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Why Sing so Many Songs? Testing the Function of Song Type Repertoires in Rock Wrens Using Playback Experiments and Behavioral Observations

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WHY SING SO MANY SONGS? TESTING THE FUNCTION OF SONG TYPE REPERTOIRES IN ROCK WRENS USING PLAYBACK EXPERIMENTS AND BEHAVIORAL OBSERVATIONS

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ABSTRACT

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Bird song has been studied extensively as a model of complex animal communication systems because of how various and flexible it is in certain species. Numerous researchers have investigated the evolutionary pressures leading to the elaboration of songs available in a bird's repertoire and this topic is most often evaluated in light of sexual selection. Intersexual selection mechanisms such as female choice are often presumed to be very important in the elaboration of song, however literature reviews on the topic find no strong correlation between female preference for larger song repertoires and the presence of the characteristic in males across all species. Another important aspect of sexual selection not often examined in regards to avian song elaboration is intrasexual selection mechanisms such as territory defense and within-sex competition. In no single study were both mechanisms of selection on a trait evaluated within the same individuals, though some studies have independently looked at both aspects in the same species. In this study I evaluated the function of song type repertoire size in a monomorphic species, the Rock Wren, in light of both inter- and intrasexual selection.

To test the function of song type repertoire in territory defense contexts, I performed playback experiments to focal male Rock Wrens and assessed their response patterns to both large and small repertoire sizes. Males responded more strongly to tracks playing large song repertoires by singing faster in response and singing more songs. They also sang shorter songs that had lower frequencies and broader bandwidth. To evaluate inter-sexual selection pressures on repertoire size, I followed males throughout the breeding season to assess various proxies of female choice, including first egg lay date, clutch size, fledge count, and male prey delivery rate. None of the measures of female choice that I evaluated were related to her mate’s repertoire size. I conclude that song repertoires must be more important for assessing rival males in territory defense scenarios than for assessing potential mates in this species. These results support intrasexual competition as an important mechanism of selection that can lead to the elaboration of communication signals in bird species, and demonstrate that elaboration of song is not necessarily driven by mate choice dynamics. Intrasexually selected increases in repertoire size provide an alternative mechanism to female choice generating complexity for animal signaling systems.

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TABLE OF CONTENTS

CHAPTER

APPENDICES

LIST OF TABLES

TABLE

LIST OF FIGURES

FIGURE

CHAPTER I

BACKGROUND AND LITERATURE REVIEW

Song Type Repertoires as Targets of Sexual Selection

Animal Communication and Complexity

Animal communication is the transfer of information using a signal from a sender that, when perceived, provides information about the sender or causes some kind of behavioral change (present or future) in the receiver (Bradbury and Vehrencamp 2011; Kaplan 2014). Animals display a wide range of communication signals for conveying information about themselves or their surroundings and also for expressing to others what has been perceived (such as a predator) (Hauser 1996). Signals are different from cues, which are properties of the outside world that are perceived and interpreted by an organism, and that correlate with conditions of interest providing information about physical, ecological, and social surroundings (Bradbury and Vehrencamp 2011). Perceiving cues, and eventually signals, is dependent on the organism's sensory system and it's sense organs. As such signals and cues are constrained by the physical medium and the vegetative density of the habitat in which an organism lives as well as its circadian rhythm and other factors (Bradbury and Vehrencamp 2011). As species evolved to perceive cues in different environments, with time they each began to manipulate some of those cues into communicatory signals to convey information to a receiver. Because of the variety of sensory systems that have evolved to perceive the world, a similar diversity of signals and signaling systems has evolved that takes advantage of the perception systems of the intended receivers (Bradbury and Vehrencamp 2011; Hauser 1996).

The earliest models of communication simplified this behavior as the transfer of signals containing encoded information from the sender to the receiver in order to trigger predetermined and rigid responses, but this is an oversimplification of the receiver's role in interpreting that signal as well as it's flexibility in response behavior (Shannon and Weaver 1964; Tinbergen 1951; Owren et al. 2010). More so, signals are used to convey (or not convey in the case of camouflage) information about a sender that is intended to cause a behavioral change or incite an action from one or several receivers (Dawkins and Krebs 1987). Signals can be differently manipulated by the sender (i.e. honest or dishonest signals) and responded to variably upon reception by the perceiver (Hauser 1996; Dawkins and Krebs 1987). Communication signals are therefore behaviors of senders that evolved to cause behavioral change and influence the actions of other individuals, rather than parcels of specific information transferred from sender to receiver (Owren et al. 2010). With this view of animal communication, senders are selected to produce signals that manipulate receiver behavior in a way that benefits themselves, while receivers are selected to only pay heed to signals that are to their own benefit (Bradbury and Vehrencamp 2011; Dawkins and Krebs 1987).

From this dynamic emerges a back and forth between senders and receivers that shapes communication signals to convey information optimally about the sender that is of interest to the receiver(s) and that successfully influences behavior in the desired way (Owren et al. 2010). Some organisms show a very restricted range of communicatory signals, while there are others that can convey and perceive a much wider array of signals (Hauser 1996). The more organisms interact in different social contexts with one or more other living things, the more important it becomes to have various communicative signals for use in those alternative situations (Kaplan 2014). Selective pressures acting on both genetic and phenotypic variation over time will favor individuals with characteristics that are more successful at causing the desired behavioral change in receivers (Bradbury and Vehrencamp 2011). Through the incremental process of cumulative selection, less complex traits can evolve into more complex traits through a series of small steps over evolutionary time (Bradbury and Vehrencamp 2011). Therefore, a simple communicative signal can increase in its complexity over time, given sufficiently strong selective pressures (Hauser 1996). This natural selection for increased signal variability is an important precursor to the evolution of complex communication systems, including our own human languages (Hauser 1996).

A critical first step to the evolution of complex communication systems is the establishment of basic communicative flexibility, or the ability to produce variable signals (including acoustic signals) and use them flexibly according to intent (Oller and Griebel 2014). Though Man is particularly adept at articulating language, Darwin in *The Descent of Man* (1871) proposed that man differs in his communication system from “lower animals” only in his seemingly infinite capacity to associate diverse sounds with abstract concepts and ideas, and is in fact not so different from other animals in the fundamentals of communication. Darwin postulated that human language originated as imitation and modification of natural sounds from the world and other animals, along with man’s own cries of pain, fear, surprise, and anger aided by signs and gestures (Hauser 1996). The same origins likely apply to animal signaling, and for this reason (and others) it is informative to study the level and use of complexity in other animal communication systems.

One of the most extensively studied forms of animal communication is avian song. This topic has been addressed at all four levels of Tinbergen's four questions: mechanistic, ontogenetic, functional, and phylogenetic perspectives (Hauser 1996; Tinbergen 1963). Birdsong is often studied due to its flexibility in form, the learning that can be involved in its acquisition, the extent of song variety between the different species of passerines, and the pressures that drive it's evolution and occasionally it's extensive elaboration (Catchpole 1980; Darwin 1871; Krebs 1977; Kroodsma 1977). A well-studied topic is the concept of song communicative complexity, which is often measured as song type repertoire size. This is measured as the total collection of all song types or distinct song units (composed of syllables) that an individual bird can produce (Catchpole and Slater 2003). Other measures of complexity used in bird song research include syllable repertoire size, syllable structure, timing and frequency features of songs, and others, depending on the singing style of the study species (Gil and Gahr 2002). Yet, if the purpose of birdsong were simply to announce one's presence or one's readiness to breed then a handful of different songs or syllables within songs would suffice to accomplish those goals. Therefore, elaborate signals like large song repertoires do not seem to be evolutionarily optimal. Hence, many researchers study repertoire size variation in light of the evolutionary pressures that would have led to the development of such a wide array of different songs (Catchpole 1980; Krebs 1977; Kroodsma 1977).

The most often proposed mechanism driving the elaboration of signals is the theory of sexual selection. Darwin (1871) stated the idea that the reproductive success of one individual over another is critical in the evolution of secondary sexual characteristics, and this happens mostly through one of two ways. Either the females are particular and choose a favorite partner based on some characteristic that is preferred over other variations of that trait present in other available mates (for example preferring more song types in the song repertoire) (Darwin 1871). This is the case of intersexual competition for mating partners. Or, members of the same sex can compete for access to mates and can fight to defend their own mate, which is the case of intrasexual competition (Darwin 1871). A classic example of sexual selection is the peacock's train, which displays features that are purported to show genetic superiority of high quality mates to interested females but are expensive to produce and do not serve males in defending territories (Loyau et al. 2007; Petrie et al. 1991; Petrie and Williams 1993; Petrie 1994; Petrie and Halliday 1994; Yasmin and Yahya 1996). However, even this widely accepted statement in biology is still a hot topic of discussion. Multiple studies have cast doubt on the original research demonstrating that peahen females have a preference for longer trains (Loyau et al. 2008; Takahashi et al. 2008) and have spurred closer examinations of what about a peacock train exactly might be selected for by females (Dakin and Montgomerie 2009; Hale et al. 2009; Petrie et al. 2009). One study found implications for the peacock tail to be used as a radiator of ultrasonic acoustic signals that causes responses in females as well as in other males and another found that males with longer trains and tarsal leg bones secured more central territories and engaged in more agonistic interactions, though more elaborate trains did gain more mating success (Freeman and Hare 2015; Loyau et al. 2005) This suggests that both inter- and intra-sexual mechanisms are involved in the evolution of the peacock train after all, even in the case of a classically female choice attributed sexual characteristic. Thus, many traits are not easily attributable to one type of sexual selection over the other, and it is important to evaluate both mechanisms as possible contributors to the evolution of traits.

Avian Song Repertoires as Signals of Male Quality

Most often, bird song repertoire size is studied in light of sexual selection by way of female choice as the most important pressure driving its evolution (Byers and Kroodsma 2009; Catchpole 1980, 1987; Catchpole and Slater 2003; Darwin 1871; Searcy 1992; Soma and Garamszegi 2011). This is not a surprising avenue of research as some passerine species, such as the Sedge Warbler, *Acrocephalus schoenobaenus*, only sing during a pre-nesting mate attraction period and cease singing for the remainder of the breeding season once they have acquired a mate (Catchpole 1980). A natural extension of this would be that mate selection pressure exerted by females is a significant force acting on this secondary sexual characteristic in that species. Several other studies have certainly shown supportive evidence for female choice being important in selection for larger repertoires in this species (Birkhead et al. 1997; Buchanan and Catchpole 1997). However, Marshall et al. (2007) found that males genetically responsible for a detected 8% extrapair fertilization rate had repertoire sizes and territory sizes that were smaller than those of the males from the social pairs. Since extrapair copulations occur later in the season when males have mostly stopped singing, females must use cues other than those related to song to assess male quality for extrapair fertilizations (Marshall et al. 2007). Perhaps males with larger repertoires suffer less loss of paternity in their brood by having larger repertoires, but the extent of cuckoldry in relation to the social mate’s repertoire size was not reported. This is another example of a well-studied signal that seems straightforward in its interpretation but that has more complexity than first apparent when investigated from multiple perspectives.

Taking a broader view, Searcy (1992) reviews female preference for larger song repertoires, taking evidence from both laboratory and field studies. Eight laboratory studies found that females uniformly prefer larger song or syllable repertoires over small ones, all of which measured copulation solicitations as the female choice response save one that evaluated nest building and egg laying (Searcy 1992). Some criticisms of lab studies are their limited power at predicting evolution of traits in a complex environment, and that many response variables quantified consist of behaviors like copulatory displays and nest building that occur post-mate choice (Searcy 1992). Field observation approaches to the question of preference in females often consider male repertoire size in relation to proxies of female choice, such as earlier pairing date, number of females mated, earlier laying date, larger clutches, or time to obtaining a new mate (Searcy 1992). The results of those studies are more variable, with some weak relationships not holding up when researchers controlled for confounding variables such as territory quality or male age (experience), but in other studies no relationships emerged at all (Searcy 1992). Only the above-mentioned Catchpole (1980) and one other field study by Mountjoy and Lemon (1996) in European Starlings, *Sturnus vulgaris*, found strong evidence of female preference selecting for large song repertoire size. A concern with field studies is that female reproductive success is closely tied to territory quality in many bird species, making territory an important factor that may overshadow any effect of male song (Searcy 1992). Most of the studies that did show small positive effects of male repertoire size lost any significance when they controlled for territory quality or male experience (Searcy 1992).

A review by Byers and Kroodsma (2009) that examined female mate choice and songbird song repertoires across 49 studies - 22 laboratory and 27 field based - also determined that results have overall been inconclusive as to whether female mate choice is actually a significant evolutionary driver behind developing a larger repertoire size in male songbirds. Soma and Garamszegi (2011) conducted a similar meta-analysis based exclusively on 39 field studies, which found only a slight positive effect of female choice on repertoire size and proposed that there is likely no universal reproductive advantage for males with larger song repertoires. Soma and Garamszegi (2011) caution that though they found a small positive effect, it was lower in studies that did control for confounding variables and thus the positive relationship may be influenced by additional factors such as territory quality, male age, or territory defense. Thus, though some model species empirically do support the hypothesis, many supporting results were confounded by other important variables (Soma and Garamszegi 2011).

From my research, at least four studies that offer important additions to this field were left out of Byers and Kroodsma's review, of which two were also excluded from Soma and Garamzsegi's meta-analysis (Hiebert et al. 1989; Horn et al. 1993; Nowicki et al. 2000; Nicholson et al. 2007). The two studies that were included in Soma and Garamszegi's meta-analysis are Hiebert et al.'s (1989) study of Song Sparrow repertoires and Horn et al.'s (1993) study of Western Meadowlark repertoires. Hiebert et al. (1989) found that Song Sparrow males with larger repertoires held territories longer and had greater annual and lifetime reproductive success than males with smaller repertoires. Horn et al. (1993) found that larger repertoire size in male Western Meadowlarks predicted earlier pairing, fledging more young, and longer wings but had correlation with territory size or mass. Both studies acknowledged though, that greater breeding success could have be achieved by attracting a mate earlier as a result of successful intersexual attraction, or might well be achieved by establishing a territory earlier as a consequence of successful intrasexual competition (Hiebert et al. 1989; Horn et al. 1993). Missing from both reviews (Byers and Kroodsma 2009; Soma and Garamszegi 2011) are Nowicki et al.'s (2000) study of nestling growth and song repertoire size in Great Reed Warblers and Nicholson et al.'s (2007) study on song sharing and repertoire size in Sedge Warblers. Nowicki et al. (2000) found that among nestlings, the length of the innermost primary feather was positively correlated with the size of its repertoire in the first year, and conclude that repertoire size may provide females with information about male quality in regards to developmental stress as a nestling. Nicholson et al. (2007) studied the classic Sedge Warbler system, in which males stop singing after pairing and male-male singing interactions are very brief and sparsely studied. Males are known to have stable repertoire sizes within a season, which is important in female selection, but can also turn over syllables within their repertoire to facilitate song sharing with nearest neighbors within a season (Nicholson et al. 2007). This study found that the repertoire size of a single bird does increase with age with the most pronounced increase in repertoire size found between first and second year birds (Nicholson et al. 2007). Thus females that choose mates with larger repertoire sizes may gain genetic benefits that allow their offspring to also survive and thrive to a more advanced age, and may benefit from mating with partners that have more chick-rearing experience (Nicholson et al. 2007).

A real challenge to determining universal patterns of the effect female choice has on song repertoire size is that most supportive studies are either from lab settings evaluating post-mating behaviors of hormonally implanted females (perhaps not the best representation of the evolution of this signal in a dynamic ecosystem), or are from species that stop singing completely after mating and represent a rarity among passerines. Another challenge is establishing whether a gain in reproductive success that is associated with larger song repertoire size is truly a direct effect of females preferring males with larger repertoires, or if it is an indirect benefit of male-male interactions that have enabled the acquisition of superior resources that allow for increased reproductive success.

Recent studies have found positive associations between female choice and song repertoire size, while trying to address some of these shortcomings. A study by Darolová et al.

