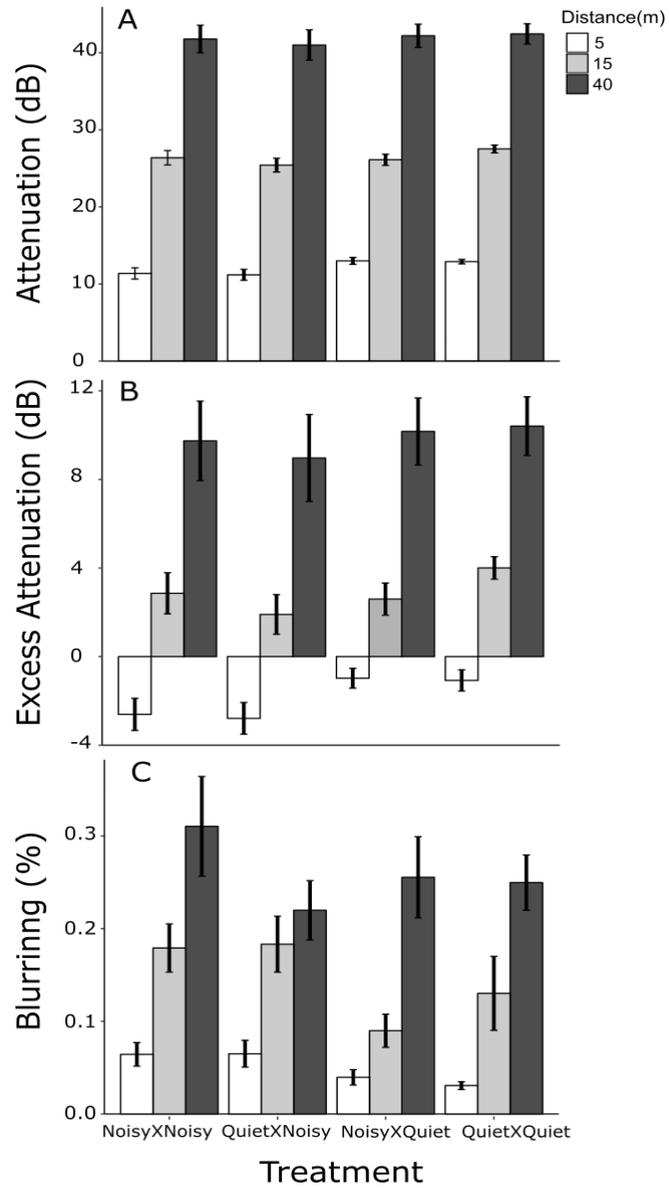
Figure 3.1: Song degradation as a function of treatment and distance. Treatment

combinations are shown as the noise environment of the recording location, followed by

the transmission test location; therefore, Quiet X Noisy would indicate a quiet recording

location and a noisy transmission test location. Bars indicate the average measurements

at each distance, relative to the 1-m microphone, with standard error bars.

Chapter 4: General Discussion

Anthropogenic noise is increasing around the world, and can have numerous

negative impacts on animals. Proximate effects include physiological stress and

interference with communication (Wright et al., 2007; Francis et al., 2009; Ripmeester et

al., 2010; Hanna et al., 2011); some ultimate effects are fragmentation of, or reduction

in, a species' geographic range, or reduced reproductive success (Wright et al., 2007;

Francis et al., 2011; Halfwerk et al., 2011b McClure et al., 2013).

I studied effects of noise on territorial songs of the Dark-eyed Junco (*Junco*

hyemalis) in Newfoundland and Labrador. I approached this study from two

perspectives. First, I confirmed that juncos increase the acoustic frequency in their songs

in response to noise, presumably to avoid spectral overlap with low-frequency

background noise (Ripmeester et al., 2010; Cardoso & Atwell, 2011; Luther & Magnotti,

2014). I then tested the assumption that songs that were altered in response to noise

remain detectable over greater distances (where detectability is defined as maintaining

signal integrity) than unaltered songs in noisy environments.

In Chapter 2, I tested whether juncos change the structure of their song in

response to noise, as reported in previous studies (Slabbekoorn et al., 2007; Cardoso &

Atwell, 2010). Most previous studies investigating noise-induced song structure changes

have been correlational (but see Verzijden et al., 2010; Hanna et al., 2011; Templeton et

al., 2016) and therefore unable to demonstrate a causal effect of noise on song

structure. It is possible that differences in song structure described in correlational

studies were due to confounding factors such as anthropogenic light or habitat

alteration, or to intrinsic differences between birds in urban versus rural environments.

