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Constraints and Context: Testing the Effects of Anthropogenic Noise and Social Factors on Avian Signaling Behavior

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Constraints and Context: Testing the Effects of Anthropogenic Noise and Social
Factors on Avian Signaling Behavior

by

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Constraints and Context: Testing the Effects of Anthropogenic Noise and Social Factors on Avian Signaling Behavior

Erin E. Grabarczyk, Ph.D.

Western Michigan University, 2019

Rapid human-generated environmental change has irrevocably altered the natural world. Understanding how animal populations respond and persist within these modified landscapes is critical for mitigating associated negative environmental impacts. For organisms that rely on vocal communication, anthropogenic noise masks signals, reducing the area over which vocalizations can be heard. For birds, noise overlaps with songs males use to defend breeding areas and attract mates, both critical aspects of reproduction. In response to noise, males adjust their song structure, but whether they actually benefit from song changes is unknown. Moreover, it remains unclear whether noise disrupts communication by affecting interactions with mates, neighbors, or intruder detection.

I tested the hypothesis that anthropogenic noise affects multiple components of communication systems, including signalers, their signals, and receivers. I studied male house wrens (*Troglodytes aedon*), asking first whether noise influences song structure, and then considering whether noise alters transmission of songs through space, and perception of singing during territorial intrusions as well as within social networks. In Chapter 1, I show that paired males adjusted their song frequency in response to noise playback, whereas unpaired males do not. Unpaired males may not change their songs

if they are constrained by female preferences for low-frequency song. In Chapter 2, I show individuals plastically adjusted some but not all song traits in response to variation in immediate noise levels, and that social context and ambient noise affected song structure across males. In Chapter 3, I show song amplitude fell below thresholds for detection and discrimination within a male's own territory under high noise conditions, suggesting that noise masking may affect house wren spatial ecology. Because noise decreases signal active space, masking may compromise the ability of males to detect and respond to intruders. In Chapter 4, noise did not influence detection of simulated territorial intruders, but did affect aggression by territorial males. Males attacked speakers broadcasting intruder song more under noisy than quiet conditions, suggesting they may be unable to assess intruders based on songs alone and rely on close encounters for discrimination. In Chapter 5, I show that although noise affected male responses to territorial intruders, social context predicted singing by neighboring males. Social networks may be resilient to short-term changes in singing by any one male and change in patterns of vocal interactions may occur over longer periods of time. My research demonstrates that noise affects each part of the communication system, beginning with the songs males produce, to their transmission, and reception by receivers. However, social factors sometimes had larger impacts on singing as they modified male responses to noise, indicating a complex interaction among factors that influence song. Thus, noise masking could act as an important selective pressure on animal signals, but its effects depend on the social context in which signal are given. My research advances our understanding of how both noise and social context affect

communication, all of which may have implications for individual fitness, population

dynamics, and ultimately the persistence of populations in urban environments.

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CHAPTER I

INTRODUCTION

Rapid human-generated environmental change has irrevocably altered the natural world. In response, some animals adaptively adjust their behaviors, enabling individuals to persist within modified landscapes. However, some individuals show maladaptive responses, either because they are unable to adjust their behaviors or because adjustments are not enough to counter the negative consequences of human-generated change (Sih 2013, Wong and Candolin 2015, Sih et al. 2016). Understanding why behavioral responses to novel environments vary is an essential step towards mitigating associated negative environmental impacts and facilitating species conservation (Sih 2013). Behavioral plasticity, or flexibility, may be a critical component for survival in human-modified environments (Sih 2013, Wong and Candolin 2015), enabling individuals to tactically adjust their behaviors in response to variation in their environment (Patricelli et al. 2016). But whether plasticity is sufficient, and how selection pressures act on plastic responses in human-modified landscapes remains poorly understood (Wong and Candolin 2015).

For birds, anthropogenic noise masks long-distance vocal signals used for mate attraction and territory defense. Masking may constrain communication if the area over which songs can be heard decreases or if signal perception by receivers changes (Patricelli and Blickley 2006). In response to noise, callers adjust the structure of their signals, but whether they benefit from song changes is poorly understood. In this introduction I will review selection pressures from the social and physical environment

that shape the structure of vocal signals, how birds adjust their vocalizations in response to human-generated noise pollution, and finally consider the costs and benefits of signaling in noise from the context of signal receivers.

Selection on animal vocal communication systems

Animal vocalizations are shaped by multiple factors, including selection pressures from the social environment (Bradbury and Vehrencamp 1998, Snijders and Naguib 2017), as well as transmission constraints from the physical environment a species inhabits (Morton 1975). Vocal signals and the information they convey are critical components for survival and reproductive success, as they function in mate attraction, territory defense, predator detection, and interactions with offspring (Catchpole and Slater 2008). For many species, social relationships are defined by vocal interactions. But in order to maintain relationships, signals must transmit through the environment and reach intended receivers with minimal loss of information. Therefore, signals undergo selection to maintain structure and intensity as they transmit through different habitats (i.e. acoustic adaptation hypothesis, Morton 1975) to signal receivers with auditory systems capable of extracting relevant information from background noise (Endler 1992). Consequently, selection on animal vocal communication systems result from co-evolution of signal senders and receivers (Endler 1992).

Social environment affects signal design

Vocal signals have multiple functions (Catchopole and Slater 2008) and signals with different functions follow design rules (Bradbury and Vehrencamp 1998) that enable transmission to potential receivers in a given environment. During the breeding season, males broadcast highly localizable, long-distance signals at high rates that simultaneously attract female breeding partners while also repelling territorial rivals (Catchpole and Slater 2008). Advertising males are constrained by the risk of predation, and therefore modulate their use of locatable signal components depending on a perceived tradeoff between mate attraction and predation risk (Ryan 1985). Once paired, males may adjust their signaling behavior in ways that could further improve reproductive success; for example, males may signal at lower amplitudes to avoid eavesdropping by other males (Johnson and Kermott 1991, Ritchison 1995, Reichard et al. 2013) or adjust song frequencies to those preferred by female breeding partners (Byers 2007, Halfwerk et al. 2011). Thus, the structure of long-distance signals and pattern of vocal behavior are influenced by the social context in which they are given.

The influence of the social environment on signaling behavior extends beyond the responses of breeding partners. Territorial males interact in complex social networks, broadcasting information about sender identity, quality, and motivation with long-distance signals. Within signaling networks, both males and females gain information about other callers via direct one-on-one signaling interactions as well as through passively by eavesdropping (McGregor and Peake 2000). Neighbors adjust their own behavior according to the information gained (Peake et al. 2002, Naguib et al.

2004, Schmidt et al. 2007, Amy et al. 2010, Snijders et al. 2017) and how signals are

perceived may influence male fitness through conspecific territorial interactions

(Schmidt et al. 2007) or female mate choice decisions (Mennill et al. 2002). Therefore,

male signaling behavior has immediate fitness consequences during one-on-one

interactions, but also indirectly affects decision-making of conspecifics that gain

information via eavesdropping.

Acoustic environment affects signal transmission

Constraints from the acoustic environment on signal transmission limit the ability

of receivers to detect, discriminate, and decode vocal signals. In order to elicit a

response from receivers, signals must be separated from other sounds that make

background noise. Therefore, the amplitude of signals must exceed those of

background sounds (Klump 1996). Frequency masking occurs when acoustic energy

from noise overlaps the energy of a signal, reducing the receiver's ability to hear and

perceive signals (Patricelli and Blickley 2006). If signals are masked by other sounds

then signal active space, or the area over which signals can be heard, decreases

(Brenowitz 1982). Among individuals, information sharing is limited by spatial proximity

to other signalers and the degree of signal masking due to the level of ambient noise in

the environment (Snijders and Naguib 2017). Changes to the acoustic environment that

alter transmission patterns through signal masking could affect social interactions, with

implications for reproductive success.

Anthropogenic noise affects avian vocalizations

Widespread anthropogenic noise has diverse effects on wildlife, including disrupting foraging (Purser and Radford 2011, Siemers and Schaub 2011, Voellmy et al. 2014, Senzaki et al. 2016), predator detection (Chan et al. 2010, Grade and Sieving 2016), and masks signals used for mate attraction and territory defense (Patricelli and Blickley 2006). In response to noise, male songbirds may change the structure of their songs, thereby increasing transmission distances and the likelihood of detection (reviewed by Brumm and Zollinger 2013). To reduce masking, signalers might increase the minimum frequency of their songs (Slabbekoorn and Peet 2003, Mockford and Marshall 2009, Potvin et al. 2011), or improve signal-to-noise ratios by increasing song amplitude (Brumm 2004, Derryberry et al. 2017). Males may also increase the probability of detection by singing more often (Slabbekoorn and den Boer-Visser 2006, Redondo et al. 2013) or by singing longer songs (Ríos-Chelén et al. 2013). For animals with complex song, shifts in spectral or temporal elements may be driven by selection of note elements used to compose a song (Halfwerk and Slabbekoorn 2009) or they might use the same notes regardless of noise, but shift their spectral characteristics (Bermudez-Cuamatzin et al. 2009). Across populations, males that breed in noisy areas structure their signals differently than males breeding under quiet noise conditions (Slabbekoorn and Peet 2003, Wood and Yezerinac 2006, Mockford and Marshall 2009, Hamao et al. 2011, Potvin et al. 2011, Luther and Derryberry 2012). Moreover, individuals may adjust songs plastically, flexibly changing their songs in response to high-amplitude bursts of noise or during periods of high noise (Halfwerk and

Slabbekoorn 2009, Gross et al. 2010, Verzijden et al. 2010, Montague et al. 2013, LaZerte et al. 2016, Gentry et al. 2017). Despite ample evidence that signalers adjust their songs in response to noise, few studies to date have shown that males benefit from signal adjustments.

Costs and benefits of singing in noise depends on social context

The benefits of singing in noise and altering signals as necessary should outweigh the costs if transmission distances are maintained and information content or signal perception remains unchanged (Patricelli and Blickley 2006, Read et al. 2014). Yet both of these conditions are contingent upon the social context in which a signal is given. Therefore, linking how noise affects signal transmission patterns in relation to signal function and social context are important for understanding behavioral changes of receivers in noise (Grabarczyk and Gill 2019). Song changes may only be beneficial for signals designed to reach receivers that are widely dispersed, such as when males advertise for female partners and signal territory ownership (Grabarczyk et al. 2018). Moreover, the cost of signal adjustments may outweigh the benefits if noise-induced signal changes alter receiver perception. Altered or masked signals could affect fitness by influencing female mate choice decisions or reducing the efficacy of repelling territorial rivals. For example, female great tits (*Parus major*) are initially attracted to males that sing less masked, high frequency songs; but females show higher fidelity to males that sing low frequency songs (Halfwerk et al. 2011). In noise, male European robins *(Erithacus rubecula)* reduce the number of low frequency notes (Zwart et al.

2016) and decrease song complexity (McMullen et al. 2014) in response to simulated

territorial intruders, which may be perceived as less aggressive by rivals (McMullen et

al. 2014). Critically, researchers must consider the consequences of noise masking on

male song, as limited evidence exists that links the effects of noise on signaler fitness

that result from altered responses and perception of signal receivers (Halfwerk et al.

2018).

Dissertation outline

      For birds, noise overlaps with signals males use to defend breeding areas and

attract mates, both critical aspects of reproduction. In response to noise, males adjust

their song structure to improve signal transmission, but whether they actually benefit

from song changes is unknown. Moreover, it remains unclear whether noise disrupts

communication by affecting interactions with neighbors and intruders, which may have

implications for individual fitness and ultimately the ability of populations to persist in

urban environments. I tested the overarching hypothesis that anthropogenic noise

affects multiple components of communication systems, including signalers, their

signals, and receivers. Therefore, my dissertation research aims to link the effects of

noise on singing behavior, signal transmission, and song perception, with the goal of

connecting each of these in analysis of communication in a social context. I

use experimental and observational methods on a common urban-dwelling passerine to

test hypotheses that 1. Context influences male House wren (*Troglodytes

aedon*) responses to noise, with males showing rapid song adjustments in noise when

advertising for mates, but not once paired; 2. Males flexibly alter their songs in response to their immediate noise environment, but that adjustments may depend on their social environment; 3. Noise differentially affects transmission of signals used for short and long-distance communication within species-typical spacing patterns; 4. Noise affects the ability of territorial males to detect and respond to intruders; and 5. Noise alters signaling interactions among territorial males.

Ultimately, if males vary in their ability to alter song, detect intruders, and attract females in relation to noise, then selection should favor males whose singing decisions in noise contribute to reproductive success. If noise-induced song changes result in increased fitness, males that alter their songs in noise will produce more offspring with similar traits, and the success of these individuals should lead to population-level change and noise-driven divergence among populations. Thus, the impacts of noise masking on song and communication could act as an important selective pressure on animal signals. Overall, my research advances our understanding of how noise-induced song changes affect communication with implications for long-term reproductive success and population change. Given current rapid land-use transformation, understanding the characteristics and behaviors that make species successful is important for identifying populations that may persist and those that are at risk of becoming excluded from noisy environments.

Literature cited

Amy, M., P. Sprau, P. de Goede, and M. Naguib. 2010. Effects of personality on territory defence in communication networks: a playback experiment with radio-tagged great tits. Proceedings of the Royal Society B-Biological Sciences **277**:3685-3692.

Bermudez-Cuamatzin, E., A. A. Rios-Chelen, D. Gil, and C. M. Garcia. 2009. Strategies of song adaptation to urban noise in the house finch: syllable pitch plasticity or differential syllable use? Behaviour **146**:1269-1286.

Bradbury, J. W., and S. L. Vehrencamp. 1998. Principles of Animal Communication. 1 edition. Sinauer Associates, Sunderland, MA.

Brenowitz, E. A. 1982. The active space of Red-winged blackbird song. Journal of Comparative Physiology **147**:511-522.

Brumm, H. 2004. The impact of environmental noise on song amplitude in a territorial bird. Journal of Animal Ecology **73**:434-440.

Brumm, H., and S. A. Zollinger. 2013. Avian vocal production in noise. Pages 187-227 *in* H. Brumm, editor. Animal communicaiton in noise. Springer Berlin Heidelberg, Seewiesen, Germany.

Byers, B. E. 2007. Extrapair paternity in chestnut-sided warblers is correlated with consistent vocal performance. Behavioral Ecology **18**:130-136.

Catchpole, C. K., and P. J. B. Slater. 2008. Bird song: Biological themes and variations. Cambridge University Press, New York.

Chan, A. A. Y., W. D. Stahlman, D. Garlick, C. D. Fast, D. T. Blumstein, and A. P. Blaisdell. 2010. Increased amplitude and duration of acoustic stimuli enhance distraction. Animal Behaviour **80**:1-5.

Derryberry, E. P., K. Gentry, G. E. Derryberry, J. N. Phillips, R. M. Danner, J. E. Danner, and D. A. Luther. 2017. White-crowned sparrow males show immediate flexibility in song amplitude but not in song minimum frequency in response to changes in noise levels in the field. Ecology and Evolution **7**:4991-5001.

Endler, J. A. 1992. Signals, Signal Conditions, and the Direction of Evolution. American Naturalist **139**:S125-S153.

Gentry, K. E., E. P. Derryberry, R. M. Danner, J. E. Danner, and D. A. Luther. 2017. Immediate signaling flexibility in response to experimental noise in urban, but not rural, white-crowned sparrows. Ecosphere **8**:e01916.

Grabarczyk, E. E., and S. A. Gill. 2019. Anthropogenic noise masking diminishes house wren (*Troglodytes aedon*) song transmission in urban natrual areas. Bioacoustics.

Grabarczyk, E. E., M. A. Pipkin, M. J. Vonhof, and S. A. Gill. 2018. When to change your tune? Unpaired and paired male house wrens respond differently to anthropogenic noise. Journal of Ecoacoustics **2**:#LHGRVC.

Grade, A. M., and K. E. Sieving. 2016. When the birds go unheard: highway noise disrupts information transfer between birds. Biology Letters **12**:20160113.

Gross, K., G. Pasinelli, and H. P. Kunc. 2010. Behavioral Plasticity Allows Short-Term Adjustment to a Novel Environment. American Naturalist **176**:456-464.

Halfwerk, W., S. Bot, J. Buikx, M. van der Velde, J. Komdeur, C. ten Cate, and H. Slabbekoorn. 2011. Low-frequency songs lose their potency in noisy urban conditions. Proceedings of the National Academy of Sciences of the United States of America **108**:14549-14554.

Halfwerk, W., B. Lohr, and H. Slabbekoorn. 2018. Impact of Man-Made Sound on Birds and Their Songs. *in* H. Slabbekoorn, R. J. Dooling, A. N. Popper, and R. R. Fay, editors. Effects of Anthropogenic Noise on Animals. Springer.

Halfwerk, W., and H. Slabbekoorn. 2009. A behavioural mechanism explaining noise-dependent frequency use in urban birdsong. Animal Behaviour **78**:1301-1307.

Hamao, S., M. Watanabe, and Y. Mori. 2011. Urban noise and male density affect songs in the great tit Parus major. Ethology Ecology & Evolution **23**:111-119.

Johnson, L. S., and L. H. Kermott. 1991. The Functions of Song in Male House Wrens (*Troglodytes aedon*). Behaviour **116**:190-209.

Klump, G. M. 1996. Bird communication in the noisy world. Cornell University Press, Ithaca, NY.

LaZerte, S. E., H. Slabbekoorn, and K. A. Otter. 2016. Learning to cope: vocal adjustment to urban noise is correlated with prior experience in black-capped chickadees. Proceedings of the Royal Society B-Biological Sciences **283**.

Luther, D., and E. P. Derryberry. 2012. Birdsongs keep pace with city life: changes in song over time in an urban songbird affects communication. Animal Behaviour **83**:1059-1066.

Marten, K., and P. Marler. 1977. Sound Transmission and Its Significane for Animal Vocalization: I. Temperate Habitats. Behavioral Ecology and Sociobiology **2**:271-290.

McGregor, P. K., and T. M. Peake. 2000. Communication networks: social envionments for receiving and signalling behavior. Acta Ethologica **2**:71-81.

McMullen, H., R. Schmidt, and H. P. Kunc. 2014. Anthropogenic noise affects vocal interactions. Behavioural Processes **103**:125-128.

Mennill, D. J., L. M. Ratcliffe, and P. T. Boag. 2002. Female eavesdropping on male song contests in songbirds. Science **296**:873-873.

Mockford, E. J., and R. C. Marshall. 2009. Effects of urban noise on song and response behaviour in great tits. Proceedings of the Royal Society B-Biological Sciences **276**:2979-2985.

Montague, M. J., M. Danek-Gontard, and H. P. Kunc. 2013. Phenotypic plasticity affects the response of a sexually selected trait to anthropogenic noise. Behavioral Ecology **24**:342-348.

Morton, E. S. 1975. Ecological sources of selection on avian sounds. American Naturalist **109**:17-34.

Naguib, M., V. Amrhein, and H. P. Kunc. 2004. Effects of territorial intrusions on eavesdropping neighbors: communication networks in nightingales. Behavioral Ecology **15**:1011-1015.

Patricelli, G. L., and J. L. Blickley. 2006. Avian communication in urban noise: Causes and consequences of vocal adjustment. Auk **123**:639-649.

Patricelli, G. L., A. H. Krakauer, and C. C. Taff. 2016. Variable Signals in a Complex World: Shifting Views of Within-Individual Variability in Sexual Display Traits. Pages 319-+ *in* M. Naguib, J. C. Mitani, L. W. Simmons, L. Barrett, S. Healy, and M. Zuk, editors. Advances in the Study of Behavior, Vol 48.

Peake, T. M., A. M. R. Terry, P. K. McGregor, and T. Dabelsteen. 2002. Do great tits assess rivals by combining direct experience with information gathered by easvedropping? Proceedings of the Royal Society B-Biological Sciences **269**:1925-1929.

Potvin, D. A., K. M. Parris, and R. A. Mulder. 2011. Geographically pervasive effects of urban noise on frequency and syllable rate of songs and calls in silvereyes (*Zosterops lateralis*). Proceedings of the Royal Society B-Biological Sciences **278**:2464-2469.

Purser, J., and A. N. Radford. 2011. Acoustic Noise Induces Anntention Shifts and Reduces Foraging Performance in Three-Spined Sticklebacks (*Gasterosteus aculeatus*). Plos One **6**:e17478.

Read, J., G. Jones, and A. N. Radford. 2014. Fitness costs as well as benefits are important when considering responses to anthropogenic noise. Behavioral Ecology **25**:4-7.

Redondo, P., G. Barrantes, and L. Sandoval. 2013. Urban noise influences vocalization structure in the House Wren *Troglodytes aedon*. Ibis **155**:621-625.

Reichard, D. G., R. J. Rice, E. M. Schultz, and S. E. Schrock. 2013. Low-amplitude songs produced by male dark-eyed juncos (Junco hyemalis) differ when sung during intra- and inter-sexual interactions. Behaviour **150**:1183-1202.

Ríos-Chelén, A. A., E. Quiros-Guerrero, D. Gil, and C. M. Garcia. 2013. Dealing with urban noise: vermilion flycatchers sing longer songs in noisier territories. Behavioral Ecology and Sociobiology **67**:145-152.

Ritchison, G. 1995. Characteristics, use and possible functions of the perch songs and chatter calls of male Common yellowthroats. Condor **97**:27-38.

Ryan, M. J. 1985. The túngara frog: a study in sexual selection and communication. University of Chicago Press, Chicago.

Schmidt, R., V. Amrhein, H. P. Kunc, and M. Naguib. 2007. The day after: effects of vocal interactions on territory defence in nightingales. Journal of Animal Ecology **76**:168-173.

Senzaki, M., Y. Yamaura, C. D. Francis, and F. Nakamura. 2016. Traffic noise reduces foraging efficeincy in wild owls. Scientific Reports **6**:30602.

Siemers, B. M., and A. Schaub. 2011. Hunting at the highway: traffic noise reduces foraging efficiency in acoustic predators. Proceedings of the Royal Society B-Biological Sciences **278**:1646-1652.

Sih, A. 2013. Understanding variation in behavioural responses to human-induced rapid environmental change: a conceptual overview. Animal Behaviour **85**:1077-1088.

Sih, A., P. C. Trimmer, and S. M. Ehlman. 2016. A conceptual framework for understanding behavioral responses to HIREC. Current Opinion in Behavioral Sciences **12**:109-114.

Slabbekoorn, H., and A. den Boer-Visser. 2006. Cities change the songs of birds. Current Biology **16**:2326-2331.

Slabbekoorn, H., and M. Peet. 2003. Ecology: Birds sing at a higher pitch in urban noise. Nature **424**:267-267.

Snijders, L., and M. Naguib. 2017. Communication in Animal Social Networks: A Missing Link? Pages 297-359 *in* M. Naguib, J. Podos, L. W. Simmons, L. Barrett, S. D. Healy, and M. Zuk, editors. Advances in the Study of Behavior, Vol 49.

Snijders, L., K. van Oers, and M. Naguib. 2017. Sex-specific responses to territorial intrusions in a communication network: Evidence from radio-tagged great tits. Ecology and Evolution **7**:918-927.

Verzijden, M. N., E. A. P. Ripmeester, V. R. Ohms, P. Snelderwaard, and H. Slabbekoorn. 2010. Immediate spectral flexibility in singing chiffchaffs during experimental exposure to highway noise. Journal of Experimental Biology **213**:2575-2581.

Voellmy, I. K., J. Purser, D. Flynn, P. Kennedy, S. D. Simpson, and A. N. Radford. 2014. Acoustic noise reduces foraging success in two sympatric fist species via different mechanisms. Animal Behaviour **89**:191-198.

Wong, B. B. M., and U. Candolin. 2015. Behavioral responses to changing environments. Behavioral Ecology **26**:665-673.

Wood, W. E., and S. M. Yezerinac. 2006. Song sparrow (*Melospiza melodia*) song varies with urban noise. Auk **123**:650-659.

Zwart, M. C., J. C. Dunn, P. J. K. McGowan, and M. J. Whittingham. 2016. Wind farm noise suppresses territorial defense behavior in a songbird. Behavioral Ecology **27**:101-108.

CHAPTER II

WHEN TO CHANGE YOUR TUNE? UNPAIRED AND PAIRED MALE HOUSE WRENS
RESPOND DIFFERENTLY TO ANTHROPOGENIC NOISE

Abstract

In response to anthropogenic noise, many bird species adjust their song frequency, presumably to optimize song transmission and overcome noise masking. But the costs of song adjustments may outweigh the benefits during different stages of breeding, depending on the locations of potential receivers. Selection might favor unpaired males to alter their songs because they sing to attract females that may be widely dispersed, whereas paired males might not if mates and neighbors are primary receivers of their song. We hypothesized male house wrens (*Troglodytes aedon*) respond differently to noise depending on their pairing status. To test our hypothesis we synthesized pink noise, which mimics anthropogenic noise, and played it at three intensities in territories of paired and unpaired focal males. We recorded their songs and analyzed whether song structure varied with pairing status and noise treatment. To validate our study design, we tested whether noise playback affected measurement of spectral song traits and changed noise levels within territories of focal males. Consistent with our predictions, unpaired males sang differently than paired males, giving longer songs at higher rates. Contrary to predictions, paired males changed their songs by increasing peak frequency during high intensity noise playback, whereas unpaired males did not. If adjusting song frequency in noise is beneficial for long-distance communication we would have expected unpaired males to change their songs in

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response to noise. By adjusting song frequency, paired males reduce masking and produce a song that is easier to hear. However, if females prefer low frequency song, then unpaired males may be constrained by female preference. Alternatively, if noise adjustments are learned and vary with experience or quality, unpaired males in our study population may be younger, less experienced, or lower quality males.

Introduction

A central premise underlying concern about the effects of anthropogenic noise on animal communication is that noise makes signals more difficult for receivers to detect and to discriminate. To improve detection probability, therefore, individuals alter their signals, increasing signal frequency to minimize masking (Slabbekoorn and Peet 2003, Slabbekoorn and den Boer-Visser 2006, Wood and Yezerinac 2006, Mockford and Marshall 2009, Ríos-Chelén et al. 2013), increasing signaling rate or duration to increase signal redundancy (Slabbekoorn and den Boer-Visser 2006, Ríos-Chelén et al. 2013), and increasing signal amplitude to improve signal-to-noise ratios (Brumm 2004, Dooling and Blumenrath 2013, Derryberry et al. 2017). Assuming any costs of such adjustments are less than associated benefits (Read et al. 2014), signal changes should be favored if they improve detection in noisy environments and the chances of attracting breeding partners and defending space. Such changes should be most beneficial for callers when active space, the area over which the signal is broadcast, is large, when receivers are widespread, and when signals are not directed towards known receivers, such as when males advertise for female partners (Bradbury and Vehrencamp 1998).

But should selection favor these same noise-induced adjustments once males have attracted females, when signal design rules (Bradbury and Vehrencamp 1998) have changed? Across songbirds, males sing differently once paired, by decreasing song rate overall or singing at high rates during shorter periods (Krebs et al. 1981, Cuthill and Hindmarsh 1985, Otter and Ratcliffe 1993), by switching to different song types (Kroodsma et al. 1989, Eens et al. 1993, Staicer 1996), and even by almost

entirely ceasing to sing (Catchpole 1973, Liu and Kroodsma 2007). Paired males may also produce low-amplitude songs that likely only transmit to mates and possibly neighbors, but not beyond (Johnson and Kermott 1991, Ritchison 1995, Reichard et al. 2013). Because paired males direct their song at mates and neighbors over a smaller active space, they may not need to change their songs in noise or they may show different responses altogether, such as signaling less or not at all when encountering noisy conditions. Whether male responses to noise differs based on pairing status is poorly known, as few studies have focused on individually marked birds of known pairing status (but see Gross et al. 2010). Unpaired male reed buntings (*Emberiza schoeniclus*) increased song frequency on noisy versus quiet days, whereas paired males did not (Gross et al. 2010), providing correlative support for the idea that males alter their responses to noise depending on pairing status and signal design rules.

Differences in response between unpaired and paired males may occur if paired males experience different costs and benefits associated with signal adjustments in noise. Although changes in song structure are assumed to be beneficial, the ratio of benefits to costs could vary with pairing status, as well as more generally across breeding. For example, singing more loudly might reduce masking and increase the distance over which signals move (Nemeth and Brumm 2010), yet additional energetic costs of such signaling (Lambrechts 1996) might only be offset for unpaired males. Moreover, song preferences of females could constrain male options to adjust signals, such as in Great tits (*Parus major*), in which females prefer and show higher fidelity to males that sing low frequency song in noise (Halfwerk et al. 2011) and may seek other

males if their partners produce altered song (Otter et al. 1999). Thus, fitness costs and benefits of noise-induced song changes may not be the same for all males. Addressing whether pairing status effects exist may reduce unexplained variation in male responses and improve our understanding of costs and benefits of signaling adjustments (e.g. Read et al. 2014).

We tested the hypothesis that male songbirds respond differently to noise depending on their pairing status. We studied a color-banded population of house wrens (*Troglodytes aedon*), a species in which males sing to attract mates (Johnson and Searcy 1996), but once paired, males produce fewer shorter songs (Tove 1988, Johnson and Kermott 1991). We therefore expected that unpaired and paired males would sing differently, regardless of noise. Tropical house wrens alter their songs based on noise conditions (Redondo et al. 2013), thus, we expected northern wrens to change their songs due to noise as well. However, we reasoned that unpaired males would experience higher costs if noise masked their songs and they failed to attract or were delayed when attracting breeding partners (Habib et al. 2007, Gross et al. 2010). Paired males direct songs towards mates (Johnson and Kermott 1991, LaBarbera et al. 2010) and nearby territorial males and nesting females (Johnson et al. 2002). Thus, we predicted unpaired males would alter their songs when exposed to noise to improve detection, but paired males either would not alter their songs at all or would sing less under noisy conditions.

To test our hypothesis, we synthesized pink noise and played it at three intensities within the territories of paired and unpaired focal males, recorded their songs,

and analyzed whether song structure varied with pairing status and noise treatment. We

also validated two assumptions of noise playback experiments. First, to test the

assumption that noise playbacks do not affect measurement of spectral features of male

songs, we sequentially played each noise treatment while simultaneously playing the

same pre-recorded house wren songs. Because pre-recorded song will not change

across treatments, any differences in song traits detected during analysis should be

attributed to the noise playback and those traits should be omitted from consideration

(Verzijden et al. 2010, Hanna et al. 2011). Second, we tested the assumption that noise

playbacks change noise levels during trials on focal male territories (Halfwerk and

Slabbekoorn 2009, LaZerte et al. 2016, Ríos-Chelén et al. 2018). If noise playbacks do

not consistently change noise levels, any song changes that occur during focal male

trials cannot be ascribed to noise treatments.

Methods

Study species and sites

We studied a migratory population of house wrens breeding in nest boxes at five

nature preserves in Kalamazoo County, Michigan, USA (42.290 N, 85.586 W). Each site

has mixed forest and open grassland preferred by house wrens, but sites varied in the

amount of surrounding urbanization (impervious surface) and distance to sources of

continuous (e.g. highways) and intermittent noise (Gill et al. 2017). We captured house

wrens by mist net and fitted them with unique combinations of three color bands and

one numbered aluminum band for individual identification. We sexed males in hand

based on their cloacal protuberance and confirmed sex by observing singing. Male

house wren song consists of two sections: an introduction composed of low amplitude notes with multiple harmonics (Rendall and Kaluthota 2013) followed by a high-amplitude, frequency modulated terminal portion (Platt and Ficken 1987). Unlike the terminal portion which transmits beyond territory boundaries (Tove 1988), the introduction is not consistently recorded even in low noise conditions (Rendall and Kaluthota 2013); therefore, we analyzed features of terminal portions only.

We checked nest boxes every three days to determine if they were occupied by males, and whether males had paired. Females can be difficult to detect during early days of pairing, therefore, we determined pairing status by observing male behavior around their boxes and stages of nest construction. Unpaired males sing at high rates from perches around 25m high, whereas paired males sing at lower heights and near their nests (Johnson and Kermott 1991). In terms of nest construction, males build nest platforms, whereas females build nest cups and add lining (McCabe 1965). Thus, we noted when cups began forming or were being lined, in addition to direct observations of females building to determine if and when males had paired. After testing, we continued to monitor focal males to determine if unpaired males eventually paired or remained unpaired over the breeding season.

Noise synthesis

Anthropogenic noise may occur continuously, such as noise generated from gas compressors or building ventilation systems, or intermittently, such as from passing cars (Gill et al. 2017). Using naturally recorded anthropogenic noise in playback experiments

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can be problematic if noise is not constant, as it is not possible to isolate whether

animals responded to fluctuations in sound pressure levels due to intermittent noise or

to mean levels of continuous noise. Given such variation, adequately describing noise

levels to which animals are exposed may be difficult (Shannon et al. 2016). For our

experiments, we therefore synthesized pink noise, which is a 1/f noise with energy

concentrated at lower frequency bands resembling continuous noise from urban

environments and traffic (Figure 2.2). We generated 5-min of pink noise in Avisoft

SASLab Pro v 5.1 (R. Specht, Berlin, Germany; 44.1 kHz sample rate, low-pass 1/f

frequency cut off at 0.20 Hz, 1-6 Hz bandpass filter) and to avoid startling the birds, we

gradually increased noise intensity over 5 s at the beginning of the recording. In

Audacity v 2.1.2, we then twice amplified the original signal by 15 dB, resulting in low,

moderate, and high intensity noise playback files. In the field, we set the lowest

amplitude playback to 50 dBA at 1 m and confirmed the remaining two at 65 and 80

dBA with a SPL meter (American Recorder Technology SPL-8810). We then applied the

same settings to all experiments.