(2012) evaluated proxies of female choice in regards to song complexity measured as syllabic strophes of song in male Marsh Warblers, *Acrocephalus palustris*. The researchers painstakingly accounted for date of first establishment of a territory by males, monitored male singing in the days leading up to female arrival, determined date of female settlement, estimated reproductive success at the egg, chick and fledgling stages, assessed parentage (particularly paternity), estimated male feeding effort relative to the female, and finally estimated ectoparasite load in the nest after fledging (Darolová et al. 2012). They found that males with more complex songs mated sooner, and had larger clutch sizes, but found no significant effects of male arrival date, nest ectoparasite load, or male feeding effort (Darolová et al. 2012). Song complexity was also positively correlated with wing length and body weight as a measure of body condition but negatively with tail length (Darolová et al. 2012). These results do somewhat support female choice of territorial males with more complex songs for social mates. However, there were no differences in song complexity between males that did suffer extra pair fertilizations (EPFs) and males with faithful mates. Unfortunately they did not compare the level of song complexity between cuckolded males and the males responsible for EPFs to see if perhaps females sought out males with larger repertoires relative to their social mates' (Darolová et al. 2012).

Further helping to distinguish between intersexual and intrasexual selection, a 9-year field study of the migratory Eastern Song Sparrow, *Melospiza melodia melodia*, concluded that song repertoire size of males was a better predictor of reproductive success than territory location (Potvin et al. 2015). This indicates that the quality of resources in a certain location is not necessarily linked to males having larger repertoires or more reproductive success, thus winning fights for the 'best' territory by out-singing opponents does not seem to be the mechanism driving song repertoire size (Potvin et al. 2015). Nonetheless, the most successful males with larger repertoires had higher territory tenure from year to year and the value of experience on a certain territory could be more important that territory quality, such that this result still blurs the lines between female choice or male-male interactions being the basis on which the reproductive benefits are gained (Potvin et al. 2015).

Avian Song Repertoires as Signals of Aggressive Intent

In light of inconclusive results when considering female choice, and because female choice may be based on success within male-male competitions, I also reviewed the available literature on territory defense and intrasexual selection as a driving evolutionary force behind large song repertoire sizes in songbirds. Avian song in general has been shown to play an important role in territory defense through speaker occupation experiments, wherein territories 'defended' by speakers playing songs of the original occupants' vocalizations were re-colonized later than silent control territories (Falls 1988; Göransson et al. 1974; Nowicki et al. 1998). In Great Tits, *Parus major*, where peak singing typically happens after pairing and males have repertoires of 2 to 8 song types, repertoire size was shown to have an important function in male territory defense and boundary maintenance through speaker replacement experiments (Krebs et al. 1978). Resident Great Tit males were captured and removed from their territories, and speakers were placed within the territory to simulate the resident male's singing. In each of three sets of experiments, one speaker broadcast a single song on the first territory, one speaker broadcast full repertoires (of different sizes in the three sets) on a second territory, and the third territory became a silent control (Krebs et al. 1978). They demonstrated that territories broadcasting full repertoires were the last to be reoccupied by a new Great Tit pair compared to single song or silent territories, and more importantly that the time to reoccupation of territories increased with repertoire size such that larger repertoires were more effective at defending territories than small ones (Krebs et al. 1978). Red-winged Blackbirds, *Agelaius phoeniceus,* were also tested for the function of song repertoire size in territory defense by speaker occupation experiments (Yasukawa 1981). Repertoire playbacks were again found to be more effective at keeping out invaders than single songs, both of which were more effective than the silent control, but this study did not vary repertoire sizes in order to examine the effect of total number of songs present in a repertoire (Yasukawa 1981).

Various other male-male interaction behaviors in relation to singing and/or song repertoire size support the hypothesis that intrasexual communication can be a strong selective force on birdsong. Males of certain species are known to repertoire-match songs with their neighbors to maintain territory boundaries, and will use type-matching of songs in contests with invaders to indicate increased aggression (Beecher et al. 1994, 1996; Ewert and Kroodsma 1994). Thus, it is reasonable to propose that increasing the number of song types or syllables in a repertoire would favor establishing and maintaining territories among various neighbors from year to year, as well as defending territory boundaries against intruders (Byers and Kroodsma 2009). Studies on Rufous Sided Towhees, *Pipilo erythrophthalmus*, Marsh Wrens, *Cistothorus palustris*, and Song Sparrows, *Melospiza melodia*, showed that resident or sedentary populations had larger or more complex repertoires than migratory populations (Ewert and Kroodsma 1994; Kroodsma and Canady 1985; Peters et al. 2000). It is hypothesized that as year-round residents, males have to associate more with neighbors and spend more time defending a single territory year-round, thus they experienced more selective pressure to develop larger repertoires useful for song-matching in neighbor and non-neighbor contexts (Ewert and Kroodsma 1994). Researchers proposed that living in higher densities, in closer quarters, and for longer periods of time (year-round) creates more opportunities for male-male interactions thereby favoring increased variety in number of song types or syllables (Ewert and Kroodsma 1994; Kroodsma and Canady 1985; Peters et al. 2000). This hypothesis however is not universally supported and at least one study in the Song Sparrow, *Melospiza melodia*, found no evidence for whole or partial song type repertoire sharing to be an important target of intrasexual selection in the evolution of song repertoire size (Hughes et al. 2007).

Another intriguing field study on the topic resulted from using the cooperatively breeding Stripe-headed Sparrow, *Peucaea r. ruficauda* (Illes 2014). In this system males have two song repertoires, where one serves for mate acquisition and one for territory defense, and females also sing but only have territory defense song repertoires. Interestingly, this species' territorial females are more implicated in resource defense than the males are, and females have larger territory defense song repertoires than males do (Illes 2014). It was strongly supported that female song is used for intra-sexual competition and aggressive interactions in Stripe-headed Sparrows from playback studies and behavioral observations (Illes 2014; Illes and Yunes-Jimenez 2009). Though these studies do not relate male song repertoire size with increased male territorial behavior, they suggest a positive correlation between these two traits in the sex that is most concerned with resource defense (Illes 2014). This lends support to the hypothesis that territory defense, independent of mate attraction, has the potential to be a strong evolutionary force behind the diversification of complex communication signals leading to large repertoire sizes in avian species.

While territory occupation studies demonstrate well that avian song repertoires are important in defending established territories, they have not thoroughly teased apart what variability in size of repertoire within the population means in male-male contexts, save Krebs et al. (1978) which found the time to recolonize was longer on territories 'defended' by larger repertoires. Playback experiments attempt to address this question by assessing established resident male responses to a simulated intruder with different sized repertoires. To date, there are only three playback experiment studies that I could find that directly tested repertoire size in relation to territory defense, all of which did not support the hypothesis that larger repertoires are more threatening. Catchpole (1989) investigated the function of repertoire size in territory defense in Sedge Warblers, *Acrocephalus schoenobaenus*, but he found no correlation between simulated repertoire size and intensity of the resident bird's response. In their research with Common Blackbirds, *Turdus meula*, Hesler et al. (2010) found no significant difference in male responses to playbacks of either a small or large repertoire size and concluded that male repertoire size must not be used to assess rival quality. However, this lack of a significant result may not necessarily prove that repertoire size has no importance. The caveat of this non-significant finding is that focal birds reacted faster than the time required to assess the full repertoire differences between the two playback types, indicating that the non-significant result was not related to differences in repertoire size (Hesler et al. 2010).

Moreover, Balsby and Dabelsteen (2001) also found that male Common Whitethroats, *Sylvia communis*, did not respond differently to different repertoire sizes in playback experiments when compared to their own repertoire sizes. However, the birds responded equally strongly to large repertoire playback and small-elongated repertoire playback when compared with small repertoire playback, suggesting that the overall length of the song was more important (Balsby and Dabelsteen 2001). It is hard to say if using playbacks that are created by elongating a small repertoire and comparing it to a naturally large repertoire is a valid representation of how birds might perceive complexity, and thus it may not be accurately testing responses to different repertoire sizes. Balsby and Dabelsteen (2001) did note a near significant change in amount of singing after the playbacks, suggesting a potential adjustment of singing behavior in response to the intrusion (Balsby and Dabelsteen 2001). They propose that though this study indicated no role of repertoire size at the moment of intrusion, it is still possible that repertoire size plays a part in modulation of singing behavior in long-term male-male establishment patterns such as in Great Tits and Redwing Blackbirds (Balsby and Dabelsteen 2001; Krebs et al. 1978; Yasukawa 1981).

So far the few playback experiments designed to test responses to variable song repertoire sizes have not lent support to the territory defense hypothesis. However, there is uncertainty in results due to problematic experimental design. In each study researchers noted that males responded before differences in the playback track repertoire sizes could be detected and were merely responding to the detection of an intruder without assessing repertoire size (Balsby and Dabelsteen 2001; Catchpole 1989; Hesler et al. 2010). As a territorial individual it is reasonable to initially respond aggressively to protect an important, sought after resource. To evaluate the importance of repertoire size in habitual interactions between establishing males however, the focal bird must first be informed about the simulated intruder's repertoire before measuring his response intensity. Therefore, measuring the response intensity to a second repetition of the same track after a period of silence would be more pertinent in evaluating differences in perceived rival quality between small and large repertoire sizes. Testing the same focal male with both a large and a small playback repertoire track would allow comparisons of each bird's responses to himself in a different intruder context, while controlling for individual differences in behavioral predispositions (i.e. being either bold or shy). Further, birds may respond differently to different repertoire sizes based on how many song types are in their own song repertoires. Modeling response patterns with a bird's own repertoire size as a possible effect may shed light on what repertoire size may be signaling to same-sex conspecifics.

Conclusion

Previous studies have found conclusive support in some passerine species for female choice influencing the evolution of avian song repertoire sizes, but there do not seem to be any consistent patterns that emerge when all studies are analyzed for trends (Byers and Kroodsma 2009; Catchpole 1980; Searcy 1992; Soma and Garamszegi 2011). These patterns are even less reliable when only field studies are considered (Searcy 1992; Soma and Garamszegi 2011). This could be in part due to loosely defined proxies of female choice, which confound different types of benefits. For example, repertoire size may signal to females that a male can successfully defend a large territory (during male-male competition) or that a male can deliver a lot of prey to chicks (indicating foraging quality of the territory). Overall, results in the field are complicated by the fact that very few studies look at both aspects of selection within the same species and none do so within the same study.

When considering song repertoire size in light of strictly intra-sexual selection pressures, the evidence again is variable and a strong trend is difficult to observe. Overall, there is not extensive literature directly testing the function of repertoire sizes in the context of intrusion and territory defense. Two studies using speaker replacement experiments found support for repertoire size being important in territory defense (Krebs et al. 1978; Yasukawa 1981). The studies that examined this with playback intrusion simulations found no evidence of stronger response to larger repertoire sizes when compared to smaller repertoire sizes, but conceded that a different result may be obtained through modified experimental procedures (Balsby and Dabelsteen 2001; Catchpole 1989; Hesler et al. 2010). Further, one of those studies did see near significant increases in singing post-playback by birds who heard larger repertoires, which suggests that a large repertoire size induces modifications in singing behavior and is indicating something to the territorial focal birds (Balsby and Dabelsteen 2001). Though this does not mount overwhelming support for the territory defense hypothesis, it does encourage further research.

It would seem that a straightforward answer for the evolutionary pressures driving song repertoire size in reference to either intersexual or intrasexual selection is unlikely. The relative importance of each force may even be different in different species depending on additional variables including mating system, migratory habits, number of songs commonly in repertoires, or even population density constraints. It may simply be that bird song is multifunctional in its essence as a communication system designed to encode multiple pieces of information. For example, male Eastern Bluebirds, *Sialia sialis*, will sing vigorously during the pre-pairing season to attract mates, but also sing during territorial disputes with conspecific males, when feeding females, nestlings, or fledglings, and when warning family members about a predator nearby (Huntsman and Ritchison 2002). Socially complex systems like territoriality, monogamy, and pair bonding create an environment ripe for strong selective pressures, potentially resulting in larger song repertoires through multiple concurrent mechanisms of evolution (Byers and Kroodsma 2009).

Rock Wren Natural History

My thesis research investigates song type repertoire size function in the Rock Wren, *Salpinctes obsoletus*. Here I provide an introduction to that species.

Species Characteristics

Rock Wrens are feisty 6-inch long songbirds weighing 15 to 18 grams with an overall dull grayish brown dorsal plumage that is finely speckled with white and black, a contrasting rufous rump patch, and a washed-out white with gray streaked throat and breast that blends into buffy unmarked flanks (Lowther et al. 2000). They have relatively long faintly-barred brown tails that end in a broad sub-terminal black band on all but the two central rectrices, that is conspicuous when spread (Lowther et al. 2000). Their faces have light brown supercilia "eyebrow" lines, and longer straight bills that add to the subtly distinctive, non-sexually dimorphic plumage of this species (Lowther et al. 2000). Fledglings share the same plumage as adults, save a darker gray dorsum and a lack of streaking on the throat and breast (Lowther et al. 2000). Rock Wrens are known for their energetic bobbing behavior as they forage, distinctive "*tic-reer*" bipartite calls, and the males' remarkable singing capacity using many song types assembled into large repertoires that according to Florence M. Bailey "at first hearing seem the drollest, most unbird-like of machine made tinklings" (1904) (Lowther et al. 2000).

Distribution and Habitat

Rock Wrens are found in western North American from Canada to Mexico (Sibley 2000). These birds are common in arid or semiarid habitats containing rocky outcrops, talus or boulder fields, scree slopes, and fractured cliffs with high densities of crevices, nooks, and crannies for foraging and nesting (Lowther et al. 2000; Warning and Benedict 2016). Populations in the northern part of the species' distribution and higher elevations are short-distance migrants flying to southern parts of the United States and to lower elevations to overwinter, whereas southern populations are sedentary and defend territories year round (Lowther et al. 2000). Typically, migratory adult males start arriving first in the breeding range in late March to early April, followed by females (Lowther et al. 2000; Benedict and Warning 2017). After the summer, adults and young of the year begin to migrate south and to lower elevations in late August and have usually retreated from the northern parts of their range (including Colorado) by the end of September (Lowther et al. 2000). East of the Rocky Mountains where winter is most severe in their northern distribution, a migratory divide between migrants and year-round residents is thought to be around the Denver area (Lowther et al. 2000). It's important to note that in coastal populations of the Pacific northwest the temperate winter conditions do allow for year round occupation at more northern latitudes such as for some Washington or Oregon populations (Lowther et al. 2000).

Systematics

Family Troglodytidae's placement within the taxonomy of Passerida has changed with time. Sibley and Ahlquist (1990) were the first to do a genetic study of wrens. They found very little resolution within Family Trogloytidae, and proposed them to be a sister-group to a clade containing gnatcatchers and gnatwrens (*Polioptila*, *Microbates* and *Ramphocaenus*) (Sibley and Ahlquist 1990). The Certhiidae (creepers: *Certhia* and *Salpornis*) were placed as outgroup to the wren/gnatchatcher/gnatwren clade, and the Sittidae (nuthatches and wallcreepers: *Sitta* and *Tichodroma*) were the outgroup to creepers (Sibley and Ahlquist 1990). Sheldon and Gill (1996) agreed with this proposed arrangement of gnatcatchers, gnatwrens, treecreepers, nuthatches and wrens with a second DNA-DNA hybridization study, as did Barker et al. (2004) using nuclear DNA sequence analyses. This monophylic arrangement of New World Certhioidea including

Polioptilidae and Troglodytidae was further confirmed by DNA sequence data in six gene regions by Barker (2017). Relationships within Family Troglodytidae remain obscure with only a handful of studies addressing the topic (Alström et al. 2006; Barker et al. 2004; Barker 2004, 2017; Mann et al. 2006; Sheldon and Gill 1996; Sibley and Ahlquist 1990). This is mostly to do with irresolution of relationships among some wren genera at the basal part of Family Troglodytidae's tree (Salpinctes, Catherpes, Hylorchilus, Microcerculus, Odontorchilus), though Barker (2017) seems to have resolved that the Salpinctes genus, within which the Rock Wren is a monotypic species, fits within a small clade that diverged from the majority of wren species near the root of the Troglodytidae tree (Figure 1). This makes Rock Wrens particularly interesting to study as they fall near the base of a large radiation of very successful bird species that can inform our understanding of other wrens.