In my study, I used complementary correlational and experimental approaches to

demonstrate that transient noise causes juncos to adjust their song structure.

I then tested the assumption that increased frequency should improve song

detectability in noisy environments (Nemeth et al., 2012). In most past research on

sound transmission, researchers have played back artificial tones or artificially adjusted

birdsong, and then measured degradation and detectability of the broadcasted signals

over distance (Halfwerk et al., 2011a; Pohl et al., 2012, but see Grabarczyk & Gill, 2019).

Nemeth et al (2012) have criticized this approach for a lack of realism; for example,

frequency shifts of manipulated signals are often greater than what is observed in

nature. Therefore, I recorded naturally produced junco songs in noisy and quiet habitats,

and then broadcast them in both naturally noisy and quiet locations to assess changes in

signal detectability. I found that songs recorded in noisy environments, and which had

higher minimum frequencies, were not detectable over greater distances than songs

recorded in quiet environments when played in noisy environments. Similarly, songs

recorded in quiet environments, and which had lower minimum frequencies, were not

detectable over greater distances in quiet environments. These findings suggest that

frequency shifts are not an adaptation for improving signal transmission and detection

in noisy habitats. I did not measure amplitude in this experiment, but the findings are

consistent with the hypothesis that increased frequency is instead a by-product of

increased amplitude (Nemeth & Brumm, 2010).

Noise-induced signal alteration can seriously affect communication, with

implications for mate choice decisions and territorial interactions. Song divergence

between anthropogenically noisy and less-disturbed, quiet habitats could lead to

population divergence as a result of disruption to mate choice (Slabbekoorn & Smith,

2002a). For instance, differing noise environments can lead to song diversification,

which can then cause females to select for habitat-dependent dialects, leading to

reproductive isolation and speciation (Slabbekoorn & Smith, 2002b). Alteration in female

preference has already been documented in some songbirds, where females prefer

males that sing low-frequency songs (Halfwerk et al., 2011a, b; Luther et al., 2016).

Hence, in anthropogenically noisy environments, noise can disrupt mechanisms of mate

choice. Alteration to song frequency can also affect male-male interactions. Previous

research has demonstrated that males react more aggressively to low-frequency songs;

however, this relationship weakened with increasing background noise (Mockford &

Marshall, 2009; Luther & Magnotti, 2014). If males in noisy areas must use songs of

perceived poorer quality to defend territories, then this may lead to more territorial

interactions and more energy expended on territory defence.

Future research on signal alteration could be advanced through long-term data

on individual birds collected near novel sources of noise (Harding et al., 2019). For

instance, does song structure permanently change after prolonged exposure to noise, or

can birds revert to previous song forms if the noise stops? Studies investigating vocal

mechanics would help determine the limits of vocal adjustments (Cardoso, 2017; Logue

et al., 2019) and whether frequency and amplitude can be adjusted independently, or

whether frequency shifts are a by-product of increasing amplitude (Nemeth & Brumm,

2010). Furthermore, genetic studies could identify genes associated with vocal flexibility

and potentially provide tools for predicting which species could and could not tolerate

noise pollution.

Future research should also investigate how noise-induced signal alteration

affects signal receivers. Previous research has measured the effectiveness of territorial

songs by the level of aggression exhibited by resident males when those songs are

played back in their territories (Catchpole, 1978; Gil, 1997; Mockford & Marshall, 2009;

Ripmeester et al., 2010; Sandoval, 2013). However, this approach has been criticized

because it is unclear whether high-quality songs should elicit aggressive or non-

aggressive responses (deKort et al., 2006). To address this issue, I conducted a remove-

and-replace experiment (Krebs et al., 1978; Nowicki et al., 1998) in which I replaced

resident males from noisy and quiet habitats with speakers broadcasting songs recorded

in noisy or quiet habitats. I then monitored the rate of territorial intrusions as a direct

measure of signal efficacy in different noise environments. Unfortunately, time

constraints and inclement weather prevented a sufficient sample size to draw

meaningful conclusions, but the experimental design and methodology proved feasible.

Full methodological details of this pilot study are provided in Appendix A.