Validation experiment

We used the automated measurement tool in Avisoft to quantify frequency traits

as recommended by recent studies (Zollinger et al. 2012, Brumm et al. 2017, Ríos-

Chelén et al. 2017). Extracting frequency traits with an automated tool without influence

from noise can be difficult, particularly with high background noise, as the program may

use input from noise rather than solely from song (Verzijden et al. 2010, Cardoso and

Atwell 2011). Therefore, to test whether noise playback influenced measurement of

spectral traits, we conducted a validation experiment during which we paired the playback of two repetitions of 10 pre-recorded house wren songs with each of four treatments: low noise, moderate noise, high noise, and control without noise (Verzijden et al. 2010). We attached one SME-AFS amplified speaker (Saul Mineroff Electronics, New York) to the top of an unoccupied nest box to simulate a singing male and set a second speaker on the ground 10m from the nest box as in our experimental trials (see below). We re-recorded songs with a Wildlife Acoustics SongMeter 2 (SM2, Maynard MA; 44.1 kHz sample frequency, 16-bit) positioned 1m in front of the nest box at speaker height. In Avisoft, we analyzed frequency traits of pre-recorded songs from each noise treatment and the control. If song frequency traits varied across treatments, we would not be able to conclude that changes in frequency traits in focal male experiments were due to adjustments by males or were artifacts of noise playbacks. Thus, for focal male experiments, we omitted frequency traits that varied with treatment in the validation experiment.

Playback experiment

We performed focal male experiments (N=31) between sunrise and 1100 (EST) and used a Wildlife Acoustics SongMeter 2 (SM2, Maynard MA; 44.1 kHz sample frequency, 16-bit) to record the songs of focal males. We attached the SM2 microphone to the focal male's nest box and connected the microphone to the recording unit using a 10-m cord. We placed an SME-AFS amplified speaker from which we broadcast noise on the ground 10m from the nest box. To minimize disturbance during trials caused by our presence, we connected the speaker to an Apple iPod (Cupertino, CA) using a 20m

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extension cable. We recorded a 5-min control period without any playback and started

the 5-min noise playback when focal males arrived within 10m of their nest boxes. We

randomized the order of noise treatments and allowed a 10-min break between trials

before presenting the next stimulus to minimize carry-over effects.

Song and noise analysis

Prior to analysis in Avisoft, we removed low- and high-frequency sounds from

recordings using a bandpass filter (1.3 – 11 kHz) on waveforms. We converted filtered

waveforms into spectrograms (Flat top window, FFT length of 512, 93.75% overlap,

0.725 ms time resolution) and extracted minimum frequency (Hz), maximum frequency

(Hz), peak frequency (Hz), and bandwidth (Hz) using automated parameter

measurements. For minimum and maximum frequency, we set a measurement

threshold of -10 dB below peak frequency on the power spectrum. We chose this

threshold because it was the lowest value that led to consistent measurement of song

rather than remaining noise that overlapped song across all noise treatments. We also

measured song length (s) and singing rate (songs/min) across treatments.

To test the assumption that noise playbacks altered sound pressure levels

(SPLs) in territories of focal males, we measured SPLs during each treatment as well as

the control period. We calibrated each microphone and SM2 unit pair by recording a 94-

dB tone generated by a Larson Davis CAL 200 sound level calibrator (Depew, NY).

Using the Calibration function in Avisoft, we set the relative amplitude of the recording to

0 dB (re 20 µPa) based on the calibration tone, then randomly selected and averaged

SPLs (RMS, dB) from five 1-sec samples of recording, not overlapped by house wren

song, from each treatment and the control.

Statistical analysis

We conducted all analysis using R statistical software v 3.3.1 (R Core

Development Team 2016). To test whether noise playback affected measurement of

frequency traits in the validation experiment, we ran a linear mixed model using the

package nlme (Pinheiro et al. 2017), with noise treatment as a fixed effect and song

number as a random effect (we played twice each of 10 unique songs in this

experiment). We tested whether noise treatments altered SPLs in focal territories by

comparing average SPLs during treatments with a linear mixed model, with treatment

as a fixed effect and male identity as a random effect to account for repeated noise

measurements. We used the package lsmeans (Lenth 2016) to run pairwise

comparisons to determine which treatments differed in each of these tests.

To test the main hypothesis that noise and pairing status influenced singing, we

analyzed only those songs clearly visible on the spectrogram window and not those

overlapped by other sounds. We calculated mean values of each song trait by individual

per treatment (control: mean = 19.7 songs, range = 2 – 59 songs; high noise: mean =

14 songs, range = 0 – 50 songs). We then used a linear mixed model, with treatment

and pairing status as fixed effects and male identity as a random effect to account for

the repeated measures design in which the songs of each individual were compared

across treatments. We explored whether an interaction term with treatment and pairing

status should be included in the final model by comparing AICc of the main effects and

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interaction models. The model with the lowest AICc was considered the best fit if AICc of the second model differed by more than 2 (Burnham and Anderson 2002). Residual plots were explored to assess model adequacy. We plotted residuals from each model against SPLs measured within individual territories to determine whether ambient SPLs may explain residual variation, but found no patterns in these plots. All figures were created with the package sciplot (Morales 2012).

Results

Validation experiments

We tested the assumption that noise playbacks have no effect on measurement of frequency traits. Minimum frequency ($F_{3,57}$ = 23.46, p = <0.0001), maximum frequency ($F_{3,57}$ = 18.93, p = <0.0001), peak frequency ($F_{3,57}$ = 3.97, p = 0.01), and bandwidth ($F_{3,57}$ = 26.59, p = <0.0001) each differed significantly among treatments. Post-hoc pairwise comparisons showed complex and somewhat unpredictable differences across traits and treatments. For example, minimum frequency was higher during high-noise playbacks and lower during low-noise playbacks than controls, but did not differ between moderate noise playbacks and controls. Minimum frequency likely increased under high noise because filtering failed to remove from the waveform all the energy generated by the playback. By contrast, peak frequency differed only between low noise and control treatments; this result appeared to be driven by two songs, which had higher peak frequency during the low noise treatment than the others. This could be due to irregular amplitude fluctuations during signal transmission, which result from

temporary shifts in wind speed or air temperature (Wiley and Richards, 1978). Noise

playbacks did not affect measurement of song rate or length.

We next tested the assumption that noise playbacks alter sound pressure levels

within focal territories. Ambient SPLs did not differ between control periods (mean $\pm$ SD

= 47.7 $\pm$ 5.07 dB, range = 36.9 – 53.7 dB) and low (mean $\pm$ SE difference between

noise and ambient SPLs: -0.7$\pm$ 0.7 dB, t_{90} = -1.00, p = 0.31) or moderate (0.7 $\pm$ 0.7 dB,

t_{90} = 0.98, p = 0.32) noise treatments. High noise treatments increased SPLs by 5.12 $\pm$

0.7 dB (t_{90} = 7.29, p = <0.0001) compared to all other treatments. We further explored

the degree to which the high noise treatment changed SPLs and noted that in 14 cases,

the high noise playback changed SPLs by < 3 dB, a difference which birds may not

detect (Dooling, 1982). In our final analysis, therefore, we omitted low and moderate

noise playbacks (which did not change SPLs relative to ambient levels) as well as high

noise playbacks that had a minor effect on SPLs (< 3 dB). This left us with 17

experiments on male house wrens for which we could compare song traits between

control and high-noise treatments (mean $\pm$ SE difference in SPL between these

treatments and controls: 8.86 $\pm$ 3.59 dB). Furthermore, we analyzed peak frequency, as

this trait did not differ between control and high-noise treatments in the validation

experiment (above).

Responses of unpaired and paired males to noise playbacks

We compared a model including the interaction between treatment and pairing

status to a main effects model without interactions and found the interaction model best

fit the data on peak frequency (ΔAICc = 3.31, AICc main effects = 449.96, AICc interaction = 446.65). During high-noise playbacks, paired males shifted their song by singing at a higher peak frequency, whereas unpaired males did not adjust peak frequency in response to noise. The peak frequency of songs of paired and unpaired males during control periods did not differ (Figure 2.3).

For temporal traits, models with and without the interaction between treatment and pairing status did not differ (rate: ΔAICc = 2.67, AICc main effects = 186.63, AICc interaction = 189.3; length: ΔAICc = 3.13, AICc main effects = 9.04, AICc interaction = 12.17), therefore, we analyzed the main effects model only. On average, unpaired males sang 4.93 $\pm$ 1.17 songs/min more than paired males (t_{15} = 4.2, p = 0.008), but noise treatment had no effect on song rate. Unpaired males sang 0.23 $\pm$ 0.1 sec longer songs than paired males (t_{15} = 2.3 1, p = 0.04), but again noise treatment had no effect on song length (Figure 2.4). Of all the unpaired males tested (N=16), 10 eventually paired and six remained unpaired during the breeding season.

Discussion

We tested the hypothesis that male house wrens adjust their song differently in response to anthropogenic noise based on whether they are unpaired or paired. Consistent with previous studies on house wrens (Tove 1988, Johnson and Kermott 1991), unpaired and paired males sang differently during experiments, with unpaired males singing longer songs at higher rates than paired males. Contrary to predictions, however, it was paired males that changed their songs in response to noise playbacks:

they increased peak frequency of their songs during high noise playback, whereas unpaired males did not. Males, regardless of pairing status, did not adjust the temporal features of their songs in response to noise.

Given that song adjustments improve detection in noise (Pohl et al. 2009, Pohl et al. 2012, LaZerte et al. 2017) and males sing to attract females from unknown locations (Bradbury and Vehrencamp 1998), unpaired males should experience greater benefits than paired males from changing their songs in noise. Accordingly, we predicted that unpaired, not paired males, would alter song frequency in noise. During control periods, paired and unpaired males produced songs with similar peak frequencies, suggesting that under ambient conditions, whether males were paired had limited or no influence on song frequency. During noise playbacks, paired males increased song frequency, but unpaired males did not, maintaining similar peak frequencies under quiet and noisy conditions (Figure 2.3). Female house wrens are attracted to male song (Johnson and Searcy 1996), but while trill consistency and vocal deviation are not used by females in mate choice decisions (Cramer 2013), other song traits, including peak frequency, have not been ruled out. Male great tits that sing at low frequencies in noisy conditions during female fertile periods are more likely to maintain paternity in their clutches (Halfwerk et al. 2011). Alternatively, male chestnut-sided warblers (*Dendroica pensylvanica*) that sing consistent songs at a high frequency fathered more extra pair offspring (Byers 2007). If peak frequency indicates male quality in house wrens, unpaired males may be constrained by female preferences to produce low frequency song regardless of noise background. Paired males in our study population could be viewed as higher quality as

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they have already paired. Moreover, because they quickly adjust their song in noise, they may be more detectable to neighboring females, allowing them to acquire additional fitness through extra-pair offspring.

How a male adjusts song frequency in response to increasing ambient noise could depend on prior experience with signaling in noise. Black-capped chickadees (*Poecile atricapillus*) in noisy habitats increased song frequency in response to noise playbacks, whereas males in quiet areas decreased song frequency, producing songs that were more difficult to detect in noise (LaZerte et al. 2016). If noise adjustments are learned and vary with experience, then the ability to adjust songs based on ambient sound conditions may improve with age. All males in our study bred in urban or peri-urban natural areas, thus we assumed they had prior exposure to anthropogenic noise. Responses were not influenced by background noise levels on male territories, but because unpaired males did not adjust songs to reduce noise masking, they could be younger and less experienced with signaling in noise. However, additional work is needed to test whether individual experience or age modifies responses of house wrens and other species to noise.

Consistent with previous studies (Tove 1988, Johnson and Kermott 1991), male house wrens structured temporal song characteristics differently based on pairing status. Unpaired males sang longer songs at a higher rate than paired males, similar to findings in other taxa, in which longer songs given at high rates are often used for long-distance communication (Nelson and Poesel 2011). For males to attract mates their signals must be detectable, but also, importantly, localizable by listening females

(Bradbury and Vehrencamp 1998). To be localizable, males sing at high rates (Bradbury and Vehrencamp 1998), which enables females to cue in on multiple renditions of songs and determine a male's location (Klump 2000). However, neither unpaired or paired males changed song rate or length due to noise playback. In noise, longer songs (Ríos-Chelén et al. 2013) and song repetition (Brumm and Slater 2006) may increase the probability of signal detection by increasing redundancy. Because unpaired males already sing long songs at high rates but do not adjust temporal song features in noise, it is possible they already sing at physiological capacity (mean $\pm$ SD unpaired male song rate: 8.02 ± 2.02 songs/min; E. Grabarczyk, unpubl. data). Perhaps unpaired males in our study signal at high rate with preferred traits; if noise is constant then their signals will likely be masked, but if noise is intermittent, then some songs may reach listening females. Assuming a cost of singing in noise, paired males may experience little or no benefit from increasing signal redundancy: their signals are likely to reach the primary receivers of their songs, their mates and neighbors. Nevertheless, paired males do produce signals that may be more detectable in noise.

Assessing song adjustments to introduced noise presents challenges different from playback studies exploring other auditory stimuli, such as songs or alarm calls. Studies using playback experiments assume that focal individuals detect a given stimulus and distinguish it from other stimuli being presented. In the case of song or alarm calls, the rather obvious change in behaviors that follow from playbacks (e.g. increased singing and approach to the speaker to songs, and fleeing or holding still to alarm calls (Gill and Sealy 2004)) signal to observers that both detection and

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discrimination have occurred. When individuals fail to respond, we assume the signal had no salient features to trigger a response in the context in which it was played, but an alternative explanation is that the signal was not detected by the intended receiver. This consideration is critical when exploring how animals alter their signals in response to anthropogenic noise using playbacks: do playbacks meaningfully change the sound environment of singing males to the point where males detect, discriminate, and then alter their songs?

We assessed two assumptions of noise playback experiments: whether noise playbacks affect measurement of spectral traits (Verzjieden et al. 2010, Hanna et al. 2011) and whether noise playback altered SPLs on focal male territories during trials (Halfwerk and Slabbekoorn 2009, LaZerte et al. 2016, Ríos-Chelén et al. 2018). Our validation experiment showed that noise playback affected minimum, maximum and frequency bandwidth values extracted with the automated parameter tool and we therefore eliminated these traits from analysis. Failure to assess if noise playback influences song measurement could result in false positives, particularly of frequency values, and changes detected could be artifacts of playbacks rather than adjustments by focal males (Brumm et al. 2017, Ríos-Chelén et al. 2017). Moreover, by extracting SPLs from recordings made using calibrated microphone and recording unit pairs, we found that only 17 out of 31 high-intensity noise playbacks effectively altered noise levels on focal male territories. Accordingly, we eliminated trials with no appreciable change in noise to focus on those that meaningfully changed the sound environment. Without quantifying the extent to which noise playbacks alter noise levels, researchers

risk misinterpreting experimental results that are needed to advance knowledge of noise effects on communication.

Anthropogenic noise has the potential to disrupt acoustic communication and could have detrimental impacts on fitness. Understanding why animals adjust their signals is critical for understanding how to mitigate negative effects of noise. Contrary to predictions, we showed that unpaired male house wrens did not alter singing in noise whereas paired males did. For unpaired house wrens, producing low frequency song at high rates may be more important for mate attraction and female choice than increasing song frequency to overcome noise masking. Paired males however may risk less by changing their song frequency as they have already mated. Thus, vocal adjustments in noise may not benefit all males in the same way within a breeding population.

Literature cited

Bradbury, J. W., and S. L. Vehrencamp. 1998. Principles of Animal Communication. 1 edition. Sinauer Associates, Sunderland, MA.

Brumm, H. 2004. The impact of environmental noise on song amplitude in a territorial bird. Journal of Animal Ecology **73**:434-440.

Brumm, H., and P. J. B. Slater. 2006. Ambient noise, motor fatigue, and serial redundancy in chaffinch song. Behavioral Ecology and Sociobiology **60**:475-481.

Brumm, H., S. A. Zollinger, P. T. Niemela, and P. Sprau. 2017. Measurement artefacts lead to false positives in the study of birdsong in noise. Methods in Ecology and Evolution **8**:1617-1625.

Burnham, K. P., and D. R. Anderson. 2002. Model Selection and Multimodel Inference. A Practical Information-Theoretic Approach. Springer Science & Business Media.

Byers, B. E. 2007. Extrapair paternity in chestnut-sided warblers is correlated with consistent vocal performance. Behavioral Ecology **18**:130-136.

Cardoso, G. C., and J. W. Atwell. 2011. On the relation between loudness and the increased song frequency of urban birds. Animal Behaviour **82**:831-836.

Catchpole, C. K. 1973. The functions of advertising song in the sedge warbler (*Acrocephalus schoenobaenus*) and the reed warbler (*A. scirpaceus*). Behaviour **46**:300-319.

Cramer, E. R. A. 2013. Physically Challenging Song Traits, Male Quality, and Reproductive Success in House Wrens. Plos One **8**.

Cuthill, I., and A. Hindmarsh. 1985. Increase in Starling song activity with removal of mate. Animal Behaviour **33**:326-327.

Derryberry, E. P., K. Gentry, G. E. Derryberry, J. N. Phillips, R. M. Danner, J. E. Danner, and D. A. Luther. 2017. White-crowned sparrow males show immediate flexibility in song amplitude but not in song minimum frequency in response to changes in noise levels in the field. Ecology and Evolution **7**:4991-5001.

Dooling, R. J., and S. H. Blumenrath. 2013. Avian sound perception in noise. Springer, Berlin Heidelberg.

Eens, M., R. Pinxten, and R. F. Verheyen. 1993. Function of the song and song repertoire in the European starling (*Sturnus vulgaris*): an aviary experiment. Behaviour **125**:51-66.

Gill, S. A., E. E. Grabarczyk, K. M. Baker, K. Naghshineh, and M. J. Vonhof. 2017. Decomposing an urban soundscape to reveal patterns and drivers of variation in anthropogenic noise. Science of the Total Environment **599**:1191-1201.

Gill, S. A., and S. G. Sealy. 2004. Functional reference in an alarm signal given during nest defence: seet calls of yellow warblers denote brood-parasitic brown-headed cowbirds. Behavioral Ecology and Sociobiology **56**:71-80.

Gross, K., G. Pasinelli, and H. P. Kunc. 2010. Behavioral Plasticity Allows Short-Term Adjustment to a Novel Environment. American Naturalist **176**:456-464.

Habib, L., E. M. Bayne, and S. Boutin. 2007. Chronic industrial noise affects pairing success and age structure of ovenbirds *Seiurus aurocapilla*. Journal of Applied Ecology **44**:176-184.

Halfwerk, W., S. Bot, J. Buikx, M. van der Velde, J. Komdeur, C. ten Cate, and H. Slabbekoorn. 2011. Low-frequency songs lose their potency in noisy urban conditions. Proceedings of the National Academy of Sciences of the United States of America **108**:14549-14554.

Halfwerk, W., and H. Slabbekoorn. 2009. A behavioural mechanism explaining noise-dependent frequency use in urban birdsong. Animal Behaviour **78**:1301-1307.

Hanna, D., G. Blouin-Demers, D. R. Wilson, and D. J. Mennill. 2011. Anthropogenic noise affects song structure in red-winged blackbirds (*Agelaius phoeniceus*). Journal of Experimental Biology **214**:3549-3556.

Johnson, L. S., B. G. Hicks, and B. S. Masters. 2002. Increased cuckoldry as a cost of breeding late for male house wrens (*Troglodytes aedon*). Behavioral Ecology **13**:670-675.

Johnson, L. S., and L. H. Kermott. 1991. The Functions of Song in Male House Wrens (*Troglodytes aedon*). Behaviour **116**:190-209.

Johnson, L. S., and W. A. Searcy. 1996. Female attraction to male song in house wrens (*Troglodytes aedon*). Behaviour **133**:357-366.

Klump, G. M. 2000. Sound localization in birds. Springer Verlag, New York.

Krebs, J. R., M. Avery, and R. J. Cowie. 1981. Effect of Removal of Mate on the Singing Behavior of Great Tits. Animal Behaviour **29**:635-637.

Kroodsma, D. E., R. C. Bereson, B. E. Byers, and E. Minear. 1989. Use of song types by the chestnut-sided warbler: evidence for both intra-and inter-sexual functions. Canadian Journal of Zoology-Revue Canadienne De Zoologie **67**:447-456.

LaBarbera, K., P. E. Llambias, E. R. A. Cramer, T. D. Schaming, and I. J. Lovette. 2010. Synchrony does not explain extrapair paternity rate variation in northern or southern house wrens. Behavioral Ecology **21**:773-780.

Lambrechts, M. M. 1996. Organization of birdsong and constraints on performance. Ecology and Evolution of Acoustic Communication in Birds:305-320.

LaZerte, S. E., H. Slabbekoorn, and K. A. Otter. 2016. Learning to cope: vocal adjustment to urban noise is correlated with prior experience in black-capped chickadees. Proceedings of the Royal Society B-Biological Sciences **283**.

LaZerte, S. E., H. Slabbekoorn, and K. A. Otter. 2017. Territorial black-capped chickadee males respond faster to high- than to low-frequency songs in experimentally elevated noise conditions. Peerj **5**.

Lenth, R. V. 2016. Least-Squares Means: The R Package lsmeans. Journal of Statistical Software **69**:1-33.

Liu, W. C., and D. E. Kroodsma. 2007. Dawn and daytime singing behavior of chipping sparrows (*Spizella passerina*). Auk **124**:44-52.

McCabe, R. A. 1965. Nest construction by house wrens. Condor **67**:229-234.

Mockford, E. J., and R. C. Marshall. 2009. Effects of urban noise on song and response behaviour in great tits. Proceedings of the Royal Society B-Biological Sciences **276**:2979-2985.

Morales M. (2012). Scientific graphic functions for factorial designs. R program software.

Nelson, D. A., and A. Poesel. 2011. Song length variation serves multiple functions in the white-crowned sparrow. Behavioral Ecology and Sociobiology **65**:1103-1111.

Nemeth, E., and H. Brumm. 2010. Birds and Anthropogenic Noise: Are Urban Songs Adaptive? American Naturalist **176**:465-475.

Otter, K., P. K. McGregor, A. M. R. Terry, F. R. L. Burford, T. M. Peake, and T. Dabelsteen. 1999. Do female great tits (*Parus major*) assess males by eavesdropping? A field study using interactive song playback. Proceedings of the Royal Society B-Biological Sciences **266**:1305-1309.

Otter, K., and L. Ratcliffe. 1993. Changes in singing behavior of male Black-capped chickadees (*Parus atricapillus*) following mate removal. Behavioral Ecology and Sociobiology **33**:409-414.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R. C. Team. 2017. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-131.

Platt, M. E., and M. S. Ficken. 1987. Organization of singing in House wrens. Journal of Field Ornithology **58**:190-197.

Pohl, N. U., E. Leadbeater, H. Slabbekoorn, G. M. Klump, and U. Langemann. 2012. Great tits in urban noise benefit from high frequencies in song detection and discrimination. Animal Behaviour **83**:711-721.

Pohl, N. U., H. Slabbekoorn, G. M. Klump, and U. Langemann. 2009. Effects of signal features and environmental noise on signal detection in the great tit, *Parus major.* Animal Behaviour **78**:1293-1300.

Read, J., G. Jones, and A. N. Radford. 2014. Fitness costs as well as benefits are important when considering responses to anthropogenic noise. Behavioral Ecology **25**:4-7.

Redondo, P., G. Barrantes, and L. Sandoval. 2013. Urban noise influences vocalization structure in the House Wren *Troglodytes aedon*. Ibis **155**:621-625.

Reichard, D. G., R. J. Rice, E. M. Schultz, and S. E. Schrock. 2013. Low-amplitude songs produced by male dark-eyed juncos (*Junco hyemalis*) differ when sung during intra- and inter-sexual interactions. Behaviour **150**:1183-1202.

Rendall, D., and C. D. Kaluthota. 2013. Song organization and variability in northern House wrens (*Troglodytes aedon parkmanii*) in Western Canada. Auk **130**:617-628.

Ríos-Chelén, A. A., C. Cuatianquiz-Lima, A. Bautista, and M. Martinez-Gomez. 2018. No reliable evidence for immediate noise-induced song flexibility in a suboscine. Urban Ecosystems **21**:15-25.

Ríos-Chelén, A. A., A. N. McDonald, A. Berger, A. C. Perry, A. H. Krakauer, and G. L. Patricelli. 2017. Do birds vocalize at higher pitch in noise, or is it a matter of measurement? Behavioral Ecology and Sociobiology **71**.

Ríos-Chelén, A. A., E. Quiros-Guerrero, D. Gil, and C. M. Garcia. 2013. Dealing with urban noise: vermilion flycatchers sing longer songs in noisier territories. Behavioral Ecology and Sociobiology **67**:145-152.

Ritchison, G. 1995. Characteristics, use and possible functions of the perch songs and chatter calls of male Common yellowthroats. Condor **97**:27-38.

Shannon, G., M. F. McKenna, L. M. Angeloni, K. R. Crooks, K. M. Fristrup, E. Brown, K. A. Warner, M. D. Nelson, C. White, J. Briggs, S. McFarland, and G. Wittemyer. 2016. A synthesis of two decades of research documenting the effects of noise on wildlife. Biological Reviews **91**:982-1005.

Slabbekoorn, H., and A. den Boer-Visser. 2006. Cities change the songs of birds. Current Biology **16**:2326-2331.

Slabbekoorn, H., and M. Peet. 2003. Ecology: Birds sing at a higher pitch in urban noise. Nature **424**:267-267.

Staicer, C. A. 1996. Honest advertisement of pairing status: Evidence from a tropical resident wood-warbler. Animal Behaviour **51**:375-390.

Tove, M. H. 1988. Patterns of singing by house wrens with respect to the breeding cycle Utah State University, Logan, UT.

Verzijden, M. N., E. A. P. Ripmeester, V. R. Ohms, P. Snelderwaard, and H. Slabbekoorn. 2010. Immediate spectral flexibility in singing chiffchaffs during experimental exposure to highway noise. Journal of Experimental Biology **213**:2575-2581.

Wood, W. E., and S. M. Yezerinac. 2006. Song sparrow (*Melospiza melodia*) song varies with urban noise. Auk **123**:650-659.

Zollinger, S. A., J. Podos, E. Nemeth, F. Goller, and H. Brumm. 2012. On the relationship between, and measurement of, amplitude and frequency in birdsong. Animal Behaviour **84**:E1-E9.

Figure 2.1 Spectrogram of house wren song, highlighting the two sections of the song.

We measured the frequency-modulated terminal section, which is capable of

transmitting beyond a male's territory (Tove, 1988).

Figure 2.2 Power spectrum of synthesized pink noise (a) shows high amplitude noise peaks at low frequencies (appx 0-2 kHz) and tapers off with increasing frequency. Pink noise reflects naturally occurring patterns of continuous anthropogenic noise (b) and eliminates intermittent peaks in SPL that may be present in recordings of anthropogenic noise.

Figure 2.3 Paired male house wrens increased the peak frequency of their songs during high noise playback compared to the control. Unpaired male house wrens did not adjust peak frequency.

Figure 2.4 Unpaired male house wrens sang longer songs (a) at a higher rate (c) than paired males, however noise treatment had no effect on song length (b) or rate (d).

CHAPTER III

WHAT DRIVES FLEXIBLE SIGNALING? ANTHROPOGENIC NOISE AND SOCIAL
CONTEXT AFFECT MALE HOUSE WREN VOCALIZATIONS

Abstract

Vocal communication shapes animal social networks, connecting multiple

individuals over space and time via information and facilitating mate attraction and

resource defense. Despite evidence that both the physical and social environment affect

signaling behavior, few studies consider variation in individual responses to rapid

environmental change within a social context. For example, in response to

anthropogenic noise, callers adjust the structure of long-distance, broadcast signals. But

change in signaling behavior may not be driven by the noise disturbance alone, and

social factors could also play an important role in song adjustments. We test the

hypothesis that male house wrens (*Troglodytes aedon*) flexibly adjust songs and song

components structured for short-and long-distance transmission in response to change

in their immediate noise environment, but that both social context and noise affect

singing patterns at the population level. We recorded paired males prior to clutch

initiation, quantified ambient noise in the moments before signaling, and defined social

context within pairs as the fertile status of females, and social context between males as

number of conspecific neighbors. Among males, patterns of adjustment varied

depending on signal transmission properties, social context, and ambient noise. In

response to immediate change in noise levels, males plastically adjust some, but not all,

song traits. Moreover, we show that not all males adjust their signals in the same way in

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response to heterogeneity in the noise environment, and that consideration of social

context and signal function are crucial for understanding variation in signal structure.

This is the first study that shows individuals adjust songs in response to natural variation

in their noise environment, and emphasizes the importance of social factors that

influence signal design.

Introduction

Communication shapes animal social networks, connecting multiple individuals over space and time via information (Snijders and Naguib 2017) and facilitating mate attraction and resource defense (McGregor and Peake 2000, Catchpole and Slater 2008). The structure of signals are shaped by selection pressures from the social environment (Bradbury and Vehrencamp 1998), as well as transmission constraints from the environment inhabited by a species (Morton 1975). Both signal senders and receivers adjust their behavior in response to human-generated changes in the environment that impede signal transfer (Endler 1992, Wiley 2013). For example, anthropogenic noise overlaps long-distance vocal signals animals use to defend breeding areas and attract mates (Brumm and Slabbekoorn 2005). Across taxa, callers modify the structure of their signals in response to noise to presumably reduce masking and thereby increase signaling space (Brumm and Zollinger 2013, Halfwerk et al. 2018, Hawkins and Popper 2018, Simmons and Narins 2018). Similarly, a signalers' social context, such as the presence of a rival or predator, often leads to changes in their signals as well (Bradbury and Vehrencamp 1998). Despite evidence that both the physical and social environment affect signaling behavior, few studies consider variation in individual responses to rapid environmental change within a social context.

Across a population, animals that inhabit noise-polluted areas structure their signals differently compared to those living under less noisy conditions (Slabbekoorn and Peet 2003, Wood and Yezerinac 2006, Mockford and Marshall 2009, Cunnington and Fahrig 2010, Parks et al. 2011, Potvin et al. 2011, Lampe et al. 2012, Luther and

Derryberry 2012, Warrington et al. 2018). Differences at the population level are driven

by change in the behavior of individuals, but the mechanism underlying patterns of

adjustment within individuals is seldom explored (Nussey et al. 2007, Dingemanse et al.

2010, Westneat et al. 2015). Vocal adjustments may be plastic, that is, individuals may

flexibly adjust their songs in response to high-amplitude shifts in noise (Halfwerk and

Slabbekoorn 2009, Gross et al. 2010, Verzijden et al. 2010, Montague et al. 2013,

LaZerte et al. 2016, Gentry et al. 2017). Vocal plasticity would enable callers, when

conditions are favorable, to sing low-frequency songs that may be preferred by females

(Halfwerk et al. 2011), but would allow for short-term, rapid song changes that increase

detectability during times of high noise, such as morning rush hour (Gross et al. 2010).

Moreover, particularly in variable noise environments, selection might favor signalers

that exhibit greater plasticity, adjusting songs as needed to improve signal transmission,

while singing songs preferred by females during periods of low noise. But whether

individuals vary in their ability to plastically adjust signals in response to change in their

immediate noise environment is unknown, as studies exploring vocal plasticity examine

male song responses to standardized noise playbacks; that is, studies to date have

asked whether signalers change their signals to one or two predetermined noise levels

(Cunnington and Fahrig 2010, Gross et al. 2010, Verzijden et al. 2010, Montague et al.

2012, LaZerte et al. 2016, Gentry et al. 2017). Critically lacking from our current

understanding on the effects of noise on animal singing behavior is whether males

rapidly change their songs according to variation in ambient noise conditions they

experience naturally. If so, do individuals vary in how they adjust their signals, that is, their plastic responses?

Plastic responses may not be driven by heterogeneity in noise alone, as social factors modify song adjustments in response to noise (McGregor and Peake 2000, Dingemanse and Araya-Ajoy 2015). Short-term adjustments by signalers in response to noise result from immediate feedback from mates (Halfwerk et al. 2012). Song changes in response to noise may also depend on conspecific male density (Hamao et al. 2011), pairing status (Gross et al. 2010, Grabarczyk et al. 2018), or breeding stage (Kight and Swaddle 2015). Moreover, long-term adjustments may occur via cultural evolution, where males copy song adjustments made by others inhabiting the same location (Cardoso and Atwell 2011, Peters et al. 2012, Potvin and Parris 2012). For example, white-crowned sparrow nestlings (*Zonotrichia leucophyrs*) copy high-frequency songs that transmit well in noisy environments, eventually crystallizing songs that are of higher pitch than those sung by their tutors (Moseley et al. 2018). Thus, the social environment could influence how individuals vary in their responses to anthropogenic noise, via short-term behavioral plasticity in response to variation in the noise environment experienced as an adult or through song learning during periods of developmental plasticity (Patricelli and Blickley 2006, Snell-Rood 2013).