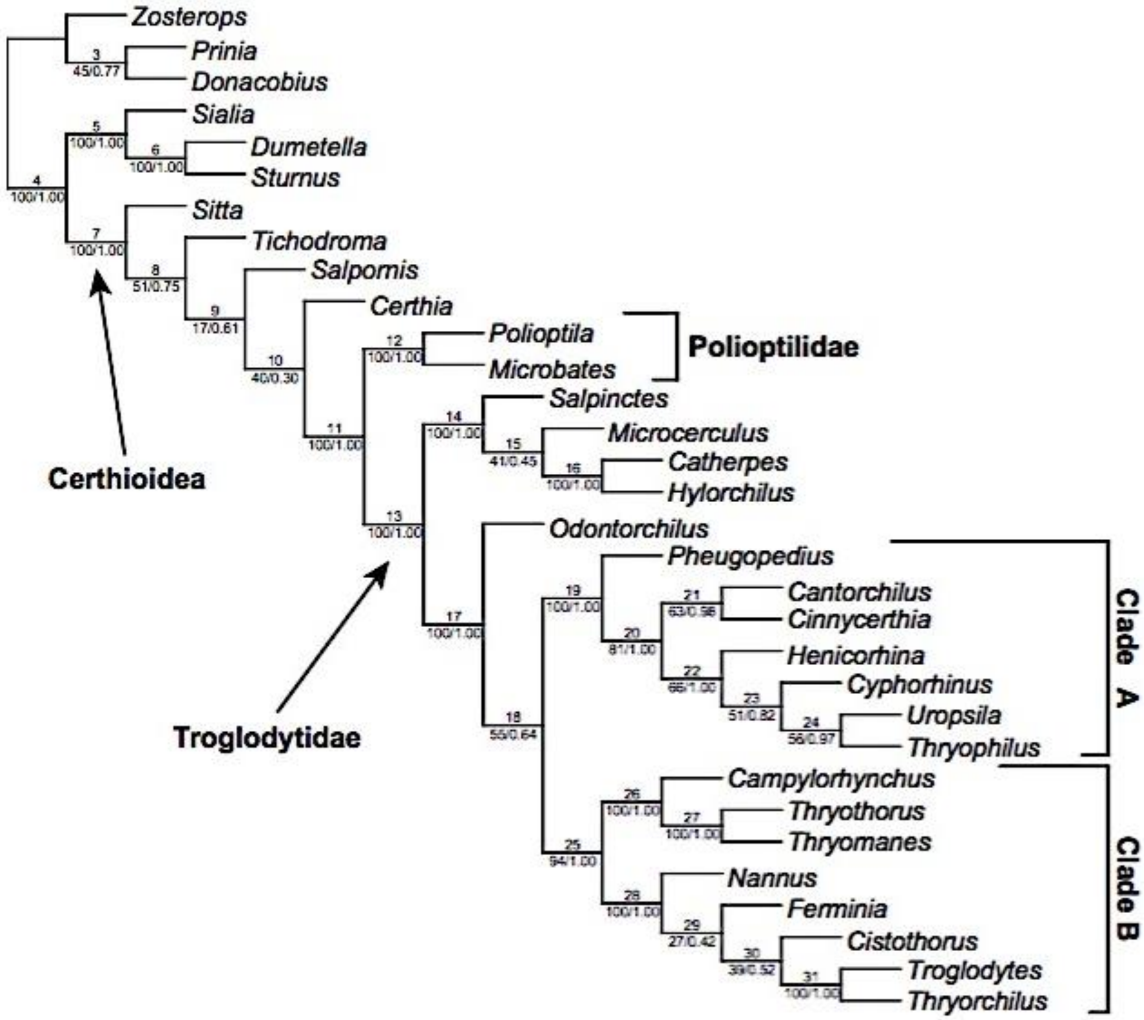


Figure 1: Family *Troglodytidae* within the monophyletic New World Superfamily *Certhioidea* (reproduced from Barker (2017)).
Best estimate of certhioid phylogeny, based on concatenated analysis of 8517 bases from five gene regions (cytb, FGB-I4, FGB-I7, RAG1, RAG2, and ZEB1). Shown is the maximum-likelihood tree from a partitioned RAxML analysis, with nonstationary partitions AGY-recoded. Bootstrap support values (from 1000 fast bootstrap replicates; left) and estimated posterior probabilities from partitioned, concatenated Bayesian analysis (right) are shown below each branch.

The Rock Wren, *Salpinctes obsoletes,* shows quite significant variation throughout its range such that it has been divided into 6-11 different subspecies (Paynter 1960; Phillips 1986). Most of the uncertainty in the systematics of this species comes from poorly understood differences in potential subspecies of Mexico and Central America that need more study

(Lowther et al. 2000). There are however five recognized (though some distinctions are debatable) subspecies that are restricted to Pacific islands, including *S. o. pulverius* from San Clemente and San Nicolas Islands near Southern California (U.S), *S. o. proximus* from San Martín Island west of Baja California (Mexico), *S. o. tenuirostris* from San Benito Island near Baja California (Mexico), *S. o. guadeloupensis* from Guadalupe Island near Baja California (Mexico), and the volcanically extirpated *S. o. exsul* from San Benedicto Island near Colima (Mexico) (Lowther et al. 2000). Three mainland subspecies recognized include *S. o. guttatus* from El Salvador, Honduras, Nicaragua, and Costa Rica, *S. o. neglectus* from central Chiapas, Mexico, and Guatemala, and finally my subspecies of interest, *S. o. obsoletus* residing from British Columbia, Alberta and Saskatchewan through the western United States to Oaxaca in Mexico (Lowther et al. 2000). This last one, *S. o. obsoletus*, is the only migratory subspecies characterized by having migratory northern populations but sedentary southern populations (Lowther et al. 2000).

Singing Behavior

Rock Wrens are more easily detected by sound than by sight, as they tend to visually blend into their habitat. While only males sing, the often heard two part "*tic-reer*" or "*tick-ear*" call is given by both males and females, as well as buzzing notes known as "chatter" that have been interpreted as courtship and nest-inspection vocalizations but need further study (Shane et al. 1997). Their songs are said to be "the sprightliest, most musical, and resonant to be heard in the entire West" (Myers 1922). Each of their different songs is known as a song type, and is characteristically a 1-2.5 second long series of repeated syllables of varying lengths creating trills to long whistles (mean 4.18 syllables/second), with an average frequency of 3.1 kHz, but ranging from 2.7kHz to 5.8kHz. Each song is typically followed by a period of silence of about 5 seconds when in a broadcast singing bout (Benedict and Warning 2017; Kroodsma 1975).

Further, Rock Wrens do not follow simple “eventual variety” patterns of song type variation while singing such as AAAAA…BBBBB…CCCCC, where each letter represents a new song type. They also are not “immediate variety” singers with singing patterns such as ABCDE…FGHIJ… This species prefers an intermediate delivery pattern, where Kroodsma (1975) described it such that song themes are sometimes repeated in succession but more often occur several times interspersed with different song types before moving onto another set of immediately varying songs, such as ABCBA...DEDEF… Eventually, Rock Wrens cycle back through all their possible songs. The total number of different song types that an individual bird sings is referred to as its song type repertoire size, though not all types are used equally and some are favored over others. It’s important to note that two song types produced by the same bird may be similar in having identical syllables and number of repetitions within the song but distinct in having different frequencies (Figure 2.A.). Alternatively, similar song types might have the same mean frequency, despite the syllables being different in form, such as being shortened or elongated (Figure 2.B.).

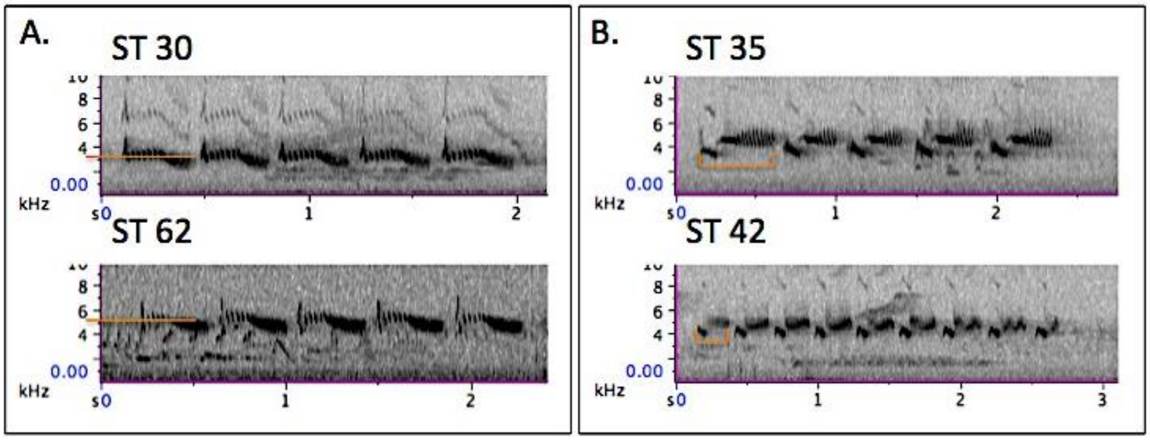


Figure 2: Four different song types (ST) from Rock Wren So39 to illustrate slight differences in similar song types.
A. ST 30 and ST 62 share overall form of syllables and number of repetitions, but ST 30 has a central frequency of 3 kHz whereas the central frequency of ST 62 is 5 kHz.
B. ST 35 and ST 42 have the same central frequencies, but the form of syllables is twice as long in ST 35 than in ST 42 and the number of repetitions is almost half.

As previously mentioned, Rock Wren populations in the northern part of their range tend to be migratory while those in the southern parts are year round residents (Lowther et al. 2000). What’s more, migrant populations of this species have repertoire sizes that are large but variable with between 50 and 120 song types, revealed progressively through time though the relative number of song types used in short subsets of time are representative of final repertoire size (Benedict and Warning 2017). The sheer number and diversity of different song types at the disposition of any male singer of this species is one of the most remarkable features of their singing behavior (Figure 3). A basic principle of evolution is that where there is variation within a population in a certain characteristic, there is room for natural selection on that characteristic, particularly in the case of secondary sexual characteristics (Hauser 1996). This was best stated by Darwin in *On the Origin of Species: By Means of Natural Selection* (1859), “No one supposes that all the individuals of the same species are cast in the same actual mould. These individual differences are of the highest importance for us, as they are often inherited, as must be familiar to every one; and they thus afford materials for natural selection to act on and accumulate". Therefore, large repertoire sizes provide an important foundation for my research to understand which sexual selection factors are influencing song type repertoire size.

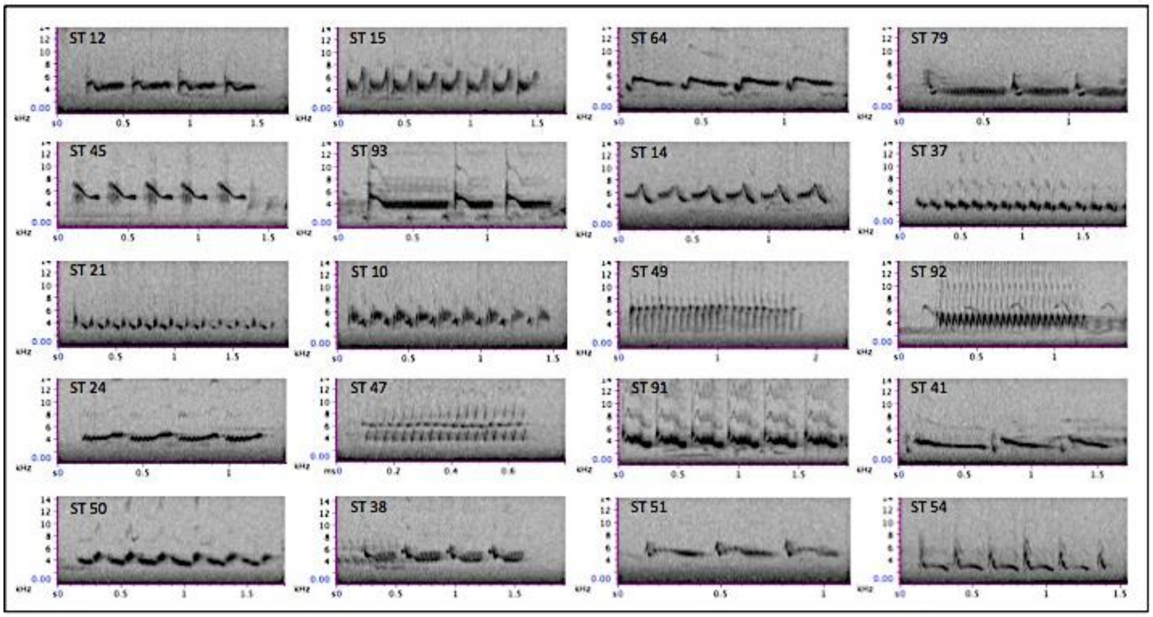


Figure 3: Selection of 20 out of 93 different song types (ST) produced after 1300 songs sampled by a single male Rock Wren studied, So 21.

Territoriality

Throughout the breeding season, males of this species are known for their fierce defense of a territory that includes foraging sites and nesting cavities, as well as of their female mates (Lowther et al. 2000). Territorial behaviors include vocalizations like calling and singing from the most prominent outcrops of a male's territory, along with the characteristic Rock Wren 'bobbing' (which females also exhibit), and sometimes even an aggressive chase of or fight with intruders. The "bob" is described as "a slow lowering and rapid raising of the bird's body", typically co-occurring with a few "*tick-ear*" calls (Tramontano 1964). If an intruder is detected, the territory resident will fly straight at the threat in an aggressive encounter and a swift chase often ensues. Boundary disputes often begin with counter singing between neighbors and will sometimes escalate to a chase. These territorial behaviors are not exhibited during the winter period and movement through less-defined home ranges becomes more fluid in the non-breeding part of the year even in sedentary populations (Lowther et al. 2000). In the Northern Colorado foothills, Rock Wren territories are estimated at 3.6 Ha (ranged 2.0 Ha to 8.1 Ha) in size with the central point of territories of nearest neighbors being 216 m ± 96 m apart (Warning and Benedict 2015a.).

Nesting Ecology

Rock Wrens build their nests in tight holes and nooks tucked away under rocks and boulders in talus fields, sometimes in crevices and existing cavities on rock walls, or even in abandoned buildings (Lowther et al. 2000). Entrances to nests often have paved walkways of small flat stones that also form the nest platform and sometimes a barricading wall at the entrance to increase cavity occlusion (Warning and Benedict 2014, Warning and Benedict 2016). Stone pavements are a multifunctional adaptation for predator deterrent, temperature control, protection from wind exposure and precipitation, and possibly anti-parasitism (Warning and Benedict 2015b.). One study found that 23 of 50 nests in West Kansas that were parasitized by at least one Brown-Headed Cowbird egg had larger cavity openings that non-parasitized nests (Matiasek 1998). This particular behavior can be viewed as complex processing of the nesting environment with subsequent environmental manipulation to fit one’s needs (Warning and Benedict 2016). Both sexes are known to bring rocks and large sticks to build the rock patio and nest base but only the female brings materials for the nest cup and it will take the pair about 4.25 days (± 0.96 SD) to prepare a nest site (Oppenheimer 1995; S. Pitt personal observations). Possibly due to the energy investment in transporting and placing stones, nest sites are usually re-used between years by the same or a new pair but not often within the same breeding season (Lowther et al. 2000).