In conclusion, I approached the question of how noise affects communication in dark-eyed juncos from the perspective of the signaller and signal alteration, and from the perspective of song transmission through the environment. I also conducted a pilot study testing a third perspective, that of signal receivers and how they perceive noise-adjusted signals. Approaching such questions from multiple perspectives will help researchers to determine how noise affects avian communities. I confirmed that juncos from noisy environments sing songs with higher frequency when compared to the songs of conspecifics in quiet populations, that this occurs through individuals flexibly altering song structure in response to noise, and that the increased frequency has minimal effects on signal detectability in noise.

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Appendix A: Methods for a pilot study entitled "Testing receiver responses to noise-altered and unaltered signals"

METHODS

I tested whether anthropogenic noise and noise-induced changes to song structure affect receiver responses, using dark-eyed juncos (*Junco hyemalis*) as a model species. I temporarily removed a male junco from his territory and replaced him with an array of speakers simulating a singing male patrolling his territory for six hours the following morning. Songs recorded from noisy and quiet habitats were broadcasted in both noisy and quiet habitats. Throughout each trial, I used a microphone array surrounding the removed male's territory to monitor intrusion rates by neighbouring males.

Study Area

This study was conducted in Pippy Park, St. John's, Canada (47.5776° N, 52.7481° W). All trial locations were within the boreal forest adjacent to the Trans-Canada Highway, which bisects Pippy Park. Noisy locations were within 300 m of the busy 4-lane highway, which has an estimated weekday rush hour traffic volume of 38 vehicles/min or 2259 vehicles/h (calculated from traffic data from 26 January 2016 between 07:00 and 10:00 h along the segment of the Trans-Canada Highway used in this study; based on information collected by the Newfoundland Government, Department of

Transportation and Works). Quiet locations were at least 500 m away from the highway, where I could no longer hear the traffic noise.

Recording Junco Songs and Comparing Song Structure

I recorded the songs of 8 unbanded male juncos living in noisy (n = 4) and quiet (n = 4) locations in Pippy Park during the 2017 and 2018 breeding seasons between 15 May and 15 July. I recorded juncos in noisy territories only on weekdays to ensure sufficient highway traffic. The territory size of dark-eyed juncos in this population, identified by following juncos and marking singing perches over a 1 – 2 hour period, ranges from 25 m to 45 m in diameter (personal observation). Therefore, to ensure that each unbanded male was a new individual, I separated recording sites by at least 300 m. Juncos were recorded opportunistically between 06:00 and 12:00 h (all recordings were of diurnal songs), when windspeed was less than 25 km/h and there was no precipitation. Subjects were recorded as they patrolled their territories and were recorded for a minimum of twenty songs from a single singing bout. Recordings were taken using a digital audio recorder (Marantz professional PMD 661 MKII solid-state recorder; WAVE format, 16-bit amplitude encoding, 44.1 kHz sampling rate; Marantz professional, Cumberland, Maryland, USA) connected to either a shotgun microphone (Sennheiser ME66; $40 - 20000$Hz frequency response; Sennheiser, Wedemark, Lower Saxtony, Germany) or a parabolic microphone (MKH20-P48 omnidirectional microphone

with a Telinga 22'' Parabola; n = 3 males, including 2 from a noisy habitat and 1 from a

quiet habitat).

I reviewed each recording as a spectrogram (Hamming window, 512-point FFT,

87.5% overlap) in Audacity (Audacity recording and editing software, version 2.3.1;

https://audacityteam.org/) and selected 20 high-quality songs from each male. Songs (±

0.25s) were exported as standalone clips (WAVE format, 16-bit amplitude encoding, 24

kHz sampling rate) using the tuneR and Seewave packages (Ligges et al., 2018; Sueur et

al., 2008) in R (R Core Team, 2018; version 3.5.1). All songs were high-pass filtered (set

at 2.5 kHz with a 24 dB cut-off) to remove background noise and then normalized to a

peak amplitude of 0 dB.

Five of the 8 junco recordings from this experiment were included in the song

structure analysis presented in Chapter 2. As a part of that analysis, I showed that songs

recorded in the presence of noise had a higher minimum frequency (average ± SE: 3.33 ±

0.11 kHz) than songs recorded in quiet habitats (minimum frequency: 2.75 ± 0.08 kHz;

frequency range average = 3.47 ± 0.10 kHz). Refer to Chapter 2 for complete details on

the structural analysis.

Playback Stimuli

For each recorded male, I used the *seewave* package in R to create a six-hour

playback sequence in which the songs of the male were organized into bouts of 20

unique songs arranged in random order (approximately 1 minute, 45 seconds per bout).