We tested the hypothesis that male house wrens (*Troglodytes aedon*) flexibly adjust songs and song components structured for short-and long-distance transmission in response to change in their immediate noise environment, but that both social context and noise affect singing patterns at the population level. Male house wrens have

complex vocal repertoires (Rendall and Kaluthota 2013) and songs consist of introduction sections, comprised of non-repeating patterns of notes that cover a broad range of frequencies, followed by terminal sections that are composed of syllables made up of frequency-modulated notes (Platt and Ficken 1987, Rendall and Kaluthota 2013). Introductions are structured for short-distance communication, as this section is not detectable beyond a male's territory, whereas terminal sections are capable of transmitting beyond a male's territory under low ambient noise conditions (Grabarczyk and Gill 2019). Noise masking differentially affects transmission of song sections (Grabarczyk and Gill 2019); thus, how males adjust their signals may depend on both social context and ambient noise, as certain adjustments may only affect transmission of short-or long-distance song sections.

Male house wrens adjust songs in response to change in noise, but their responses also depend on social factors (Grabarczyk et al. 2018; Grabarczyk and Gill, in review; see also Stuart et al. 2019). Males increase peak frequency of the terminal section of songs in response to simulated intruders, which may improve signal transmission during interactions with rivals related to resource defense (Grabarczyk and Gill, in review). Low frequency signals may be important for mate attraction and may be preferred by females, as paired but not unpaired males increase peak frequency in response to noise playback (Grabarczyk et al. 2018). Male wrens decrease their rate of singing as females approach egg laying (Johnson and Kermott 1991, Rendall and Kaluthota 2013); thus, within-pair social context may affect variation in signal structure.

To explore variation in male song structure and signaling plasticity, we recorded paired males at one breeding stage (prior to clutch initiation) across a gradient of noise while exploring the influence of social factors. We took a hierarchical approach, examining songs and components of male song structured for short-and long-distance transmission and quantifying both ambient noise at the moment of signaling as well as the male's social environment on the day of recording. For each focal male, we considered noise variation within- and among-males. We used a behavioral reaction-norm approach to test a suite of random regression mixed-effects models, exploring whether patterns of song adjustments are consistent across males (among-male effects) and whether individual males plastically adjust their songs in response to change in their noise environment (within-male effects). We tested whether patterns of plasticity detected within-males were similar to patterns at the population levels and finally, for traits found to be plastically adjusted, we use a random slopes model to determine whether males varied in the level of plasticity (Nussey et al. 2007, van de Pol and Wright 2009). If males flexibly adjust spectral or temporal traits within songs, we expect to see different patterns of song structure within males as a function of heterogeneity in their signaling environment. If males vary in their level of plasticity, then selection should favor males that adaptively adjust signals in such a way that reduces signal masking and facilitates communication.

Methods

Field methods

We studied house wrens breeding in nest boxes at three natural areas in Kalamazoo County, Michigan, USA (42.290° N, 85.586° W) from 2016 – 2017. Sites varied in proximity to local roads, highways, trains, and airways, and thus, the source and level of anthropogenic noise differed both among sites, but also between-male territories within each site (Gill et al. 2017, Authors, unpublished data). Within sites, nest boxes were placed in areas of open grassland and mixed forest edge. We manipulated the number of potential neighbors by placing boxes in 18 6-box circles or networks. Within circles, boxes were positioned 45-50m from the next closest box at a 60° angle. We separated each circle of nest boxes by at least 150m to limit signal transmission between networks (Grabarczyk and Gill 2019).

We monitored box use and breeding progress in a color-banded population of house wrens, checking activity at boxes every three days. We captured house wrens with mist nets and fitted them with three plastic color-bands and 1 USGS numbered aluminum band. Males were sexed in hand based on cloacal protuberance and sex was confirmed by observing singing. Thirty-four out of 36 focal males (94%) included in this study were color-banded. Once a male was confirmed at a box, we determined whether he had paired, either through observation of a female bringing nesting material to the box or if nest lining had been added to the forming cup (Grabarczyk et al. 2018). We recorded clutch initiation, hatch, and fledging dates for each nesting attempt.

We recorded focal males at their nest boxes using Wildlife Acoustics Sound Meter 2 units (SM2; Maynard, MA; 44.1 kHz sample frequency, 16-bit, .wav format), by

attaching a microphone directly to the nest box pole and connecting the microphone to

SM2 unit with a 3-m cord. Units were pre-programmed to begin recording one hour

before sunrise (Eastern Standard Time, EST) and to continue recording for five hours.

For our recording protocol, we recorded males over multiple days at the nest box. This

enabled us to record high-fidelity songs, when males were at or near their nest boxes,

such that we recorded both the lower amplitude introduction sections of the songs as

well as louder terminal sections (Grabarczyk and Gill 2019). For analysis, we randomly

selected one day of recordings for each focal male, confirming from our nest

observations that all males were paired and that observations occurred prior to clutch

initiation.

Acoustic analysis

In Avisoft SASLab Pro v5.2 (R. Specht, Berlin, Germany), we labeled every song

sung by a focal male at his nest box between 6:00 – 8:30 AM (EST) that was not

overlapped by the songs of other birds. We were interested in analyzing songs recorded

with high fidelity and of similar recording quality among males. Therefore, we used the

sig2noise function in warbleR package (Araya-Salas and Smith-Vidaurre 2017) in R

program software v3.3.3 (R Core Development Team) to calculate the signal-to-noise

ratio (SNR) for each marked song. SNR was calculated as the ratio between relative

ambient noise 1-sec prior to each marked song (dB, rms) divided by signal amplitude

(dB, rms). To determine the SNR cut-off of songs to be considered for analysis, we first

analyzed SNR of all songs sung by 20 males in our dataset (N = 2,437 songs). We

calculated measures of central tendency for song SNR (mean ± SD: 6.9 ± 5.5, range: -13.4 – 32, where negative values indicate that background amplitudes exceeded signal amplitude) and selected a SNR greater than or equal to 4 as a cut-off. This ensured we had high quality songs, including introduction sections, from all males, but the cut-off was not too stringent that we would have less than 25 songs per male to analyze (see below). From 36 focal male recordings, we labeled 2,358 songs with SNR > 4 (mean ± SD of all songs with SNR > 4: 12.9 ± 7.8). From this list, we used a random number generator to randomly select 25 songs from each male to be included in analysis, except for one focal male for which we had only 23 songs. In total, we analyzed 898 songs from 36 focal males (SNR mean ± SD of songs used in analysis: 12.4 ± 8.1).

After randomly selecting 25 songs per male, we used Avisoft to insert section labels marking each song section (introduction and terminal), and within terminal sections, we additionally marked all syllables on the spectrogram window (Flat top window, 512 FFT length, 93.75% overlap, 0.725 ms time resolution). Song sections were defined based on note structure; introductions were identified by non-repeating patterns of broadband frequency notes, often with harmonics, whereas terminal sections were consistently composed of frequency modulated patterns of repeating notes (Rendall and Kaluthota 2013, dos Santos et al. 2016). Syllables were classified following Rendall and Kaluthota (2013) and dos Santos et al. (2016) as consistent units of note combinations detected among-males in our population (Figure 3.1).

To remove high- and low-frequency sounds from our recordings, we used a bandpass filter (1.2-11kHz) on all recordings. We used the automated parameter

window (-20 dB threshold) to extract minimum frequency (Hz), maximum frequency (Hz), peak frequency (Hz), bandwidth (Hz), and song duration (s) from each song, song section, and syllable. Despite selecting high-fidelity songs for analysis, the amplitudes of ambient noise between 0-2 kHz approached or exceeded those of the lower amplitude introduction sections. This was problematic, as the automated parameter tool would then calculate values for minimum frequency that were equal to the frequency of the high-pass cutoff (1.2 kHz). In these instances, we could not rule out whether ambient SPLs biased calculations of minimum frequency or other frequency traits for introduction sections (see also Grabarczyk et al. 2018). One possible solution would be to increase the cut-off frequency of the bandpass filter, but doing so eliminated portions of the introduction from calculations. Therefore, for analysis that included introduction sections (entire song and introduction section), we only included temporal traits, whereas we analyzed both temporal and spectral traits of the higher amplitude terminal section.

Quantifying ambient noise

To determine whether within- or among-male noise effects predict vocal adjustments, we measured ambient noise 1-sec prior to the start of each focal male song. To allow for amplitude measurements from song recordings, we calibrated microphone and SM2 unit pairs with a Larson Davis 200 sound level calibrator (Depew, NY) by recording a 1-kHz 94-dB tone. In Avisoft, we used the calibration function to set the recording amplitude to 0 dB (re 20 μPa) based on our recorded calibration tone. The frequency range over which noise occurs may affect how males adjust song, therefore, we extracted ambient SPLs in two ways that capture different masking frequencies.

First, we applied a low-pass filter set to 0-2 kHz, which are frequencies typical of continuous anthropogenic noise, and then extracted SPLs. Next, we calculated the mean minimum and maximum frequency (Hz) of all 25 songs sung by the 36 focal males. We then defined the high-pass cut off (low end of the bandwidth spectrum) as mean minimum frequency minus one standard deviation and the low-pass cut off as mean maximum frequency plus one standard deviation. To measure the frequencies of noise that mask male house wren song we removed the low-pass filter and applied a bandpass filter of 1.6-8.9 kHz, which was based on the average frequency range of house wren song (hereafter masking noise). We inserted a 1-sec section label before the start of each song and used the automated parameter tool to extract ambient SPLs (dB, rms).

Social factors

We determined social factors for each focal male from our nest records. For analysis, we categorized whether the social mates of focal males were fertile on the day of recording; we defined the fertile period as beginning five days prior to clutch initiation (Johnson and Kermott 1991, Stuart et al. 2019) We quantified the number of males breeding within 100m of focal males on the day of recording (0-4 males). By analyzing recordings of males prior to clutch initiation, we reduced variation in male vocal responses due to breeding stage.

Statistical analysis

Estimating within-and among-male vocal plasticity. We tested the hypotheses that patterns of song adjustments in response to noise and social factors are detectable at the population level (among-male effects) and that males plastically adjust their songs in response to change in their noise environment (within-male effects). We also tested whether males varied in the degree of plasticity by considering among-male variation in within-male responses. To test our hypotheses, we fit up to three random regression mixed-effects models exploring whether: 1) change in song traits vary within males (i.e. are responses plastic), 2) if vocal adjustments are consistent across males, 3) whether individual male slopes differ from the population slope, and 4) do males vary in the level of plasticity expressed (i.e. are some males more plastic than others). We used a behavioral reaction norm approach, comparing change in song trait intercepts (i.e. among-male effects) and slopes (i.e. within-male effects) as a function of noise and social environment. For model testing, we partitioned variation in ambient noise for focal males three ways by calculating: 1) grand mean across all males and songs, 2) among-male effects, and 3) within-male effects. We quantified among-male effects of noise by calculating mean ambient noise level for each male (N = 36 males) and within-male noise effects as each male's mean noise value subtracted from each observation (N = 25 noise observations per male), for both anthropogenic and masking noise, respectively. We centered each noise parameter prior to random regression mixed-effects modeling.

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For model testing, we used lme4 (Bates et al. 2015) and report test estimates and 95% bootstrapped confidence intervals (N = 2,000 simulations). For each song trait, we separately fit random intercept models for the two noise predictors, anthropogenic or masking. We included both within-and among-male effects (either anthropogenic or masking noise), female fertile status (two levels; yes or no), number of conspecific male neighbors (range: 0-4 neighbors), and the interaction between number of neighbors and among-male variation in noise as fixed effects and male identity as a random effect.

If random intercepts models indicated that changes in songs traits vary within-males (i.e. males plastically adjust their songs), we next tested whether males differed in the level of song plasticity in noise by separately fitting random slopes models for each respective song trait and noise predictor (van de Pol and Wright 2009). We included within-and among-male noise effects, female fertile status, number of neighbors, and the interaction between among-male noise variation and number of neighbors as fixed effects. We defined the random effects term as within-male noise given male identity. We determined the conditional repeatability of adjustments, which estimates the amount of variance attributed to the differences within and among individuals (Nakagawa and Schielzeth 2010, Dingemanse and Dochtermann 2013) as well as covariance, which is a measurement of how the vocal responses of individuals in the average ambient noise environment relates to its level of plasticity (Araya-Ajoy and Dingemanse 2017). We used log-likelihood tests to determine whether random intercepts models differed from the random slopes models and we reported residual variance from the random slopes models. For all models, if residual plots showed

heteroscedascity, we log transformed our response variable, which improved model fit

(Zuur et al. 2010). We plotted residuals from each song trait model against Julian date

and year, but found no patterns, thus did not include either as co-variates in our models.

Results

Among-male song adjustments vary with noise levels and social factors

Among males, change in duration of songs and song components depended on

signal function, social factors, and average ambient noise levels. Males decreased the

duration of their entire song with an increasing number of conspecific neighbors (95%

CI: -0.2, -0.03; Figure 3.2A). No change to the introduction section, which is the section

structured for short-distance communication, was detectable at the population level.

Males also did not alter the duration of terminal section of their songs in relation to

either average anthropogenic or masking noise. Instead, males adjusted the duration of

syllables that made up the terminal section, although this change depended on an

interaction between the number of conspecific neighbors as well as variation among

males in masking and anthropogenic noise (i.e. average noise conditions; among-male

anthropogenic noise levels x number of neighbors 95% CI: -0.03, - 0.01; Figure 3.2B;

among-male masking noise x number of neighbors 95% CI: -0.04, -0.002; Figure 3.2C,

Table 3.1). Males adjusted the number of syllables within terminal sections depending

on an interaction between number of conspecific neighbors and anthropogenic noise

(95% CI: 0.01, 0.09; Table 3.3).

Spectral adjustments in the terminal section among males were predicted by social context; males sang terminal sections with lower minimum frequencies when their mates were fertile (95% CI: -200.3, -10.7; Figure 3.3A, Table 3.2). Males increased peak frequency of the terminal section as well as syllables with increasing number of neighbors when modeled with average masking noise levels (terminal section 95% CI: 4.1, 173.3, Table 3.2; syllables 95% CI: 8.3, 145.3; Figure 3.3B, Table 3.3). Among males, there were no significant predictors of maximum frequency or bandwidth of terminal sections (Table 3.2) nor of minimum frequency or maximum frequency of syllables. (Table 3.3).

Noise affects plastic changes to song structure

Next, we tested whether individual males adjusted their songs based on noise before signaling (i.e. within-male effect of noise). We found males plastically adjust the structure of songs in response to immediate fluctuations in anthropogenic noise. Variation in anthropogenic noise predicted individual male changes to both the duration of the entire song and the introduction section (entire song 95% CI: -0.04, -0.0001; Figure 3.4A; introduction section 95% CI: -0.02, -0.004; Figure 3.4B, Table 3.1). In addition, the bandwidth of syllables varied within males depending on anthropogenic noise levels in the moments before signaling (95% CI: -29.5, -3.8; Figure 3.3C, Table 3.3).

Within males, we found evidence for plastic changes in duration of the entire song and introduction section as well as the bandwidth of syllables. Therefore, to

determine whether if males varied in their plastic responses, for each trait we tested

whether there was a significant difference among males in the within-male effect. Males

varied in their slopes; that is, males varied in how they adjusted the duration of their

entire song in noise, with some males increasing entire song duration with increasing

noise but others doing the opposite (X^2 = 12.3, p = 0.002, Figure 3.2A, Table 3.1).

Despite evidence for plastic changes of the duration of introduction section and syllable

bandwidth within males we found no evidence that males varied in the degree of

plasticity in these traits (Table 3.1 and 3.3). For all song traits, repeatability was low

(range: 0.03 – 0.4), which suggests that males sing many different songs and song

components over a short time period. This is not an unexpected result because male

house wrens have complex repertoires, and the ceiling on the number of different song

types a male sings, based on the terminal section alone, is unknown (Rendall and

Kaluthota 2013, dos Santos et al. 2016).

Discussion

        Our study shows that constraints from the ambient noise environment affect

patterns of signal adjustments among and within-males, but that adjustments are

influenced by social context and signal transmission properties. Variation in ambient

noise levels affected plastic responses to the duration of entire songs and introduction

sections as well as syllable bandwidth of terminal sections. Males varied in the level of

plasticity of adjustments to the duration of their entire song in response to immediate

fluctuations in anthropogenic noise. Yet by further examining song components used to

compose entire songs, we found that adjustments to the duration of entire songs were the result of complex variation in caller social context, ambient noise, and signal transmission properties. Among males, social factors, but not noise, affected the spectral features of the song components used for long-distance transmission. Critically, we show that not all males adjusted their signals in the same way in response to heterogeneity in the noise environment, suggesting that consideration of social context and signal function are crucial for understanding additional variation in signal structure. Our study is the first to reveal how individuals respond to natural variation in their noise environment using a behavioral reaction norm approach, and emphasizes the importance of social factors that influence signal design.

The duration of songs often reflects a callers social context, early in the breeding season males produce longer signals that are locatable by prospective females, but decrease the duration of signals as the season progresses (Bradbury and Vehrencamp 1998). Among male house wrens, social factors affected the duration of entire songs. Males decreased the duration of their entire song if they had conspecific neighbors (Figure 3.2A). Males that produce longer songs may increase the probability of overlapping the songs of neighbors, which in many species is thought to be an aggressive signal (Naguib and Mennill 2010). Thus, decreasing the duration of songs by paired males with increasing number of neighbors may be a means to reduce the risk of aggression between established neighbors.

Plastic signaling within-males may enable individuals to cope with anthropogenic noise masking, but changes may depend on social context. Male house wrens showed

plastic song adjustments that were attributable to immediate changes in their noise

environment. Males flexibly adjusted their entire song and introduction section duration

in response to changes in anthropogenic noise, and further, males varied in the level of

plastic changes to the duration of their entire songs; that is, males adjusted song length

in different ways. To achieve adjustments to the entire song requires change to the

components that compose an entire song. But males did not vary in the level of plastic

responses of temporal traits for other song components. Instead, changes to the

introduction and terminal section resulted from a combination of population level social

factors as well as average and immediate ambient noise levels. This suggests flexibility

in entire song duration may result from underlying social factors that interplay with

ambient noise levels. For example, differences in social factors, such as increased male

breeding density in noise-polluted habitats (Nemeth and Brumm 2009), could result in

males adjusting the structure or timing of their signals differently.

Signal plasticity among and within individuals can vary over different temporal

scales (Patricelli et al. 2016). Differences in the duration of song components structured

for short-and long-distance transmission may be affected at different temporal scales.

Males plastically adjusted the duration of the introduction section in response to

variation in anthropogenic noise. High ambient noise significantly diminishes signaling

distance of the introduction section, such that this part of the song may not detectable

even within a male's own territory (Grabarczyk and Gill 2019). Therefore, this section

may not be an effective signal under high noise, unless signal receivers are in very

close proximity to the caller (approximately <15m; Grabarczyk and Gill 2019). In

variable noise environments, males may depend on feedback from mates (Halfwerk et al. 2012), rapidly modulating the duration of the introduction depending on immediate social interactions. For example, under high noise conditions, males might increase the duration of the introduction section when female breeding partners are present at nest boxes, but limit the use of this section when females are outside the range of detection. Males did not alter the duration of song terminal sections, the section of song structured for long-distance transmission, according to ambient noise, female fertile status, or number of conspecific. But additional social factors are important predictors of terminal section duration. Males sing longer songs during early stages of breeding (Tove 1988, Johnson and Kermott 1991, Grabarczyk et al. 2018), and increase terminal section duration in response to simulated conspecific territorial intruders (Grabarczyk and Gill, in review). We may not have detected variation in the duration of the terminal section because we analyzed male songs during one stage of breeding. Thus, male house wrens may vary in the degree of plasticity of terminal section duration over a longer temporal scale, such as across entire nesting attempts, whereas adjustments to the introduction section may be rapidly modulated to increase the likelihood of received signals in response to immediate social feedback or context.

In response to noise, birds with complex songs might adjust their signals by singing different song types (Halfwerk and Slabbekoorn 2009), or by singing the same song type but adjusting the structure of components used to compose a song (Bermudez-Cuamatzin et al. 2009). Male house wrens do not alter the structure of terminal sections of songs in response to noise, but do adjust features of syllables,

which are the components used to compose terminal sections. The interaction between among-male variation in ambient noise (both anthropogenic and masking frequencies) and the number of conspecific neighbors predicted patterns of syllable duration and the number of syllables sung within terminal sections. Whether males increased or decreased syllable duration with changing noise was highly variable (Figure 3.2 B-C). Males plastically adjusted syllable bandwidth as a function of variation in anthropogenic noise, but males changed bandwidth in similar ways, indicating a lack of among-male variation in plasticity (Figure 3.4C). Together, patterns of change in syllable duration, number of syllables per song, and bandwidth may suggested that males switch between syllable types as a function of both noise and social factors. In a population of southern house wrens (*Troglodytes aedon chilensis*), the diversity of syllables males use to compose the terminal sections of songs positively correlated with female clutch initiation date and number of eggs laid (dos Santos et al. 2018). This suggests that high-quality females may be attracted to males that sing with high song complexity, but whether diversity of syllables or the degree of plasticity in syllable composition also increases male fitness (i.e. within-clutch and extra-pair paternity) has not been tested.

Animals may be more likely to adjust certain song traits if the trait itself is not under strong selection pressures by mates and rivals. Social factors predicted variation in the spectral traits of the terminal section of songs; males sang songs at lower minimum frequencies if their mate was fertile and increased peak frequencies with an increase in the number of conspecific neighbors. The finding that song minimum frequency is affected by social factors, and not noise, contrasts with many studies that

show population-level increases in minimum frequency in noise-polluted areas

(Slabbekoorn and Peet 2003, Wood and Yezerinac 2006, Mockford and Marshall 2009,

Potvin et al. 2011, Hamao et al. 2011, Luther & Derryberry 2012, Warrington et al.

2018) as well as those that demonstrate increases in minimum frequency in response to

noise playbacks (Gross et al. 2010, Verzijden et al. 2010, Bermudez-Cuamatzin et al.

2011, Goodwin and Podos 2013, McLaughlin and Kunc 2013, LaZerte et al. 2016). The

presumption of these studies is often that an increase minimum frequency benefits a

male caller by reducing noise masking, yet the costs of adjustments are seldom

considered (Read et al. 2014). Female great tits (*Parus major*) prefer and show higher

fidelity to males that sang lower pitch song, but under high noise conditions, songs with

a higher minimum frequency were more effective at initial mate attraction (Halfwerk et

al. 2011). Because male house wrens sing songs at lower minimum frequencies during

a females' fertile period, but do not adjust minimum frequency in response to noise,

suggests that males may be constrained by female preference for low frequency song.

If so, males may benefit from maintaining low frequency songs during a female's fertile

period, and from adjusting other song traits, such as the duration of songs and song

components, that might increase the probability of detection (Ríos-Chelén et al. 2013),

but which are not under selection via female preference and therefore have little impact

on reproductive success. Males increased the peak frequency of terminal sections and

syllables with an increase in the number of conspecific neighbors. In this population of

house wrens, males increase song peak frequency in response to territorial intruders,

but peak frequency responses are weakened by noise (Grabarczyk and Gill, in review).

In response to noise playback, paired but not unpaired males adjust peak frequency, possibly because unpaired males may also be constrained by female preference for low frequency song during initial mate attraction (Grabarczyk et al. 2018). One possible explanation is that female choice, male competition, or both favor particular frequencies in house wrens and therefore constrains adjustment due to noise. Whether frequency adjustments in response to environmental change (i.e. anthropogenic noise) affect selection on frequency traits are critical for understanding whether adjustments are adaptive in nature.

Regarding the effects of anthropogenic noise on male signaling behavior, the general interpretation assumes that in response to anthropogenic noise, all males respond in the same way to reduce masking and that signals changes result in a net fitness benefit. This concept is supported by many studies that show populations of animals inhabiting noise-polluted areas structure their signals differently than populations inhabiting quiet environments, or that animals shift their vocalizations in response to noise playbacks. Most studies to date then focus analyses to explore average male responses to average environmental conditions, but neglect to consider possible variation of individual responses to environmental change (i.e. phenotypic plasticity; Nussey et al. 2007). Despite our consideration of additional factors that affect the structure of vocalizations (i.e. social factors and signal transmission distance), more variation in natural signaling behavior exists. Song changes may be due to prior experience with noise (LaZerte et al. 2016, Gentry et al. 2017), or could result of developmental plasticity (Mosley et al. 2018). Adjustments may be the result of

immediate interactions with mates or neighbors, or could vary more generally across the

breeding season. Crucially, further work must consider adaptive phenotypic change in a

social context as well as evolutionary consequences of song adjustments in response to

noise.

Literature cited

Araya-Ajoy, Y. G., and N. J. Dingemanse. 2017. Repeatability, heritability, and age-dependence of seasonal plasticity in aggressiveness in a wild passerine bird. Journal of Animal Ecology **86**:227-238.

Araya-Salas, M., and G. Smith-Vidaurre. 2017. warbleR: an r package to streamline analysis of animal acoustic signals. Methods in Ecology and Evolution **8**:184-191.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. . Journal of Statistical Software **67**:1-48.

Bermudez-Cuamatzin, E., A. A. Rios-Chelen, D. Gil, and C. M. Garcia. 2009. Strategies of song adaptation to urban noise in the house finch: syllable pitch plasticity or differential syllable use? Behaviour **146**:1269-1286.

Bermudez-Cuamatzin, E., A. A. Rios-Chelen, D. Gil, and C. M. Garcia. 2011. Experimental evidence for real-time song frequency shift in response to urban noise in a passerine bird. Biology Letters **7**:36-38.

Bradbury, J. W., and S. L. Vehrencamp. 1998. Principles of Animal Communication. 1 edition. Sinauer Associates, Sunderland, MA.

Brumm, H., and H. Slabbekoorn. 2005. Acoustic communication in noise. Pages 151-209 *in* P. J. B. Slater, C. T. Snowdon, H. J. Brockmann, T. J. Roper, and M. Naguib, editors. Advances in the Study of Behavior, Vol 35.

Brumm, H., and S. A. Zollinger. 2013. Avian vocal production in noise. Pages 187-227 *in* H. Brumm, editor. Animal communicaiton in noise. Springer Berlin Heidelberg, Seewiesen, Germany.

Cardoso, G. C., and J. W. Atwell. 2011. Directional cultural change by modification and replacement of memes. Evolution **65**:295-300.

Catchpole, C. K., and P. J. B. Slater. 2008. Bird song: Biological themes and variations. Cambridge University Press, New York.

Cunnington, G. M., and L. Fahrig. 2010. Plasticity in the vocalizations of anurans in response to traffic noise. Acta Oecologica **36**:463-470.

Dingemanse, N. J., and Y. G. Araya-Ajoy. 2015. Interacting personalities: behavioural ecology meets quantitative genetics. Trends in Ecology & Evolution **30**:88-97.

Dingemanse, N. J., and N. A. Dochtermann. 2013. Quantifying individual variation in behaviour: mixed-effect modelling approaches. Journal of Animal Ecology **82**:39-54.

Dingemanse, N. J., A. J. N. Kazem, D. Reale, and J. Wright. 2010. Behavioural reaction norms: animal personality meets individual plasticity. Trends in Ecology & Evolution **25**:81-89.

dos Santos, E. B., P. E. Llambias, and D. Rendall. 2016. The structure and organization of song in Southern House Wrens (*Troglodytes aedon chilensis*). Journal of Ornithology **157**:289-301.

dos Santos, E. B., P. E. Llambias, and D. Rendall. 2018. Male song diversity and its relation to breeding success in southern house wrens *Troglodytes aedon* chilensis. Journal of Avian Biology **49**.

Endler, J. A. 1992. Signals, Signal Conditions, and the Direction of Evolution. American Naturalist **139**:S125-S153.

Gentry, K. E., E. P. Derryberry, R. M. Danner, J. E. Danner, and D. A. Luther. 2017. Immediate signaling flexibility in response to experimental noise in urban, but not rural, white-crowned sparrows. Ecosphere **8**:e01916.

Gill, S. A., E. E. Grabarczyk, K. M. Baker, K. Naghshineh, and M. J. Vonhof. 2017. Decomposing an urban soundscape to reveal patterns and drivers of variation in anthropogenic noise. Science of the Total Environment **599**:1191-1201.

Goodwin, S. E., and J. Podos. 2013. Shift of song frequencies in response to masking tones. Animal Behaviour **85**:435-440.

Grabarczyk, E. E., and S. A. Gill. 2019. Anthropogenic noise masking diminishes house wren (*Troglodytes aedon*) song transmission in urban natrual areas. Bioacoustics.

Grabarczyk, E. E., M. A. Pipkin, M. J. Vonhof, and S. A. Gill. 2018. When to change your tune? Unpaired and paired male house wrens respond differently to anthropogenic noise. Journal of Ecoacoustics **2**:#LHGRVC.

Gross, K., G. Pasinelli, and H. P. Kunc. 2010. Behavioral Plasticity Allows Short-Term Adjustment to a Novel Environment. American Naturalist **176**:456-464.

Halfwerk, W., S. Bot, J. Buikx, M. van der Velde, J. Komdeur, C. ten Cate, and H. Slabbekoorn. 2011. Low-frequency songs lose their potency in noisy urban

conditions. Proceedings of the National Academy of Sciences of the United States of America **108**:14549-14554.

Halfwerk, W., S. Bot, and H. Slabbekoorn. 2012. Male great tit song perch selection in response to noise-dependent female feedback. Functional Ecology **26**:1339-1347.

Halfwerk, W., B. Lohr, and H. Slabbekoorn. 2018. Impact of Man-Made Sound on Birds and Their Songs.*in* H. Slabbekoorn, R. J. Dooling, A. N. Popper, and R. R. Fay, editors. Effects of Anthropogenic Noise on Animals. Springer.

Halfwerk, W., and H. Slabbekoorn. 2009. A behavioural mechanism explaining noise-dependent frequency use in urban birdsong. Animal Behaviour **78**:1301-1307.

Hawkins, A. D., and A. N. Popper. 2018. Effects of Man-Made Sound on Fishes.*in* H. Slabbekoorn, R. J. Dooling, A. N. Popper, and R. R. Fay, editors. Effects of Anthropogenic Noise on Animals. Springer, New York, NY.

Johnson, L. S., and L. H. Kermott. 1991. The Functions of Song in Male House Wrens (*Troglodytes aedon*). Behaviour **116**:190-209.

Kight, C. R., and J. P. Swaddle. 2015. Eastern Bluebirds Alter their Song in Response to Anthropogenic Changes in the Acoustic Environment. Integrative and Comparative Biology **55**:418-431.

Lampe, U., T. Schmoll, A. Franzke, and K. Reinhold. 2012. Staying tuned: grasshoppers from noisy roadside habitats produce courtship signals with elevated frequency components. Functional Ecology **26**:1348-1354.

LaZerte, S. E., H. Slabbekoorn, and K. A. Otter. 2016. Learning to cope: vocal adjustment to urban noise is correlated with prior experience in black-capped chickadees. Proceedings of the Royal Society B-Biological Sciences **283**.

Luther, D., and E. P. Derryberry. 2012. Birdsongs keep pace with city life: changes in song over time in an urban songbird affects communication. Animal Behaviour **83**:1059-1066.

McGregor, P. K., and T. M. Peake. 2000. Communication networks: social envionments for receiving and signalling behavior. Acta Ethologica **2**:71-81.

McLaughlin, K. E., and H. P. Kunc. 2013. Experimentally increased noise levels change spatial and singing behaviour. Biology Letters **9**.

Mockford, E. J., and R. C. Marshall. 2009. Effects of urban noise on song and response behaviour in great tits. Proceedings of the Royal Society B-Biological Sciences **276**:2979-2985.

Montague, M. J., M. Danek-Gontard, and H. P. Kunc. 2013. Phenotypic plasticity affects the response of a sexually selected trait to anthropogenic noise. Behavioral Ecology **24**:342-348.

Morton, E. S. 1975. Ecological sources of selection on avian sounds. American Naturalist **109**:17-34.

Moseley, D. L., G. E. Derryberry, J. N. Phillips, J. E. Danner, R. M. Danner, D. A. Luther, and E. P. Derryberry. 2018. Acoustic adaptation to city noise through vocal learning by a songbird. Proceedings of the Royal Society B-Biological Sciences **285**.

Naguib, M., and D. J. Mennill. 2010. The signal value of birdsong: empirical evidence suggests song overlapping is a signal. Animal Behaviour **80**:E11-E15.

Nakagawa, S., and H. Schielzeth. 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biological Reviews **85**:935-956.

Nemeth, E., and H. Brumm. 2009. Blackbirds sing higher-pitched songs in cities: adaptation to habitat acoustics or side-effect of urbanization? Animal Behaviour **78**:637-641.

Nussey, D. H., A. J. Wilson, and J. E. Brommer. 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. Journal of Evolutionary Biology **20**:831-844.

Parks, S. E., M. Johnson, D. Nowacek, and P. L. Tyack. 2011. Individual right whales call louder in increased environmental noise. Biology Letters **7**:33-35.

Patricelli, G. L., and J. L. Blickley. 2006. Avian communication in urban noise: Causes and consequences of vocal adjustment. Auk **123**:639-649.

Patricelli, G. L., A. H. Krakauer, and C. C. Taff. 2016. Variable Signals in a Complex World: Shifting Views of Within-Individual Variability in Sexual Display Traits. Pages 319-+ *in* M. Naguib, J. C. Mitani, L. W. Simmons, L. Barrett, S. Healy, and M. Zuk, editors. Advances in the Study of Behavior, Vol 48.

Peters, S., E. P. Derryberry, and S. Nowicki. 2012. Songbirds learn songs least degraded by environmental transmission. Biology Letters **8**:736-739.

Platt, M. E., and M. S. Ficken. 1987. Organization of singing in House wrens. Journal of Field Ornithology **58**:190-197.

Potvin, D. A., and K. M. Parris. 2012. Song convergence in multiple urban populations of silvereyes (*Zosterops lateralis*). Ecology and Evolution **2**:1977-1984.