The Rock Wren nest cup itself is a soft construct built from twigs and grass, lined with moss and hair, placed atop a paved platform (Harrison 1979). It contains from 4 to 10 eggs (with 5 being the average) laid daily one at a time, and incubation is thought to begin after the last egg is laid and takes 12-14 days on average (Merola 1995). The young hatch in succession, altricial and constrained to the nest, weighing one and a half grams (Lowther et al. 2000). They begin to respond to light with eyes closed and yellow gapes wide open at about four days old and weighing around five grams (Lowther et al. 2000). By the ninth day they are capable of thermoregulation and weigh 12 grams (Oppenheimer 1995). The chicks are big enough to leave the nest by the 13th to 16th day after hatching, getting coaxed out by adults using food and vocalizations. They immediately fly to a nearby rock to hide under, with all siblings of a nest fledging to the same hideout over the course of a few minutes to several hours (Lowther et al. 2000). Once fledged, young of the year remain on the parental territory and are looked after primarily by the male for about three to four weeks, mostly foraging for themselves but also begging from their parents (Lowther et al. 2000). If early enough in the season, the female will often initiate a second nesting attempt during this post-fledging period (Lowther et al. 2000). Pairing early and choosing a high quality mate are therefore important for reproductive success in Rock Wrens as females are second brooders and males provide an important amount of fledgling care. Nest initiation date and male prey delivery effort are thus informative proxies for female choice in this species.

In regards to parental investment, the female incubates the eggs alone, though the mate will bring her occasional food items and will continue to broadcast sing while she incubates

(Lowther et al. 2000). The female will take short forays off the eggs during incubation; on average inattentive periods were 17.4 minutes long and attentive periods were 45.2 minutes long for six nests measured at 3000m of elevation in the Sierra Nevada Mountains (Oppenheimer and Morton 2000). Apart from a few deliveries to the female on the nest, the male spends most of the incubation period defending his territory and broadcast singing (Lowther et al. 2000). Once the eggs have hatched, both parents partake in feeding chicks and removing fecal sacs from the nest cavity (Lowther et al. 2000). Though both sexes feed the young in a nest, the relative amounts contributed by each is not known (Merola 1995). A study of a single nest in California noted 298 nests visits, of which 240 were with a food item (unidentified) but feeding rates were variable between hours of observation (Wolf et al. 1985). In another study of 96 feeding events at nests in west Kansas the majority were short-horned grasshoppers (44), moths (15), field crickets (9), and some long-horned grasshoppers, ground beetles, and spiders (Matiasek 1998). Much information about the nesting ecology of Rock Wrens is still unknown, including rates of feeding by each parent, peak feeding hours, or the main types of prey delivered in the Colorado Front Range.

Feeding Habits

As a stouter insectivorous wren with fairly long tarsal bones and bills, Rock Wrens will forage on a wide variety of insects, including grasshoppers and crickets (Orthoptera), leaf-hoppers and aphids (Homoptera), bugs (Hemiptera), caterpillars (Lepidoptera), ants (Hymenoptera), and beetles (Coleoptera) (Knowlton and Harmston 1942; Tramontano 1964). Usually they forage on the ground, gleaning insects from rocks or soil or from low vegetation, bobbing around and probing into cracks and crevices and disappearing under rocks and into cavities (Lowther et al. 2000). On a few occasions Rock Wrens have been seen to glean flying insects from the air on the wing, diving from a nearby cliff or leaping up from the ground in short flight bouts (Lowther et al. 2000; S. Pitt - Personal observations).

Predation

The main agents of Rock Wren nest predation in the United States are snakes, including western hognose (*Heterodon nasicus*), racers (black racer *Coluber constrictor;* striped racers *Masticophis taeniatus*), and adders (unspecified) that have been seen leaving active nest sites or taking chicks out of nests. Other likely culprits include woodrats, ground squirrels, and chipmunks (Hardy 1945; Lowther et al. 2000). In one study of 50 nests in West Kansas in 1998, eight were depredated (Matiasek 1998). Adults off the nest have the most to fear from smaller birds of prey, particularly the American Kestrel (*Falco sparverius*) (Cameron 1907).

Research Goals

With the research reported here I provide an investigation into what factors might be driving the evolution of large song repertoire sizes among Rock Wrens. I evaluated Rock Wren song repertoire size in regards to territory defense as well as in regards to female choice. I provide a robust test of the importance of intrasexual selection on song repertoire size in this species, and potentially whether this single aspect of communication behavior may also function in other contexts such as female choice. This study is unique in assessing a focal male’s response to a small and a large simulated repertoire, with knowledge of the target male’s own repertoire size. To accomplish this research, I performed territory intrusion playback experiments followed by continued field and camera-trap behavioral observations. I playback tested, banded, and continued to monitor a total of 27 male Rock Wrens from natural areas in Larimer County, Colorado over two summer field seasons. Of those males I confirmed that 21 were successfully mated, and I monitored eight nests using field camera traps. My specific research hypotheses and predictions for the study are as follows.

Research Hypotheses and Predictions

Chapter II

H1 A large song repertoire size in Rock Wrens is a threatening intrasexual signal used by males to assess rivals and defend territories.

P1 When presented with playback simulating large and small song repertoire sizes, males will respond more aggressively to large song repertoire playbacks by a) approaching faster, and singing more songs, and b) responding with a higher diversity of song types using longer songs of lower frequencies.

Chapter III

H2 Large song repertoire size in Rock Wrens indicates male quality and can be used as an intersexual signal by females when assessing potential mates.

P2 Males with larger song repertoire sizes will a) have larger home ranges, b) be in better body condition, c) will deliver more food to the nest. Females paired with these males will indicate more investment by laying clutches earlier with more eggs per clutch.

CHAPTER II

TESTING THE TERRITORY DEFENSE FUNCTION OF SONG TYPE REPERTOIRE SIZE IN ROCK WRENS USING PLAYBACK EXPERIMENTS

Introduction

Avian Song Repertoire Size In Male-Male Interactions

Song type repertoires represent a measure of birdsong complexity that is defined by the total number of different song forms, or distinct song units that a single bird can produce (Catchpole and Slater 2003). For the vast majority of bird species, having one to a few different song types suffices the communicatory needs of that bird in different social contexts (Benedict and Warning 2017; Catchpole and Slater 2003; Krebs and Kroodsma 1980). However, there are a few species of bird that present a remarkably wide variety of different sounds that they can produce when singing, such as members of the Mimidae (mocking birds, catbirds, and thrashers) (Borror 1964; Thompson and Jane 1969; Wildenthal 1965) which can have thousands of song types or Troglodytidae (wrens) which can have hundreds of song types (Dos Santos et al. 2016; Kroodsma 1975; Kroodsma et al. 2002; Rendall and Kaluthota 2013; Sosa-López and Mennill 2014).

Having a large song repertoire size does not seem evolutionarily optimal unless redundancy in the signal confers some sort of advantage to the singer in manipulating receiver responses (Guilford and Dawkins 1991; Hauser 1996; Krebs and Kroodsma 1980). Animals in agnostic interactions often signal before engaging in physical contests to inform opponents about strength or motivation to fight (Benedict et al. 2012), and more variety in signaling allows for more nuanced communication. Various avian species are known to adjust their singing behaviors in neighbor and non-neighbor contexts and will either match or avoid matching song types as indications of increased aggression (Beecher et al. 1994, 1996, 2000; Burt et al. 2001; Ewert and Kroodsma 1994; Vehrencamp 2001; Vehrencamp et al. 2007). A larger repertoire would allow for more facultative song use as needed to match or not match based on interactions with neighbors or strangers. Furthermore, having a large song type repertoire allows the potential for more flexibility in behavioral responses by differential song use (Benedict and Warning 2017), such that some song types could be used in certain social contexts and others used for different interactions.

Often, the elaboration of song repertoire size is evaluated in light of the evolutionary pressures that would have encouraged this trait and Darwin's (1871) theory of sexual selection forms the basis for most hypotheses (Byers and Kroodsma 2009). These hypotheses state that song repertoire size increases may be driven by female mates actively preferring males that sing larger song repertoires (Birkhead et al. 1997; Buchanan and Catchpole 1997; Catchpole 1980, 1986; Hasselquist et al. 1996; Horn et al. 1993; Hosoi et al. 2005; Mountjoy and Lemon 1996), and/or may be driven by males having increased success at defending better territories if they can sing larger repertoires (Hiebert et al. 1989; Krebs et al. 1978; Lapierre et al. 2011; Yasukawa et al. 1980).

Many early studies considered female choice as the primary selective pressure on repertoire sizes and this was supported in certain species (Birkhead et al. 1997; Catchpole 1980; Buchanan and Catchpole 1997; Darolová et al. 2012; Horn et al. 1993; Marshall et al. 2003) but a recent review (Byers and Kroodsma 2009) and meta-analysis (Soma and Garamszegi 2011) did not find overwhelming support for female choice being an important selective driver across all bird species, particularly not among field studies. Support for male-male interactions being an important sexual selection force in the evolution of song repertoire sizes is starting to accumulate and is getting more attention from researchers (Beecher et al. 1994, 1996; Ewert and Kroodsma 1994; Kroodsma and Canady 1985; Krebs et al. 1978; Potvin et al. 2015; Yasukawa 1981).

Several speaker occupation experiments have demonstrated the importance of repertoires in territory defense (Falls 1988; Göransson et al. 1974; Nowicki et al. 1998). Two speaker replacement studies demonstrated that repertoires were more effective than single song or no song at all (Krebs et al. 1978; Yasukawa 1981), of which one study was able to demonstrate that larger repertoires were more effective than smaller ones (Krebs et al 1978). Three experiments using playback have not detected any differences in behavioral responses to either a small or a large playback track (Balsby and Dabelsteen 2001; Catchpole 1989; Hesler et al. 2010). However, each of the studies had some methodology problems that could have contributed to their non-significant findings. In Hesler et al.'s (2010) study, the focal birds responded to both playback tracks before the difference in repertoire could be determined, Balsby and Dabelsteen's (2001) results may have been confounded by overall length of the experimental songs rather than actual differences in repertoire size. Catchpole's (1989) study was conducted in a species that stops singing after pairing and though males do respond to conspecific playback they do not use song in territory defense contexts, and as such male-male interactions would likely not influence the evolution of its singing characteristics. The uncertainty of the results found in these three studies encourages further testing of song repertoire size differences in territory defense contexts.

In light of inconclusive results teasing apart the function of small or large repertoire size due to flaws in experimental design, the purpose of my research was to test for selection on the secondary sexual characteristic of song type repertoire by male-male competition. I investigated this question with a species that uses song in male-male interactions and has a particularly large song type repertoire size, the Rock Wren (*Salpinctes obsoletus*).

Target Species and Singing Behavior

As territorial songbirds with large song type repertoires that migrate and establish breeding territories in the Northern part of their range, Rock Wrens (*Salpinctes obsoletus*) provide an ideal system in which to study the significance of repertoire size (Benedict and Warning 2017; Lowther et al. 2000). Males Rock Wrens sing to establish territories early in the breeding season, will aggressively chase other males from within their territories, are known to counter-sing with neighbors and will sometimes song type match during contests at boundary lines (Kroodsma 1975; Lowther et al. 2000). Males continue to sing through the early nesting phase while females incubate eggs, and some will still sporadically sing through feeding nestlings (Lowther et al. 2000; S. Pitt unpublished data). In migratory populations of the Northern United States, Rock Wrens exhibit repertoire sizes that are large but variable, including from 50 to 120 different song types (Benedict and Warning 2017). The presence of this variation suggests that selective forces could have a strong impact on the evolution of this characteristic if it confers a benefit to the singer to have a larger song type repertoire.

My research used Rock Wrens to test whether a large song type repertoire is a more threatening intrasexual signal than a small song repertoire in territory defense contexts. I assessed focal male Rock Wren responses to playback tracks simulating an intruder with either a small or large repertoire. I predicted that males would respond more strongly by approaching faster, singing in response faster, and singing more songs in response to large song repertoire playback tracks than in response to small ones. I further predicted that they would respond by singing longer songs with lower frequencies and using a higher diversity of song types after large repertoire stimulus tracks than small ones.

Methods

Study Location

This study surveyed migratory populations of Rock Wrens on public lands of Larimer County in the foothills region of Northern Colorado. Specific field sites included Devil's Backbone Natural Area (Loveland, CO), Bobcat Ridge Natural Area (Fort Collins, CO), Pine Ridge Natural Area (Fort Collins, CO), and Lory State Park (Bellevue, CO). In this region, male migrants establish territories in the breeding season of about 3.6 ha in size (Warning and Benedict 2015a.). Breeding habitats include rocky outcrops, escarpments, cliffs with crevices, rock talus fields, and large boulders, with slopes approaching cliffs that are typically quite steep (15-32°) (Warning and Benedict 2015a.). Mountain Mahogany (*Cercocarpus montanus*), Wax Currant (*Ribes cereum*), Three Leaf Sumac (*Rhus trilobata*), Rabbitbrush (*Chrysothamnus nauseosus*), Yucca (*Yucca glauca*), Ponderosa Pine (*Pinus ponderosa* var. *scopulorum*), and Rocky Mountain Juniper (*Juniperus scopulorum*) are the characteristic vegetation encountered in these habitats.

Focal Bird Selection and Individual Identification

I located male Rock Wrens singing on established territories on public lands of Larimer County between May and June of 2016 and 2017. Individuals were subject to playback experiments and monitored through each breeding season. A combination of reliable territory fidelity and preferred singing outcrops allowed the identification of particular male individuals at study sites prior to testing with playback, and unique color banding of individuals allowed identification after playback testing. Agreeable behavior towards a conspecific in the same territory indicated the female mate of each male. A total of 27 male wrens were tested using playback experiments, 12 during the 2016 field season and 15 in 2017. All tested males were regularly visited following playback in order to record more songs (up to an approximate 800 sampled per bird), to determine if they were mated, and (if females were present) to track breeding progress through the season.

Playback Experiments

Territory locations. After locating a male Rock Wren defending a territory on two consecutive visits, he would be targeted for playback experiments. Playback experiments were designed to test the hypothesis that song repertoire size is an important vocal communication signal in male-male interactions during territory defense, with larger song repertoires predicted to simulate a more threatening intruder (Figure 4). Where possible, neighboring males were tested to establish dynamics in the immediate area and compare neighbor repertoire sizes. Each territory holding male was tested on two subsequent days with constructed experimental playback tracks from different original singers, once with a large and once with a small repertoire stimulus, with presentation order randomized.