The 0.25 s padding at each end of a given song was attenuated using the linear attenuation function in Audacity to gradually soften the transition from silence to the background noise preceding and following each song. Songs within a bout were separated by 3.5, 4.5, or 5.5 seconds of silence (selected randomly) to mimic natural variation in singing rates. Bouts within the 6-h recording were separated by 24 minutes of silence.

Three different 6-hour playback sequences were created using the same 20 songs so that a different sequence could be played through each of the three speakers used to simulate the singing male during the playback trial. Speakers were Bliiq Infinite X (frequency response: $120 - 5000$Hz; Juneed Technology Co., Ltd, GuangHao International Genter, Shenzchen, China) or IconntechsIT speakers (frequency response: $80 - 18000$ Hz; Piron Supply Chain Management Co., Ltd., Shenzhen, China) with an internal microSD card for song storage. The playback sequences were designed so that a bout would play once every 8 minutes and 20 seconds (plus or minus 1 minute due to variation created by the random selection of inter-song intervals) from one of the three speakers. The first song bout would start at speaker one, followed by 8 minutes and 20 seconds of silence (± 1 min), followed by a bout from speaker two, silence, a bout from speaker three, and then more silence. The bouts would continue to cycle among the three speakers for the entire six-hour playback period. Bout length, song rate within bouts, and interbout length were based on an analysis of singing behaviour of dark-eyed juncos recorded as a part of a previous project (Hennigar et al., 2019).

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To correct for the possible deterrent effect of speaker playback in general, and to test the effectiveness of unfamiliar, heterospecfic song, I also conducted control trials in which I broadcast the songs of common yellowthroats (*Geothlypis trichas*), which are not present in the study area. The control recordings were provided by the Macaulay Library. All control trials used different recordings, and from each recording twenty songs were selected and assembled into a playback sequence following the same procedure as for the junco songs.

Remove and Replace Experiment

Playback trials were conducted following a randomized complete block design from May to mid-July of the 2017 and 2018 avian breeding seasons. Each block included six different treatments: songs from a noisy habitat played back in a noisy habitat (noisy X noisy, n = 2), noisy X quiet (n = 2), quiet X noisy (n = 3), quiet X quiet (n = 1), a control playback in a noisy habitat (control X noisy, n = 2), and control X quiet (n = 2). All trials within one block were completed in a random order before trials from the next block began. Each playback trial location was at least 300 m away from where the songs in the playback were recorded to ensure the songs were unfamiliar to the neighbouring males.

Before beginning a trial, I mapped the territory of the male junco targeted for removal. I opportunistically targeted males in either a noisy or quiet habitat. I observed the targeted male and recorded the singing perches with a handheld GPS (Garmin GPS map 78s; Kansas, USA). I continued this observation until the junco circled its core

territory at least twice (this typically took 45 min to 1.5 h of observation). At this time, the targeted male junco was not banded; therefore, if I lost sight of the bird momentarily, I continued marking singing perches once it was re-sighted, but waited until the junco returned to a previous perch before concluding it was the same bird.

The afternoon before the trial began, I mounted the three speakers on tree branches in an equilateral triangle around the edges of the core territory. Speakers were programmed to delay playback until the following morning. The locations of each speaker were recorded using a survey-grade global navigation satellite system (GNSS; Trimble Geo 7X with 10-cm accuracy; Trimble Geospatial, St. John's NL; see Figure 4.1). I also set up an array of four audio recorders (Song Meter, model SM3; Wildlife Acoustics, Concord, MA, USA) arranged in a 40 X 40m square that encompassed the core territory (see figure A.1 for a typical microphone array set-up). These recorders can detect songs anywhere within the array and up to 10 m outside the array (Ethier & Wilson, 2019; Hennigar et al., 2019), which ensured that the entire core territory was within range of the recorders. Each recorder was attached to a tree with its left internal microphone pointed towards the centre of the territory. I attached a GPS to each recorder to synchronize their internal clocks, which is a requirement for localizing sounds with a microphone array. I programed the recorders to record continuously and to create 2-h stereo WAVE files (sampling rate of 24 kHz, 16-bit, and with a gain setting of 10 dB). I used the survey-grade GNSS to record the locations of the left internal microphone of

each recorder, which is also a requirement of localizing sounds recorded with a microphone array.