Potvin, D. A., K. M. Parris, and R. A. Mulder. 2011. Geographically pervasive effects of urban noise on frequency and syllable rate of songs and calls in silvereyes (*Zosterops lateralis*). Proceedings of the Royal Society B-Biological Sciences **278**:2464-2469.

Read, J., G. Jones, and A. N. Radford. 2014. Fitness costs as well as benefits are important when considering responses to anthropogenic noise. Behavioral Ecology **25**:4-7.

Rendall, D., and C. D. Kaluthota. 2013. Song organization and variability in northern House wrens (*Troglodytes aedon parkmanii*) in Western Canada. Auk **130**:617-628.

Ríos-Chelén, A. A., E. Quiros-Guerrero, D. Gil, and C. M. Garcia. 2013. Dealing with urban noise: vermilion flycatchers sing longer songs in noisier territories. Behavioral Ecology and Sociobiology **67**:145-152.

Simmons, A. M., and P. M. Narins. 2018. Effects of Anthropogic Noise on Amphibians and Reptiles.*in* H. Slabbekoorn, R. J. Dooling, A. N. Popper, and R. R. Fay, editors. Effects of Anthropogenic Noise on Animals. Springer, New York, NY.

Slabbekoorn, H., and M. Peet. 2003. Ecology: Birds sing at a higher pitch in urban noise. Nature **424**:267-267.

Snell-Rood, E. C. 2013. An overview of the evolutionary causes and consequences of behavioural plasticity. Animal Behaviour **85**:1004-1011.

Snijders, L., and M. Naguib. 2017. Communication in Animal Social Networks: A Missing Link? Pages 297-359 *in* M. Naguib, J. Podos, L. W. Simmons, L. Barrett, S. D. Healy, and M. Zuk, editors. Advances in the Study of Behavior, Vol 49.

Stuart, C. J., Grabarczyk, E. E., Vonhof, M. J., and S. A. Gill. 2019. Social factors, not anthropogenic noise or sky glow, influence onset of dawn singing in a common songbird. Auk.

Tove, M. H. 1988. Patterns of singing by house wrens with respect to the breeding cycle Utah State University, Logan, UT.

van de Pol, M. V., and J. Wright. 2009. A simple method for distinguishing within- versus between-subject effects using mixed models. Animal Behaviour **77**:753-758.

Verzijden, M. N., E. A. P. Ripmeester, V. R. Ohms, P. Snelderwaard, and H. Slabbekoorn. 2010. Immediate spectral flexibility in singing chiffchaffs during experimental exposure to highway noise. Journal of Experimental Biology **213**:2575-2581.

Warrington, M. H., C. M. Curry, B. Antze, and N. Koper. 2018. Noise from four types of extractive energy infrastructure affects song features of Savannah Sparrows. Condor **120**:1-15.

Westneat, D. F., J. Wright, and N. J. Dingemanse. 2015. The biology hidden inside residual within-individual phenotypic variation. Biological Reviews **90**:729-743.

Wiley, R. H. 2013. Signal Detection, Noise, and the Evolution of Communication. Brumm, H. edition. Springer, Berlin Heidelberg.

Wood, W. E., and S. M. Yezerinac. 2006. Song sparrow (*Melospiza melodia*) song varies with urban noise. Auk **123**:650-659.

Zuur, A. F., E. N. Ieno, and C. S. Elphick. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution **1**:3-14.

Table 3.1 Estimates and bootstrapped 95% CI (N = 2,000 simulations) for random regression mixed effects models testing whether variation in ambient noise (anthropogenic; 0-2 kHz and masking; 1.2-7.6 kHz) and social factors affect plastic adjustments to the duration of entire songs, introduction, terminal, and syllable variation within- and among-males. For song traits that show a significant within-male effect of noise in the random intercepts model, we additionally compared this model to a random slopes model to determine if males vary in the level of plasticity. We report estimates and CI based on log-likelihood and AIC comparison for the best fit model. Except for the duration of entire songs, all model estimates and CI are from the random intercepts model.

	Entire song	Introduction	Terminal	Syllable
		Anthropogenic noise effects (0-2 kHz)		
Fixed effects				
Intercept	2.53 (2.3, 2.8)	0.99 (0.78, 1.21)	1.35 (0.32, 0.36)	-1.32 (0.99, 1.04)
Among-male noise	0.01 (-0.03, 0.05)	0.007 (-0.02, 0.04)	-0.01 (-0.04, 0.02)	0.04 (0.01, 0.08)
Within-male noise	-0.02 (-0.04, -0.001)	-0.01 (-0.02, -0.004)	0.001(-0.01, 0.01)	-0.01 (-0.02, 0.01)
Fertile status	-0.001 (-0.2, 0.2)	0.07 (-0.01, 0.24)	-0.04 (-0.19, 0.12)	-0.17 (-0.34, 0.02)
No. neighbor	-0.13 (-0.23, -0.03)	-0.02 -0.09, 0.05)	-0.05 (-0.13, 0.03)	-0.02 (-0.11, 0.08)
Among-male noise * No. neighbors	-0.003 (-0.02, 0.01)	-0.005 (-0.02, 0.01)	0.01 (-0.003, 0.02)	-0.02 (-0.03, -0.01)
Variance				
Among-male	0.09 (0.21, 0.37)	0.05 (0.15, 0.26)	0.05 (0.14, 0.25)	0.06 (0.15, 0.28)
Within-male	0.001 (0.01, 0.04)			

Residual	0.16 (0.37, 0.42)	0.12 (0.33, 0.36)	0.12 (0.33, 0.36)	1.0 (-1.57, -1.07)
Covariance	-0.64 (-1, -0.05)			
Conditional repeatability	0.41	0.31	0.33	0.07

	Masking noise effects (1.2 – 7.6 kHz)			
Fixed effects				
Intercept	2.43 (2.14, 2.72)	0.98 (0.76, 1.2)	1.4 (1.6, 1.6)	-1.29 (-1.53, -1.02)
Among-male noise	0.004 (-0.05, 0.04)	0.001 (-0.03, 0.03)	-0.001 (-0.03, 0.03)	0.04 (0.001, 0.07)
Within-male noise	0.0002 (-0.005, 0.005)	0.003 (-0.001, 0.007)	-0.002 (-0.01, 0.002)	0.003 (-0.003, 0.01)
Fertile status	0.03 (-0.18, 0.24)	0.08 (-0.07, 0.23)	-0.06 (-0.20, 0.10)	-0.15 (-0.33, 0.03)
No. neighbor	0.09 (-0.20, 0.01)	-0.02 (-0.1, 0.06)	-0.05 (-0.13, 0.03)	-0.04 (-0.13, 0.06)
Among-male noise * No. neighbors	0.01 (-0.02, 0.03)	0.0004 (-0.01, 0.01)	0.005 (-0.01, 0.02)	-0.02 (-0.04, -0.002)
Variance				
Among-male	0.09 (0.20, 0.35)	0.05 (0.15, 0.26)	0.05 (0.15, 0.26)	0.06 (0.15, 0.29)
Residual	0.16 (0.38, 0.42)	0.12 (0.33, 0.36)	0.12 (0.32, 0.35)	1.04 (0.99, 1.05)
Conditional repeatability	0.41	0.31	0.34	0.07

Table 3.2 Estimates and bootstrapped 95% CI (N = 2,000 simulations) for random regression mixed effects models testing whether variation in ambient noise (anthropogenic; 0-2 kHz and masking; 1.2-7.6 kHz) and social factors affect plastic adjustments to the terminal section peak frequency (Hz), minimum frequency (Hz), maximum frequency (Hz), and bandwidth (Hz). For all traits, the random intercepts model fit the data better than the random slopes model, and therefore report estimates and CI from the random intercepts model.

	Peak frequency	Minimum frequency	Maximum frequency	Bandwidth
		Anthropogenic noise effects (0-2 kHz)		
Fixed effects				
Intercept	3691.1 (3461.1, 3916.7)	2303.7 (2180.0, 2428.5)	6051.1 (5629.1, 6458.9)	3750.4 (3306.8, 4189.9)
Among-male noise	-5.5 (-38.2, 26.2)	6.2 (-11.5, 24.5)	-0.15 (-60.1, 55.8)	-3.2 (-62.0, 58.2)
Within-male noise	-2.6 (-15.9, 10.6)	-0.86 (-7.8, 5.9)	-9.6 (-29.5, 11.5)	-9.4 (-29.3, 12.5)
Fertile status	83.1 (-160.1, 170.2)	-105.2 (-200.3, -10.7)	16.9 (-296.4, 313.9)	133.8 (-180.7, 451.6)
No. neighbor	6.9 (0.12, 165.5)	9.98 (-37.9, 56.7)	69.4 (-80.2, 220.4)	51.5 (-114.0, 210.2)
Among-male noise * No. neighbors	-0.3 (-15.6, 14.4)	-5.95 (-14.2, 2.2)	-2.1 (-28.1, 25.6)	2.2 (-27.3, 29.8)
Variance				
Among-male	50173 (136.7, 268.9)	16678 (81.8, 153.8)	172524 (264.3, 490.4)	196193 (287.0, 520.4)
Residual	277261 (501.1, 550.4)	73987 (258.0, 284.6)	623042 (751.5, 828.4)	660963 (771.9, 850.3)
Conditional repeatability	0.18	0.22	0.23	0.25

	Masking noise effects (1.2 – 7.6 kHz)			
Fixed effects				
Intercept	3673.3 (3446.7, 3902.2)	2291.1 (2145.8, 2422.2)	6114.2 (5712.7, 6525.2)	3822.3 (3385.0, 4255.2)
Among-male noise	-9.3 (-44.1, 26.8)	-1.77 (-22.3, 18.8)	32.5 (-32.4, 93.8)	34.3 (-30.2, 97.8)
Within-male noise	-0.2 (-6.9, 6.4)	1.06 (-2.36, 4.43)	-0.31 (-10.6, 9.9)	-1.0 (-11.6, 9.0)
Fertile status	10.9 (-155.4, 175.3)	-94.5 (-189.3, 0.89)	5.4 (-281.6, 300.4)	113.3 (-183.8, 439.1)
No. neighbor	88.9 (4.1, 173.3)	13.1 (-37.6, 64.6)	48.8 (-102.5, 197.9)	28.8 (-125.5, 190.4)
Among-male noise * No. neighbors	4.5 (-12.7, 21.9)	-0.77 (-10.9, 9.5)	-19.0 (-48.6, 11.2)	-17.4 (-47.5, 13.9)
Variance				
Among-male	51043 (136.1, 269.0)	18320 (86.5, 160.1)	164330 (260.9, 480.3)	189294 (277.4, 508.8)
Residual	277304 (501.3, 551.0)	73870 (258.8, 285.2)	623686 (748.7, 831.7)	661548 (772.9, 852.6)
Conditional repeatability	0.18	0.22	0.23	0.25

Table 3.3 Estimates and bootstrapped 95% CI (N = 2,000 simulations) for random regression mixed effects models testing whether variation in ambient noise (anthropogenic; 0-2 kHz and masking; 1.2-7.6 kHz) and social factors affect plastic adjustments to the peak frequency (Hz), minimum frequency (Hz), maximum frequency (Hz), and bandwidth (Hz) of syllables.

	Peak frequency	Minimum frequency	Maximum frequency	Bandwidth
		Anthropogenic noise effects (0-2 kHz)		
Fixed effects				
Intercept	4231.6 (4036.5, 4430.8)	3022.5 (2803.9, 3246.6)	5915.5 (5658.7, 6170.1)	2875.6 (2619.6, 3144.1)
Among-male noise	0.6 (-25.8, 26.9)	10.6 (-37.2, 22.5)	9.2 (-26.6, 44.5)	16.0 (-19.3, 53.5)
Within-male noise	-2.6 (-17.8, 13.1)	-8.0 (-7.3, 28.9)	-6.0 (-23.9, 12.0)	-15.6 (-29.5, -3.8)
Fertile status	-13.4 (-158.5, 125.9)	-29.9 (-191.6, 126.6)	-104.0 (-290.9, 83.6)	-68.6 (-258.5, 134.9)
No. neighbor	69.1 (-6.8, 138.2)	25.7 (-53.4, 106.1)	11.3 (-83.6, 106.4)	-6.9 (-103.5, 87.9)
Among-male noise * No. neighbors	-0.5 (-13.0, 11.9)	1.2 (-13.3, 15.5)	-3.6 (-20.4, 13.4)	-4.4 (-21.3, 12.3)
Variance				
Among-male	27551 (71.3, 206.7)	37500 (92.2, 239.7)	54724 (168.7, 322.0)	71088 (172.4, 313.3)
Residual	1296117 (1108.9, 1167.3)	1455751 (1175.0, 1238.6)	1659068 (1256.1, 1321.4)	820517 (883.8, 929.0)
Conditional repeatability	0.03	0.03	0.04	0.09
		Masking noise effects (1.2 – 7.6 kHz)		
Fixed effects				

Intercept	4211.7 (4021.0, 4395.8)	3022.5 (2784.5, 3244.8)	5951.4 (5665.4, 6164.5)	2924.9 (2653.6, 3172.4)
Among-male noise	-7.1 (-35.5, 21.8)	10.6 (-37.6, 26.2)	17.2 (-24.3, 42.1)	22.7 (-15.5, 59.7)
Within-male noise	-2.5 (-10.4, 5.1)	-8.0 (-10.3, 6.4)	1.2 (-24.0, 12.2)	4.1 (-2.0, 10.4)
Fertile status	-8.6 (-140.3, 124.2)	-29.9 (-193.4, 130.8)	-106.5 (-292.1, 72.0)	-72.0 (-246.2, 107.7)
No. neighbor	76.2 (8.3, 145.3)	25.7 (-53.7, 112.2)	-2.7 (-81.6, 102.9)	-26.8 (-122.9, 74.4)
Among-male noise * No. neighbors	4.5 (-9.8, 18.2)	1.2 (-10.6, 21.6)	-11.4 (-19.4, 12.2)	-17.1 (-35.5, 1.4)
Variance				
Among-male	27000 (70.3, 205.1)	37000 (92.5, 235.7)	51178 (119.3, 284.8)	62428 (157.2, 295.8)
Residual	1295998 (1108.5, 1168.1)	1456417 (1174.7, 1237.5)	1659352 (1257.1, 1322.2)	821815 (882.4, 929.4)
Conditional repeatability	0.03	0.03	0.04	0.08

Figure 3.1 House wren song is composed of an introduction section with non-repeating notes that cover a broad frequency range, followed by a frequency-modulated terminal section. The terminal section is composed of syllables, that are repeated combinations of notes consistently sung across males (Rendall and Kaluthota 2013, dos Santos et al. 2016).

Figure 3.2 Among-male or population-level responses. (A) Males decreased the duration of their entire song if they had neighbors. Variation in syllable duration was attributable to the interaction between average or standardized anthropogenic noise (B) as well as masking noise (C) and number of conspecific neighbors. Males show more variation in syllable duration under high-masking noise conditions.

Figure 3.3 Social factors predicted among-male spectral adjustments. (A) Prior to clutch initiation males sing songs with lower minimum frequencies (Hz) when mates are fertile, and (B) increase terminal section peak frequency (Hz) depending on the number of conspecific neighbors.

Figure 3.4 Behavioral reaction norm plots of song traits show within-male or plastic

responses (black lines) and the population average (blue line). Within-males,

anthropogenic noise moments before signaling affected adjustments to (A) the entire

song and (B) introduction section duration (s). Males varied in the level of plasticity of

adjustments to the duration of their entire song. (C) Males adjusted the bandwidth of

syllables (Hz) in response to anthropogenic noise in the moments before signaling, but

males did not vary in the degree of their plastic responses.

CHAPTER IV

ANTHROPOGENIC NOISE MASKING DIMINISHES HOUSE WREN (*TROGLODYTES AEDON*) SONG TRANSMISSION IN URBAN NATURAL AREAS

Abstract

Anthropogenic noise changes the acoustic environment in which avian signals have evolved, possibly decreasing active space, or the area over which signals may be detected and discriminated by receivers. Linking signal transmission patterns to signal function and species' spatial ecology is important for understanding behavioural changes of receivers in noise. We tested whether varying levels of ambient noise affects transmission of two structurally distinct sections of male house wren (*Troglodytes aedon*) song used for short- and long-distance communication. We placed our experiment in an ecological context by measuring signal degradation and attenuation in relation to species-typical spacing patterns to investigate whether song structure is maintained within (short-distance within-pair communication) and between territories (long-distance male-male and extra-pair communication) depending on noise levels. Songs experienced more masking and fell below thresholds for detection and discrimination at shorter distances under noisier conditions. Decay of signal-to-noise ratios and cross-correlation factors in noise were so pronounced that song components used for both short- and long-distance communication did not transmit beyond average territory boundaries. Noise masking could affect species ecology: if signals are not

detected by intended receivers in noisier habitats, settlement, space use and social

interactions may be fundamentally altered compared to those in quieter environments.

Introduction

Animal signals have multiple functions (Catchpole and Slater 2008) and are shaped by selection to increase information transfer (Bradbury and Vehrencamp 1998). As signals travel through the environment from sender to receiver the frequency and temporal characteristics degrade and the signal attenuates, resulting in a loss of intensity or amplitude. Therefore, selection should favor signals that minimize decay, increasing the likelihood that the signal reaching receivers contains salient information. Signal transmission is influenced by the environment through which the signal travels (Morton 1975, Marten and Marler 1977, Derryberry 2009). Accordingly, signal structures are in part shaped by the environment a species inhabits; species living in open areas often produce trills, which resist degradation due to atmospheric turbulence, whereas species inhabiting forest utter lower frequency tonal calls, which withstand absorption and scattering caused by vegetation (Morton 1975; Marten and Marler 1977).

Anthropogenic noise changes the acoustic environment in which signals have evolved, which may alter transmission patterns through masking. Frequency masking occurs when acoustic energy from noise overlaps a signal, reducing the signal receiver's ability to detect and discriminate signals from noise (Patricelli and Blickley 2006). Signal masking due to anthropogenic noise reduces signal active space (Lohr et al. 2003, Nemeth and Brumm 2010, Parris and McCarthy 2013, LaZerte et al. 2015)and may constrain acoustic communication if receivers do not detect a signal (Slabbekoorn and Ripmeester 2008). As noise increases in an environment, the active space of a signal decreases, increasing degradation and making it both less likely that signals

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reach intended receivers and that the signal content is accurately conveyed (Lohr et al. 2003).

How noise affects active space can be inferred by assessing patterns of degradation and attenuation of signals with increasing distance from the sender (Mockford et al. 2011, Kight et al. 2012, LaZerte et al. 2015). As sound travels through space, it spreads, resulting in 6 dB loss of energy with every doubling of distance (Wiley and Richards 1978), and degrades resulting in a loss of signal structure with increasing distance. Whether information contained within a signal is decoded occurs only if the signal is detected (i.e. heard) and discriminated (distinguished from other sounds) by receivers. For birds, a receiver can generally detect a signal if its amplitude is greater than 3 dB above ambient background noise (Brenowitz 1982a, Dooling 1982) and discriminate if greater than 2-7 dB above detection thresholds (Dooling 1982; Lohr et al. 2003). In noisy environments, birds that experience high levels of signal masking and decreased signal active space may need to change their vocalization behaviours, for example increasing song amplitude to improve signal-to-noise ratios (Brumm 2004, Dooling and Blumenrath 2013, Derryberry et al. 2017) or song frequency to reduce masking (Slabbekoorn and Peet 2003, Wood and Yezerinac 2006, Mockford and Marshall 2009). Birds breeding in noisy areas may also adjust species-specific spacing patterns and social interactions in order for signals to be detected, which could lead to higher breeding densities over smaller areas in noisy habitats (Nemeth and Brumm 2009).

Signals used for long compared to short-distance communication differ in structure and transmission properties (Dabelsteen et al. 1993, Balsby et al. 2003, Nemeth and Brumm 2010, Rek 2013, Vargas-Castro et al. 2017). Songbirds broadcast long-distance signals for mate attraction and territory defense over wide areas, with signals structured to be detectable beyond the caller's own territory boundary (Brenowitz 1982a, Naguib et al. 2008, Barker et al. 2009). To increase transmission distance, broadcast songs are often given at low frequencies that attenuate slower than high frequencies (Morton 1975; Marten and Marler 1977; Wiley and Richards 1978). Signals are given at an amplitude high enough relative to environmental noise to minimize masking (Bradbury and Vehrencamp 1998). By contrast, signals used for short-distance communication often contain high frequency and broad bandwidth notes, which degrade faster (Dabelsteen et al. 1993; Balsby et al. 2003; Naguib et al. 2008; Rek 2013; Vargas-Castro et al. 2017). This results in a smaller active space, which is advantageous for within-pair communication because the likelihood of eavesdropping by conspecific rivals (McGregor 1993) or predators (Dabelsteen et al. 1998) decreases as well.

Some animals have components of both long and short-distance transmission properties within the same vocalization and selection may favor the use of particular notes based on transmission characteristics (Brenowitz 1982b, Balsby et al. 2003). For example, male túngara frogs (*Physalaemus pustulosus*) add a low frequency chuck note preferred by females to their signals (Ryan 1985). However, males are constrained by predation risk, as chuck notes are highly localizable by both predators and females;

therefore, males modulate their use of chuck notes based on predation risk (Ryan 1985). Male Red-winged blackbirds (*Agelaius phoeniceus*) give introductory notes for short-distance communication and individual recognition, followed by a lower frequency trill portion for long-distance signaling and male-male aggressive interactions (Brenowitz 1982b). Similarly, house wrens (*Troglodytes aedon*) have complex vocal repertoires (Rendall and Kaluthota 2013) and their song is composed of two structurally distinct components: an introduction section, which appears to be used during within-pair interactions in which short-distance signaling would be advantageous, followed by a terminal section that transmits beyond a male's territory (Tove 1988) and functions for both primary and extra-pair mate attraction and territory defense (Johnson and Kermott 1991).

We tested whether ambient noise influences degradation and attenuation of male house wren song and song sections at four natural areas that varied in levels of anthropogenic noise. We conducted transmission experiments during which we broadcast male house wren song from a loudspeaker and re-recorded it at four ecologically relevant distances. That is, we placed this experiment in an ecological context by considering patterns of degradation and attenuation in relation to species-typical patterns of spacing. We investigated the extent to which song structure is maintained both within territories (for short-distance within-pair communication) and amongst territories (for longer distance male-male and extra-pair communication) in different noise environments.

Characteristics of the two sections of house wren songs differ, such that the song parts should transmit differently. The introduction section covers a broad frequency range with many notes containing high frequency components (Rendall and Kaluthota 2013), unlike the terminal section. The introduction will likely undergo greater frequency-dependent attenuation with increasing distance (Morton 1975; Marten and Marler 1977; Wiley and Richards 1978). By contrast, the terminal section, composed of frequency modulated notes (Platt and Ficken 1987), should transmit better over greater distances. Further, how each section transmits could have implications for communication in noisy environments such that we might expect to see selection against song portions that do not transmit effectively in noise compared to those that do. Over time, notes or song portions that do not transmit effectively may be lost from vocal repertoires of species living in noisy compared to quiet habitats.

Methods

Study sites

We conducted transmission experiments at four natural areas (Table 4.1) in southwest Michigan, USA (42.290 N, 85.586 W), from April 9 – 17, 2017, one to two weeks before males arrived at their breeding grounds and prior to leaf out. The timing of experiments was a compromise; we avoided broadcasting songs when males had returned to breeding sites and were setting up territories (during which time our playbacks would likely elicit song from nearby breeding males resulting in overlapped songs in our recordings), and avoided periods of high insect sounds that overlap the

playback signal post breeding season. All sites consisted of mixed forest and open

grassland and each recording location is part of ongoing long-term research monitoring

song and breeding success of house wrens in nest boxes across an urban noise

gradient (Grabarczyk et al. 2018); Stuart et al. in press; authors unpublished data). Land

use surrounding sites varied from urban to rural, and each of our transects differed in

their proximity to roads and highways, both of which are major sources of anthropogenic

noise (Gill et al. 2017).

Playback recordings

We created playbacks by selecting songs from 30 breeding males recorded in

southwest Michigan in 2015 and 2016. We recorded males during dawn chorus with

Wildlife Acoustics Sound Meter 2 units (Maynard, MA, 44.1 kHz, 16-bit sample rate,

.wav format) by attaching a microphone directly to the focal male's nest box. Prior

research on house wren vocalizations has failed to record the introduction section, as

researchers recorded songs with hand-held shotgun microphones as far as 10-30 m

(Johnson and Kermott 1991; Rendall and Kaluthola 2013). Our recording set-up

enabled us to obtain high-fidelity recordings of male song, including the introduction

section, with males singing closer than 1 m to the microphone. For playbacks, songs

were used if both introduction and terminal sections were clearly visible and not

degraded in the spectrogram window, not overlapped by other sounds, and represented

a unique song type (Figure 4.1). Song types were defined based on the order and

composition of introductory and terminal notes (E. Grabarczyk, in prep). We filtered

songs selected for playbacks (bandpass: 1.2 – 11 kHz) in Avisoft SASLab Pro v5.1 (R. Specht, Berlin, Germany) in the waveform window to remove high- and low-frequency noise outside the range of frequencies in house wren songs. We adjusted song amplitude (rms) in Audacity v. 2.1.2 with the amplify function, setting new peak amplitude to 0.0 dB and did not select the option to allow for clipping. In Avisoft, we checked song amplitudes (rms) to ensure all songs had similar ($\pm$ 1 SD) amplitudes. For playbacks, each song was repeated five times with a 2-sec interval between individual songs and a 5-sec interval between song types. We randomly determined song order to create four unique playback loops, one for each experiment location. Thus, at each of the four sites, the same 30 songs were broadcast five times.

Sound transmission experiment

We re-recorded song playbacks at five distances along a horizontal transect selected in relation to typical house wren spacing patterns within and amongst territories. Males in southwest Michigan nest no closer than 25 m to their nearest neighbor, more often 40 – 100 m apart (EEG, unpublished data). To explore signal transmission patterns for short-distance within-pair communication, we placed microphones at 12.5 m and 25 m from the speaker to document transmission; these distances correspond to positions within an average male's territory and at its edge, respectively. To capture how noise affects long-distance signals used for male-male and extra-pair communication we placed microphones at 50 m and 100 m, representing a neighboring male's territory and the opposite edge of a neighboring male's territory,

respectively. For each transect, we placed an additional microphone 1 m from the speaker and in line with the rest of the transect; recordings at this microphone acted as reference sounds for analysis.

We broadcast song playback from a SME-AFS amplified speaker (Saul Mineroff Electronics, New York) mounted on a step ladder approximately 2.5 m above the ground. Before each experiment, we adjusted speaker volume to play songs at 76 dBA measured at 1 m with a SPL meter, fast averaging (American Recorder Technology SPL-8810). We determined approximate song peak amplitudes by measuring singing males at their nest boxes with a SPL meter from known distances (E. Grabarczyk, unpublished data). We used high-amplitude horn to signal the start of each experiment, which later enabled us to synchronize onset of experiments in recordings for analysis. During each experiment, song playbacks were simultaneously re-recorded on three SM2 units (44.1 kHz, 16-bit sample rate, .wav format) with their microphones (two microphone ports per unit) positioned in a linear transect relative to the playback speaker. We attached microphones to the tops of plastic poles 2 m above the ground using 10 – 50 m cables. At this height, we anticipated little to no effect of ground absorption (Marten and Marler 1977, Holland et al. 1998). All transects were positioned in open habitat (i.e. no trees or shrubs between microphones that might impact transmission) on flat ground with low (<1 m) vegetation parallel to the nearest roadway or highway. Experiments took place on days when wind speeds were low.

Acoustic analysis

We analyzed recordings in Avisoft SASLab Pro by labeling every whole song, introduction, and terminal section in each reference recording. Labels were exported from the waveform window and saved in text file format. We time synced recordings from each distance of the transect (12.5, 25, 50 and 100 m) to 1-m reference signals recorded at each site by identifying the horn blast in each and then imported text labels into the remaining recordings. We scanned each recording and excluded songs overlapped by ambient sounds from analysis.

To determine the frequency range of house wren songs used for playback, we measured minimum and maximum frequency (kHz) of songs re-recorded at 1 m at our quietest site using the automated parameter window (Flat top window, 512 FFT length, 93.75% overlap, −20 dB threshold, 0.725 ms time resolution). Song frequency ranged between 1.2 – 7.6 kHz, and we used this range to set our bandpass filter, which we applied in the waveform window to recordings from the transmission experiments before analysis.

As a measure of overall degradation, we determined amplitude cross-correlation factor for each song and song section between the reference sound (defined as songs recorded at the 1 m reference microphone at each site) and transmitted songs (defined as those recorded at the remaining four distances). Amplitude cross-correlation measures degradation based on comparison of the amplitude envelope of the test signal to that of the reference signal. This is similar to the blur ratio (Dabelsteen et al. 1993), but is a measure of overall similarity rather than the difference (Apol et al. 2017).

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In Avisoft, we saved each labeled song re-recorded at each distance and site as individually numbered .wav files (0.0 s margin) with the Avisoft Label settings function. We used .wav files of reference sounds recorded at 1 m as templates for cross-correlation. We used the Avisoft Correlator tool to run amplitude cross-correlation on each song type against the song model template. This tool generated a cross-correlation coefficient for each song, with values ranging from 1 (no degradation) to 0 (fully degraded).

We extracted song amplitude (rms, dB) and ambient sound pressure levels (SPLs, rms, dB) from each recording. We calibrated each SM2 unit and respective microphone pairs with a Larson Davis CAL 200 sound level calibrator (Depew, NY) by recording a 1-kHz 94 dB tone. We set the recording relative amplitude to 0 dB (re 20 µPa) based on the calibration tone in Avisoft using the Calibration function. Amplitude for whole song, introduction, and terminal section was extracted from the waveform window (bandpass filter = 1.2 – 7.6 kHz) for each song at each distance. To measure ambient SPLs during each experiment, we extracted and log averaged two 1-sec samples of recording for each song type at each recording distance. Samples were taken 1 sec before and after each five-song sequence. We were interested in noise that overlapped male song, or masking SPLs, thus we extracted SPLs in the same frequency range as house wren song (1.2 – 7.6 kHz); thus, our calculations of ambient SPLs exclude low-frequency sounds, which contribute significantly to full spectrum (0 – 22 kHz) SPL measurements (Gill et al. 2017). We calculated the signal-to-noise ratio

(SNR), which reflects the amplitude of the song above ambient noise, by subtracting ambient SPL from song amplitude.

Statistical analysis

Variation in ambient SPL with distance and by site. Statistical analyses were completed in R program software v3.3.3 (R Core Development Team 2017). We used a generalized linear model to compare average ambient masking SPLs (1.2 – 7.6 kHz) measured at each microphone along the transect at each site taken before and after each song type. For our model, we included microphone distance and site as explanatory variables and masking SPLs as the response variable. The residual plots of the fitted model showed a bimodal distribution, which corresponded with whether sites were on average quieter or noisier. Therefore, we centered the data by taking the difference between each SPL measure (rms, dB) and the mean SPL value (rms, dB) for all measures and refit the model.

Effects of noise on song attenuation and degradation. To test the hypothesis that ambient noise affects transmission of male song and song sections, we calculated log averages of signal-to-noise using the R package seewave (Sueur et al. 2008) and mean amplitude cross-correlation factors for each whole song and song section by distance at each location tested. For both measures, we separately fit linear mixed effects models with the package lme4 (Bates et al. 2015) to analyze transmission of whole song and song section traits; for hypothesis testing, we approximated p-values with the package

lmerTest (Kuznetsova et al. 2017) using a Satterthwaite approximation. For analysis of whole song signal-to-noise ratio, we included site (4 levels), distance (5 levels), and the interaction between site and distance as fixed effects and song type (30 unique types) as a random effect. Initial data exploration showed a curvilinear relationship between signal-to-noise ratio and cross-correlation with distance, therefore for all models we included a quadratic polynomial term for distance, which improved model fit based on residual plots and the corrected Akaike information criterion (AIC_c) model selection. We tested for inclusion of interaction terms and selected final models as best fit if ΔAIC_c was less than 2 (Burnham and Anderson 2002). For models comparing transmission of introduction and terminal sections, we included an additional fixed effect for section type (2 levels, introduction or terminal). In the song section model, we tested two-way interactions between site and distance, site and section, and distance and section, as well as a three-way interaction between site, distance, and song section.

For amplitude cross-correlation factor, which we used as a measure of overall song degradation, we used a linear mixed effects model with site, distance, and two-way interaction between site and distance, and site as fixed effects and song type as a random effect. For the song section model comparing cross-correlation factors, we included song section as a fixed effect, and thus tested two-way interactions, as well as a three-way interaction between site, distance, and song section. We used fit values plotted against model residuals and normality plots to assess model adequacy. All figures were created with the package effects (Fox 2003) and ggplot2 (Wickham 2016).