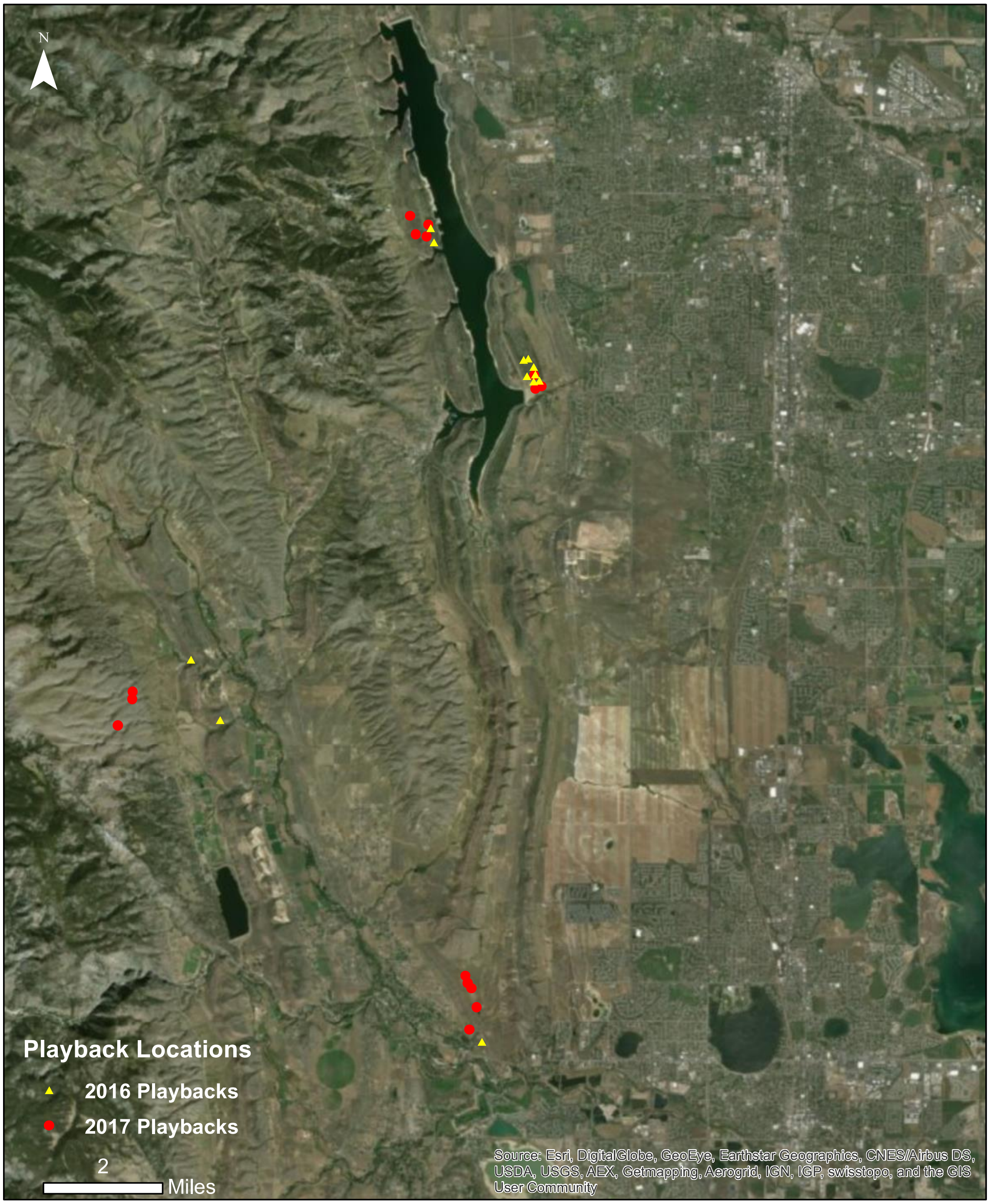
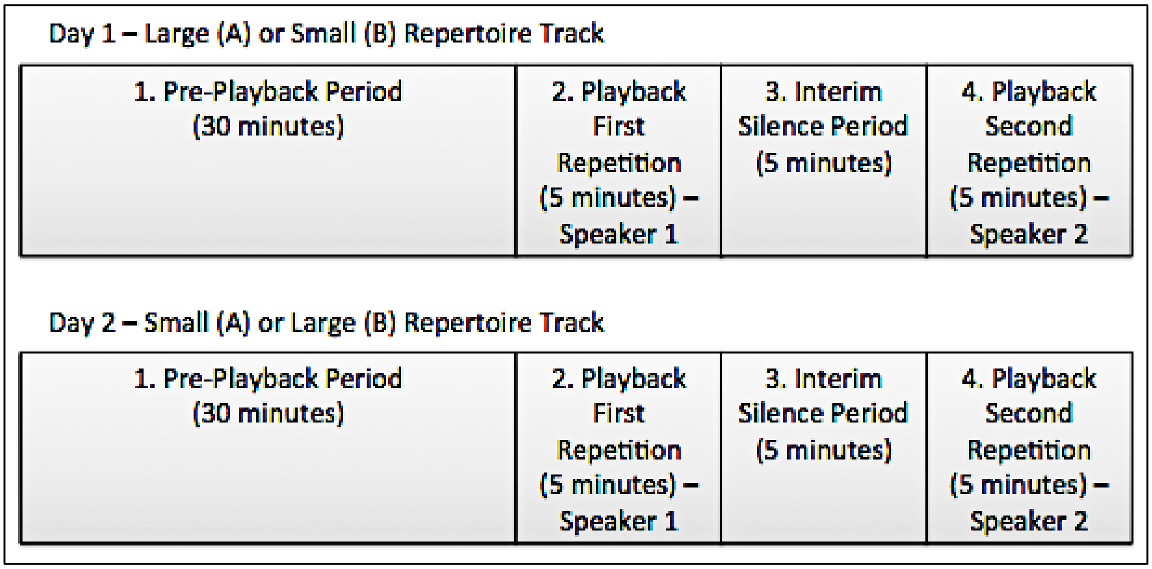


Figure 4: Map of playback experiment locations in both 2016 and 2017 field seasons. Natural Areas include Lory State Park (North cluster), Pine Ridge Natural Area (North West), Bobcat Ridge Natural Area (South East), and Devil's Backbone Natural Area (South West) all in Larimer County, Colorado. Large water body is Horsetooth Reservoir just West of Fort Collins, Colorado. Yellow triangles are playback experiments conducted in 2016 and red circles were conducted in 2017.

Playback stimuli. Experimental tracks were built in RavenPro 1.3 Sound Analysis Software (Cornell Laboratory of Ornithology, NY, USA) using recordings collected from 12 birds from Larimer County in 2013 and 2014. Each track was a total of five minutes long with a song heard every 6th second for a total of 50 songs, which is considered an appropriate singing rate for this species based on previous data (Benedict and Warning 2017; Kroodsma 1975). Songs in experimental tracks were presented with a natural delivery pattern: song type A; A; B; C; B; C; D; E; D; E; presented within one minute. From the 12 previously recorded birds both a small and a large repertoire playback track were built (but were not used together when testing focal males). A small repertoire playback track consisted of five total different song types presented in the first minute and repeated five times for a total track length of five minutes. A large repertoire was built in the same manner, however each minute consisted of a new set of five song types such that the full five-minute track contained 25 total different song types but was identical to the small repertoire track in all other regards.

Playback experimental design. On the first day of playback experiments, two portable speakers (Radio Shack model 277-1008) were set up within a focal male's territory, approximately 10 meters apart with the second speaker placed in a location favorable for mist netting. Each experiment consisted of 1) a 30-minute pre-experimental control period, 2) a 5-minute playback of either a large or small repertoire playback track from the first speaker (Speaker 1), 3) a 5-minute interim period of silence before 4) a second repetition of the same stimulus track for another 5-minute period from the second speaker (Speaker 2) (Figure 5). The second repetition of the experimental track is an important addition to the protocol as it allowed me to assess the difference in response to either a large or a small simulated repertoire, after the focal male has already heard the full track once and had the chance to assess its repertoire size.

The focal male's response to the second repetition of the stimulus should reveal its perceived level of threat based on information gathered about repertoire size over the first repetition.



Day 1 – Large (A) or Small (B) Repertoire Track

1. Pre-Playback Period (30 minutes)	2. Playback First Repetition (5 minutes) – Speaker 1	3. Interim Silence Period (5 minutes)	4. Playback Second Repetition (5 minutes) – Speaker 2

Day 2 – Small (A) or Large (B) Repertoire Track

1. Pre-Playback Period (30 minutes)	2. Playback First Repetition (5 minutes) – Speaker 1	3. Interim Silence Period (5 minutes)	4. Playback Second Repetition (5 minutes) – Speaker 2

Figure 5: Schematic of playback experiment timing layout.

All playback experiments were conducted between 0545 and 0745 hours Mountain Standard Time and the first playback repetition began exactly one hour after sunrise. No playback was used on any day before the experimental period to prevent subject habituation. The bird's vocalizations were recorded for the entirety of the pre- experimental period and during the experiment itself using a Sennheiser MKH-60 shotgun microphone connected to a Marantz PMD 671 solid state digital recorder in mono at a sampling frequency of 48 kHz and a 16-bit sample depth. Throughout the playback experiment, behavioral observations of males and mates were dictated into the microphone for later analysis. Dictation included data about the subject's latency to approach the speaker, distance from the speaker, and the bird's closest approach distance to the speaker, along with other general behaviors including, but not limited to, copulation events, foraging, or prey exchanges between mates. On the first day of experiments, each bird’s singing response to the playback was opportunistically recorded post-experiment to acquire more song samples for subsequent song type repertoire estimations.

The second day of playback used a track of opposite repertoire size constructed from a different original bird (to control for bird identity) and was identically set up (Figure 4), with the addition of a mist net (Avinet, Dryden, NY) placed and opened above the second speaker. If conditions were not favorable for mist netting due to precipitation or wind, the second day of experiments was conducted on the next day that returned to favorable conditions. The second day of experiments concluded either when the bird was caught in the mist net or after failure to catch the bird with repeated playback for a half hour post-experiment. When the bird was caught, a unique combination of plastic color bands and a United States Fish and Wildlife Services metal band (Gey, Norristown, PA) were affixed to its legs to allow for individual identification. Blood samples, morphological measurements, and spread wing and tail photographs were taken before releasing the male back on his territory. All procedures were conducted in accordance with the University of Northern Colorado Institutional Animal Care and Use Committee (Protocol No. 1506C-LB-Birds-18), and all banding was performed under the license of Dr. Lauryn Benedict, Federal Bird Banding Permit number 23741 and 23741c, with special authorization to mist net, band, trap, and take blood samples that do not exceed 1% of body mass.

Response measures. I analyzed song output and behavior to assess response to playbacks. For all response measures, analyses were run both for response to the first speaker and response to the second speaker. I predicted that response to speaker 1 should not vary between large or small playback tracks since the responder does not know the track repertoire size in advance, but response to speaker 2 should vary between small or large repertoire treatment types as the responder is now considered to be informed. Recordings from playback experiments were meticulously scored for bird approach response variables that included 1) latency from playback start to first approaching the speaker during playback repetitions 1 (speaker 1) and 2 (speaker 2) (sec), 2) closest approach distance (m) to both speaker 1 and speaker 2 during playback repetitions 1 and 2, and 3) time spent within 10m of the speaker for both the first 5-minute playback track and in the 5-minute interim silence period. For this last measure, as well as some of the singing measures detailed below I was not able to include the 5 minute 2nd playback repetition because many birds were caught in my mist net before the 5 minute period concluded and as such these were not included in statistical comparisons.

All recordings were visualized in spectrograms using Raven Pro 1.5 Sound Analysis Software (Cornell Laboratory of Ornithology, NY, USA) as Hanning type spectrograms with a discrete Fourier transform of 256 samples, a frequency resolution of 188 Hz, and a time resolution of 2.67 ms (Setting 1) (Figure 5). Recordings from playback experiments were visually scored using Raven Pro 1.5 (Setting 1) for bird singing response variables to both speaker 1 and speaker 2. This was accomplished by visually scanning through the tracks and listening to the dictation in order to quantify all songs produced by the focal male, noting the time of initiation of playback tracks, and noting the timing of response variables relative to the onset of the playback track. Response variables include the behavioral measures listed above, plus vocal measures: time to first song (seconds), time to first call (seconds). I also quantified singing amount as the number of songs and number of calls produced in the 5-minute period before, 5-minute period during playback repetition 1, and 5-minute period of interim silence between playback repetitions. Comparing the number of songs in the period of silence directly following the first playback repetition measures the focal male’s singing motivation in response to a perceived intruder, after he has fully assessed the repertoire size of the simulated intruder. I calculated singing rates by dividing the number of songs produced in each 5-minute period by five to get a number of songs per minute.

Song Analyses

Song repertoire size. In order to examine how focal male birds sang in response to different simulated intruders, I built song type repertoires for as many focal birds as possible. As an avian species with a wide selection of different song types in its repertoire, a minimum sampling of over 800 songs per bird was required in order to accurately approximate each wren's song type repertoire size (Kroodsma 1975, Benedict and Warning 2017). I was successful in obtaining 800+ songs for 15 of my 27 tested males. Of the 12 remaining males, six produced between 500 and 800 songs, five produced between 300 and 500 songs, and one produced fewer than 300 songs (range: 133 - 2,369). This variation is attributable to individual variation in singing motivation, reasons for which were not evaluated in this study. All songs collected were used for repertoire estimation, including those sung during the pre-playback periods, the playback experiments themselves, and those collected opportunistically on subsequent visits.

A total of 27,000 songs were sampled from 27 birds representing a mean ± standard deviation of 1000 ± 557.6 songs per bird, of which 11,850 have been identified to song type within 12 constructed individual repertoires. Song type repertoires for each bird were built using a simple enumeration technique that is possible with the stereotyped songs of Rock Wrens (Kroodsma 1975; Botero et al. 2008), where I visualized every song for each individual and assigned it to a song type by eye using the above-mentioned Raven Pro settings. Song type accumulation curves were calculated by quantifying the number of new songs types presented after a certain number of songs have been heard, counted at 10, 20, 30, 40, 50, and 100 songs and then every subsequent 50 songs heard for all sampled songs. This builds a graph plotting the number of new songs types presented over the total number of songs heard for each bird. Previous research has demonstrated that the final number of song types in the repertoire of a bird is only positively correlated statistically for the first 500 songs heard and the relationship looses significance the more songs sampled. In order to make comparisons with birds that produced fewer than 800 songs, repertoire size at 500 songs was considered as a representative of final repertoire size distribution and was used in behavioral response analyses.

Song measurements. As an elaborate signal, birdsong responses to a more important threat could be much more subtle than simply singing more songs. Therefore I measured different features of song relating to song duration and audio frequency. For each song in the 5-minute period before the start of the experiment, during the first 5-minute playback track, and during the 5-minute interim silence period I measured: 1. Highest frequency (Hz), 2. Center frequency (Hz), 3. Lowest frequency (Hz), 4. Frequency band-width (Hz), 5. Song duration (s). I visually assessed spectrograms to assign the songs to the song type used.

By breaking down the songs used into component measurements, I was able to assess how each of these components of song features were used differently between broadcast and defensive singing, and if there was any difference in these features in response to playback of a large or a small repertoire size. All songs were measured in Raven Pro 1.5 Sound Analysis Software (Cornell Laboratory of Ornithology, NY, USA) using the same settings as reported above but in a cool color scale rather than gray scale and zoomed in closer so as to accurately measure song features (Setting 2) (Figure 6).

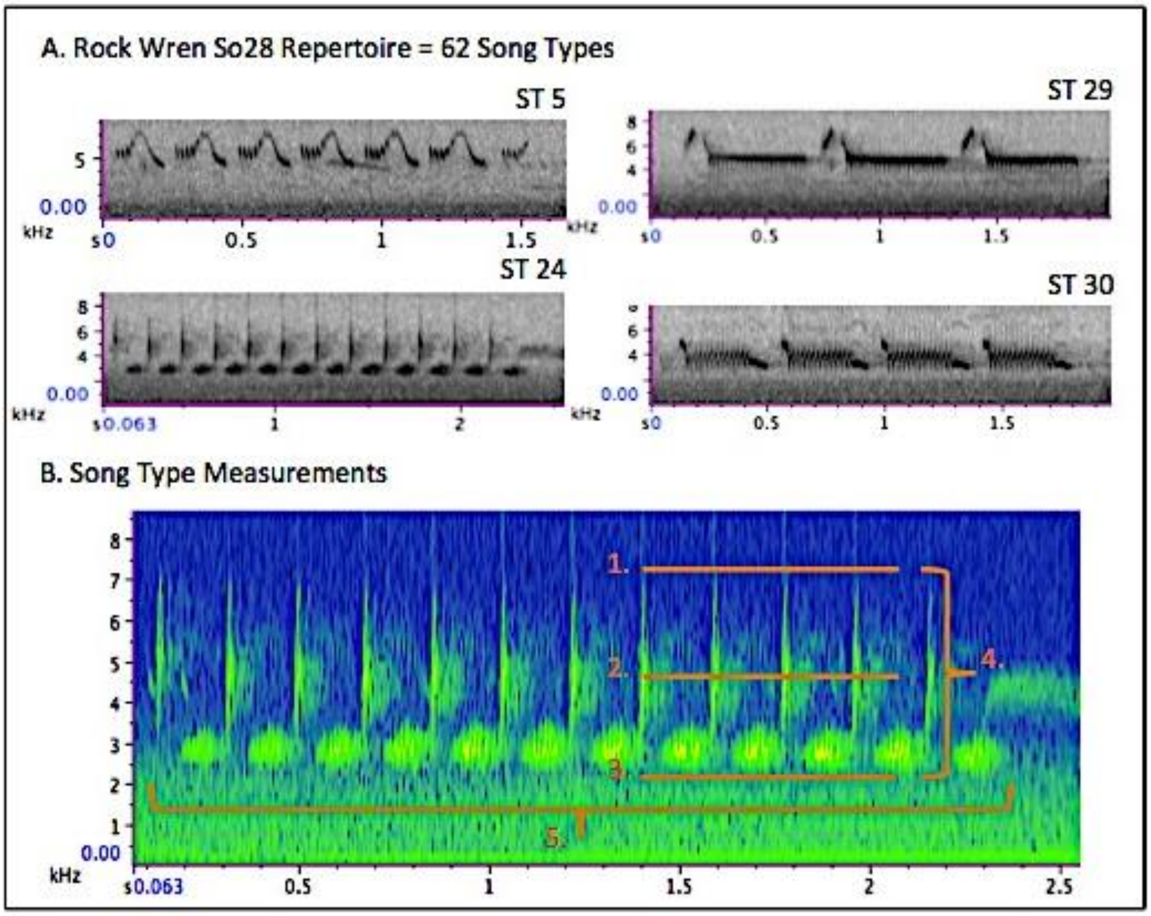


Figure 6: Spectrograms of different Rock Wren song types with one indicating song measurements taken.
A. Four different Song Types (ST) from Rock Wren So28, with a total repertoire size of 62 different STs. This view is ‘Setting 1’ described above of Raven Pro.
B. Diagram of measurements taken on songs illustrated by ST 24, except inter-song intervals. This view is ‘Setting 2’ of Raven Pro.