After setting up the speakers and microphone array, I captured the resident male junco in a mist net placed in the centre of his territory. He was lured to the net by broadcasting unfamiliar junco songs from a speaker placed under the net. Juncos were captured between 19:00 and 21:00h to make use of a small evening singing peak (found to be present based on observational data collected in Labrador; Ethier & Wilson, 2019; Hennigar et al., 2019). Catching birds in the evening was intended to minimize the amount of time the male was removed from his territory before beginning the playback trial the following morning. After a bird was captured, he was transported to a nearby outdoor aviary, where he was banded and measured. The total handling time, from when the junco flew into the mist net until it was released into the aviary, was 45 minutes or less. Juncos were provided water and a songbird seed mixture containing suet and mealworms.

Playback began at 05:00 h the morning after the subject was captured and continued until 11:00 h that same day. After each trial (i.e. within 18 hours of capture), the junco was caught in the aviary with a hand-held net and released at its site of capture.

Future Research: Analysis of Territorial Intrusion Rates

To measure the rate of territorial intrusions by neighbouring males during the six-hour playback, I would identify all junco songs produced by both the speaker and neighbouring males by visually inspecting sonograms of the microphone array recordings in Audacity (Hamming window, 512-point FFT, 87.5% overlap). Songs broadcasted by the speakers would then be excluded from subsequent analyses. I would localize each remaining junco song in two-dimensional space using a customized MATLAB program developed for this purpose (Ethier & Wilson, 2019). I could then use the average distance of vocalizations from the territory centre to quantify the degree of territorial intrusion.

Intrusion rate would be compared between conspecific and heterospecific treatments using a linear mixed effects model, with distance of detected songs from the territory centre as the dependent variable, species of the playback stimulus as a fixed factor, and trial number as a random factor. If junco songs were more effective than common yellowthroat songs at deterring territorial intrusion, then a second linear mixed-effects model would investigate the effects of noise and noise-induced changes to song structure on deterring territorial intruders among the junco trials. Playback location (noisy versus quiet), stimulus recording location (noisy versus quiet), and the two-way interaction between playback and recording location would be included as fixed factors, and trial number as a random factor.

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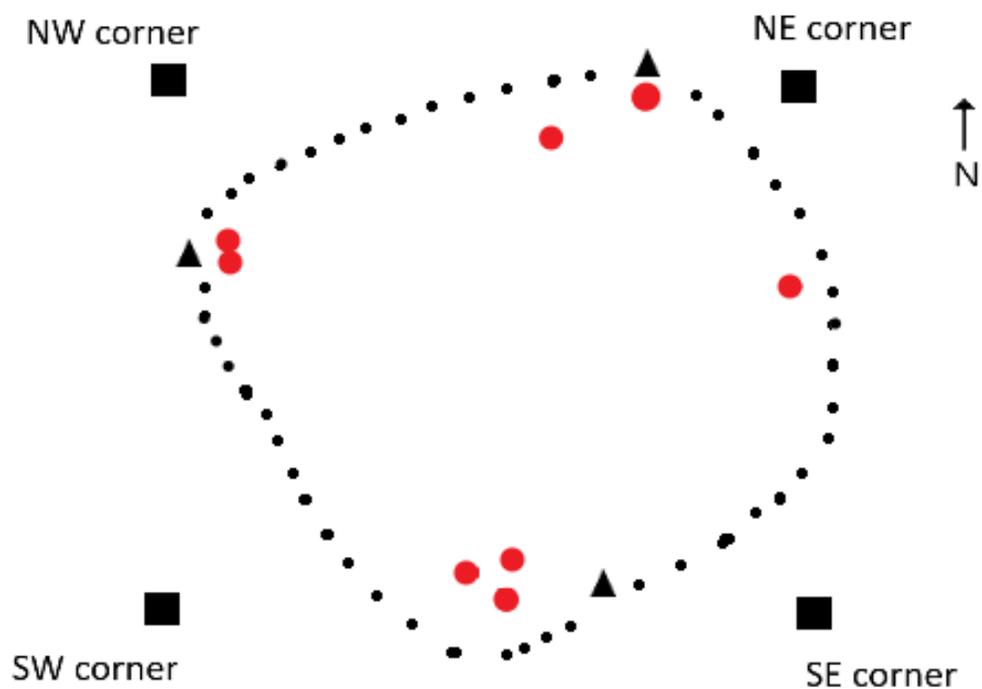
Figure A.1: A typical microphone array set-up. Red circles indicate known singing perches, triangles indicate speaker locations, and squares indicate microphone locations. The dotted line approximates the male's territory boundary. Distance between microphones is approximately 40 m.