Results

Variation in ambient SPL with distance and by site

Because anthropogenic noise varies over space and time (Gill et al. 2015) and could affect transmission, we tested whether ambient SPLs levels varied with distance and site during our experiments. Ambient noise that occurred in the same frequency range as house wren song (1.2 – 7.6 kHz; hereafter masking SPLs) varied with distance, but the estimate was small (Estimate $\pm$ SE: -0.006 $\pm$ 0.002, t = -2.5, p = 0.01). Singing birds may not be able to detect differences in noise fluctuations less than 3 dB (Brenowitz 1982a; Dooling 1982), and therefore differences in masking SPLs among microphones may not be biologically relevant. However, small differences in ambient SPLs could affect calculation of degradation and attenuation values. Thus, for calculation of signal-to-noise ratios, we used ambient SPLs taken at each distance within 1-sec of re-recorded songs. Comparing among sites, ambient SPLs of frequencies that mask male song fell into two categories: SPLs at two sites were on average quieter (mean $\pm$ SD: 33.6 $\pm$ 9.2 dB; site 1: Estimate $\pm$ SE: -4.46 $\pm$ 0.2, t = -21.1, p = <0.0001; site 2: Estimate $\pm$ SE: -1.04 $\pm$ 0.2, t = -4.0, p = <0.0001) and two sites were on average noisier (mean $\pm$ SD: 53.1 $\pm$ 2.9 dB; site 3: 0.17 $\pm$ 0.3, t = 0.7, p=0.5; site 4 (intercept): Estimate $\pm$ SE: 11.7 $\pm$ 0.2, t = 68.0, p = <0.0001; Table 4.1).

Effects of noise on song attenuation and degradation

We tested predictions that transmission, measured by signal-to-noise ratio and cross-correlation factors, would differ across sites for whole song (i.e. a site x distance

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interaction) and for song sections (i.e. song section x site x distance interaction). Based on AIC_c selection, full models with the predicted interactions best fit the data describing whole song and song section transmission patterns for signal-to-noise ratio and cross-correlation factors (Table 4.2). Signal-to-noise ratios of whole songs showed a significant site x linear distance interaction at site 1 (Estimate ± SE: -192.7 ± 12.1, t = 16.0, p < 0.0001) and site 2 (Estimate ± SE: -221.3 ± 11.9, t = -18.7, p = <0.0001), but not site 3 (Estimate ± SE: -16.6 ± 14.6, t = -1.1, p = 0.3) compared to site 4 (intercept), suggesting signal-to-noise ratios decayed more slowly at quiet sites (1 and 2) compared with noisy sites (3 and 4; Figure 4.1, Table 4.3). A significant interaction between quadratic distance and site occurred for site 1 only (Estimate ± SE: 31.0 ± 12.0, t = 2.6, p = 0.01), indicating that the overall shape of the curve is convex and the effect of distance on signal-to-noise ratios changed with increasing distance. The interaction between quadratic distance and site did not differ between site 2 (quiet) and site 4 (intercept) (Estimate ± SE: 20.3 ± 12.0, t = 1.7, p = 0.09). This means while overall steepness of the curve differs between these sites, the effect of distance on signal-to-noise ratios did not change over the curve for either site. In summary, over space, signal-to-noise ratios remained consistently higher in lower ambient noise compared to sites with high ambient noise levels (Figure 4.2). At the two noisy sites, signals recorded at 25 m experienced considerable masking and fell below the detection threshold (3dB), which indicates ambient SPLs were either similar to signal amplitude or signals were lower than ambient SPLs but were masked and presumably could not be detected by another bird (Figure 4.2).

We predicted the effects of noise masking on transmission patterns between two song sections with distinct frequency structure would differ in decay with distance. For signal-to-noise ratios of song sections, we found a significant three-way interaction between song section x site x quadratic distance at site 1 (Estimate ± SE: -64.1 ± 24.8, t = -2.6, p = 0.01) and site 2 (Estimate ± SE: -81.2 ± 24.8, t = -3.2, p = 0.001), but not site 3 (Estimate ± SE: -7.7 ± 24.8, t = -0.3, p = 0.7) compared to site 4 (intercept). This suggests the effect of distance on signal-to-noise ratio differs between song sections and changes with increasing distance, and that decay with distance is slower at quiet sites (1 and 2) compared with noisy sites (3 and 4; Figure 4.4 A-D, Table 4.4). Across sites signal-to-noise ratios were lower for the introduction compared to terminal sections and decayed more quickly with distance at sites with higher ambient noise (Figure 4.4 A-D, Table 4.4).

We predicted noise masking could affect signal degradation due to change in the amplitude structure, and that this pattern of degradation would increase with distance. For songs recorded at 1m, cross-correlation values were approximately 1, indicating little or no degradation, but cross-correlation values decayed towards 0 with increasing distance (Figure 4.3). Cross-correlation factors of whole songs showed both a significant site x linear distance interaction at site 1 (Estimate ± SE: 15.3 ± 0.3, t = 54.2, p = <0.0001), site 2 (Estimate ± SE: 9.3 ± 0.3, t = 35.0, p = <0.0001), and site 3 (Estimate ± SE:-5.1 ± 0.3, t = -15.3, p = <0.0001), as well as a significant site x quadratic interaction for site 1 (Estimate ± SE: -4.3 ± 0.3, t = -15.7, p = <0.0001), site 2 (Estimate ± SE: -6.4 ± 0.3, t = -23.8, p = <0.0001), and site 3 (Estimate ± SE: -4.6 ±

0.3, t = -14.0, p = <0.0001) compared to site 4 (intercept). This means the overall

steepness of the curves differed between sites and the effect of distance on cross-

correlation factors changed with increasing distance. At sites with higher ambient noise,

songs degraded faster and showed overall more degradation at each distance (Figure

4.3, Table 4.3). This pattern changed with distance, as signals at quiet sites retained

their structure over distance compared with noisy sites and then degraded more at the

farther distances.

Due to the dissimilarities in structure, we predicted noise masking would affect

song section degradation patterns differently. Cross-correlation of song sections

showed a significant three-way interaction between site x song section x linear distance

at site 1 (Estimate ± SE: 3.8 ± 0.4, t = 8.7, p = <0.0001), site 2 (Estimate ± SE: 2.0 ±

0.4, t = 4.4, p = <0.0001), but not site 3 (Estimate ± SE: -0.6 ± 0.4, t = -1.4, p = 0.2), as

well as the three-way interaction between site x song section x quadratic distance for

site 1 (Estimate ± SE: 1.8 ± 0.4, t = 4.1, p = <0.0001), site 2 (Estimate ± SE: 1.3 ± 0.4, t

= 2.9, p = 0.004), but not site 3 (Estimate ± SE: -0.5 ± 0.4, t = -1.2, p = 0.2) compared to

site 4 (intercept). Following whole song cross-correlation patterns, the overall steepness

of song section curves differed between sites with distance, and the effect of distance

on cross-correlation changed with increasing distance. Further, degradation patterns

were different between song sections, as introduction sections degraded more quickly

than terminal sections and this pattern was more pronounced at noisy sites. With

increasing distance, eventually all songs degraded but decay started at closer distances

to the source at noisy sites.

Discussion

We tested whether ambient noise influenced the transmission of house wren song within and amongst typical male territory spacing at sites with varying levels of anthropogenic noise. At high ambient noise levels, songs experienced considerably more masking and fell below thresholds for detection and discrimination at shorter distances. Our results show that the introduction section, structured similarly to songs used for short distance communication in other bird species (Dabelsteen et al. 1993; Balsby et al. 2003; Naguib et al. 2008; Rek 2013; Vargas-Castro et al. 2017), attenuated and degraded faster resulting in a smaller active space compared to the terminal section. Degradation patterns were so pronounced under high noise that song components used for short-distance within-pair communication but also those for long-distance communication for mate attraction and territory defense did not transmit beyond the boundaries of an average territory.

Noise masking could affect bird species' ecology if space-use patterns change due to altered transmission and reduced signal active space. Signal-to-noise ratios were significantly lower and degraded faster for both whole song and song sections at sites with higher noise compared to those with lower noise levels, resulting in a smaller active space within and amongst a typical male territory (Figure 4.2). At our noisy sites, the terminal section of song, which is used as a long-distance signal (Johnson and Kermott 1991; Rendall and Kaluthota 2013), attenuated and began to degrade within the area of a typical male's territory. The rapid decay of signals could lead to decreases in the size of breeding territories with high ambient noise levels, as songs of territory holders may

not be detected by other males and non-territorial males could perceive an area is available for settlement (Nemeth and Brumm 2009). Alternatively, males may spend more time defending and broadcasting song along territory edges, and less time at the core of their territories leaving nest and mate unguarded. Further, males may be may be slower to detect conspecific intruders (Kleist et al. 2016) or may alter their responses (Phillips and Derryberry 2018) if noise interferes with territory defense. As vegetation develops on male territories, sound attenuation patterns will change as a result of absorption and scattering, decreasing signal active space of male songs and dampening ambient noise with increasing distance at the source. Males may be more constrained by noise early in the breeding season when establishing their territories, but less so during the summer when advertising for extra-pair fertilizations or secondary mates.

Vocalizations produced at lower amplitudes, such soft songs (Rek 2013; Vargas-Castro et al. 2017), or those structured with broadband frequencies, such as introductory sections (Brenowitz 1982b), transmit shorter distances than long-distance signals; however, these signals are also subject to noise masking. We found the introduction section of house wren song had a smaller transmission range and degraded faster compared to the terminal section. Transmission distance of the introduction was further reduced at sites with higher ambient noise (Figure 4.4). Male house wrens respond more aggressively towards introduction than terminal sections (Tove 1988), possibly because the introduction does not typically transmit beyond a male's territory boundary and could suggest to the focal male his territory is being

invaded. Thus, because introduction sections transmit typically with little degradation within a male's own territory but not beyond, this section may primarily function for within-pair communication, similarly to whisper or soft songs of other species (Dabelsteen et al. 1993; Balsby et al. 2003; Vargas-Castro et al. 2017). The functional significance of introduction note composition is unknown, as this section is difficult to record with high fidelity (Rendall and Kaluthota 2013). However, whether introduction composition or complexity functions in individual recognition, courtship behaviour, or mate choice decisions in house wrens warrants further study, particularly because noise limits transmission of introductory notes, which could have fitness impacts if within-pair communication fails.

As urban areas continue to grow and noise masking becomes a more prominent and pervasive disturbance in natural areas, selection should favor males that structure their song to increase transmission distances. In noise, males may benefit by adjusting their songs to increase their signal-to-noise ratio by altering their song amplitude (Brumm 2004; Nemeth and Brumm 2010) or minimize masking by increasing signal frequency (Slabbekoorn and Peet 2003; Wood and Yezeriniac 2006; Mockford and Marshall 2009). Prior to clutch initiation, paired male house wrens increase song peak frequency whereas unpaired males do not (Grabarczyk et al. 2018), possibly because unpaired males may be constrained by female preference for low frequency song. However, if low frequency songs are not detected by prospective females, males may fail to attract or will be delayed in attracting breeding partners (Habib et al. 2007, Gross et al. 2010). Over time, we might expect to see selection against song portions that do

not transmit effectively, leading to the loss of note or syllable types in noise, or the use of high frequency or high amplitude song components that transmit effectively under noisy conditions.

High levels of anthropogenic noise significantly increased masking and accelerated degradation of songs of male house wrens. By investigating transmission patterns within and amongst typical male territory spacing, we show song components used for short- as well as long-distance communication may not be detected beyond typical territory boundaries under high ambient noise. To be detected by prospecting females and non-territorial males, males may need to increase the amplitude of their songs, which has been documented in nightingales (*Luscinia megarhynchos*, Brumm 2004) and white-crowned sparrows (*Zonotrichia leucophrys*, Derryberry et al. 2017), and which may drive frequency changes across populations and species (Nemeth and Brumm 2010). Additional work exploring how noise influences signal receiver behaviour, settlement decisions, and reproductive success will be critical for understanding detrimental effects of reduced signal active space for this and other urban dwelling species.

Literature cited

Apol, C. A., C. B. Sturdy, and D. S. Proppe. 2017. Seasonal variability in habitat structure may have shaped acoustic signals and repertories in the black-capped and boreal chickadees. Evolutionary Ecology **32**:57-74.

Balsby, T. J. S., T. Dabelsteen, and S. B. Pedersen. 2003. Degradation of whitethroat vocalisations: Implications for song flight and communication network activities. Behaviour **140**:695-719.

Barker, N. K. S., T. Dabelsteen, and D. J. Mennill. 2009. Degradation of Male and Female Rufous-and-White Wren Songs in a Tropical Forest: Effects of Sex, Perch Height, and Habitat. Behaviour **146**:1093-1122.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. . Journal of Statistical Software **67**:1-48.

Bradbury, J. W., and S. L. Vehrencamp. 1998. Principles of Animal Communication. 1 edition. Sinauer Associates, Sunderland, MA.

Brenowitz, E. A. 1982a. The active space of Red-winged blackbird song. Journal of Comparative Physiology **147**:511-522.

Brenowitz, E. A. 1982b. Long-range communication of species identity by song in the Red-winged blackbird. Behavioral Ecology and Sociobiology **10**:29-38.

Brumm, H. 2004. The impact of environmental noise on song amplitude in a territorial bird. Journal of Animal Ecology **73**:434-440.

Burnham, K. P., and D. R. Anderson. 2002. Model Selection and Multimodel Inference. A Practical Information-Theoretic Approach. Springer Science & Business Media.

Catchpole, C. K., and P. J. B. Slater. 2008. Bird song: Biological themes and variations. Cambridge University Press, New York.

Dabelsteen, T., O. N. Larsen, and S. B. Pedersen. 1993. Habitat-induced degradation of sound signals: Quantifying the effects of communication sounds and bird location on blur ratio, excess attenuation, and signal-to-noise ratio in blackbird song. Journal of the Acoustical Society of America **93**:2206-2220.

Dabelsteen, T., P. K. McGregor, H. M. Lampe, N. E. Langmore, and H. J. 1998. Quiet song in song birds: an overlooked phenomenon. Bioacoustics **2**:98-105.

Derryberry, E. P. 2009. Ecology Shapes Birdsong Evolution: Variation in Morphology and Habitat Explains Variation in White-Crowned Sparrow Song. American Naturalist **174**:24-33.

Derryberry, E. P., K. Gentry, G. E. Derryberry, J. N. Phillips, R. M. Danner, J. E. Danner, and D. A. Luther. 2017. White-crowned sparrow males show immediate flexibility in song amplitude but not in song minimum frequency in response to changes in noise levels in the field. Ecology and Evolution **7**:4991-5001.

Dooling, R. J. 1982. Auditory perception in birds. Acoustic communication in birds **1**:95–130.

Dooling, R. J., and S. H. Blumenrath. 2013. Avian sound perception in noise. Springer, Berlin Heidelberg.

Fox, H. 2003. Effect Displays in R for Generalised Linear Models. Journal of Statistical Software **32**:1-24.

Gill, S. A., E. E. Grabarczyk, K. M. Baker, K. Naghshineh, and M. J. Vonhof. 2017. Decomposing an urban soundscape to reveal patterns and drivers of variation in anthropogenic noise. Science of the Total Environment **599**:1191-1201.

Gill, S. A., J. R. Job, K. Myers, K. Naghshineh, and M. J. Vonhof. 2015. Toward a broader characterization of anthropogenic noise and its effects on wildlife. Behavioral Ecology **26**:328-333.

Grabarczyk, E. E., M. A. Pipkin, M. J. Vonhof, and S. A. Gill. 2018. When to change your tune? Unpaired and paired male house wrens respond differently to anthropogenic noise. Journal of Ecoacoustics **2**:#LHGRVC.

Gross, K., G. Pasinelli, and H. P. Kunc. 2010. Behavioral Plasticity Allows Short-Term Adjustment to a Novel Environment. American Naturalist **176**:456-464.

Habib, L., E. M. Bayne, and S. Boutin. 2007. Chronic industrial noise affects pairing success and age structure of ovenbirds *Seiurus aurocapilla*. Journal of Applied Ecology **44**:176-184.

Holland, J., T. Dabelsteen, S. B. Pedersen, and O. N. Larsen. 1998. Degradation of wren *Troglodytes troglodytes* song: implications for information transfer and ranging. Journal of the Acoustical Society of America **103**:2154-2166.

Johnson, L. S., and L. H. Kermott. 1991. The Functions of Song in Male House Wrens (*Troglodytes aedon*). Behaviour **116**:190-209.

Kight, C. R., M. K. Hinders, and J. P. Swaddle. 2012. Acoustic space is affected by anthropogenic habitat features: implications for avian vocal communication. Ornithological Monographs **74**:47-62.

Kleist, N. J., R. P. Guralnick, A. Cruz, and C. D. Francis. 2016. Anthropogenic noise weakens territorial response to intruder's songs. Ecosphere **7**.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. Journal of Statistical Software **82**:1-26.

LaZerte, S. E., K. A. Otter, and H. Slabbekoorn. 2015. Relative effects of ambient noise and habitat openness on signal transfer for chickadee vocalizations in rural and urban green-spaces. Bioacoustics **24**:233-252.

Lohr, B., T. F. Wright, and R. J. Dooling. 2003. Detection and discrimination of natural calls in masking noise by birds: estimating the active space of a signal. Animal Behaviour **65**:763-777.

Marten, K., and P. Marler. 1977. Sound Transmission and Its Significane for Animal Vocalization: I. Temperate Habitats. Behavioral Ecology and Sociobiology **2**:271-290.

McGregor, P. K. 1993. Signalling in Territorial Systems: A Context for Individual Indentification, Ranging and Eavesdropping. Philosophical Transactions of the Royal Society of London Series B **340**:237-244.

Mockford, E. J., and R. C. Marshall. 2009. Effects of urban noise on song and response behaviour in great tits. Proceedings of the Royal Society B **276**:2979-2985.

Mockford, E. J., R. C. Marshall, and T. Dabelsteen. 2011. Degradation of Rural and Urban Great Tit Song: Testing Transmission Efficiency. Plos One **6**.

Morton, E. S. 1975. Ecological sources of selection on avian sounds. American Naturalist **109**:17-34.

Naguib, M., R. Schmidt, P. Sprau, T. Roth, C. Florcke, and V. Amrhein. 2008. The ecology of vocal signaling: male spacing and communication distance of different song traits in nightingales. Behavioral Ecology **19**:1034-1040.

Nemeth, E., and H. Brumm. 2009. Blackbirds sing higher-pitched songs in cities: adaptation to habitat acoustics or side-effect of urbanization? Animal Behaviour **78**:637-641.

Nemeth, E., and H. Brumm. 2010. Birds and Anthropogenic Noise: Are Urban Songs Adaptive? American Naturalist **176**:465-475.

Parris, K. M., and M. A. McCarthy. 2013. Predicting the Effect of Urban Noise on the Active Space of Avian Vocal Signals. American Naturalist **182**:452-464.

Patricelli, G. L., and J. L. Blickley. 2006. Avian communication in urban noise: Causes and consequences of vocal adjustment. Auk **123**:639-649.

Phillips, J. N., and E. P. Derryberry. 2018. Urban sparrows respond to a sexually selected trait with increased aggression in noise. Scientific Reports **8**.

Platt, M. E., and M. S. Ficken. 1987. Organization of singing in House wrens. Journal of Field Ornithology **58**:190-197.

Rek, P. 2013. Soft calls and broadcast calls in the corncrake as adaptations to short and long range communication. Behavioural Processes **99**:121-129.

Rendall, D., and C. D. Kaluthota. 2013. Song organization and variability in northern House wrens (*Troglodytes aedon parkmanii*) in Western Canada. Auk **130**:617-628.

Ryan, M. J. 1985. The túngara frog: a study in sexual selection and communication. University of Chicago Press, Chicago.

Slabbekoorn, H., and M. Peet. 2003. Ecology: Birds sing at a higher pitch in urban noise. Nature **424**:267-267.

Slabbekoorn, H., and E. A. P. Ripmeester. 2008. Birdsong and anthropogenic noise: implications and applications for conservation. Molecular Ecology **17**:72-83.

Sueur, J., T. Aubin, and C. Simonis. 2008. Seewave: a free modular tool for sound analysis and synthesis. Bioacoustics **18**.

Tove, M. H. 1988. Patterns of singing by house wrens with respect to the breeding cycle Utah State University, Logan, UT.

Vargas-Castro, L. E., L. Sandoval, and W. A. Searcy. 2017. Eavesdropping avoidance and sound propagation: the acoustic structure of soft song. Animal Behaviour **134**:113-121.

Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Wiley, R. H., and D. G. Richards. 1978. Physical constraints on acoustic communication in the atmosphere: implications for the evolution of animal vocalisations. Behavioral Ecology and Sociobiology **3**:69-94.

Wood, W. E., and S. M. Yezerinac. 2006. Song sparrow (*Melospiza melodia*) song varies with urban noise. Auk **123**:650-659.

Table 4.1 Ambient masking SPL levels, or frequencies that overlap with male house wren song varied amongst sites. Sites differed in their proximity to roads and highways. To estimate distance to nearest road and highway, we used ArcGIS 10.5 software (ESRI Redlands, California, USA). We collected GPS locations ($\pm$ 3 m accuracy) for each site using a Garmin handheld GPS unit (GPSmap 60CSx). Michigan country, road, and highway frameworks were downloaded from the Michigan Center for Geographic Information (michigan.gov/cgi).

	Mean ± SD masking SPL (dB)	Distance to nearest roadway (m)	Distance to nearest highway (m)
Site 1[1]	35.5 ± 8.7	207	1,816
Site 2[2]	31.0 ± 3.4	52	4,801
Site 3[3]	52.8 ± 1.7	12	186
Site 4[4]	53.3 ± 3.2	56	382

[1] Site 1 (Chipman Preserve, 42.305° N, -85.458° W).
[2] Site 2 (Hidden Pond Preserve, 42.592° N, -85.413° W).
[3] Site 3 (Western Michigan University Parkview campus, 42.252° N, -85.641° W).
[4] Intercept/Site 4 (Asylum Lake Preserve, 42.264° N, -85.648° W).

Table 4.2 Based on AIC*c* comparisons full models including predicted interactions best fit data describing whole song and song section patterns for evaluating signal-to-noise ratios (SNR) and amplitude cross-correlation factors (CCF).

	Candidate models	AIC*c*	ΔAIC$_c$	weight
Whole song SNR	Full model	5865.69	0.00	1
	Next competing[1]	6303.06	437.37	0
	Null	8156.55	2290.86	0
Song section SNR	Full model	6872.12	0.00	1
	Next competing[2]	6926.88	54.70	0
	Null	9624.52	2752.34	0
Whole song CCF	Full model	-1334.61	0.00	1
	Next competing[3]	-854.64	479.96	0
	Null	364.02	1698.63	0
Song section CCF	Full model	-2551.21	0.00	1
	Next competing[4]	-2412.59	138.62	0
	Null	769.59	3320.80	0

[1] Next competing model dropped distance*site interaction.
[2] Next competing model dropped the 3-way interaction between distance*section*site.
[3] Next competing model dropped distance*site interaction.
[4] Next competing model dropped the 3-way interaction between distance*section*site.

Table 4.3 Results of linear mixed models comparing signal-to-noise ratio (SNR) and amplitude cross-correlation factor (CCF) at 4 nature reserves at 5 increasing distances.

Predictor	SNR			CCF		
	Estimate ± SE	t_{df}	p-value	Estimate ± SE	t_{df}	p-value
(Intercept)[1]	6.4 ± 0.3	23.8_{259}	<0.0001	0.47 ± 0.01	58.9_{87}	<0.0001
distance1	-175.2 ± 8.4	-20.9_{996}	<0.0001	-7.13 ± 0.1	-49.1_{553}	<0.0001
distance2	170.3 ± 8.4	20.2_{996}	<0.0001	3.4 ± 0.1	23.4_{553}	<0.0001
Site 1	13.4 ± 0.4	35.4_{996}	<0.0001	0.3 ± 0.01	41.3_{555}	<0.0001
Site 2	12.6 ± 0.4	33.4_{996}	<0.0001	0.1 ± 0.01	14.9_{555}	<0.0001
Site 3	0.1 ± 0.5	0.2_{996}	0.8	-0.03 ± 0.001	-3.8_{555}	0.0001
dist1*Site 1	-192.7 ± 12.1	-16.0_{996}	<0.0001	3.3 ± 0.2	16.1_{553}	<0.0001
dist2*Site 1	31.0 ± 12.0	2.6_{996}	0.01	-3.5 ± 0.2	-17.2_{553}	<0.0001
dist1*Site 2	-221.3 ± 11.9	-18.7_{996}	<0.0001	0.4 ± 0.2	2.1_{553}	0.04
dist2*Site 2	20.3 ± 12.0	1.7_{996}	0.09	-2.4 ± 0.2	-11.7_{553}	<0.0001
dist1*Site 3	-16.6 ± 14.6	-1.1_{996}	0.3	-0.1 ± 0.2	-0.3_{553}	0.8
dist2*Site 3	3.3 ± 14.6	0.2_{996}	0.8	-0.1 ± 0.2	-0.6_{553}	0.5

Table 4.4 Results of linear mixed models examining signal-to-noise ratio (SNR) and amplitude cross-correlation factors (CCF) at 4 nature reserves at 5 increasing distances for 2 male house wren song sections (introduction and terminal).

Predictor	SNR			CCF		
	Estimate ± SE	t_{df}	p-value	Estimate ± SE	t_{df}	p-value
(Intercept)	4.5 ± 0.3	12.4_{1176}	<0.0001	0.4 ± 0.008	50.2_{164}	<0.0001
distance1	-138.5 ± 12.4	-11.1_{1176}	<0.0001	-9.5 ± 0.2	$-43,1_{1147}$	<0.0001
distance2	147.2 ± 12.4	11.1_{1176}	<0.0001	5.8 ± 0.2	26.3_{1147}	<0.0001
Site 1	11.1 ± 0.5	22.0_{1176}	<0.0001	0.4 ± 0.01	40.4_{1147}	<0.0001
Site 2	9.7 ± 0.5	19.1_{1176}	<0.0001	0.2 ± 0.01	21.3_{1147}	<0.0001
Site 3	-0.2 ± 0.5	-0.4_{1176}	0.7	-0.03 ± 0.01	-3.2_{1147}	0.001
Section (terminal)	3.0 ± 0.5	6.0_{1176}	<0.0001	0.1 ± 0.01	14.6_{1147}	<0.0001
dist1*Site 1	-227.8 ± 17.5	-13.0_{1176}	<0.0001	2.0 ± 0.3	6.5_{1147}	<0.0001
dist2*Site 1	75.5 ± 17.5	4.3_{1176}	<0.0001	-5.8 ± 0.3	-18.5_{1147}	<0.0001
dist1*Site 2	-223.3 ± 17.5	-12.8_{1176}	<0.0001	-0.7 ± 0.4	-2.3_{1147}	0.02
dist2*Site 2	74.8 ± 17.5	4.2_{1176}	<0.0001	-4.3 ± 0.3	-13.8_{1147}	<0.0001
dist1*Site 3	-11.0 ± 17.5	-0.6_{1176}	0.5	0.3 ± 0.3	1.0_{1147}	0.3
dist2*Site 3	5.1 ± 17.5	0.3_{1176}	0.7	0.1 ± 0.3	0.3_{1147}	0.8
dist1*section	-82.6 ± 17.5	-4.7_{1176}	<0.0001	-0.8 ± 0.3	-2.7_{1147}	0.007
dist2*section	55.5 ± 17.5	3.1_{1176}	0.0002	-1.9 ± 0.3	6.0_{1147}	<0.0001

Site 1*section	3.0 ± 0.7	4.2_{1176}	<0.0001	0.05 ± 0.01	-3.8_{1147}	0.0001
Site 2*section	3.8 ± 0.7	5.3_{1176}	<0.0001	0.09 ± 0.01	-6.7_{1147}	<0.0001
Site 3*section	0.2 ± 0.7	0.3_{1176}	0.7	0.003 ± 0.01	-0.3_{1147}	0.8
dist1*Site 1*section	28.7 ± 24.8	1.2_{1176}	0.2	3.8 ± 0.4	8.7_{1147}	<0.0001
dist2*Site 1*section	-64.1 ± 24.8	-2.6_{1176}	0.01	1.8 ± 0.4	4.1_{1147}	<0.0001
dist1*Site 2*section	-12.9 ± 24.8	-0.5_{1176}	0.6	2.0 ± 0.4	4.4_{1147}	<0.0001
dist2*Site 2*section	-81.2 ± 24.8	-3.2_{1176}	0.001	1.3 ± 0.4	2.9_{1147}	0.004
dist1*Site 3*section	-2.7 ± 24.8	-0.1_{1176}	0.9	-0.6 ± 0.4	-1.4_{1147}	0.2
dist2*Site 3*section	-7.7 ± 24.8	-0.3_{1176}	0.7	-0.5 ± 0.4	-1.2_{1147}	0.2

Figure 4.1 Spectrogram image of male house wren song. Typical songs contain two structurally distinct sections: the introduction composed of broad bandwidth frequency notes which degrade within a male's territory followed by the frequency modulated terminal section used for long-distance signaling (Johnson and Kermott 1991, Rendall and Kaluthota 2013).

Figure 4.2 In high noise, signals used for both short- and long-distance communication fell below detection thresholds within the typical boundaries of a male's territory. The dotted horizontal line represents an estimated discrimination threshold at 10 dB (Dooling 1982) and the solid horizonal line at 3 dB represents an estimated detection threshold (Brenowitz 1982). Vertical lines estimate the distance at which songs reach estimated detection and discrimination thresholds. Curves represent model estimates including a polynomial quadratic term for distance, generated using the effects package. As signal-to-noise ratios approach 0 dB, or no difference detected between signal and ambient background noise near 100 m, estimate curves increase slightly due to the polynomial term. Estimates less than 0 indicate ambient noise is greater than the signal itself.

Figure 4.3 Song cross-correlation factors values range from 1 (no degradation) at 1m and decay towards 0 (fully degraded) with distance. In noise signal degradation or loss of signal structure decayed faster with increasing distance. Lines were estimated using the effects package (model estimates) and show a significant site x linear distance and site x quadratic distance interactions.

Figure 4.4 Introduction and terminal sections differ in transmission patterns. Noise accelerates patterns of attenuation and degradation with distance on both song sections. A-D: Signals fell below estimated detection (dotted horizontal line) and discrimination thresholds (solid horizonal line). E-H: Introduction sections degrade faster than terminal sections, and noise significantly increases the amount of degradation on both sections.

CHAPTER V

ANTHROPOGENIC NOISE AFFECTS MALE HOUSE WREN RESPONSE TO BUT
NOT DETECTION OF TERRITORIAL INTRUDERS

Abstract

Anthropogenic noise decreases signal active space, or the area over which male

bird song can be detected in the environment. For territorial males, noise may make it

more difficult to detect and assess territorial challenges, which in turn may increase

defense costs and influence whether males maintain territory ownership. We tested the

hypothesis that noise affects the ability of male house wrens (*Troglodytes aedon*) to

detect intruders and alters responses to them near an active nest. We broadcast pre-

recorded male song and pink noise on territories to simulate intrusions with and without

noise, as well as to noise alone. We measured detection by how long males took to sing

or approach the speaker after the start of a playback. To measure whether playbacks

changed male behavior, we compared their vocal responses before and during

treatments, as well as compared mean vocal responses and the number of flyovers and

attacks on the speaker during treatments. Noise did not affect a male's ability to detect

an intruder on his territory. Males altered their responses to simulated intruders with and

without noise compared to the noise-only treatment by singing longer songs at faster

rates. Males increased peak frequency of songs during intrusions without noise

compared to noise-only treatments, but frequency during intruder plus noise treatments

did not differ from either. When confronting simulated intruders in noise, males

increased the number of attacks on the speaker compared to intruders without noise,

possibly because they were less able to assess intruders via songs and relied on close

encounters for information. Although noise did not affect intruder detection, noise

affected some aspects of singing and aggressive responses, which may be related to

the challenge of discriminating and assessing territorial threats under elevated noise.

Introduction

Selection on animal communication systems favors signalers that structure their signals to transmit with minimal degradation through the environment, as well as receivers with auditory systems capable of extracting relevant information from background noise (Endler 1992, Wiley 2013). To elicit a response, a signal must first be detected, or separated from other sounds that make up background noise. Detection, however, is less likely to occur when background sounds occupying similar frequencies overlap with or mask the signal (Klump 1996). Sources of masking may be biological (calls of birds, anurans, or insects), geophysical (wind, rain, or thunder), or anthropogenic (traffic, airplanes, or industrial generators) in origin. When background sounds are intermittent, animals may adjust the timing of their signals and vocalize during silent gaps (Brumm 2006, Egnor et al. 2007, Proppe and Finch 2017). But when sounds are continuous and masking is constant, the probability that receivers will detect a signal decreases. Anthropogenic noise sources produce sounds that are often continuous, high volume, and low frequency (Gill et al. 2015). Animals that vocalize at low frequencies experience more noise masking, which makes their signals harder to detect (Pohl et al. 2009, Pohl et al. 2012, LaZerte et al. 2017) and constrains communication (Brumm and Slabbekoorn 2005).

The consequences of noise masking on vocal communication are wide ranging. Predation may increase if noise minimizes detection of alarm calls by adults (Kern and Radford 2016, Templeton et al. 2016) or offspring (McIntyre et al. 2014). If noise masks advertisement signals, males may fail to attract a mate, leading to reproductive failure

(Bee and Swanson 2007). Even if males succeed in attracting mates, noise masking could be costly for birds when they are defending active nests. Threats within or at the territory core pose greater risks to resident males compared to intruders on or near territory edges, as resident males risk losing mates, eggs, or nestlings if they are unable to repel an intruder (Bradbury and Vehrencamp 1998). Accordingly, males respond more aggressively to simulated intruders near the core part of their territory compared to territory edges by approaching more closely and increasing the number of flights towards the intruder (Stoddard et al. 1991). Thus, if noise affects detection of and response to intruders around nests, males could suffer reproductive failure as well. However, little is known as to whether noise compromises intruder detection or response at active nests.