Song type use. I further examined how many different song types were being used by birds before and after the first repetition of the playback and standardized this measure across birds by taking the number of different song types over the number of songs sung in 5-minutes pre- and post- the first playback. This gave me a ratio between 0 and 1 that I multiplied by 100 to get a song type diversity percentage with 0% indicating the same song was used in the whole 5-minute period and 100% indicating every song was different over 5-minutes.

Statistical Analyses

Approach responses. Distance of closest approach (m) and time to first flight approach (s) were modeled to see if the birds were approaching the sound of an intruder simulating a small repertoire size differently than one simulating a large one. I tested for fixed effects of type of playback track heard, repertoire size at 500 songs, and mating status using linear mixed models with the standard least squares method (that included bird identity as a random factor) to evaluate these three factors' influence on a bird's approaching or singing behavior. Mating status was also assessed and included in analyses because mated and unmated males may show distinct differences in territory defense behavior. Responses to both the first repetition of the playback track from speaker 1 and to the second repetition from speaker 2 were evaluated to test whether a difference existed between informed and uninformed responses.

Singing responses. Similarly, I wanted to model time to first singing response (s) and the number of songs sung in 5 minutes between track repetitions for differences in singing responses between a small and a large playback track. I used the same three fixed effects of type of playback track heard, repertoire size at 500 songs, and mating status, again using linear mixed models with the standard least squares method (that included bird identity as a random factor) to test their effects on these two singing response patterns. Responses to both the first and second repetition of the playback track were evaluated for the time to first singing in response (s). This did not apply to the number of songs sung because I evaluated their singing motivation in the 5-minute silence period between the two repetitions after they have been informed by hearing the entire first track.

Song repertoire size. Using the simple enumeration method mentioned, I was able to estimate the total number of song types in focal male bird's own repertoire and assess whether a bird's own repertoire size had any effect on either approach or singing response patterns. Previous research has demonstrated that Rock Wren's final repertoire size is significantly positively correlated with the number of songs sampled only in the first 500 songs and the statistical significance of the relationship disappears as more songs are sampled (N. Najar, unpublished data). Because not all males were motivated to sing more than 500 songs even with multiple days of recording, repertoire size at 500 songs was used as a representative distribution of final repertoires.

Song measurements. I compared songs during the 5-minute periods before and after the first playback track to assess whether any of song measurements changed differently in response to playback in general, to discover what a habitual Rock Wren response is to an intruder. The song type measurements included were lowest frequency (Hz), highest frequency (Hz), center frequency (Hz), frequency bandwidth (Hz) (the difference between the high and low frequencies), and song length (s). I also compared songs in the 5-minute period after the first playback track repetition to evaluate if within behavioral responses to a simulated intruder, whether birds responded differently to either a small or a large repertoire size. For these comparisons, I constructed a generalized linear mixed model (GLMM) for each song type variable measured pre- and post-playback of the first repetition, while setting playback track type as a fixed factor and including bird identity as a random factor. Male age was unknown and male size is not predicted to correlate with repertoire size, thus were not included in the model as covariables.

Song type use. I calculated average percent diversity of song types by taking the total number of song types used and dividing that by the total number of songs in the 5-minute time period both before and in-between playbacks. I compared average percent diversity of song types used pre- and post-playback of the first repetition by type of playback heard. I compared responses using the same linear mixed model with the standard least squares method, with bird identity as a random factor, and type of playback track heard, repertoire size at 500 songs, and mating status included as fixed effects to evaluate whether birds were using a higher diversity of song types in response to a simulated intruder, and whether they did so differently in response to a small or a large playback track.

Results

Playback Experiments

A total of 27 paired field experiments were conducted with independent male Rock Wrens. Of these, 18 were successfully banded at the conclusion of playback trials and two were banded in 2015 and had returned to the same general area that they had been originally banded at for a second breeding season. For the remaining seven birds we attempted to capture them by mist net a minimum of twice on two separate mornings: the first attempt was directly after the second day of playback experiments and a second was made if unsuccessful a few days later. Overall, males responded as expected to playback experiments by approaching or at least vocalizing once in response to both speakers. Five attempted trials were excluded from the sample because either the focal male had disappeared from the area and did not respond on the second day or, as in two instances, though a male had been present singing during a scouting trip the male was never seen on the first day of trials and no responses were detected. This was likely due to males not having been firmly established yet on that territory and having moved elsewhere between scouting and playback testing. Among the 27 tested males, 21 were seen interacting cooperatively with a second wren likely or confirmed by observed copulation to be the female mate, either on one of the days of experiments or on a subsequent behavioral observation. Of the 21 mated males, I confirmed a nesting attempt for 12 pairs of which nine fledged at least one young.

Approach responses. Linear mixed models were run for both the first playback track repetition and the second track repetition for approach response variables of time to first flight approach (s) and closest approach distance (m). There were no differences in time to first flight approach (s) response between a small or a large playback repertoire track during either the first or the second track repetition (Tables 1.A. and 1.B.).

Table 1: Linear mixed model results for time to first flight approach.

A. 1st Repetition	**β ± SE**	***df***	***F***	***P***
Playback Repertoire	-11.950 ± 19.397	1, 11.45	0.3795	0.5499
Mating Status	41.004 ± 22.01	1, 10.16	3.4707	0.0916
Bird (500) Repertoire	-1.852 ± 1.258	1, 11.82	2.1689	0.1670

B. 2nd Repetition	**β ± SE**	***df***	***F***	***P***
Playback Repertoire	-30.458 ± 21.949	1, 11.00	1.9256	0.1927
Mating Status	8.945 ± 25.304	1, 9.00	0.1249	0.7319
Bird (500) Repertoire	-1.473 ± 1.445	1, 9.00	1.0399	0.3345

Note: Results of linear mixed model testing the of playback repertoire type (small or large), mating status (mated or unmated), and bird’s own repertoire (at 500 songs) on the time to first flight approach (s) to first track repetition (A.) and second track repetition (B.) (N = 27).

Similarly, males did not approach small or large playback tracks any differently during either the first or the second repetitions of the stimulus track (Tables 2.A. and 2.B.).

Table 2: Linear mixed model results for closest approach distance.

A. 1st Repetition	**β ± SE**	***df***	***F***	***P***
Playback Repertoire	-4.006 ± 3.086	1, 11.45	1.6852	0.2198
Mating Status	4.203 ± 4.693	1, 10.44	0.8018	0.3907
Bird (500) Repertoire	-0.268 ± 0.262	1, 11.58	1.0479	0.2198

B. 2nd Repetition	**β ± SE**	***df***	***F***	***P***
Playback Repertoire	-3.167 ± 1.981	1, 11.00	2.5564	0.1382
Mating Status	3.610 ± 5.500	1, 9.00	0.4308	0.5280
Bird (500) Repertoire	-0.099 ± 0.314	1, 9.00	0.0989	0.7603

Note: Results of linear mixed model testing the effects of playback repertoire type (small or large), mating status (mated or unmated), and bird’s own repertoire (at 500 songs) on the closest approach distance (m) to speaker for first track repetition (A.) and second track repetition (B.) (N = 27).

Song Analyses

Song repertoire size. Repertoires at more than 1000 songs were enumerated for seven birds and for an additional five birds at more than 500 songs. Full repertoires can take even more than 1000 songs to determine, however previous research has demonstrated that final repertoire size was only statistically correlated with the number of songs delivered for the first 500 songs heard and then loses significance with more songs sampled (N. Najar, unpublished data). Although 14 of the tested males sang well over 1000 songs, some males had a lower motivation to sing for unknown reasons and six never sang more than 500 songs (only one of which sang less than 300). Therefore, I will report only comparable repertoire sizes at 500 songs heard that are a valid representation of final repertoire size, which ranged from 35 different song types to 92.

Singing responses. I found that the time (s) to first singing was not different between small or large playback treatments in response to the first repetition of playback track but that in response to the second repetition it was significantly faster in response to a large playback track when compared to small playbacks. The most predictive fixed effect of this response pattern was the size of the playback track heard (p = 0.0177*) and neither the birds own repertoire size or mating status had any effect (p = 0.6567 and p = 0.8985) (Tables 3.A. and 3.B.).

Table 3: Linear mixed model results for time to first singing.

A. 1st Repetition	**β ± SE**	***df***	***F***	***P***
Playback Repertoire	-4.652 ± 15.292	1, 11.00	0.0925	0.7667
Mating Status	19.035 ± 15.423	1, 9.61	1.5232	0.2464
Bird (500) Repertoire	-1.381 ± 0.894	1, 11.46	2.3894	0.1493

B. 2nd Repetition	**β ± SE**	***df***	***F***	***P***
Playback Repertoire	-11.458 ± 4.110	1, 11.00	7.7726	0.0177*
Mating Status	3.137 ± 6.825	1, 9.00	0.2112	0.6567
Bird (500) Repertoire	-0.051 ± 0.390	1, 9.00	0.0172	0.8985

Note: Results of linear mixed model testing the effects of playback repertoire type (small or large), mating status (mated or unmated), and bird's own repertoire (at 500 songs) on the time to first singing (s) in response to playback for first track repetition (A.) and second track repetition (B.) (N = 27).

In regards to singing motivation after they have heard the first repetition, I found that large song repertoires provoked responders to sing significantly more songs in the following five minutes when compared to small repertoires (ANOVA p = 0.0408*) (Table 5) and the type of playback repertoire heard best predicted this response behavior (p = 0.0299*) (Table 4). Though not significant, mating status did show a trend for unmated males to actually sing more songs than mated males did after hearing playback (p=0.0950) (Table 4, Table 5).

Table 4: Linear mixed model results for the number of songs sung between track repetitions.

# Songs Between	**β ± SE**	***df***	***F***	***P***
Playback Repertoire	9.472 ± 3.832	1, 11.67	6.1096	0.0299*
Mating Status	-8.350 ± 4.548	1, 10.42	3.3712	0.0950
Bird (500) Repertoire	0.214 ± 0.259	1, 11.19	0.6813	0.4253

Note: Results of linear mixed model testing the effects of playback repertoire type (small or large), mating status (mated or unmated), and bird's own repertoire (at 500 songs) on the number of songs sung between track repetitions (N = 27).

Table 5: Mean number of songs sung between playback track repetitions by playback repertoire type.

	Mated Mean ± SD	**Unmated Mean ± SD**	**Total Mean ± SD**
Large Playback	34.1905 ± 19.5720	40.8333 ± 26.2482	35.6767 ± 20.8585
Small Playback	22.7143 ± 17.1293	30.8333 ± 21.6464	24.5185 ± 18.1009
Average	35.8333 ± 5.8012	28.4524 ± 3.1009	

Note: Standard deviation shown for mated and unmated males by large or small playback tracks (Mated males N = 21; Unmated mates N = 6; Total N = 27).

Song measurements. Rock Wrens responded to a simulated intruder by lowering the low frequency and increasing the high frequency resulting in a song with a larger frequency bandwidth, as well as shorten their songs (Table 6). The central frequency also decreases slightly but was not a significant decrease (Table 6).

Table 6: Mean song measurements in 5-minute periods pre- and post- first playback repetition and results of a generalized mixed model.

	Pre-PB Mean ± SD	**Post-PB Mean ± SD**	**β ± SE**	***df***	***F***	***P***
Low Frequency (Hz)	2658.81 ± 638.414	2556.00 ± 790.049	-64.880 ± 790.049	1, 1168	9.6195	0.0020*
High Frequency (Hz)	5437.04 ± 1299.890	5693.21 ± 1566.630	108.446 ± 42.910	1, 1169	6.3873	0.0116*
Center Frequency (Hz)	3757.50 ± 671.240	3705.57 ± 775.228	-37.797 ± 21.668	1, 1168	3.0429	0.0814
Frequency Bandwidth (Hz)	2778.24 ± 1173.690	3137.21 ± 1480.370	172.177 ± 39.557	1, 1169	18.9460	<0.0001*
Song Length (s)	1.40945 ± 0.372	1.32513 ± 0.406	-0.032 ± 0.011	1, 1168	8.1102	0.0045*

* Indicate a significant difference between Pre- and Post-playback song measurement variables.

Note: Mean song measurements of lowest, highest, center frequencies (Hz), frequency bandwidth (delta frequency) (Hz), and song length (s) in 5-minute periods pre- and post- first playback repetition (Pre-playback songs measured N = 500; Post-playback songs measured N = 671; Total songs measured N = 1171; Songs measured from 10 different birds), and results of a generalized linear mixed model testing the effects of small or large conspecific playback on song measurement variables of territory holding male Rock Wrens.

Lowest frequency (Hz) was significantly lower in response to a large playback repertoire track whereas highest frequency (Hz) was not, and frequency bandwidth (Hz) was also significantly larger to a large playback repertoire track (Table 7). The center frequency however was not statistically different but tending to be lower in response to large playback tracks (Table 7). In regards to song length (s), it was significantly shorter in response to a large playback repertoire track than it was to a small playback repertoire track, however, this statistical difference was also present before the playback was heard (Table 7).

Table 7: Mean song measurements in 5-minute periods pre- and post- first playback repetition by playback repertoire type and results of a generalized mixed model.

	Post-Small PB Mean ± SD	**Post-Large PB Mean ± SD**	**β ± SE**	***df***	***F***	***P***
Low Frequency (Hz)	2569.74 ± 7988.790	2548.00 ± 785.750	-87.503 ± 33.088	1, 657.8	6.9937	0.0084*
High Frequency (Hz)	5620.90 ± 1504.770	5735.34 ± 1601.81	64.598 ± 67.430	1, 547.9	0.9178	0.3385
Center Frequency (Hz)	3753.04 ± 756.256	3677.92 ± 785.631	-54.002 ± 33.357	1, 434.2	2.6209	0.1062
Frequency Bandwidth (Hz)	3051.16 ± 1345.01	3187.34 ± 1553.14	152.848 ± 62.918	1, 635.9	5.9017	0.0154*
Song Length (s)	1.37373 ± 0.391	1.29681 ± 0.413	-0.037 ± 0.017	1, 665.5	4.8184	0.0285*

* Indicate a significant difference between Post-Small and Post-Large playback song measurement variables.

Note: Mean song measurements of lowest, highest, center frequencies (Hz) frequency bandwidth (delta frequency) (Hz), and song length (s) post-first playback repetition by large and small playback tracks (Post-small playback songs measured N = 247; Post-large playback songs measured N = 424; Total post-playback songs measured N = 671; Songs from N = 10 birds), and results of a generalized linear mixed model testing the effects of small or large conspecific playback on song measurement variables post-playback of territory holding male Rock Wrens.

Song type use. There are no significant differences in the diversity of song types used by male Rock Wrens before, during, or after the first repetition regardless of the playback being a large or a small track (Figure 7).

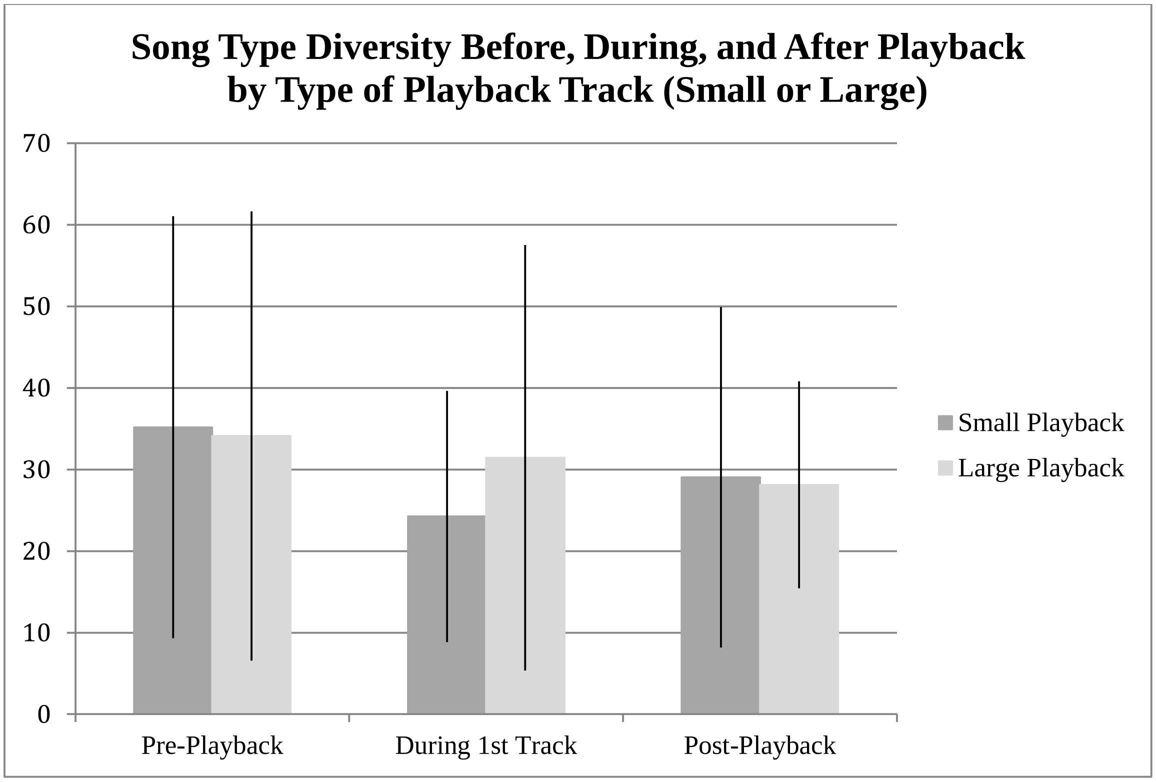


Figure 7: Graph of average percent song type diversity in response to small or large playback tracks before, during, and after playback, with standard deviations shown (N = 13).

No statistically significant change in the number of different song types used after playback was determined in the linear mixed model, though this only includes 13 paired experiments of 27 (Tables 8.A. and 8.B.).

Table 8: Linear mixed model results of song type diversity use.

A. Pre-Playback	**β ± SE**	***df***	***F***	***P***
Playback Repertoire	1.0955 ± 4.028	1, 11.00	0.0740	0.7907
Mating Status	4.1000 ± 6.849	1, 9.00	0.3583	0.5642
Bird (500) Repertoire	0.5679 ± 0.391	1, 9.00	2.1098	0.1803

B. Post-Playback	**β ± SE**	***df***	***F***	***P***
Playback Repertoire	-1.5506 ± 4.003	1, 11.00	0.1500	0.7059
Mating Status	-5.4497 ± 2.985	1, 9.00	3.3325	0.1012
Bird (500) Repertoire	-0.1163 ± 0.170	1, 9.00	0.4658	0.5121

Note: Results of linear mixed model testing the effects of playback repertoire type (small or large), mating status (mated or unmated), and bird's own repertoire (at 500 songs) on the Song type diversity (percentage of different song types in a 5-minute period) both pre-playback (A.) and post-playback (B.) (N = 13).

Discussion

Behavioral Responses

Rock Wren males defend their territories vigorously during the breeding season as an important resource that provides foraging grounds and appropriate nesting habitat (Lowther et al. 2000; Merola 1995). My main research question in this study was to assess whether or not large song repertoire size in Rock Wrens is a threatening intrasexual signal used by males to assess rivals when defending their territories. I expected birds to not show any behavioral differences in response to the first repetition of the track from speaker 1 in any instance since all territorial birds should respond to the detection of an intruder on the territory (Howard et al. 1920). All results to the first repetition of tracks were not significant as expected (Tables 1A, 2A, and 3A). I further hypothesized that when a territorial male hears a second repetition of a simulated intruder with a large song repertoire, he will respond more aggressively than when he hears a second repetition of a small song repertoire. Indeed conspecific playback incited aggressive responses from territorial birds, however not always in the way that I had predicted.

Both measured approach responses of time to approach (s) and closest approach distance (m) showed no significant differences in responses to either large or small repertoires. Results showed that male Rock Wrens investigate the sound of an intruder present within their territory equally quickly and closely regardless of the size of the song repertoire of that intruder (Table 1B and 2B). This supports the idea that song provoked a behavioral response and has an important function for Rock Wrens in a male-male context, but does not elucidate any differences based on repertoire size.

Singing response patterns on the other hand did show significant differences between small and large playback repertoires. The time to first singing in response to large repertoire playback tracks was significantly faster than in response to small repertoire playback tracks. This differs from previous playback experiments that detected no differences in response patterns (Balsby and Dabelsteen 2001; Catchpole 1989; Hesler et al. 2010). However, they only assessed responses to one repetition of the playback track, which I consider an uninformed response, and for a territorial species I expect the uninformed response to an intruder to be identical. In fact, my data from the first repetition of each playback match the results of multiple studies (Balsby and Dabelsteen 2001; Catchpole 1989; Hesler et al. 2010). I also found that time to first singing at the first hearing of large and small repertoires did not differ (Table 3A). To get at the idea that a large repertoire track presents a more important intrusion threat it is critical to assess an informed response, or the response to a repetition of the track. When informed about the intruder Rock Wrens showed a significant difference in singing latency that was predicted by the type of repertoire heard (Table 3B).

Additionally, the number of songs Rock Wrens sang after they heard the full playback track once was significantly higher in response to a large playback track than it was to a small playback track (Table 4). This indicates an increased motivation to sing when a rival's repertoire is higher, which is a more aggressive response to conspecific song (Benedict and Warning 2017). The energetic cost of singing more songs is likely compensated by the benefits gained through successfully defending a highly important resource, the territory (Eberhardt 1994; Szymkowiak and Kuczyński 2017). Though not statistically significant, mated males unexpectedly sang less in response to a both playback repertoires than unmated males did. This trend was surprising but does not go against the conclusion that song repertoire size is relevant to males in assessing potential rivals in territory defense context. This might be explained because unmated males would perceive intruders on their territory as a bigger threat to their chances of securing a female mate and their potential success at reproduction for the breeding season. Or perhaps mated males are more preoccupied with finding prey to deliver to incubating females or nestlings and do not invest as much energy in counter-singing with an intruder.

After male Rock Wrens have detected an intruder on their territory, they respond by altering their songs in various ways. Song low frequency got lower, highest frequency increased slightly, the overall bandwidth became larger, and the song was shortened, all significantly so (Table 6). This follows predictions of the motivation-structural hypothesis that animals in a hostile interaction will use vocalizations that are harsh and with lower frequencies (Morton 1977). Low frequency vocalizations are also associated with larger body size in some avian species and can thus be an honest indicator of fighting ability (Forstmeier et al. 2009; Galeotti et al. 1997; Geberzahn and Aubin 2014; Hall et al. 2013) but this correlation is not universal (Cardoso et al. 2008). Broadband frequency sounds are also effective aggressive signals in other species (Järvi et al. 1980; Trillo and Vehrencamp 2005). Similar response patterns after playback were detected in the song features of Canyon Wrens and could represent characteristic song modifications in response to detecting an intruder in the Troglodytids and other species (Benedict et al. 2012; Linhart and Fuchs 2015).

When considering how song features change after playback between small repertoire playback tracks or large ones, male Rock Wrens were found to alter certain features of their songs even more in response to large repertoires. In response to the large versus small repertoire playback track, males sang with significantly lower lowest frequency of their songs, wider frequency bandwidth, and decreased song duration (Table 7). The highest frequency and the center frequency of the songs however, were not significantly any different in the two experimental treatments (Table 7). Therefore, this frequency bandwidth change was only due to the fact that birds were singing songs with lower lowest frequencies. As this response followed the overall pattern of response to simulated intrusion but was even more pronounced in response to large repertoires than to small ones, it builds support for larger repertoires presenting a more important threat in male-male interactions. This singing response pattern could potentially be a common singing response to intruders for small-bodied songbirds of North America, to honestly indicate their body size and sound more aggressive (Benedict et al. 2012; Forstmeier et al. 2009; Galeotti et al. 1997; Järvi et al. 1980; Linhart and Fuchs 2015; Trillo and Vehrencamp 2005). No other studies have examined whether large versus small song repertoires incite changes to song form in responding individuals.

In avian species with repertoires, signaling aggressive intent could be mediated by adjusting song delivery patterns in various ways (Searcy and Beecher 2009). Some avian species adjust song type delivery patterns but deliver each song type with consistent timing and frequency parameters, while others like Canyon Wrens modify the timing and frequency parameters of songs to adjust delivery (Benedict and Warning 2017; DuBois et al. 2009; Price et al. 2006; Trillo and Vehrencamp 2005). In regards to the results obtained here, it would seem that though Rock Wrens have numerous song types to choose from in their repertoires, they do not significantly change their song type diversity in response to a simulated intruder regardless of whether it was a small or a large playback (Table 9). This did not match my prediction, as a natural extension of having a large repertoire is that birds will use it when they need to indicate quality, as would be expected during territory defense (Darolová et al. 2012; Hiebert et al. 1989; Hill et al. 2018; Horn et al. 1993; Potvin et al. 2015; Searcy et al. 1985). Perhaps as the males are responding by singing faster and singing songs with lower frequencies and longer bandwidths, they are more restricted in the song types they can use from their repertoire.

Overall, these results build strong support for the idea that song type repertoire size is important in male-male agonistic interactions in the Rock Wren and provides information about the level of threat an intruder presents. Since the breeding territory is crucial to this species' reproductive success, by offering habitat to forage for insects and appropriate locations for building nests, it is not surprising that males are heavily invested in defending their territories (Lowther et al. 2000; Merola 1995). Unexpectedly males did not respond differently in their approach to a small or a large repertoire playback track, but they did vary their singing behavior. Males responded to conspecific playback more aggressively by singing faster in response and singing more songs with wider bandwidths and lower frequencies after they had heard the large repertoire playback stimulus.

Avian species use a variety of behaviors in response to conspecific intrusion on their territory, but few studies have established how singing behaviors are modulated to express aggressive intent (Benedict et al. 2012; Cardoso 2011; Searcy and Beecher 2009). My results indicate that large repertoires do incite more aggressive singing responses from territorial males, who 1) respond with potentially honest indications of their body size by singing low frequency songs (Hall et al. 2013), 2) demonstrate their motivation and energetic aptitude to fight by singing more songs (Szymkowiak and Kuczyński 2017), and 3) express their aggression with harsher, wider bandwidth songs (Geberzahn and Aubin 2014; Moynihan 1962). Intriguingly, the diversity of song types used after a simulated intrusion did not seem to be significantly related to the level of aggressive response, but this could be due to restrictions in song types available to signal increased aggression. These behavioral responses to a conspecific intruder provide evidence for various aggressive signaling theories, including for example the motivational-structural concept (Morton 1977). Future work investigating whether certain song types are used only in agonistic interactions could determine whether or not there is compartmentalization of song types potential adjustment of song type delivery patterns to signal aggressive intent (Trillo and Vehrencamp 2005).

Overall, it would seem that male-male interactions of territory defense would be an important selective pressure on the elaboration of song repertoire size in the Rock Wren, signaling more aggressive intent and inciting faster and more vigorous singing responses from resident males. They do not seem to be using more song types in response to a simulated intruder however, and thus how larger song type repertoires are used may be more subtle than simply showing off how many a male can sing, but might be more related to how many song types he can use for specific social contexts.

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CHAPTER III

USING BEHAVIORAL OBSERVATION TO ASSESS ROCK WREN SONG TYPE REPERTOIRE SIZE IN RELATION TO MALE QUALITY AND PARENTAL INVESTMENT

Introduction

Avian Song Repertoire Size in Female-Male Interactions

Many avian species produce just a few different song types that are enough for their communicatory needs in social interactions (Marler and Tamura 1962; Zann 1996; Jaška et al. 2015), however there are other species that demonstrate a wide variety of vocalizations (Borror 1964; Dos Santos et al. 2016; Kroodsma 1975; Kroodsma et al. 2002; Rendall and Kaluthota 2013; Sosa-López and Mennill 2014; Thompson and Jane 1969; Wildenthal 1965). Sexual selection by way of female choice has long been considered an important driver of song repertoire elaboration in avian species (Birkhead et al. 1997; Buchanan and Catchpole 1997; Catchpole 1980, 1987; Darwin 1871; Mountjoy and Lemon 1996; Searcy 1992; Hasselquist et al. 1996). Song repertoire size has been positively correlated with various proxies for female choice including the singing male’s territory size (Searcy et al. 1985), territory tenure (Hiebert et al. 1989), body size (Horn et al. 1993; Searcy et al. 1985), pairing date (Horn et al. 1993), fledgling success (Gil and Slater 2000; Hiebert et al. 1989; Horn et al. 1993), and lifetime reproductive success (Hiebert et al. 1989; Reid et al. 2004) among others.

Since females invest more in the production of each gamete and often have more to lose from choosing a poor mate (especially if she is entirely dependent on that mate for any reproductive success), it is reasonable that females should be careful to select a mate that will contribute either directly (i.e. parental effort) or indirectly (i.e. good genes) to the success of the offspring (Catchpole 1986; Darolová et al. 2012; Hasselquist et al. 1996). However, a review paper (Byers and Kroodsma 2009) and a meta-analysis (Soma and Garamszegi 2011) of multiple studies that evaluated song repertoire size in regards to female choice did not find compelling evidence that intersexual selection mechanisms alone are driving the elaboration of song repertoires across all passerine species. Numerous more recent studies investigated this aspect of the evolution of song repertoire size and have found some support for the idea that females prefer mates with larger repertoires (Crawford et al. 2015; Darolová et al. 2012; Hesler et al. 2010; Hill et al. 2018; Krištofík et al. 2014; Potvin et al. 2015). Thus, female choice could play an important role in selection for elaborate song repertoires across species; I therefore evaluated this in the Rock Wren, *Salpinctes obsoletus*.

Target Species and Nesting Ecology

Rock Wrens, *Salpinctes obsoletus*, are energetic territorial songbirds that are migrants in the Northern part of their range, with Colorado lying right in the migratory-sedentary transition zone and populations in Larimer County being migratory (Lowther et al. 2000). Males arrive earlier than females in the breeding season to establish territory boundaries between males and broadcast sing to attract females (Lowther et al. 2000). Rock Wrens defend breeding territories in rocky areas along canyons, hogbacks, and rocky slopes in Colorado and are relatively common across the Western United States (Lowther et al. 2000). They can be found bouncing along rocky scree foraging for insects, and males spend time singing from high points with a distinctive litany of buzzy musical song types interspersed with a few seconds of silence, while females build and incubate nests tucked in tight rock crevices (Kroodsma 1975, Merola 1995).