Whereas noise has the potential to lead to reproductive failure, the consequences of noise masking may be more subtle: noise could delay the detection of intruders and mask intruder signals thereby influencing male responses (Kleist et al. 2016, Zwart et al. 2016). In areas of chronic noise, territorial males respond more slowly to intruders: masking delays detection of an intruder's song (Kleist et al. 2016). Once detected, how males respond to intruders may influence whether he maintains ownership of his territory. In response to simulated intruders in quiet conditions, European robins (*Erithacus rubecula*) sing more low frequency notes, whereas in noisy conditions males eliminate low frequency notes (Zwart et al. 2015) and decrease song complexity (McMullen et al. 2014). Song changes by male robins in noisy conditions may be perceived as less aggressive (Zwart et al. 2015). If noise modifies how intruders

perceive territory holders, they may experience more intrusions and takeover attempts by other males.

We hypothesized that noise affects male responses to territorial intruders near their nests by decreasing the ability of males to detect intruders and by altering male responses to them. We tested this idea on male house wrens (*Troglodytes aedon*), a species in which neighbors sneak onto territories seeking extra-pair copulations (Johnson and Kermott 1989) and non-territorial birds challenge resident males for nest sites and mates (Johnson and Kermott 1990). During such challenges, intruders often sing, which triggers resident males to chase intruders within their territory (Johnson and Kermott 1990). Takeover attempts have been reported in 10 – 13 % of nesting attempts (Freed 1986, Johnson and Kermott 1990)and occur when males are mate limited (Freed 1986). Risk of territorial takeovers vary across the season; early takeover attempts are more successful than those later in the season (Johnson and Kermott 1990). Nearly half of all challenges are successful, following which new territory holders typically kill the prior male's offspring and re-initiate broods with the resident female (Johnson and Kermott 1990). During early breeding stages, males experience the added risk of paternity loss from intruders, whereas males during later stages risk loss of their entire reproductive investment. Accordingly, the responses of males to intruders may vary seasonally, and therefore we tested whether noise has similar effects on detection and responses to intruders near active nests early and late in the nesting cycle.

To test our hypothesis, we broadcast pre-recorded male song and pink noise, which mimics anthropogenic noise, on territories to simulate intrusions with and without

noise, as well as to noise alone. We tested color-banded males either prior to clutch

initiation (early) or during incubation (late) at primary active nests. During trials, we

recorded male vocal responses (song rate, duration, and peak frequency) and counted

the number of fly overs and attacks on the playback speaker. To determine whether

noise affected intruder detection, we measured from recordings the time elapsed from

start of playbacks to the first song produced as well as latency to approach within 2 m of

the speaker broadcasting song. To measure whether playbacks changed male

behavior, we compared their vocal responses before and during treatments. Finally, we

compared focal males' vocal and aggressive responses to determine whether male

responded differently to intruders depending on the presence of noise.

Methods

      Study sites and species

      We studied a color-banded population of house wrens breeding in nest boxes at

four urban to peri-urban natural areas in Kalamazoo County, Michigan, USA (42.290 N,

85.586 W). We arranged nest boxes (N = 108) in open habitat near forest edge, where

house wrens prefer to nest. We checked nest boxes every three days to monitor use

and breeding activity. We determined if a nest box was occupied by a male if sticks

were present and he was singing to attract a mate. We considered a male paired once

lining was added to the nest cup or we observed a female nest building. House wrens in

our population typically lay 5 – 7 eggs (mean ± SD: 5.9 ± 1.0; EEG, unpublished data);

therefore, we waited at least 1 day after clutches were completed to test males during

the incubation stage. We captured adult house wrens by mist net and banded them with a USGS aluminum band and 3 plastic bands for individual identification. We sexed individuals by cloacal protuberance (male) or presence of a brood patch (female), and confirmed sex by observing singing. Of the 45 males included in this study, 35 were banded; 28 before experiments and 7 captured (3 – 16 days) after experiments but within the same breeding attempt. During mist netting, we first attempted to capture males without playback. If we were unsuccessful, we broadcast male or female house wren song recorded from a different population. Songs played during capture attempts were different from those used as experimental playback stimuli.

Playback recordings

To create playbacks simulating a conspecific intruder, we selected songs recorded from 28 male house wrens during 2015 – 2016 in six natural areas in southwest Michigan. We recorded males during the dawn chorus using a Wildlife Acoustics Sound Meter 2 (SM2) recording unit (Maynard, MA, 44.1 kHz, 16-bit sample rate, .wav format), connecting the SM2 microphone to the unit with a 10-m cord and then attaching the microphone to the tops of nest boxes. Although house wrens have large repertoires with an unknown number of song types (Rendall and Kaluthota 2013), they sing with eventual variety (Kroodsma 1977, Rendall and Kaluthota 2013), repeating the same song type multiple times before gradually switching to a new type. Therefore, for song playback simulating a male intruder, we used a single song type, and repeated the same song every 15-sec for 10-min (approximately 4 songs/min), which compares

to natural singing rates of males prior to clutch initiation in our populations (mean $\pm$ SD: 3.47 $\pm$ 2.0 songs/min; N = 20 males recorded from 630-730 (EST)). We selected songs for playback if they represented a unique song type, had a high signal-to-noise ratio, and fell within the range of song averages for duration and peak frequency (male song prior to clutch initiation (N = 1,124 songs from 45 males); duration, mean $\pm$ SD: 2.2 $\pm$ 0.5 s; peak frequency, mean: $\pm$ SD: 3.9 $\pm$ 0.6 kHz, EEG, unpublished data; playback duration, mean $\pm$ SD: 2.2 $\pm$ 0.4 s; peak frequency, mean $\pm$ SD: 4.0 $\pm$ 0.8 kHz). Song types or exemplars were defined as unique combinations of introduction and terminal note types (EEG, unpublished data). For each playback, songs were filtered in the waveform window (bandpass 1.3 – 11 kHz) to remove high- and low-frequency sounds. We standardized song peak amplitudes with the amplify function (did not allow for clipping) in Audacity v2.1.2 in order for all songs to play at the same volume. During trials, we randomly selected a song playback until all 28 exemplars were played once before resampling from all possible playbacks (N = 45 trials). Individual focal males received the same song exemplar during treatments simulating an intruder with and without noise.

Noise playback experiments vary in the type of noise stimuli presented; some studies have broadcast pre-recorded traffic with peak amplitudes standardized across recordings (Bermudez-Cuamatzin et al. 2009, Gross et al. 2010, Verzijden et al. 2010), whereas others synthesize a constant noise signal (Hanna et al. 2011, Gentry et al. 2017, LaZerte et al. 2017, Grabarczyk et al. 2018). Using pre-recorded traffic noise introduces complexity into field experiments as the focal bird may respond to amplitude

peaks from passing cars as opposed to increased ambient SPLs (Shannon et al. 2016).

To create a noise playback that mimics continuous anthropogenic noise, we created a

pink noise signal, which like traffic has energy concentrated at 0 – 2 kHz that gradually

decays with increasing frequency. We synthesized a 10-min pink noise signal in Avisoft

SASLab Pro v 5.2 (R. Specht, Berlin, Germany; 44.1 kHz sample frequency, lowpass

1/f, frequency cut off at 0.20 Hz) and added 5-sec of fade in at the start of the signal to

minimize startling focal males at the onset of the playback. Prior to field experiments,

we determined the speaker volume necessary to play noise and male song at 76 dBA

measured with fast averaging at 1m with a SPL meter (American Recorder Technology

SPL-8810); we applied this setting to all males. To approximate song peak amplitudes

used for playback, we measured males while singing at their nest boxes with a SPL

meter from known distances (EEG, unpublished data). We selected an amplitude of 76

dBA for noise playbacks because noise played at higher amplitudes (> 80 dBA)

completely masks male songs, which prevents extraction and analysis of song traits, but

noise played below 76 dBA may not change the ambient noise environment

(Grabarczyk et al. 2018). As a reference point, at 24 recording points on an urban

campus with microphones positioned near roads and a major thoroughfare, the

logarithmic mean SPL was 52.6 dBA (i.e. A-weighted dB) and logarithmic mean

maximum SPL was 76.8 dBA (Gill et al., unpublished data). Therefore, pink noise

played at 76 dBA is higher in amplitude than would be expected of typical continuous

ambient SPLs in an urban landscape.

Playback experiments

We performed focal male experiments (N = 45 males; 19 prior to clutch initiation (8 unpaired and 11 paired) and 26 during incubation) between sunrise and 1100 (EST) between June 6 – July 21, 2016 and April 28 – July 2, 2017. To simulate a male intruder, we played song from an amplified SME-AFS speaker (Saul Mineroff Electronics, New York) placed 5 – 10m from the focal male's nest box, positioned 0.5 – 1.5-m above ground in vegetation. To simulate a noise disturbance on the focal male's territory, we broadcast noise from a second speaker placed approximately 10 m in the opposite direction of the nest box on the ground. We separated the noise playback speaker from the song playback speaker because we wanted to document whether song structure changed in response to the treatments. Males were attracted to the speaker playing song and if the speaker playing noise was nearby or noise was played from the same speaker as song, noise would mask the songs by focal males, making it impossible to extract song frequency (Grabarczyk et al. 2018). Moreover, singing male birds move away from intense noise sources (SAG, unpublished data), such that the separation of intruder and noise may be more likely to mimic the location of an actual intruder relative to a noise source in the territory. To record focal male songs, we placed a Sound Meter 2 recording unit (44.1 kHz, 16-bit sample rate, .wav format) between the nest box and speaker simulating an intruding male. To minimize disturbance during trials, we attached an Apple iPod (Cupertino, CA) using 20 m extension cords to each speaker and controlled onset of experiment at a distance.

We randomly selected the order of treatments to present. Each treatment

consisted of a 10-min control period without playback followed by a 10-min treatment

with a 10-min break between successive treatments. We chose to present all playback

treatments on the same day, rather than on different days as male singing behavior

changes as breeding progresses (Johnson and Kermott 1991; Rendall and Kaluthota

2013; Grabarczyk et al. 2018). During trials, we quantified two behavioral responses:

the number of times a male physically attacked the speaker (attack) and the number of

times a male flew over the speaker (fly over), as well as the time (s) from start of

playbacks for males to approach within 2 m of the speaker broadcasting intruder song.

From our recordings, we measured focal male vocal response time, or the time (s) from

the beginning of a playback (song playback for intruder with and without noise, or the

start of noise playback for noise alone) until a male sang.

Acoustic analysis

We used a bandpass filter (1.3 – 11 kHz) to remove high and low frequency

sounds from our recordings. In Avisoft, we used section labels to mark every focal male

and intruder playback song recorded on the spectrogram window (Flat top window, 512

FFT length, 93.75% overlap, 0.725 ms time resolution). We quantified vocal responses

of focal males to playbacks as rate of singing (song/min), song duration (s) and peak

frequency (Hz), with the latter measured using automated parameter window, and

compared vocal and behavioral responses for each 10-min control and stimulus. We

analyzed song duration and peak frequency analysis only if songs were not overlapped

by songs of another bird or playback. Across trials, we analyzed 8,495 focal male songs (during the control mean ± SD: 25.3 ± 22.9 songs per male and experimental playback periods mean ± SD: 36.1 ± 25.3 songs per male). Due to naturally occurring high ambient noise (which often exceeded song amplitudes) at some recording locations, we were unable to extract minimum frequency using automated parameter measures (see Grabarczyk et al. 2018). Avisoft consistently measured minimum frequency as the noise floor (i.e. the frequency of the high-pass filter) rather than the lowest frequencies of songs of focal males. We did not increase the cut-off frequency of the high-pass filter setting, as this adjustment would have eliminated the lowest frequency portions of focal male song.

To test whether ambient noise levels affected male response to treatments, we extracted sound pressure levels (SPLs) from focal male recordings. Each microphone and SM2 unit pair was calibrated with a Larson Davis CAL 200 sound level calibrator (Depew, NY) by recording a 1 kHz tone played at 94 dB. From the calibrated tone, we set the relative amplitude to 0 dB (re 20 µPa) in Avisoft for each recording. SPL measurements were taken from the first 10-min of focal male recordings by randomly selecting and averaging five 1-sec full spectrum noise samples that were not overlapped by house wren song.

Statistical analysis

Detection. We used R program software v3.3.3 (R core development team, 2017) for all statistical analyses. To test whether treatments affected focal male response time, we compared how long males took to vocally respond after the start of each playback treatment and approach (s) the playback speaker within 2 m. We ran a linear mixed effects model using the package lme4 (Bates et al. 2015) to test if response time differed among treatments and included treatment, breeding stage, and the interaction between treatment and breeding stage as fixed effects and male identity and song exemplar as random effects. We approximated p-values with the package lmerTest (Kuznetsova et al. 2017) using a Satterthwaite approximation for hypothesis testing. Because we presented successive treatments with only 10-min breaks between them, an earlier treatment could have influenced a later one (i.e. a carry-over effect); therefore, for all models (detection and response), we initially included sequence of playback as an additional fixed effect. If playback sequence was significant predictor of male response, we could not isolate behavioral responses to the current treatment alone from the influence of an earlier treatment. We found sequence to be a significant predictor of male responses to our treatments (data not shown); therefore, we reanalyzed all models using only the first playback treatment and eliminated the second and third treatments for each male. Focal males did not approach the intruder playback speaker during any noise-alone trials, therefore we excluded this treatment from models exploring approach latency. Based on residual plots, the data showed heteroscedasticity, therefore we log transformed response time (vocal response time

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and approach latency) for the final model, which eliminated patterns of heteroscedasticity (Zuur et al. 2010). Male response time could have been influenced by ambient noise conditions; therefore, to explore whether ambient noise conditions influenced male detection, we plotted model residuals response time (s) against ambient noise (dB). We found no patterns that would indicate a relationship between noise and unexplained variation in the response time model.

Male response to a territorial intruder. If males recognized the song playback as an intruder, we expected a change in vocal behavior from the pre-playback to the playback period. To demonstrate playbacks altered male singing, we calculated the mean difference between song traits in the 10-min control period without playback from 10-min playback stimulus average. To determine if treatment affected male response during playback, we calculated mean responses for song rate (song/min), song duration (s), and peak frequency (Hz) during each 10-min playback treatment. We used linear mixed effects models to test whether males changed their songs differently depending on treatments (i.e. before versus during treatments) and whether mean song traits produced during trials differed among treatments. We included treatment, breeding stage, and the interaction between treatment and breeding stage as fixed effects, and male identity and song playback exemplar as random effects. We detected a carry-over effect in male song length and rate of singing to subsequent treatments, therefore to be consistent, we analyzed only song traits (song length, rate, and peak frequency) from the first treatment presented to each male.

For linear mixed effects models, we compared model fit with and without interaction terms using AICc values and ΔAICc. Models with a change in AICc of 2 or less were selected for analysis. If models with and without the interaction term did not differ, we selected the interaction model for analysis (Table 5.1). We used residual plots to test model adequacy and ran pairwise comparisons with the package lsmeans (Lenth 2016) to determine whether song traits differed between treatments. For each model, we plotted residuals against year tested (2016 or 2017), Julian date, and ambient noise, but found no dependencies for these factors and did not include any in final models. During initial data exploration, we used box plots to assess variation in responses of paired versus unpaired males prior to clutch initiation; these plots revealed little or no differences in behavior between paired and unpaired males in our sample and therefore we did not include pairing status in our models.

In addition to vocal traits, change in male response to an intruder could also include movement behavior such as fly overs or attacks near the playback speaker. For non-vocal behavior during trials, we used a permutation test of independence to compare the number of fly overs and attacks during a simulated intruder with and without noise. In addition, we used a Fisher exact test to determine whether the proportion of males that responded during trials with either fly overs or attacks differed between simulated intrusions with and without noise. During playbacks with noise only, we recorded no fly overs or attacks from any focal male tested, thus eliminated this treatment from analysis. Based on data plots, sequence of presentation was significant,

therefore we compared the number of fly overs and attacks to the first playback treatment during an intrusion (N = 14) and to an intruder with noise (N = 12).

Results

Intruder detection

If noise masks intruder signals resident males may be slower to detect intruders on their territories. We found treatment had no effect on intruder detection measured as the duration from start of first playback to a male's first song (intruder treatment: Estimate ± SE: 0.8 ± 0.6, t = 1.3, p = 0.2; intruder + noise treatment: Estimate ± SE: 0.07 ± 0.5, t = 0.1, p = 0.9; Figure 5.1). Breeding stage was not a significant predictor of a male's first vocal response (stage early: Estimate ± SE: –0.9 ± 0.5, t = -1.6, p = 0.1). Similarly, males may be slower to approach an intruder if noise disrupted detection. However, treatment did not affect how quickly males approached the playback speaker within 2 m (intruder + noise treatment: Estimate ± SE: -0.3 ± 0.5, t = -0.5, p = 0.6). Breeding stage was not a significant predictor of a male's latency to approach the playback speaker (stage early: Estimate ± SE: -0.9 ± 0.6, t = -1.6, p = 0.1).

Male response to a territorial intruder

Change in male vocal behavior before and during treatments. To determine the effect of playbacks on male behavior, we tested whether males changed their songs from the pre-playback to the playback period. Overall, males show an increase in song peak frequency in response to intruders (Figure 5.2A), although, change in song peak

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frequency did not statistically differ among treatments (Table 5.2). Males increased song rate, singing on average 4 songs more per minute in response to a simulated intruder, regardless of noise, and on average slightly decreased song rate in response to noise alone (Figure 5.3A). The interaction between breeding stage and treatment was a significant predictor of change in song duration (Table 5.2, Figure 5.4). Males in later breeding stages increased their song duration in response to intruder playback stimulus with and without noise, whereas males in early stages did not alter their song duration (Figure 5.4).

Do males respond differently to simulated intruders depending on noise? If noise affects how territorial males respond to intruders, then the average vocal response of males should differ between intrusions with and without noise. Males sang on average at a higher peak frequency during intruder playback without noise compared to treatments with noise alone (Figure 5.2B, Table 5.3). Average peak frequency responses to an intruder with noise did not differ from either the noise only or intruder only treatment, suggesting male responses are intermediate between intruder only and noise only treatments (Figure 5.2B). Breeding stage did not affect mean song peak frequency during treatments (Table 5.3). Males sang longer songs (Figure 5.5A) at a higher rate (Figure 5.3B) in response to intruders regardless of noise compared to noise alone (Table 5.3). Males sang longer songs regardless of treatment during early compared to late breeding stages (Figure 5.5B). Breeding stage did not affect mean song rate.

Non-vocal behavioral responses of males to an intruder. To test whether males differed in their non-vocal response towards the speaker during a simulated intrusion with and without noise, we compared the total number of fly overs and attacks on the speaker during each treatment. For analysis, we included fly over and attack data for only the first treatment presented to each male in response to an intruder (N = 14) without and with noise (N = 12). Males attacked the playback speaker significantly more times in response to an intruder with noise compared to without noise (Z = –2.01, p = 0.04, Figure 5.6A). The number of male fly overs did not differ between treatments (Z = 1.1, p = 0.3, Figure 5.6B). The proportion of males to attack or fly over the speaker during simulated intrusions with and without noise did not differ (Fisher exact test: attack, p = 0.5; fly over, p = 0.6). Of the 27 males included in analysis, 40.7% (N = 11) attacked the playback speaker during song treatments (N = 6 out of 12 males during intrusion with noise and N = 5 out of 14 males during an intrusion without noise). Of the 26 males, 84.6% of males (N = 22) responded by flying over the speaker.

Discussion

Anthropogenic noise masking reduces the active space of male long-distance signals used for mate attraction and territory defense (Lohr et al. 2003). Noise may be particularly costly to males when defending territories if they are less able detect territorial intruders or if noise alters their ability to assess intruders. We tested the hypothesis that anthropogenic noise affects male house wrens' detection of and responses to conspecific territorial intruders near active nests. Males first sang and

approached the speaker with similar delay from the onset of playbacks regardless of noise, suggesting that noise treatments did not affect the ability of males to detect territorial intruders. However, during simulated intrusions paired with noise playback, males attacked the playback speaker more compared to intrusions without noise, suggesting that noise alters aggressive responses to intruders. Males sang longer songs more often to simulated intruders regardless of noise playback, compared to the noise alone. Males increased song peak frequency in response to intruders. Responses to an intruder with noise were intermediate between the noise only and intruder only treatments, suggesting that noise did not compromise vocal responses to intruders, but did dampen male peak frequency responses.

Under high ambient noise conditions, resident males could be delayed or fail to detect intruders on their territory if noise alters the probability of intruder detection. With increasing ambient noise, spotted towhees (*Pipilo maculatus*) and chipping sparrows (*Spizella passerina*) more slowly approach a playback speaker broadcasting intruder song (Kleist et al. 2015), whereas Nuttall's white-crowned sparrow (*Zonotrichia leucophrys nuttalli*) approach more quickly (Phillips and Derryberry 2018). The differences in response time may be attributed to where the playback took place on the male's territory, as Phillips and Derryberry (2018) explicitly identified focal male territories and simulated intrusions at the territory core, whereas Kleist et al. (2016) did not identify territory boundaries. By contrast, noise did not influence response time by male house wrens, as the time elapsed from start of playback to first song and latency to approach the playback speaker did not differ by treatment. We performed playback

experiments near active primary nests, which may have facilitated detection by males

regardless of treatment, as males are more likely to spend time at or near active nests

during the breeding season.

Noise could affect the ability of territorial males to adequately assess the threat

posed by intruder. Such an effect could occur if noise masks intruder signals, resulting

in more intense responses under noisy conditions. During simulated intrusions with

noise, focal males attacked the speaker more frequently compared to intrusions without

noise (Figure 5.6A). Our results are similar to patterns in male Nuttall's white-crowned

sparrows, which respond aggressively by approaching intruders more closely with

increasing ambient noise levels, likely enabling males to better discriminate the threat

(Phillips and Derryberry 2018). During actual intrusions, closer approaches and

increased attacks on or towards an intruder might lead to interactions escalating more

quickly, which could be physically costly to both participating males. However, less than

half of the males in our study responded by physically attacking the speaker during

playbacks, and only five out of 14 males responded with attacks during a simulated

intrusion without noise. This result, based on simulated intrusions with playbacks, differs

from interactions with actual intruders in which males immediately approach and attack

intruding males (Johnson and Kermott 1990). In response to actual intrusions, males

may also rely on visual cues and movements in addition to acoustic detection as part of

their territorial response.

During intrusions, the information conveyed via signals to neighboring birds may

be important if neighbors eavesdrop thereby passively gaining information about

aggressive encounters. Male house wrens increased song peak frequency during intrusions regardless of noise, but male responses to an intruder with noise were intermediate between the noise only and intruder only treatments (Figure 5.2B). This pattern suggests noise may weaken male frequency responses to a territorial intruder. Song frequency adjustments to intruders could be an indication of aggressive intent (Cardoso 2012) and may reflect signaler body size or condition (Morton 1977). The degree to which males adjust song frequency during intrusions may affect their reproductive success if females eavesdrop on territorial interactions and use information regarding male performance for mate choice decisions (Otter et al. 1999, Mennill et al. 2002). Neighboring males might also eavesdrop on territorial interactions and use information gained passively to guide their own responses to territorial intruders (Schmidt et al. 2007).

Longer songs given at high rates improve the likelihood of detection by increasing redundancy (Pohl et al. 2013), but could also be used as an aggressive signal to overlap with an intruders' song (Naguib and Mennill 2010). Focal males sang longer songs at higher rates during early breeding stages compared to later ones, a finding consistent with prior studies (Tove 1988, Johnson and Kermott 1991, Grabarczyk et al. 2018). However, these early breeding males did not adjust song duration to playbacks, whereas males at the later breeding stage sang longer duration songs in response to intruders. During early stages of breeding, male songbirds broadcast mate attraction signals at high rates that transmit over large distances and are locatable by females, but also function to repel conspecific male competitors

(Bradbury and Vehrencamp 1998). For house wrens, song duration may therefore play an important role in both territorial defense and mate attraction signaling. In addition to repelling intruders, focal males may adjust temporal song traits to mask or overlap their challenger's signal. By simply increasing song rate and duration, the probability of overlap also increases. However, further testing is necessary to determine whether song rate and duration adjustments by intruders elicit a gradient of responses by focal males. This, in combination with evidence males adjust the timing of their signals relative to playback, could be evidence of an aggressive signal in response to territorial intruders.

Anthropogenic noise pollution is a widespread and increasingly common feature in urban natural areas. For birds, we might expect that species inhabiting noisy areas are those that are able to adjust their behaviors such that they reduce the cost of breeding in noise (Read et al. 2014, Fischer et al. 2015). In this study, we used playback simulations to test whether noise altered territorial male responses to an intruder around active nests. Importantly, we provide additional support that at territory cores, noise does not delay intruder detection (Phillips and Derryberry 2018). Masking may however hinder the ability of males to discriminate intruder signals, as suggested by the closer approaches (see Phillips and Derryberry 2018) and an increased number of attacks (this study) elicited by playbacks in noise. Males show an intermediate song peak frequency response to an intruder with noise compared to the noise only and intruder only treatments, suggesting noise affects some aspects of singing. We show focal males adjusted their song length and rate to an intruder similarly in both quiet and

noisy conditions. Spectral and temporal vocal adjustments in response to an intruder

may increase the active space of focal male signals, which could be important if

neighboring conspecifics eavesdrop to gain information on aggressive interactions. In

summary, responses of male house wrens to intruders differed depending on noise, but

were not completely compromised by noise. Presumably, noise has been a persistent

disturbance within our study areas, and the responses we measured from males

breeding in these established urban natural areas may be learned behaviors as a result

of past environmental change, rather than maladaptive responses to a novel

environmental disturbance (Sih et al. 2016).

Literature cited

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. . Journal of Statistical Software **67**:1-48.

Bee, M. A., and E. M. Swanson. 2007. Auditory masking of anuran advertisement calls by road traffic noise. Animal Behaviour **74**:1765-1776.

Bermudez-Cuamatzin, E., A. A. Rios-Chelen, D. Gil, and C. M. Garcia. 2009. Strategies of song adaptation to urban noise in the house finch: syllable pitch plasticity or differential syllable use? Behaviour **146**:1269-1286.

Bradbury, J. W., and S. L. Vehrencamp. 1998. Principles of Animal Communication. 1 edition. Sinauer Associates, Sunderland, MA.

Brumm, H. 2006. Signalling through acoustic windows: nightingales avoid interspecific competition by short-term adjustment of song timing. Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology **192**:1279-1285.

Brumm, H., and H. Slabbekoorn. 2005. Acoustic communication in noise. Pages 151-209 *in* P. J. B. Slater, C. T. Snowdon, H. J. Brockmann, T. J. Roper, and M. Naguib, editors. Advances in the Study of Behavior, Vol 35.

Cardoso, G. C. 2012. Paradoxical calls: the opposite signaling role of sound frequency across bird species. Behavioral Ecology **23**:237-241.

Egnor, S. E. R., J. G. Wickelgren, and M. D. Hauser. 2007. Tracking silence: adjusting vocal production to avoid acoustic interference. Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology **193**:477-483.

Endler, J. A. 1992. Signals, Signal Conditions, and the Direction of Evolution. American Naturalist **139**:S125-S153.

Fischer, J. D., S. C. Schneider, A. A. Ahlers, and J. R. Miller. 2015. Categorizing wildlife responses to urbanization and conservation implications of terminology. Conservation Biology **29**:1246-1248.

Freed, L. A. 1986. Territory takeover and sexually selected infanticide in tropical House wrens. Behavioral Ecology and Sociobiology **19**:197-206.

Gentry, K. E., E. P. Derryberry, R. M. Danner, J. E. Danner, and D. A. Luther. 2017. Immediate signaling flexibility in response to experimental noise in urban, but not rural, white-crowned sparrows. Ecosphere **8**:e01916.

Gill, S. A., J. R. Job, K. Myers, K. Naghshineh, and M. J. Vonhof. 2015. Toward a broader characterization of anthropogenic noise and its effects on wildlife. Behavioral Ecology **26**:328-333.

Grabarczyk, E. E., M. A. Pipkin, M. J. Vonhof, and S. A. Gill. 2018. When to change your tune? Unpaired and paired male house wrens respond differently to anthropogenic noise. Journal of Ecoacoustics **2**:#LHGRVC.

Gross, K., G. Pasinelli, and H. P. Kunc. 2010. Behavioral Plasticity Allows Short-Term Adjustment to a Novel Environment. American Naturalist **176**:456-464.

Hanna, D., G. Blouin-Demers, D. R. Wilson, and D. J. Mennill. 2011. Anthropogenic noise affects song structure in red-winged blackbirds (*Agelaius phoeniceus*). Journal of Experimental Biology **214**:3549-3556.

Johnson, L. S., and L. H. Kermott. 1989. Territorial Intrusions in the House Wren *Troglodytes aedon*: Evidence for the Sperm Competition Hypothesis. Ornis Scandinavica **20**:89-92.

Johnson, L. S., and L. H. Kermott. 1990. Possible Causes of Territory Takeovers in a North-Temperate Population of House Wrens. Auk **107**:781-784.

Johnson, L. S., and L. H. Kermott. 1991. The Functions of Song in Male House Wrens (*Troglodytes aedon*). Behaviour **116**:190-209.

Kern, J. M., and A. N. Radford. 2016. Anthropogenic noise disrupts use of vocal information about predation risk. Environmental Pollution **218**:988-995.

Kleist, N. J., R. P. Guralnick, A. Cruz, and C. D. Francis. 2016. Anthropogenic noise weakens territorial response to intruder's songs. Ecosphere **7**.

Klump, G. M. 1996. Bird communication in the noisy world. Cornell University Press, Ithaca, NY.

Kroodsma, D. E. 1977. Correlates of Song Organization Among North American Wrens. American Naturalist **111**:995-1008.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. Journal of Statistical Software **82**:1-26.

LaZerte, S. E., H. Slabbekoorn, and K. A. Otter. 2017. Territorial black-capped chickadee males respond faster to high- than to low-frequency songs in experimentally elevated noise conditions. Peerj **5**.

Lenth, R. V. 2016. Least-Squares Means: The R Package lsmeans. Journal of Statistical Software **69**:1-33.

Lohr, B., T. F. Wright, and R. J. Dooling. 2003. Detection and discrimination of natural calls in masking noise by birds: estimating the active space of a signal. Animal Behaviour **65**:763-777.

McIntyre, E., M. L. Leonard, and A. G. Horn. 2014. Ambient noise and parental communication of predation risk in tree swallows, *Tachycineta bicolor*. Animal Behaviour **87**:85-89.

McMullen, H., R. Schmidt, and H. P. Kunc. 2014. Anthropogenic noise affects vocal interactions. Behavioural Processes **103**:125-128.

Mennill, D. J., L. M. Ratcliffe, and P. T. Boag. 2002. Female eavesdropping on male song contests in songbirds. Science **296**:873-873.

Morton, E. S. 1977. On the occurrence and significance of motivation-structural rules in some bird and mammal sounds. American Naturalist **111**:855-869.

Naguib, M., and D. J. Mennill. 2010. The signal value of birdsong: empirical evidence suggests song overlapping is a signal. Animal Behaviour **80**:E11-E15.

Otter, K., P. K. McGregor, A. M. R. Terry, F. R. L. Burford, T. M. Peake, and T. Dabelsteen. 1999. Do female great tits (*Parus major)* assess males by eavesdropping? A field study using interactive song playback. Proceedings of the Royal Society B-Biological Sciences **266**:1305-1309.

Phillips, J. N., and E. P. Derryberry. 2018. Urban sparrows respond to a sexually selected trait with increased aggression in noise. Scientific Reports **8**.

Pohl, N. U., E. Leadbeater, H. Slabbekoorn, G. M. Klump, and U. Langemann. 2012. Great tits in urban noise benefit from high frequencies in song detection and discrimination. Animal Behaviour **83**:711-721.

Pohl, N. U., H. Slabbekoorn, G. M. Klump, and U. Langemann. 2009. Effects of signal features and environmental noise on signal detection in the great tit, *Parus major*. Animal Behaviour **78**:1293-1300.

Pohl, N. U., H. Slabbekoorn, H. Neubauer, P. Heil, G. M. Klump, and U. Langemann. 2013. Why longer song elements are easier to detect: threshold level-duration functions in the Great Tit and comparison with human data. J Comp Physiol A Neuroethol Sens Neural Behav Physiol **199**:239-252.

Proppe, D. S., and E. Finch. 2017. Vocalizing during gaps in anthropogenic noise is an uncommon trait for enhancing communication in songbirds. Journal of Ecoacoustics **1**:TLP16D.

Read, J., G. Jones, and A. N. Radford. 2014. Fitness costs as well as benefits are important when considering responses to anthropogenic noise. Behavioral Ecology **25**:4-7.

Rendall, D., and C. D. Kaluthota. 2013. Song organization and variability in northern House wrens (*Troglodytes aedon parkmanii*) in Western Canada. Auk **130**:617-628.

Schmidt, R., V. Amrhein, H. P. Kunc, and M. Naguib. 2007. The day after: effects of vocal interactions on territory defence in nightingales. Journal of Animal Ecology **76**:168-173.

Shannon, G., M. F. McKenna, L. M. Angeloni, K. R. Crooks, K. M. Fristrup, E. Brown, K. A. Warner, M. D. Nelson, C. White, J. Briggs, S. McFarland, and G. Wittemyer. 2016. A synthesis of two decades of research documenting the effects of noise on wildlife. Biological Reviews **91**:982-1005.

Sih, A., P. C. Trimmer, and S. M. Ehlman. 2016. A conceptual framework for understanding behavioral responses to HIREC. Current Opinion in Behavioral Sciences **12**:109-114.

Stoddard, P. K., M. D. Beecher, C. L. Horning, and S. E. Campbell. 1991. Recognition of individual neighbors by song in the Song Sparrow, a species with song repertoires. Behavioral Ecology and Sociobiology **29**:211-215.

Templeton, C. N., S. A. Zollinger, and H. Brumm. 2016. Traffic noise drowns out great tit alarm calls. Current Biology **26**:R1173-R1174.

Tove, M. H. 1988. Patterns of singing by house wrens with respect to the breeding cycle Utah State University, Logan, UT.

Verzijden, M. N., E. A. P. Ripmeester, V. R. Ohms, P. Snelderwaard, and H. Slabbekoorn. 2010. Immediate spectral flexibility in singing chiffchaffs during experimental exposure to highway noise. Journal of Experimental Biology **213**:2575-2581.

Wiley, R. H. 2013. Signal Detection, Noise, and the Evolution of Communication. Brumm, H. edition. Springer, Berlin Heidelberg.

Zuur, A. F., E. N. Ieno, and C. S. Elphick. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution **1**:3-14.

Zwart, M. C., J. C. Dunn, P. J. K. McGowan, and M. J. Whittingham. 2016. Wind farm noise suppresses territorial defense behavior in a songbird. Behavioral Ecology **27**:101-108.

Table 5.1 Comparison of competing models for change in male response and mean response with and without the interaction term using AICc and ΔAICc.

Analysis	Model[a]	AICc	ΔAICc
Change in peak frequency (Hz) – all treatments	Main effects	1783.44	0.00
	Interaction	1784.74	1.3
Change in song duration (s) – first treatment only	Main effects	25.32	0.37
	Interaction	24.95	0.00
Change in song rate (songs/min) – first treatment only	Main effects	224.07	0.00
	Interaction	227.88	3.81
Mean peak frequency (Hz) – all treatments	Main effects	1914.53	0.00
	Interaction	1917.42	2.89
Mean song duration (s) – all treatments	Main effects	81.04	0.00
	Interaction	85.28	4.24
Mean song rate (songs/min) – first treatment only	Main effects	225.93	0.00
	Interaction	230.13	4.2

[a] Main effects models included treatment, breeding stage, and sequence of treatment presentation as main effects, with male identity and song exemplar a random effects. Interaction models included the interaction between treatment and breeding stage. If sequence of presentation was a significant predictor of a song trait, the model was reanalyzed including only the first treatment presented to each focal male.

Table 5.2 Change in song trait by male house wrens in response to a simulated intruder with and without noise, and to noise alone.

Analysis	Parameter	Estimate $\pm$ SE	$t_{(df)}$	p-value
Change in peak frequency (Hz) – all treatments[a]	Intercept	−91.8 $\pm$ 136.5	$-0.7_{(104)}$	0.5
	Treatment: intruder	387.5 $\pm$ 107.0	$3.6_{(75)}$	0.0005
	Treatment: intruder + noise	290.9 $\pm$ 106.8	$2.7_{(75)}$	0.008
	Breeding stage: prelaying	216.2 $\pm$ 146.6	$1.5_{(97)}$	0.1
	Sequence	-41.5 $\pm$ 42.9	$-1.0_{(77)}$	0.4
	Intruder*prelaying	−170.6 $\pm$ 173.5	$-1.0_{(76)}$	0.3
	Intruder + noise*prelaying	−318.5 $\pm$ 174.1	$-1.8_{(77)}$	0.07
Change in song duration (s) – all treatments[a]	Intercept	0.2 $\pm$ 0.09	$2.5_{(93)}$	0.01
	Treatment: intruder	0.1 $\pm$ 0.06	$2.2_{(79)}$	0.03
	Treatment: intruder + noise	0.1 $\pm$ 0.06	$1.7_{(80)}$	0.09
	Breeding stage: prelaying	−0.1 $\pm$ 0.06	$-2.1_{(42)}$	0.04
	Sequence	−0.1 $\pm$ 0.03	$-3.2_{(80)}$	0.002
Change in song duration (s) – first treatment only[b]	Intercept	−0.03 $\pm$ 0.2	$-0.2_{(27)}$	0.8
	Treatment: intruder	0.3 $\pm$ 0.2	$1.4_{(27)}$	0.2
	Treatment: intruder + noise	0.6 $\pm$ 0.2	$2.8_{(27)}$	0.009
	Breeding stage: prelaying	−0.02 $\pm$ 0.2	$-0.1_{(25)}$	0.9
	Intruder*prelaying	−0.4 $\pm$ 0.3	$-1.2_{(26)}$	0.2
	Intruder + noise * prelaying	−0.6 $\pm$ 0.3	$-2.0_{(26)}$	0.05
Change in song rate (songs/min) – all treatments[a]	Intercept	0.6 $\pm$ 0.7	$0.9_{(129)}$	0.4
	Treatment: intruder	3.9 $\pm$ 0.5	$7.3_{(107)}$	0.0000
	Treatment: intruder + noise	3.2 $\pm$ 0.5	$5.9_{107)}$	0.0000
	Breeding stage: prelaying	1.2 $\pm$ 0.5	$-0.4_{(85)}$	0.7
	Sequence	−0.6 $\pm$ 0.3	$-2.2_{(107)}$	0.03
Change in song rate (songs/min) – first treatment only[b]	Intercept	0.6 $\pm$ 0.8	$0.7_{(41)}$	0.5
	Treatment: intruder	3.4 $\pm$ 1.0	$3.4_{(37)}$	0.002
	Treatment: intruder + noise	3.7 $\pm$ 2.0	$3.8_{(38)}$	0.0005
	Breeding stage: prelaying	−1.7 $\pm$ 0.8	$-2.0_{(37)}$	0.05

[a] We first analyzed all treatments presented to males and included treatment, breeding stage, and sequence of presentation as fixed effects, male identity and song exemplar as random effects.

[b] For models where sequence was a significant predictor of the change in male response we eliminated the second and third treatments, and reanalyzed the model including only the first treatment presented.

Table 5.3 Mean song trait response by male house wrens in response to a simulated

intruder with and without noise, and to noise alone.

Analysis	Parameter	Estimate ± SE	$t_{(df)}$	p-value
Mean peak frequency (Hz) – all treatments[a]	Intercept	3878.3 ± 112.7	$34.4_{(105)}$	<0.00001
	Treatment: intruder	221.7 ± 74.7	$3.0_{(85)}$	0.004
	Treatment: intruder + noise	201.7 ± 74.4	$2.7_{(85)}$	0.009
	Breeding stage: prelaying	− 26.7 ± 85.9	$-0.3_{(82)}$	0.8
	Sequence	33.8 ± 37.3	$0.9_{(55)}$	0.4
Mean song duration (s) – all treatments[a]	Intercept	1.9 ± 0.09	$20.1_{(73)}$	0.000
	Treatment: intruder	0.1 ± 0.05	$2.4_{(81)}$	0.02
	Treatment: intruder + noise	0.1 ± 0.05	$2.7_{(80)}$	0.009
	Breeding stage: prelaying	0.1 ± 0.07	$1.6_{(96)}$	0.1
	Sequence	0.03 ± 0.03	$1.2_{(80)}$	0.2
Mean song rate (songs/min) – all treatments[a]	Intercept	2.0 ± 0.7	$2.9_{(126)}$	0.005
	Treatment: intruder	2.3 ± 0.5	$4.8_{(87)}$	0.0000
	Treatment: intruder + noise	2.0 ± 0.5	$4.0_{(87)}$	0.0000
	Breeding stage: prelaying	1.0 ± 0.7	$1.5_{(43)}$	0.1
	Sequence	0.9 ± 0.2	$3.6_{(87)}$	0.0004
Mean song rate (songs/min) – first treatment only[b]	Intercept	1.8 ± 0.8	$2.3_{(41)}$	0.03
	Treatment: intruder	3.1 ± 1.0	$3.0_{(41)}$	0.003
	Treatment: intruder + noise	4.0 ± 1.0	$4.0_{(41)}$	0.0002
	Breeding stage: prelaying	1.2 ± 0.8	$1.4_{(41)}$	0.2

[a] Models describing mean song duration and peak frequency males included treatment, breeding stage, and sequence of presentation as fixed effects, male identity and song playback exemplar as random effects.
[b] For models describing mean song rate during treatments sequence was a significant predictor therefore we eliminated the second and third treatments, and reanalyzed and interpret the model including only the first treatment presented.

Figure 5.1 Male house wrens did not differ in the delay from beginning of playbacks to their first songs based on treatment.

Figure 5.2 In response to an intruder, (a) male house wrens increase song peak

frequency (Hz) during an intrusion with (N = 13) and without noise (N = 12), and but

decreased peak frequency in response to noise alone (N = 8) compared to pre-playback

control periods. (b) On average males sing at a higher peak frequency during an

intrusion without noise (N = 13) compared to the noise only treatment (N = 14). Male

responses to an intruder with noise (N = 13) did not differ from either the noise only or

intruder only treatment. Breeding stage was not a significant predictor of song peak

frequency.

Figure 5.3 Male house wrens increased singing rate (a) in response to an intruder with (N = 12) and without noise (N = 15), but they did not change singing rate in noise (N = 18) compared to pre-playback control periods. (b) On average males sang at a higher rate when an intruder was present, regardless of whether or not noise was played, than during noise playback alone.

Figure 5.4 The interaction between treatment and breeding stage was a significant predictor of change in song duration. During later breeding stages, males increase their song duration in response to intruders (N = 13 intruder alone, N = 12 intruder with noise), whereas males during early stages overall do not increase their song duration.

Figure 5.5 (A) Mean song duration was longer in response to an intruder with (N = 13) and without noise (N = 13) compared to noise alone (N = 14). (B) During early breeding stages (N = 16) males sang longer songs compared to later stages (N = 24).

Figure 5.6 Males attacked the playback speaker more during an intrusion with noise (N = 14) compared to an intruder alone (N = 12; (A)), treatment did not affect the number of fly overs towards the speaker (B).

CHAPTER VI

SOCIAL CONTEXT, NOT ENVIRONMENTAL CONTSTRAINTS, PREDICT
SIGNALING BEHAVIOR AMONG MULTIPLE TERRITORIAL MALE HOUSE WRENS

Abstract

Territorial animals interact in complex signaling networks, broadcasting
information with long-distance vocalizations. Vocal signals mediate social relationships,
and among groups of callers, individuals adjust signal production in response to
changes in the social environment. Additional constraints on signal transmission, such
as anthropogenic noise masking or distance-related signal attenuation may weaken
social ties if information sharing via acoustic signals is limited. But whether patterns of
vocal interactions among multiple territorial males are similarly affected by social and
environmental factors remains poorly known. Therefore, we test the hypothesis that
signaling interactions among territorial male house wrens (*Troglodytes aedon*) vary with
social context, but interactions may be constrained by limitations on signal transmission
from the environment. We used playback experiments to simulate territory intrusions
with and without noise playbacks as well as to noise alone on focal territories, while
simultaneously recording the vocal responses of all males breeding within signaling
distance of simulated intruders. We used randomization tests to determine whether
social or environmental factors affected singing coordination between focal and
neighbors as well as temporal patterns of singing across males. Focal males increased
singing rate in response to simulated intruders and sang longer bouts with more songs
per bout in response to intruder-only treatments compared to controls. Focal male

responses during intruder treatments plus noise did not differ from either the intruder-only treatment or the control, suggesting that noise may dampen male territorial responses. The vocal behavior of neighboring males was predicted by their breeding stage, with males singing at higher rates during early stages of breeding. During early and late breeding stages neighbors sang longer song bouts with more songs per bout, but treatment, ambient noise, and spatial proximity to focal males had no effect on song bouts. We found no evidence of vocal coordination between focal and neighboring males. Fluctuations in ambient noise affected how male house wrens structure their songs, but we found no evidence that short-term environmental change on focal territories influenced the singing behavior of neighbors. The effects of noise on multiple callers within signaling networks may occur over longer periods of time, but and is intricately linked to the social environment.

Introduction

Animals give vocalizations structured for long-distance transmission to convey information regarding sender identity, quality, and motivation (Bradbury and Vehrencamp 1998). Vocal signals mediate social relationships, linking signal senders and receivers in complex signaling networks (McGregor and Dabelsteen 1996, McGregor and Peake 2000, McGregor 2005, Peake et al. 2005). Within signaling networks, territorial males acquire information regarding other males through countersinging or passively through eavesdropping (McGregor 2005, Peake et al. 2005), adjusting their own behavior according to information gained (Peake et al. 2002, Naguib et al. 2004, Schmidt et al. 2007, Amy et al. 2010). Simultaneously, females eavesdrop on and alter their behavior in response to male counter-signaling interactions (Snijders et al. 2017), using acquired information to guide mate choice decisions (Otter et al. 1999, Mennill et al. 2002). Evidence of eavesdropping may be detected among groups of territorial animals; male common yellowthroats (*Geothlypis trichas*) increase their song output when a neighboring female is fertile, but sing fewer songs as their breeding partner approaches egg laying (Taff et al. 2014). Thus, male signaling has immediate fitness consequences during one-on-one interactions and indirectly affects decision-making and vocal behavior of conspecifics that obtain information via eavesdropping. Accordingly, changes in the social environment will likely affect patterns of vocal interactions among the members of signaling networks.

Constraints on signal transmission that affect the ability of receivers to detect, discriminate, and decode signals may weaken exchanges among individuals, altering

the structure of signaling networks and social relationships. Acoustic signals attenuate over space, and become further degraded as they are absorbed by reflective surfaces or scatter through the environment with increasing distance from the signaler (Morton 1975, Marten and Marler 1977, Wiley and Richards 1978). Additionally, frequency masking from other sounds increases degradation by decreasing the area over which the signal can be detected (Klump 1996), limiting the ability the receivers to hear and perceive signals (Patricelli and Blickley 2006). Adding to these natural causes of signal degradation is human-generated noise pollution, which further limits information sharing due to frequency masking. In noise-polluted areas, vocal interactions among territorial males may change; for example, individuals may move closer together which may increase the likelihood of detecting signals (Owens et al. 2012) or neighbors may not interact at all if receivers miss information as a result of frequency masking, signal attenuation, or a combination of both. Anthropogenic noise therefore may fundamentally alter the structure of social interactions within signaling networks, although impacts on noise on signaling networks remain largely unexplored.

The extent to which environmental constraints combined with social context affects patterns of vocal interactions in signaling networks remains poorly known. Within signaling networks, vocal coordination, or patterns of signal overlap and alternation between males (Masco et al. 2016, Araya-Salas et al. 2017, Fernandez et al. 2017), as well as total song output in response to simulated intruders can be used to disentangle the nature of social relationships (McGregor and Peake 2000, Fitzsimmons et al. 2008, Foote et al. 2008, Amy et al. 2010, Snijders and Naguib 2017). Patterns of vocal coordination among groups of males depend on spatial proximity to other callers

(Araya-Salas et al. 2017), but are also influenced by social conditions, such as group size and presence of females (Fernandez et al. 2017). Perceived aggression levels and spatial proximity to simulated intruders affects the singing behavior of neighbors; male black-capped chickadees (*Poecile atricapillus*) breeding near two simulated intruders (i.e. two strangers) increase song output, singing at higher song rates (Fitzsimmons et al. 2008) for longer periods of time (Foote et al. 2011) than those breeding further away. Thus, spatial proximity to other callers and differences in social context may influence patterns of signaling behavior and vocal coordination.

Among individuals, information sharing may be limited by spatial proximity to other signalers and the degree of signal masking due to the level of ambient noise in the environment (Snijders and Naguib 2017). Noise may disrupt communication by affecting interactions with mates, neighbors, or intruder detection. Masked advertisement signals may result in delayed pairing (Habib et al. 2007, Gross et al. 2010) or failure to attract a mate (Bee and Swanson 2007). Signal degradation due to noise may decrease the ability of females to recognize breeding partners based on songs (Swaddle and Page 2007), which could affect within-pair interactions. Interactions between territorial males may be affected if noise masking delays intruder detection (Kleist et al. 2016) or alters male responses to intruders (Zwart et al. 2016, Phillips and Derryberry 2018). While dyadic vocal relationships uncover important behavioral interactions, realistically all individuals breeding within a signaling network may hear and respond to each other (McGregor 2005). Therefore, measuring change in patterns of signaling interactions among multiple callers is crucial for revealing the underlying structure of relationships in a social network context and can lead to a better understanding of the consequences of

anthropogenic noise on social interactions between mates and neighbors (McGregor and Horn 2015).

We tested the hypothesis that social context and environmental conditions affect signaling interactions and vocal coordination among territorial male house wrens (*Troglodytes aedon*). To test our hypothesis, we used playbacks to simulate territorial intrusions with and without noise, as well as to noise alone, on territories of established males and simultaneously recorded singing behavior of neighbors. We predicted that focal males would adjust their singing behavior in response to simulated intruders, that detectability of these changes by neighbors would vary based on ambient noise and spatial proximity to simulated intruders, and as a result, neighbors would also change their singing behavior. Therefore, we measured male singing rate, duration of bouts of singing, number of songs per bout and estimated vocal coordination by determining the probability of overlap or alternation between focal and neighboring males during treatments. That is, we assess the temporal nature of signals and signaling interactions patterns in response to a simulated intruder, not information contained within the focal males' signal itself (e.g. aggressive intent or male quality) in order to identify the underlying structure of social relationships between focal males and neighbors. We analyzed whether treatments changed the behavior of focal males, and then asked whether changes in focal male signaling altered the singing behavior of neighbors. We factored in noise levels at nest boxes of neighbors to determine whether background noise constrained vocal interactions within signaling networks and measured the distance between focal male and neighbor territories.

Male house wrens sing throughout the breeding season (Johnson and Kermott 1991, Rendall and Kaluthota 2013), but total song output varies with different stages of breeding (Tove 1988, Johnson and Kermott 1991, Rendall and Kaluthota 2013, Grabarczyk et al. 2018). Males sing to attract primary breeding partners (Johnson and Searcy 1996) and once paired direct songs towards mates and neighbors (Johnson and Kermott 1991, Johnson et al. 2002, LaBarbera et al. 2010). Both social context and environmental conditions affect the structure of male songs; in response to anthropogenic noise playbacks, paired males increase the peak frequency of their songs, whereas unpaired males do not (Grabarczyk et al. 2018). In some species, low frequency songs are preferred by female breeding partners (Halfwerk et al. 2011). In addition, male house wrens increase song frequency during territorial interactions, indicating that this trait signals aggression in some contexts (Grabarczyk and Gill, in review). Thus, change in ambient noise affects short-term signal structure of male song, whereas the social environment could have immediate and longer term effects on patterns of singing.

Methods

Study sites and species

We monitored a color-banded population of house wrens breeding in nest boxes at three natural areas in Kalamazoo County, Michigan, USA (42.290 N, 85.586 W). Prior to their arrival on the breeding grounds, we arranged nest boxes (N = 96) into 16 networks, each containing six nest boxes in areas of open habitat near a forest edge.

Within networks, boxes were arranged into hexagons, placing adjacent boxes at a 90°

angle and separating them by 45-50m. Networks were separated by at least 150m to

minimize vocal interactions between networks. With this arrangement, high-amplitude

song sections could transmit more than 50m into the territory of neighboring males

under low ambient noise conditions, whereas under high ambient noise conditions, the

same signals would not transmit outside a male's own territory (Grabarczyk and Gill

2019). Networks varied in spatial proximity to anthropogenic noise sources such as

local roads and highways (Grabarczyk and Gill 2019), and therefore differed in ambient

noise levels.

Field recordings and playback experiment

We ran playback experiments between June 6 – July 21, 2016, and April 28 –

July 2, 2017, on 18 focal territories while simultaneously recording all males breeding in

each network (N = 43 neighbors breeding within 100m). Each network was tested at

most once per year. In networks occupied by two or more males, we placed Wildlife

Acoustics Sound Meter 2 units (SM2; Maynard, MA, 44.1kHz sample rate, 16-bit, .wav

format) at each active nest box. Males were recorded at their nest boxes by attaching to

the nest box pole a microphone that was connected to SM2 units with a 3, 10, or 50m

cord. Units were pre-programmed to begin recording 1h before to 4 h after sunrise

(Eastern Standard Time, EST) in 30-min recording increments. Experiments took place

on days with minimal wind speed and no precipitation and follow the same procedure

used by Grabarczyk and Gill (in review). Briefly, we created playbacks from recordings

of male house wrens breeding in southwest Michigan during 2015-2016. Males sing

with eventual variety, repeating the same terminal section several times before

gradually transitioning to a new song type (Kroodsma 1977, Rendall and Kaluthota

2013). Therefore, to mimic natural singing patterns, a single song type was repeated

every 15-sec for 10-min, similar to natural rates of singing (Grabarczyk and Gill, in

review). For noise playbacks, we created a pink noise signal in Avisoft SASLab Pro v5.2

(R. Specht, Berlin, Germany). Pink noise is continuous noise that has more energy at

low frequencies (0-2 kHz), similar to anthropogenic noise (44.1 kHz sample frequency,

lowpass 1/f, frequency cut off at 0.20 Hz).

Each focal male received three consecutive treatments; intruder only, intruder

plus noise, and noise only. We randomly selected the order of treatments. Each

stimulus consisted a 10-min control period, a 10-min playback, followed by a 10-min

break before starting the next control period. To simulate a territorial intruder, an

amplified SME-AFS speaker (Saul Mineroff Electronics, New York) was placed 5 – 10m

from the focal male's nest box. We broadcast noise from a second speaker that was

placed 10m in the opposite direction. Both pink noise and simulated intruder playback

were broadcast at 76 dBA measured with a SPL meter at 1 m, fast averaging (American

Recorder Technology SPL-8810).

Acoustic analysis

Because network recordings during trials were made on two to six SM2 units with

time manually set, we needed to ensure that the relative time differences between units

was accounted for before extracting data. Therefore, after playback experiments, units

were removed from the field and synchronized. To synchronize recordings, we set all units side-by-side, programmed them to start at the same time and record for 5 min during which we played pre-recorded house wren song through an iPhone. From recordings, we determined the time offset between units, which ranged from a few seconds to several minutes, and adjusted the start of treatments during our field experiments within 0.01 ms accordingly. In Avisoft, we inserted point labels indicating the start and end of each 10-min experimental period on all recordings (i.e. before, during, and after each of the 3 treatments).

House wren song consists of two parts, a low-amplitude introduction section that is structured for short-distance transmission, followed by the high-amplitude terminal section capable of transmitting beyond the typical boundaries of a male's territory under low-noise conditions (Grabarczyk and Gill 2019). On recordings, we marked the terminal section of all songs recorded for each male with section labels (N = 17,231 songs). We defined the control period as the first 10-min of recording, prior to treatment stimuli. Across treatments and the control period, we analyzed 7,588 songs that were recorded during the control and treatments, excluding post playback periods (during the control, mean ± SD: 20.3 ± 17.9 songs per male; during treatments, mean ± SD: 34.0 ± 29.9 songs per male).

We predicted that focal males would alter their singing behavior in response to a simulated intrusion, that songs changes would be detectable to neighbors, and as a result that neighbors would also change their singing behavior. Therefore, to quantify signaling behavior and interactions among territorial males, we measured rate of

singing, the duration of bouts of singing, the number of songs per bout sung, and the

total number of bouts during each treatment and the control for focal and neighboring

males. To define song bouts, we measured the inter-song duration (time (s) from the

end of a song until the beginning of the next song) for all non-focal males during the

control period. We next calculated the average inter-song duration based on

all sequential songs sung with gaps in singing no longer than one minute (mean ± SD:

6.8 ± 7.5 sec) and defined a single bout as all songs falling within the mean plus two

standard deviations (21.8 sec) of this average and consisting of at least three

consecutive songs. Then, for all males within networks, we determined the number of

bouts sung, bout duration, and number of songs per bout during each treatment and the

control.

To explore vocal coordination between focal males and each of their neighbors,

we approximated the probability of song overlaps and alternation between pairs of

males. From Avisoft, we exported label metadata, including the start and end time of

each male song that was used for calculation of coordination estimates. We used the

coor.test function in warbleR (Araya-Salas and Smith-Vidaurre 2017) in R program

software v.3.3.3 (R Core Development Team). This function uses Monte Carlo

randomization tests to determine whether males overlap or alternate songs more than

what is expected by chance. Pairs of males breeding in the same network were

included in analysis if males each sang more than 20 songs during each 10-min

treatment. We reasoned that if males sang less than 20 songs during a 10-min time

period, we could not be sure whether they were countersinging with focal males,

eavesdropping on them, or they were simply not present on their territories during treatments.

Quantifying environmental variables

Males breeding in the same network may experience different noise conditions at any given moment, as ambient noise varies over space and time (Gill et al. 2015). We wanted to assess the influence of noise an signaling interactions and assumed that ambient noise on focal and neighboring male territories would affect responses. Therefore, we quantified ambient noise on each male's territory during treatment and control periods. We used a Larson Davis CAL 200 sound level calibrator (Depew, NY) to calibrate SM2 unit and microphone pairs by recording a 1-kHz 94 dB tone. Using the Calibration function in Avisoft, we set the recording amplitude to 0 dB (re 20 µPa) based on the recorded calibration tone. The frequency at which ambient sounds occur may affect the transmission of signals, therefore, we explore ambient noise occurring in two frequency bands: 1) sounds associated with low frequency anthropogenic noise (0-2 kHz) and 2) sounds that completely overlap or mask male house wren songs (1.2-7.6 kHz; Grabarczyk and Gill 2019). To determine average ambient noise levels, we randomly selected five 1-sec samples from each 10-min experimental period and the control. We used the automated parameter tool in Avisoft to extract amplitudes (rms, dB) for each 1-sec noise sample from the two frequency bandwidths and calculated a log-average for each treatment and the control.

Signals degrade as they travel over space, therefore the distance between focal

and neighboring male territories may affect signal detectability. To determine the

location of each nest box, we used a Garmin handheld unit (GPSmap 60CSx) to

collected GPS locations ($\pm$ 3 m accuracy). To estimate the distance between focal and

neighboring male territories we used ArcGIS 10.5 software (ESRI Redlands, California, USA).

Nesting stage

Throughout the breeding season nest boxes were checked every three days and

from this record we defined social context as the nesting stage of males. We grouped

stages into three main categories based on typical singing behavior of male house

wrens during each nesting stage. Both paired and unpaired males prior to clutch

initiation sing long songs at high rates (Johnson and Kermott, 1991, Rendall and

Kaluthota 2013, Grabarczyk et al. 2018) and were categorized as early stage. Males

decrease singing rates as females approach clutch initiation, and almost cease singing

entirely during the period when females are laying (Johnson and Kermott 1991, EEG

personal observation). Thus, we categorized as 'laying' males that were recorded on a

day when their partner was laying (mean $\pm$ SD eggs in this population: 5.9 $\pm$ 1.0; EEG,

unpublished data). After egg-laying, singing behavior is more variable (Johnson and

Kermott 1991, Rendall and Kaluthota 2013), but in general males increase song output

during incubation and nestling stages, and were thus categorized as late stage.

Statistical analysis

Do treatments affect focal male singing behavior? We first determined whether

focal males changed their behavior in response to treatments. In R program software,

we used a general linear mixed effect model to test whether treatments and sequence

of playback presentation affected focal male rate of singing. Because we presented

consecutive treatments on the same day, an earlier treatment could affect male

responses during later treatments, resulting in a carry-over effect. For model testing, we

included treatment and the sequence in which treatments were presented as fixed

effects and male identity as a random effect. We used lme4 (Bates et al. 2015) for

model testing and used a Satterthwaite approximation to generate p-values with the

package lmerTest (Kuznetsova et al. 2017). We assessed model adequacy with

residual plots and log transformed response variables if residual plots indicated

heteroscedasticity (Zuur et al. 2010). We used the package lsmeans (Lenth 2016) to run

pairwise comparisons to determine whether male responses differed by fixed effects.

Vocal responses of neighboring males. We used general linear mixed effects

models to test whether neighboring males adjusted their rate of singing, number of

songs per bouts, and length of song bouts in response to changes in the focal male

vocal behavior due to a simulated intruder. We also explored whether dyadic vocal

interactions of neighbors and focal males resulted in vocal overlap, alternation, or if

neighbors were uncoordinated in their singing behavior. We separately fit models

exploring effects of anthropogenic and masking noise on neighbor responses. For

models, we included nesting stage (3 levels; early, laying, and late), distance to focal

male (m), ambient noise (dB, rms), and focal male song rate (songs/min), and a three-

way interaction between distance to focal male, ambient noise, and treatment as fixed

effects and male identity nested within network identity as a random effect. We

compared model fit with and without interaction terms using AICc values and ΔAICc.

Models with a change in AICc of 2 or less were selected for analysis (Burnham and

Anderson 2004).

Results

       Focal males adjust singing in response to treatments

       We first tested whether treatments affected the behavior of focal males and

whether successive treatments resulted in a carry-over effect. Sequence of treatment

presentation was a significant predictor of focal male vocal response. Males increased

their rate of singing with each consecutive intruder treatment and continued singing at

high rates after simulated intruder playbacks ended (data not shown). Therefore, for all

analyses, we report model results that included only the first treatment presented to

each network (Table 6.1).

       Focal males increased song rates in response to intruders, regardless of noise

treatment (Estimate ± SD, intruder only: 3.3 ± 1.0, t=3.4, p = 0.003, N = 7; intruder +

noise: 3.8 ± 0.9, t=4.0, p = 0.0005, N = 8), but rate of singing during the noise-only

treatment did not differ from control periods (noise: 0.3 ± 1.4, t=0.2, p = 0.8, N = 3;

Figure 6.1A; Table 6.1). Because noise playback did not change the rate of singing by

focal males, we did not expect to detect any changes in singing behavior of neighboring males during noise-only treatments. Therefore, we omitted noise treatment and focused analysis on the first presentation of intruder trials only, considering responses of focal and neighbor males to presentation of intruder playbacks with and without noise (N = 9 networks that received intruder only, N = 9 networks with intruder and noise treatment, Table 6.2). Focal males produced longer bouts with more songs in response to the intruder-only treatment compared to the control, whereas in response to the intruder plus noise treatment male responses did not differ from either intruder-only or the control (Figure 6.1 B-C).

Nesting stage predicts signaling behavior in neighboring males

Although playback treatments altered singing behavior on focal male territories, they did not affect the duration of song bouts or the number of songs per bout by neighboring males (Table 6.3 and 6.4). The singing rate of neighbors during treatments did not differ from the control (Table 6.3 and 6.4). Whereas ambient noise levels experienced by the neighbor males did not affect vocal responses of neighboring males during simulated intruder treatments on focal male territories, distance to focal male territories did, as neighbor males increased singing rates with increasing distance to focal territories (Figure 6.2B).

Instead, singing behavior of neighbor males was predicted by their own social context. Neighbors sang at higher rates during early stages of nesting (Figure 6.2A; Table 6.3 and 6.4). Neighbors sang longer bouts with more songs per bout during early

and late nesting stages compared to males recorded when their mates were laying (Table 6.3 and 6.4). We found no significant predictors of vocal coordination between focal and neighboring males (Table 6.2), suggesting that the timing of vocal signals between pairs of males is uncoordinated.

Discussion

We explored whether patterns of signaling interactions among territorial male house wrens in response to a simulated intruder on an established male's territory varied with social context or environmental conditions. Focal males increased their rate of singing in response to an intruder with and without noise playbacks. Focal males sang longer bouts with more songs per bout in response to the intruder-only treatment compared to the control, whereas the response to the intruder plus noise treatments focal male singing patterns was intermediate between intruder-only treatment and the control. The singing behavior of neighboring males was predicted by nesting stage, as males sang at higher rates during early nesting stages compared to males during later and laying stages. Males during early and late nesting stages sang longer song bouts with more songs per bout, but treatment, ambient noise, and spatial proximity to focal males had no effect on song bouts. We found no evidence of vocal coordination, measured as the probability of song overlap or alternation between focal males and neighbors. Thus, noise dampens the vocal responses of focal males to simulated intruders, but did not influence singing by neighbors. Moreover, ambient noise on

neighboring territories did not influence singing by neighbors. Instead, social context, specifically the male's own nesting stage, predicted singing by neighboring males.

Focal males adjusted their singing behavior in response to simulated intruders, but noise-only treatments did not affect singing patterns. Males responses to the intruder-only treatment differed from the control, but responses to the intruder plus noise treatment did not differ from either the intruder-only treatment or the control. In another study on the same population of house wrens, males increased their rate of singing by approximately 4 songs/min during simulated intrusions with noise (Grabarczyk and Gill, in review), similar to our findings in this study. Males also increased the number of attacks on the playback speaker, possibly because they were unable to assess the threat imposed by intruders due to noise masking (Grabarczyk and Gill, in review). In this study, we did not analyze the number of attacks on the speaker by focal males, as non-vocal behaviors of focal males are unlikely to affect the behavior neighboring males. In the present study, male responses during the intruder plus noise treatment did not differ from the control, suggesting that noise may dampen male responses. This may be due to males interrupting their singing to attack the speaker.

Despite evidence that focal males adjusted their songs in response to intruder treatments, we did not find evidence that neighboring males also adjust their vocal responses according to change in focal male song bout patterns. Neighbor males did not coordinate their singing with focal males, nor did they adjust other aspects of signaling. To date, evidence of vocal coordination among groups of birds is limited to species in which inter-individual spacing is considerably lower than in the present study.

Lekking male long-billed hermits (*Phaethornis longirostris*) alternate songs during bouts of coordinated singing with other males that are advertising in close proximity, but the probability of overlap increases with increasing distance between males (Araya-Salas et al. 2017). Despite patterns of alternation, not all song bouts were between pairs of hermits were coordinated, and evidence suggests that males actively adjust the timing of songs during less than 50% of recorded bouts (Araya-Salas et al. 2017). Captive non-breeding zebra finches (*Taeniopygia guttata*) coordinate bouts of singing depending on the size of the group test and pairing status (Fernandez et al. 2017), suggesting that social factors play a key role in patterns of vocal behavior. In both studies, all males were tested during the same breeding stage (Araya-Salas et al. 2017; Fernandez et al. 2017), whereas in this study, we measured males across breeding stages and found that breeding stage predicts neighboring male vocal behavior. Male house wrens did not adjust their behavior during the short duration of our treatments, regardless of environmental constraints. Patterns of vocal coordination may only occur during early stages of breeding. For example, males may to attend to the temporal patterns of other males and adjust their own singing patterns while they are advertising for mates, as females may compare vocalizations between males when selecting a breeding partner (Mennill et al. 2002), but additional energy expenditure to maintain coordinated song patterns with neighbors may not benefit males once paired. Moreover, given that change in social and environmental conditions vary across different time scales, the temporal window that we consider in this study, blocks of 10-min periods of singing, may be too small to detect vocal coordination, as evidence of coordination

patterns have been found among groups of birds recorded over several hours and days (Taff et al. 2014, Araya-Salas et al. 2017, Fernandez et al. 2017).

Although neighbor males did not adjust their vocal behavior, they may instead alter their movements and use of their territories during intrusions on neighboring territories (Naguib et al. 2004, Amy et al. 2010, Snijders et al. 2017). Depending on the strength of focal male responses, neighboring males may move away from shared territory boundary, avoiding costly physical interactions with a male that has recently protected his territory. Neighboring male great tits respond to intrusions on focal male territories by moving away from the simulated intrusion, and stronger responses from focal males resulted in stronger repulsion behaviors by neighbors (Snijders et al. 2017). Spatial responses may also depend on personality type, male great tits with high exploration scores respond more quickly to simulated intruders, and the responses of neighbors depends on the personality type of the territorial male experiencing an intrusion (Amy et al. 2010). Alternatively, neighboring male house wrens may not have responded vocally, if males were positioned outside the range of focal male signal active space. Of all neighboring males tested (N = 43), only 15 were breeding in nest boxes directly adjacent to the focal male (i.e. within 50 m). Under high levels of ambient noise, male house wren songs undergo significant degradation, falling below thresholds for detection and discrimination within a males own territory (Grabarczyk and Gill 2019). Therefore, we may not have detected differences in the singing patterns of neighbors, because they were simply too far away to detect simulated intruders.

Anthropogenic noise affects the structure of songs in male house wrens, but social factors play an important role male singing behavior more generally. In response to heterogeneity in the ambient noise environment, male house wrens adjust their signaling behavior (Grabarczyk et al. 2018, in review), but variation in individual song changes depends on social factors, such as pairing status (Grabarczyk et al. 2018), the density of conspecific neighbors, and female fertile status (Grabarczyk et al. in prep). Social factors, including breeding stage and conspecific male density, predict the onset of dawn song, yet anthropogenic noise and artificial light had no influence on the timing of dawn chorus (Stuart et al. 2019). In this species, environmental conditions influence song structure in the moments before signaling, but social factors sometimes have a larger impact on singing behavior, indicating complex interactions among factors.

Anthropogenic noise masks vocal signals birds use for mate attraction and territory defense. Despite evidence that males adjust their signaling behavior in response to noise (Brumm and Zollinger 2013), much less is known regarding perception of signals in noise and whether masking affects singing behavior of among groups of callers. In this study, we considered both environmental and social conditions and found that added noise in the territories of the focal males affected their responses to simulated intruders, but altered singing of focal males didn't translate into changes in singing by neighboring males. Instead, social context predicts singing patterns in neighbors. Males do not immediately alter singing in response to intrusions on neighboring territories, but they might change singing patterns instead in the days following an intrusion (Foote et al. 2011, Schmidt et al. 2007). Thus, environmental

constraints on signal detection may affect neighbor responses, but changes in singing

behavior may be detected only over longer periods of time.

Literature cited

Amy, M., P. Sprau, P. de Goede, and M. Naguib. 2010. Effects of personality on territory defence in communication networks: a playback experiment with radio-tagged great tits. Proceedings of the Royal Society B-Biological Sciences **277**:3685-3692.

Araya-Salas, M., and G. Smith-Vidaurre. 2017. warbleR: an r package to streamline analysis of animal acoustic signals. Methods in Ecology and Evolution **8**:184-191.

Araya-Salas, M., K. Wojczulanis-Jakubas, E. M. Phillips, D. J. Mennill, and T. F. Wright. 2017. To overlap or not to overlap: context-dependent coordinated singing in lekking long-billed hermits. Animal Behaviour **124**:57-64.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. . Journal of Statistical Software **67**:1-48.

Bee, M. A., and E. M. Swanson. 2007. Auditory masking of anuran advertisement calls by road traffic noise. Animal Behaviour **74**:1765-1776.

Bradbury, J. W., and S. L. Vehrencamp. 1998. Principles of Animal Communication. 1 edition. Sinauer Associates, Sunderland, MA.

Brumm, H., and S. A. Zollinger. 2013. Avian vocal production in noise. Pages 187-227 *in* H. Brumm, editor. Animal communicaiton in noise. Springer Berlin Heidelberg, Seewiesen, Germany.

Burnham, K. P., and D. R. Anderson. 2004. Model Selection and Multimodel Inference. A Practical Information-Theoretic Approach. Springer Science & Business Media.

Fernandez, M. S. A., C. Vignal, and H. A. Soula. 2017. Impact of group size and social composition on group vocal activity and acoustic network in a social songbird. Animal Behaviour **127**:163-178.

Fitzsimmons, L. P., J. R. Foote, L. M. Ratcliffe, and D. J. Mennill. 2008. Eavesdropping and communication networks revealed through playback and an acoustic location system. Behavioral Ecology **19**:824-829.

Foote, J. R., L. P. Fitzsimmons, D. J. Mennill, and L. M. Ratcliffe. 2008. Male chickadees match neighbors interactively at dawn: support for the social dynamics hypothesis. Behavioral Ecology **19**:1192-1199.

Foote, J. R., L. P. Fitzsimmons, D. J. Mennill, and L. M. Ratcliffe. 2011. Male black-capped chickadees begin dawn chorusing earlier in response to simulated territorial insertions. Animal Behaviour **81**:871-877.

Gill, S. A., J. R. Job, K. Myers, K. Naghshineh, and M. J. Vonhof. 2015. Toward a broader characterization of anthropogenic noise and its effects on wildlife. Behavioral Ecology **26**:328-333.

Grabarczyk, E. E., and S. A. Gill. 2019. Anthropogenic noise masking diminishes house wren (*Troglodytes aedon*) song transmission in urban natrual areas. Bioacoustics.

Grabarczyk, E. E., M. A. Pipkin, M. J. Vonhof, and S. A. Gill. 2018. When to change your tune? Unpaired and paired male house wrens respond differently to anthropogenic noise. Journal of Ecoacoustics **2**:#LHGRVC.

Gross, K., G. Pasinelli, and H. P. Kunc. 2010. Behavioral Plasticity Allows Short-Term Adjustment to a Novel Environment. American Naturalist **176**:456-464.

Habib, L., E. M. Bayne, and S. Boutin. 2007. Chronic industrial noise affects pairing success and age structure of ovenbirds *Seiurus aurocapilla*. Journal of Applied Ecology **44**:176-184.

Halfwerk, W., S. Bot, J. Buikx, M. van der Velde, J. Komdeur, C. ten Cate, and H. Slabbekoorn. 2011. Low-frequency songs lose their potency in noisy urban conditions. Proceedings of the National Academy of Sciences of the United States of America **108**:14549-14554.

Johnson, L. S., B. G. Hicks, and B. S. Masters. 2002. Increased cuckoldry as a cost of breeding late for male house wrens (*Troglodytes aedon*). Behavioral Ecology **13**:670-675.

Johnson, L. S., and L. H. Kermott. 1991. The Functions of Song in Male House Wrens (*Troglodytes aedon*). Behaviour **116**:190-209.

Johnson, L. S., and W. A. Searcy. 1996. Female attraction to male song in house wrens (*Troglodytes aedon*). Behaviour **133**:357-366.

Kleist, N. J., R. P. Guralnick, A. Cruz, and C. D. Francis. 2016. Anthropogenic noise weakens territorial response to intruder's songs. Ecosphere **7**.

Klump, G. M. 1996. Bird communication in the noisy world. Cornell University Press, Ithaca, NY.

Kroodsma, D. E. 1977. Correlates of Song Organization Among North American Wrens. American Naturalist **111**:995-1008.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. Journal of Statistical Software **82**:1-26.

LaBarbera, K., P. E. Llambias, E. R. A. Cramer, T. D. Schaming, and I. J. Lovette. 2010. Synchrony does not explain extrapair paternity rate variation in northern or southern house wrens. Behavioral Ecology **21**:773-780.

Lenth, R. V. 2016. Least-Squares Means: The R Package lsmeans. Journal of Statistical Software **69**:1-33.

Marten, K., and P. Marler. 1977. Sound Transmission and Its Significane for Animal Vocalization: I. Temperate Habitats. Behavioral Ecology and Sociobiology **2**:271-290.

Masco, C., S. Allesina, D. J. Mennill, and S. Pruett-Jones. 2016. The Song Overlap Null model Generator (SONG): a new tool for distinguishing between random and non- random song overlap. Bioacoustics **25**:29-40.

McGregor, P. K. 2005. Animal communication networks. Cambridge University Press, Cambridge, UK.

McGregor, P. K., and T. Dabelsteen. 1996. Communication Networks.*in* D. E. Kroodsma and E. H. Miller, editors. Ecology and Evolution of Acoustic Communication in Birds. Cornell University Press, Ithaca, New York.

McGregor, P. K., and A. G. Horn. 2015. Communication and Social Networks.*in* J. Krause, R. James, D. W. Franks, and D. P. Croft, editors. Animal Social Networks. Oxford University Press, New York, NY.

McGregor, P. K., and T. M. Peake. 2000. Communication networks: social envionments for receiving and signalling behavior. Acta Ethologica **2**:71-81.

Mennill, D. J., L. M. Ratcliffe, and P. T. Boag. 2002. Female eavesdropping on male song contests in songbirds. Science **296**:873-873.

Morton, E. S. 1975. Ecological sources of selection on avian sounds. American Naturalist **109**:17-34.

Naguib, M., V. Amrhein, and H. P. Kunc. 2004. Effects of territorial intrusions on eavesdropping neighbors: communication networks in nightingales. Behavioral Ecology **15**:1011-1015.

Otter, K., P. K. McGregor, A. M. R. Terry, F. R. L. Burford, T. M. Peake, and T. Dabelsteen. 1999. Do female great tits (*Parus major*) assess males by eavesdropping? A field study using interactive song playback. Proceedings of the Royal Society B-Biological Sciences **266**:1305-1309.

Owens, J. L., C. L. Stec, and A. O'Hatnick. 2012. The effects of extended exposure to traffic noise on parid social and risk-taking behavior. Behavioural Processes **91**:61-69.

Patricelli, G. L., and J. L. Blickley. 2006. Avian communication in urban noise: Causes and consequences of vocal adjustment. Auk **123**:639-649.

Peake, T. M., P. K. McGregor, and T. Dabelsteen. 2005. Song type matching, song type switching and eavesdropping in male great tits. Animal Behaviour **69**:1063-1068.

Peake, T. M., A. M. R. Terry, P. K. McGregor, and T. Dabelsteen. 2002. Do great tits assess rivals by combining direct experience with information gathered by easvedropping? Proceedings of the Royal Society B-Biological Sciences **269**:1925-1929.

Phillips, J. N., and E. P. Derryberry. 2018. Urban sparrows respond to a sexually selected trait with increased aggression in noise. Scientific Reports **8**.

Rendall, D., and C. D. Kaluthota. 2013. Song organization and variability in northern House wrens (*Troglodytes aedon parkmanii*) in Western Canada. Auk **130**:617-628.

Schmidt, R., V. Amrhein, H. P. Kunc, and M. Naguib. 2007. The day after: effects of vocal interactions on territory defence in nightingales. Journal of Animal Ecology **76**:168-173.

Snijders, L., and M. Naguib. 2017. Communication in Animal Social Networks: A Missing Link? Pages 297-359 *in* M. Naguib, J. Podos, L. W. Simmons, L. Barrett, S. D. Healy, and M. Zuk, editors. Advances in the Study of Behavior, Vol 49.

Snijders, L., K. van Oers, and M. Naguib. 2017. Sex-specific responses to territorial intrusions in a communication network: Evidence from radio-tagged great tits. Ecology and Evolution **7**:918-927.

Stuart, C. J., Grabarczyk, E. E., Vonhof, M. J., and S. A. Gill. 2019. Social factors, not anthropogenic noise or sky glow, influence onset of dawn singing in a common songbird. Auk.

Swaddle, J. P., and L. C. Page. 2007. High levels of environmental noise erode pair preferences in zebra finches: implications for noise pollution. Animal Behaviour **74**:363-368.

Taff, C. C., G. L. Patricelli, and C. R. Freeman-Gallant. 2014. Fluctuations in neighbourhood fertility generate variable signalling effort. Proceedings of the Royal Society B-Biological Sciences **281**.

Tove, M. H. 1988. Patterns of singing by house wrens with respect to the breeding cycle Utah State University, Logan, UT.

Wiley, R. H., and D. G. Richards. 1978. Physical constraints on acoustic communication in the atmosphere: implications for the evolution of animal vocalisations. Behavioral Ecology and Sociobiology **3**:69-94.

Zuur, A. F., E. N. Ieno, and C. S. Elphick. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution **1**:3-14.

Zwart, M. C., J. C. Dunn, P. J. K. McGowan, and M. J. Whittingham. 2016. Wind farm noise suppresses territorial defense behavior in a songbird. Behavioral Ecology **27**:101-108.

Table 6.1 Results of linear mixed effects models testing the responses of focal male to the first treatments presented (intruder only N = 7, intruder + noise N = 8, noise only N = 3).

Analysis	Parameter	Estimate ± SE	$t_{(df)}$	p-value
Rate (songs/min)	Intercept	1.9 ± 0.5	$3.5_{(31)}$	0.001
	Treatment: noise	0.3 ± 1.4	$0.2_{(28)}$	0.8
	Treatment: intruder	3.3 ± 1.0	$3.4_{(23)}$	0.003
	Treatment: intruder + noise	3.8 ± 0.9	$4.0_{(22)}$	0.0005
Bout length (s)	Intercept	4.2 ± 0.2	$18.6_{(37)}$	<0.0001
	Treatment: noise	-0.1 ± 0.5	$-0.2_{(43)}$	0.8
	Treatment: intruder	0.8 ± 0.4	$2.0_{(53)}$	0.05
	Treatment: intruder + noise	0.7 ± 0.3	$2.0_{(53)}$	0.05
Number of songs per bout	Intercept	3.0 ± 0.1	$21.9_{(24)}$	<0.0001
	Treatment: noise	0.02 ± 0.3	$-0.1_{(50)}$	0.9
	Treatment: intruder	1.1 ± 0.2	$4.0_{(50)}$	<0.0001
	Treatment: intruder + noise	0.7 ± 0.1	$5.7_{(44)}$	<0.0001
Number of bouts	Intercept	2.4 ± 0.2	$10.9_{(35)}$	<0.0001
	Treatment: noise	-0.2 ± 0.5	$-0.3_{(42)}$	0.8
	Treatment: intruder	1.0 ± 0.4	$2.6_{(54)}$	0.01
	Treatment: intruder + noise	0.7 ± 0.3	$2.2_{(52)}$	0.03

Table 6.2 Results of linear mixed effects models testing the responses of focal males to the first intruder treatments (N = 9 intruder only, N = 9 intruder + noise).

Analysis	Parameter	Estimate ± SE	$t_{(df)}$	p-value
Rate (songs/min)	Intercept	1.9 ± 0.6	$3.1_{(33)}$	0.004
	Treatment: intruder	4.2 ± 1.1	$4.0_{(33)}$	0.0003
	Treatment: intruder + noise	3.6 ± 1.1	$3.4_{(33)}$	0.002
Bout length (s)	Intercept	4.3 ± 0.2	$18.7_{(37)}$	<0.0001
	Treatment: intruder	1.0 ± 0.4	$2.6_{(52)}$	0.01
	Treatment: intruder + noise	0.6 ± 0.3	$1.9_{(52)}$	0.06
Number of songs per bout	Intercept	2.3 ± 0.2	$9.7_{(35)}$	<0.0001
	Treatment: intruder	1.2 ± 0.4	$3.1_{(53)}$	0.003
	Treatment: intruder + noise	0.6 ± 0.3	$2.0_{(50)}$	0.05
Number of bouts	Intercept	1.0 ± 0.1	$14.8_{(26)}$	<0.0001
	Treatment: intruder	0.02 ± 0.1	$0.2_{(54)}$	0.8
	Treatment: intruder + noise	0.1 ± 0.1	$2.0_{(44)}$	0.05

Table 6.3 Results of linear mixed effects models testing the responses of neighboring males to first presentation of all treatments (intruder only, intruder + noise, noise only).

Analysis	Parameter	Estimate $\pm$ SE	$t_{(df)}$	p-value
Rate (songs/min)	Intercept	3.5 $\pm$ 0.5	7.4 $_{(28)}$	<0.0001
	Anthropogenic noise	-0.2 $\pm$ 0.3	-0.9$_{(63)}$	0.4
	Distance to focal male	0.4 $\pm$ 0.2	1.7$_{(29)}$	0.09
	Treatment: noise	-0.2 $\pm$ 0.6	-0.4$_{(49)}$	0.7
	Treatment: intruder	0.7 $\pm$ 0.4	1.7$_{(48)}$	0.1
	Treatment: intruder + noise	-0.04 $\pm$ 0.4	-0.1$_{(41)}$	0.9
	Stage: Late	-1.8 $\pm$ 0.5	-3.4$_{(41)}$	0.002
	Stage: Laying	-3.4 $\pm$ 0.7	-4.9$_{(40)}$	<0.0001
Bout length (s)	Intercept	4.8 $\pm$ 0.4	11.8$_{(48)}$	<0.0001
	Anthropogenic noise	-0.1 $\pm$ 0.1	-0.5$_{(34)}$	0.6
	Distance to focal male	-0.01 $\pm$ 0.004	-1.3$_{(70)}$	0.2
	Treatment: noise	-0.3 $\pm$ 0.3	-1.1$_{(104)}$	0.3
	Treatment: intruder	-0.1 $\pm$ 0.2	-0.3$_{(70)}$	0.9
	Treatment: intruder + noise	-0.02 $\pm$ 0.2	-0.1$_{(131)}$	0.9
	Stage: Late	-0.03 $\pm$ 0.2	-0.3$_{(39)}$	0.8
	Stage: Laying	-0.7 $\pm$ 0.3	-2.1$_{(61)}$	0.04
Number of songs per bout	Intercept	2.9 $\pm$ 0.4	7.7$_{(41)}$	<0.0001
	Anthropogenic noise	-0.1 $\pm$ 0.1	-0.7$_{(30)}$	0.5
	Distance to focal male	-0.005 $\pm$ 0.004	-1.2$_{(59)}$	0.2
	Treatment: noise	-0.3 $\pm$ 0.2	-1.3$_{(90)}$	0.2
	Treatment: intruder	-0.02 $\pm$ 0.2	-0.1$_{(33)}$	0.9
	Treatment: intruder + noise	0.04 $\pm$ 0.2	0.2$_{(131)}$	0.9
	Stage: Late	-0.2 $\pm$ 0.2	-0.9$_{(35)}$	0.4
	Stage: Laying	-0.8 $\pm$ 0.3	-2.5$_{(55)}$	0.02
Number of bouts	Intercept	0.2 $\pm$ 0.3	0.5$_{(54)}$	0.6
	Anthropogenic noise	-0.003 $\pm$ 01	-0.03$_{(40)}$	0.9
	Distance to focal male	0.007 $\pm$ 0.004	1.7$_{(57)}$	0.09
	Treatment: noise	0.02 $\pm$ 0.2	0.1$_{(55)}$	0.9
	Treatment: intruder	-0.1 $\pm$ 0.2	-0.5$_{(52)}$	0.8
	Treatment: intruder + noise	0.1 $\pm$ 0.2	0.5$_{(52)}$	0.6
	Stage: Late	-0.1 $\pm$ 0.2	0.6$_{(45)}$	0.6

	Stage: Laying	-0.3 ± 0.2	$-1.3_{(52)}$	0.2
Vocal coordination	Intercept	-0.1 ± 0.1	$-1.2_{(36)}$	0.2
	Anthropogenic noise	0.04 ± 0.06	$0.7_{(41)}$	0.5
	Distance to focal male	0.06 ± 0.06	$1.0_{(27)}$	0.3
	Treatment: intruder	0.2 ± 0.1	$1.4_{(58)}$	0.2
	Treatment: intruder + noise	-0.01 ± 0.1	$-0.1_{(57)}$	0.9
	Stage: Late	0.2 ± 0.1	$1.5_{(26)}$	0.1
	Stage: Laying	-0.2 ± 0.2	$-1.0_{(37)}$	0.3

Table 6.4 Results of linear mixed effects models testing the responses of neighboring

males to the first intruder treatments.

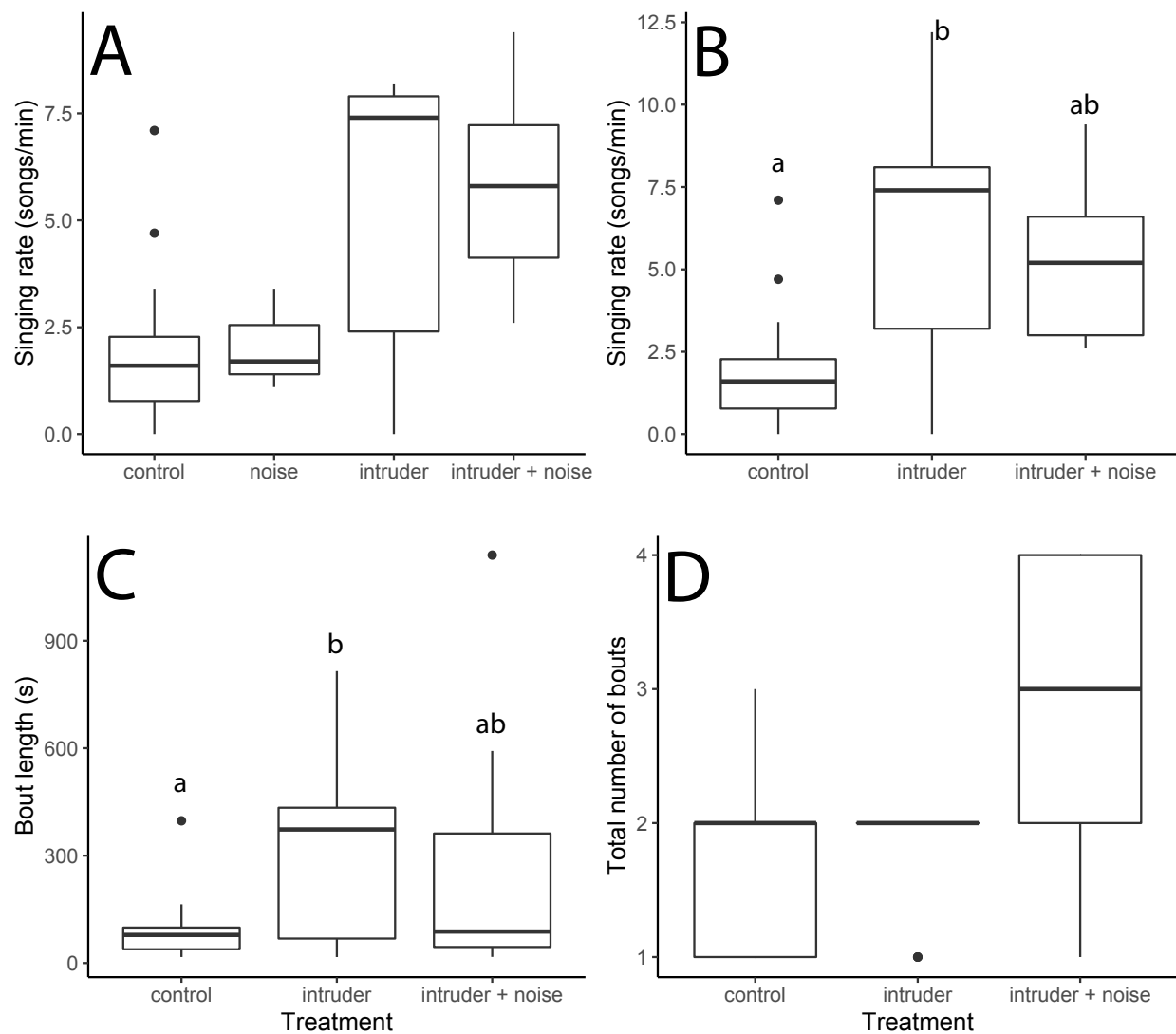| Analysis	Parameter	Estimate ± SE	$t_{(df)}$	p-value
Rate	Intercept	1.3 ± 0.1	$9.0_{(29)}$	<0.0001
(songs/min)	Anthropogenic noise	-0.08 ± 0.07	$-1.0_{(50)}$	0.3
	Distance to focal male	0.2 ± 0.07	$2.6_{(31)}$	0.01
	Treatment: intruder	0.004 ± 0.1	$0.03_{(44)}$	0.9
	Treatment: intruder + noise	-0.01 ± 0.1	$-0.09_{(43)}$	0.9
	Stage: Late	-0.5 ± 0.2	$-2.7_{(39)}$	0.009
	Stage: Laying	-0.9 ± 0.2	$-4.3_{(40)}$	<0.0001
Bout length (s)	Intercept	4.3 ± 0.1	$30.2_{(30)}$	<0.0001
	Anthropogenic noise	0.02 ± 0.09	$0.3_{(25)}$	0.8
	Distance to focal male	-0.1 ± 0.09	$-1.3_{(62)}$	0.2
	Treatment: intruder	-0.3 ± 0.2	$-1.5_{(94)}$	0.1
	Treatment: intruder + noise	-0.1 ± 0.2	$-0.7_{(132)}$	0.5
	Stage: Late	0.08 ± 0.2	$0.4_{(18)}$	0.7
	Stage: Laying	-0.7 ± 0.3	$-2.1_{(52)}$	0.04
Number of	Intercept	2.4 ± 0.1	$18.9_{(12)}$	<0.0001
songs per bout	Anthropogenic noise	0.01 ± 0.08	$0.2_{(20)}$	0.8
	Distance to focal male	-0.09 ± 0.08	$-1.1_{(54)}$	0.3
	Treatment: intruder	-0.3 ± 0.2	$-1.6_{(86)}$	0.1
	Treatment: intruder + noise	-0.1 ± 0.2	$-0.5_{(131)}$	0.6
	Stage: Late	-0.06 ± 0.2	$-0.3_{(15)}$	0.7
	Stage: Laying	-0.7 ± 0.3	$-2.4_{(46)}$	0.02
Number of	Intercept	0.7 ± 0.1	$5.0_{(19)}$	<0.0001
bouts	Anthropogenic noise	-0.1 ± 0.8	$-0.1_{(35)}$	0.9
	Distance to focal male	0.06 ± 0.08	$0.7_{(31)}$	0.5
	Treatment: intruder	0.1 ± 0.2	$0.6_{(40)}$	0.5
	Treatment: intruder + noise	-0.02 ± 0.2	$-0.1_{(40)}$	0.9
	Stage: Late	-0.2 ± 0.2	$-1.2_{(28)}$	0.2
	Stage: Laying	-0.4 ± 0.3	$-1.6_{(34)}$	0.1

Figure 6.1 Focal males increased the rate of singing in response to an intruder with and without additional noise playback (A-B), but rate of singing during the noise only treatment did not differ from the control. Focal males sang longer bouts with more songs per bout in response to the intruder only treatment (C), but the total number of bouts was not affected by treatment (D).
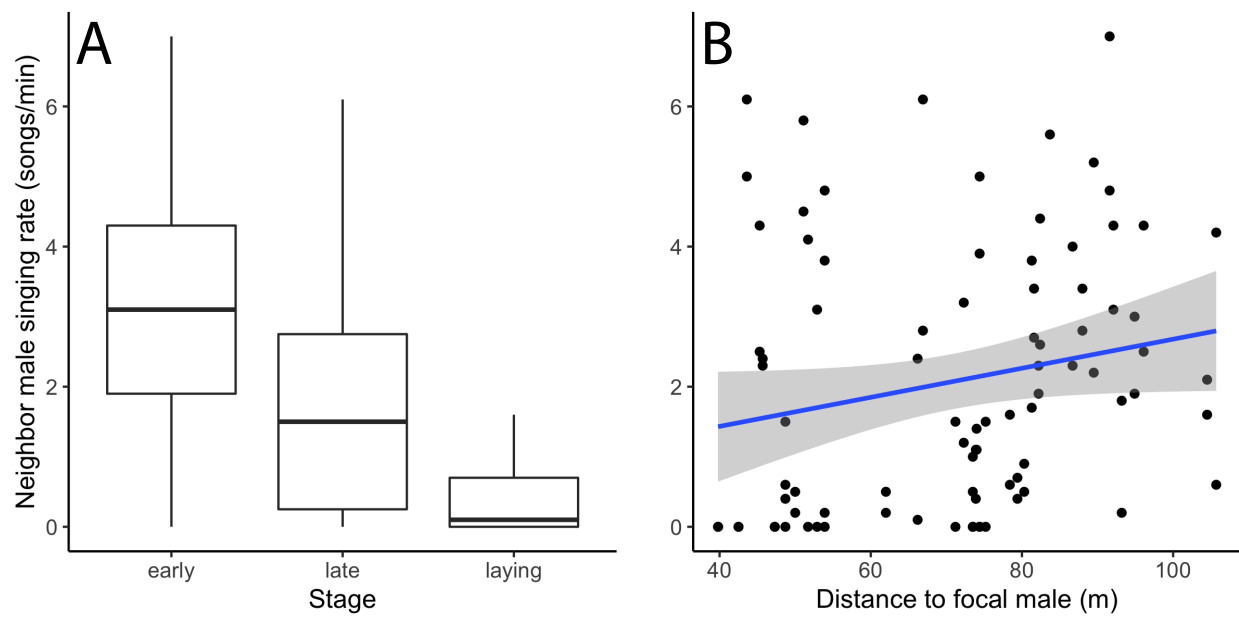
Figure 6.2 Neighboring male house wrens sang at the highest rate of singing during early breeding stages and the lowest rate of singing if their partner was laying (A). Males sang at higher song rates with increasing distance from the focal male (B).

Appendix

WESTERN MICHIGAN UNIVERSITY

Institutional Animal Care and Use Committee

ANNUAL REVIEW OF VERTEBRATE ANIMAL USE

PROJECT OR COURSE TITLE: Integrating Approaches From Behavior And Engineering To Explore How Male Songbirds Respond To Anthropogenic Noise

IACUC Protocol Number: 16-01-01
Date of Review Request: 01/18/18   Date of Last Approval: 12/22/16
Purpose of project (select one): ☐ Teaching   ☒ Research   ☐ Other (specify):

PRINCIPAL INVESTIGATOR OR ADVISOR
Name: Sharon Gill   Title: Associate Professor
Department: BIOS   Electronic Mail Address: sharon.gill@wmich.edu
CO-PRINCIPAL OR STUDENT INVESTIGATOR
Name: Maarten Vonhof   Title: Professor
Department: BIOS Electronic Mail Address: maarten.vonhof@wmich.edu

1. The research, as approved by the IACUC, is completed:
    ☐ Yes (Continue with items 4-5 below.)   ☒ No (Continue with items 2-5 below.)

If the answer to any of the following questions (items 2-4) is "Yes," please provide a detailed explanation on an attached sheet of paper. Include details of any modifications made to the protocol based on new findings or publications, adverse events or mortalities.

2. Have there been any changes in Principal or Co-Principal Investigators?   ☐ Yes   ☒ No
3. Have there been any new findings or publications relative to this research that require you to alter your study?   ☐ Yes   ☒ No
    Describe the sources used to determine the availability of new findings or publications:
    ☐ No search conducted (Please provide a justification on an attached sheet.)
    ☐ Animal Welfare Information Center (AWIC)
    ☒ Search of literature databases (select all applicable)
        ☐ AGRICOLA   ☐ Current Research Information Service (CRIS)
        ☐ Biological Abstracts   ☐ Medline
        ☒ Other (please specify): Web of Science, Google Scholar
        Date of search: ongoing   Years covered by the search:
        Key words: playback experiment, noise, birds, blood collection, blood sampling, fitness, reproductive succes, urban ecology, anthropogenic noise, avian blood parasites, avian microbiome
    ☐ Additional search strategy narrative:
4. Are there any adverse events, in terms of animal well-being, or mortalities to report as a result of this research?   ☐ Yes   ☒ No
    Cumulative number of mortalities:

5. Animal usage: Number of animals used during   Cumulative number of
                         this quarter (3 months):   animals used to date: 1300

✏ _____   1/30/18
   Principal Investigator/Faculty Advisor Signature   Date

✏ _____   01/20/2018
   Co-Principal or Student Investigator Signature   Date

IACUC REVIEW AND APPROVAL