Rock Wren nest entrances and walkways are often paved with small, flat stones that sometimes partially barricade the cavity entrance. This cavity occlusion is postulated to serve multiple functions including predator deterrence, temperature control, protection from the elements, keeping nests dry, keeping nestlings from falling out, and possibly nest recognition (Bailey 1904; Merola 1995; Oppenheimer and Morton 2000; Ray 1904; Smith 1904; Warning and Benedict 2015b.). Main predators of Rock Wren nests in the United States are snake species, including western hognoses (*Heterodon nasicus*), racers (black racers *Coluber constrictor;* striped racers *Masticophis taeniatus)*, and adders (unspecified) that have been seen leaving active nest sites or taking chicks out of nests (Hardy 1945; Lowther et al. 2000). Other likely predators include woodrats, ground squirrels, and chipmunks (Hardy 1945; Lowther et al. 2000).

Rock Wren clutch size typically ranges from 4 to 10 eggs with an average of 5, incubation takes between 12 and 14 days, and nestlings are in the nest for about 13 to 16 days (Lowther et al. 2000; Merola 1995). After fledging, males typically continue to feed and take care of young fledglings for about two weeks while the female initiates a second nest if early enough in the breeding season (Lowther et al. 2000). Initiating nests early and pairing with males that are highly motivated to deliver prey would be advantageous to females and thereby should be strongly selected for.

I hypothesize that song features indicate male quality to potential mates. This species' characteristic songs are around 1.5 seconds long, range between 3kHz and 6kHz, and make up large song type repertoires (approximately 50 to 120 different songs) that are variable in size within migrant populations (Kroodsma 1975; Lowther et al. 2000). This variation in repertoire size might be the subject of evolution by sexual selection and may confer advantages to males with larger repertoire sizes in relation to female choice (Hauser 1996).

My research aims to investigate whether a large song type repertoire size indicates male quality and might confer benefits to male Rock Wrens through intersexual mechanisms of female choice (Catchpole and Slater 2003; Darwin 1871; Searcy 1992). I evaluated multiple proxies of male quality and female choice in regards to song type repertoire size including home range size, body size, body condition, date of first egg laying, clutch size, number of fledglings, overall prey delivery rate of males, and male prey delivery effort relative to his mate. I expected males with larger song repertoires to be preferred by females as indicated by earlier nest initiation and larger clutches. I also expected that high quality males would have large repertoire sizes in conjunction with larger home ranges, better body condition, higher fledging success, higher prey delivery rates, and increased parental effort.

Methods

Study Location

Study sites were located on public lands of Larimer County in Northern Colorado including Lory State Park (Bellevue), Pine Ridge Natural Area (Fort Collins), Bobcat Ridge Natural Area (Fort Collins), and Devil’s Backbone Open Space (Loveland). These habitats are vegetated by common species of the Rocky Mountain foothills including Mountain Mahogany (*Cercocarpus montanus*), Wax Currant (*Ribes cereum*), Three Leaf Sumac (*Rhus trilobata*), Rabbitbrush (*Chrysothamnus nauseosus*), Yucca (*Yucca glauca*), Pinus Ponderosa (*Pinus ponderosa* var. *scopulorum*), and Rocky Mountain Juniper (*Juniperus scopulorum*). Ideal breeding habitat for Rock Wrens provided by these study sites includes rock talus fields with relatively steep slopes of 15-32° scattered with large boulders and approaching cliffs with rocky outcrops and pitted with narrow crevices (Warning and Benedict 2015a.).

Focal Pair Selection and Individual Identification

This study examined a migratory population of monogamous and territorial Rock Wrens that establish breeding home ranges of approximately 3.6 ± 1.6 hectares (Warning and Benedict 2015a.). From among 27 male Rock Wrens tested earlier in the 2016 and 2017 breeding seasons with playback experiments, I determined that 21 males had secured a female mate and I regularly monitored their progression through the breeding seasons. For 16 of these birds, I affixed a unique color combination of three colored bands in addition to a metal USFWS band to their legs following playback and mist-netting that allowed for individual identification. All banding was performed under the Federal Bird Banding Permit number 23741 and Colorado State Permit TRb2041. I took multiple measurements of caught wrens including weight, wing chord (distance from the wrist joint to the longest primary flight feather), secondary feather (length of the first secondary flight feather), tail (length from rump to the tip of the longest tail feather), culmen (length of the upper mandible), and tarsus (length of the tarsometatarsal bone), The five remaining paired birds were not successfully lured into mist nets for banding with unique color combinations. However, as they were geographically isolated, territorially reliable, and behaviorally consistent to singing perches, they were repeatedly identifiable as known individuals. Further, no area with an unbanded bird was reused in the second field season to ensure that I was not retesting unbanded birds across years. Conspecifics seen within the home range that were favorably tolerated by the resident male were counted as female mates, often confirmed by copulations, mate feeding displays, and inter-pair soft song contact calls.

I monitored all 21 pairs through the end of each field season in order to locate nesting cavities, evaluate nest progress, and estimate reproductive outcomes, and I kept checking that unmated males had still not found a mate. On visits to territories, I took GPS coordinates (UTM, NAD 1983, Zone 13N) using handheld Garmin GPS units (Garmin International Inc., Olathe, KS, USA) accurate to within 3 m. I sometimes also collected GIS data immediately following the behavioral watch using Google Earth to mark locations in inaccessible locations, such as rock walls. These data were used to estimate home range sizes. Although the experiments reported in Chapter II confirm that these birds were actively defending territories, I do not know the defended boundary in every direction and therefore I report space use as home ranges rather than territories. I plotted coordinates using ArcMap 10.5 and constructed Maximum Convex Polygons (MCPs) using those points to estimate home range areas in hectares. I used MCPs rather than more advanced approaches such as Kernel analysis due to my opportunistic sampling technique which focused on marking the extent of space the birds were seen using regularly, rather than systematically collecting data points throughout the behavioral watch.

Song Type Repertoire Size

Focal males were recorded on several different occasions, including during playback experiments, to collect a sample of their songs. I attempted to record a minimum of 500 songs from all birds tested during playback experiments, unfortunately some birds (6) never sang more than 500 songs across multiple recording days for unknown reasons. Using a simple enumeration technique, songs were ascribed to song type using Raven Pro 1.5 Sound Analysis Software (Cornell Laboratory of Ornithology, NY, USA) to build individual male repertoires (Botero et al. 2008). Previous research has demonstrated that the number of song types found within a male Rock Wren’s repertoire only statistically positively correlated with the total number of songs sampled for the first 500 songs, but looses significance with more songs sampled (N. Najar, unpublished data). Thus, the song repertoire size at 500 sampled songs is a valid proxy for the complete repertoire size, and male song type repertoire size at 500 songs was used in all statistical analyses. Repertoire size at 500 songs was compared between mated and unmated males to see if there were any differences.

Behavioral Observations and Nest Monitoring

In order to assess whether a male's song type repertoire size correlated with his direct reproductive success or reproductive effort, several variables were evaluated over the course of this project. Through regular behavioral observations of focal males through the breeding season, I endeavored to determine each one's nest location, date first egg was laid, number of eggs laid in clutch, number of chicks hatched, number of chicks fledged, and fledging date. Nesting pairs were monitored between May 1st and August 1st 2016 and May 1st and August 3rd in 2017. Rock Wrens characteristically build their nests in tight cracks and crevices in rocky habitats, and they often further decrease the width of the cavity opening by paving and barricading nest entrances with small stones (Warning and Benedict 2015b.). This makes monitoring nests, eggs, and chicks challenging. I circumvented this problem by using a plumbing inspection camera (Medit Flexible Inspection Camera, fiberscope.net) to check on the nest status about once a week early in the nesting attempt and every four days on average when approaching hatching, and through the nestling phase for all monitored nests. This allowed me to accurately track the progress of nests from a full clutch of eggs through the nestling and fledgling stages. I recorded number of eggs present on each visit, maximum clutch sizes, number of nestlings present each visit, number of eggs that failed to hatch (one occurrence), and whether the nest was empty.

Camera Trapping

Camera monitoring. When a nest was found in a favorable location (N = 8), I installed a Reconyx Hyperfire HC600 covert camera (Holmen, WI) three to four feet away from the nest entrance. Cameras were ideally placed with a side view of the nest entrance so as to get optimal views of parents entering and exiting the rock crevice, and to identify what they were carrying in their bills. The cameras were camouflaged as a rock cairns and were left to monitor nest activity 24 hours a day through to fledging (Figure 8).

Figure 8: Reconyx Hyperfire Trail Camera HC600 (Holmen, WI) set up near the entrance of a Rock Wren nest.

Note:
A. Reconyx Hyperfire Trail Camera HC600 (Holmen, WI).
B.i. Camera camouflaged as rock cairn, placed at 4 feet from nest entrance.
B.ii. Camera revealed, nest entrance indicated with a red arrow.
C. Image produced by camera of Rock Wren So28 with a cricket in his bill (bands: Right leg: white over green / Left leg: yellow over USGS silver) at Pine Ridge Natural Area, Fort Collins, Colorado, June 12th 2016.

Approximately once a week the batteries and SD cards were switched out to keep the cameras running. A total of eight covert cameras were placed in front of nests over two field seasons, three cameras watching four nests in 2016 and five cameras watching five nests in 2017.

Photograph processing. All images were downloaded to Dropbox (San Francisco, CA) organized in folders by focal pair and period of time covered. This allowed me to monitor nest activity without influencing bird behavior, to get estimates of prey delivery types and rates, and to gather information on fledging count and date or possible nest predation events. This provided valuable real-time insight into ongoing field observations, such as confirming that a nest was abandoned when a female was nowhere to be found in the field. These photos also provided the material for follow-up processing that estimated numbers and rates of prey delivery.

Following the field season, undergraduate researchers meticulously went through all photographs collected (N = 92,071) using Adobe Bridge (San Jose, CA) organized by nest and tagged relevant photographs using the following keywords: Rock Wren (banded female, banded male, female unbanded, male unbanded, juvenile, unknown), Prey Delivery (female to chicks, male to chicks, male to female), Prey Type (beetle, caterpillar, grasshopper, praying mantis, grub, moth, dragonfly, butterfly, fly, spider, snail, centipede, unknown prey), Something In Bill (fecal sac, nesting material, rock, unknown item), and if needed Predation (mammal, reptile, unknown).

Each independent prey delivery or nest attendance event was recorded once with presence of male and/or female parent and type of prey delivered, such that if two parents were present each with a prey item at the same time, then two separate images would be marked: one for the female parent and her prey type and one for the male parent and his prey type. Since cameras were set to continuously photograph when motion was detected, prey delivery or nest attendance events were considered independent if it was clear that the parent had left and returned but not when the camera recorded multiple pictures of the same bird approaching or leaving the nest. Undergraduate assistants further marked if there was a prey pass-off between adults (male passing a prey item to the female), and entering or exiting the nest where possible. Prey passed from one parent to another and subsequently to chicks was only counted once as a prey delivery event and was attributed to the original parent with the prey item. All nest predation events were tagged and predators noted. Other animal visitors at the nest, as well as other interesting behavior by the wrens not related to prey deliveries, including bringing in nesting material or stones for the characteristic rock patio and nest cavity paving, were also noted. I personally verified all tagged photos a second time for quality control and to make final decisions about prey types.

All experimental procedures were conducted in accordance with the University of Northern Colorado Institutional Animal Care & Use Committee (Protocol No. 1506C-LB-Birds-18).

Statistical Analyses

Measures of reproductive investment and success. I calculated average clutch size from nests where eggs could be seen using the inspection camera (N = 7), the average number of chicks in the nest (N = 8), the average number of fledglings for pairs that fledged at least one chick (N = 9), and the average number of fledglings for all pairs with confirmed nests (N = 12). I assessed overall fledging success by taking the number of nests where at least one fledgling was observed over the total number of pairs to get a percentage of successful nests. I assumed that mated pairs that were not confirmed to have eggs in a nest had indeed at least attempted to breed but had failed if not detected in order to calculate the minimum possible fledging success.

Repertoire size. Repertoire size at 500 songs was compared between mated (N = 21) and unmated (N = 6) males using a Wilcoxon Signed Rank test to see if there were any differences. For mated males, I ran linear regressions between focal male song type repertoire sizes and various proxies of male quality and female choice including home range size, male size (tarsus length, tail length, wing chord, weight), and male body condition (weight over tarsus). I also ran linear regressions between repertoire size and measures of breeding success including first egg date, clutch size, and fledgling count.

Measures of prey delivery. The proportion of each type of prey was calculated overall (grasshopper, beetle, unknown prey, etc.). I counted the number of prey delivered in the first five days post-hatching by males, by females, and by an unidentified parent. I calculated the proportion of prey contributed in that time frame by each parent at each nest. I evaluated amount of prey delivered by males in the first 5 days post hatching in relationship to number of female prey deliveries, male repertoire size, home range size, and fledging success.

Results

Behavioral Observations And Nest Monitoring

Of 27 males tested in previous playback experiments, 21 were mated and all pairs were suspected to have nests though not all nests were found or were accessible. Twelve pairs (six in 2016 and six in 2017) were confirmed to have nests with eggs present or confirmed feeding young fledglings within their territory. Of the 12 discovered nests, one was abandoned and nine (five in 2016 and four in 2017) had at least one fledging survive at least two weeks after emergence from the nesting cavity (Table 9). In the table below, I also included the one nest that was abandoned after five eggs were laid (So35) but it was not included in any average measurements since it is unknown if the clutch was complete, or if the nest was abandoned because of placing the covert camera (Table 9). Therefore, final sample size for discovered nests will be for 11 nests.

The nesting behavior of the remaining nine pairs is unknown, however no fledglings were ever detected within the vicinity of the playback area or in the presence of banded males tested in that area. It is possible that these pairs attempted to breed but failed, in which case the 21 pairs had a minimum overall reproductive success of 42.857%. It is also possible that some of these pairs fledged chicks, which I failed to detect, and therefore the nest success rate for this population is likely slightly higher than reported here.

I found nests at various stages, from eggs to nestling chicks. When considering only nests that successfully made it through the breeding stage in question (through incubation period, through nestling rearing, or past fledging), I found complete clutch sizes between 4 and 7 eggs per nest (N = 7), and nests with between 3 and 4 hatched chicks (N = 8) (Table 9). For those nests that did successfully fledge young (N = 9), an average of 2.667 ± 0 (SD) fledglings survived at least 2 weeks, with between 2 and 3 fledglings per family (Table 1). Overall minimum fledgling survival (including all 21 mated pairs) was 0.1270 ± 1.3887 (SD) chicks per nest (N = 21). Overall fledgling survival from the 11 located nests was 0.198 ± 1.1677 (SD) when including the two nests that suffered total predation of the nest (N = 11).

First egg lay dates were variable and estimated between the 13th of May and 26th of June with an average date of June 2nd ± 13.4 days, which included two suspected second nest attempts (Table 9). Estimated hatch dates were between the 30th of May and 28th of June, including one suspected second nest. Estimated fledging dates were between the 13th of June and 14th of July including one suspected second nest attempt.

Table 9: Reproductive measures of nesting pairs confirmed to have a nest.

Bird ID	Field Site	First Egg Date	Clutch Size	Number Hatched	Number Fledged	Male (500) Repertoire	Camera ?
LB17	BRNA	8-Jun-16	5	4	2	81	Yes
LB19	LSP	14-May-16	Unk.	Unk.	3		No
So21	LSP	10-Jun-16	7	3	2	72	Yes
So28	PRNA	17-May-16	Unk.	Unk.	3	54	Yes
So30	PRNA	30-May-16	4	3	3	87	Yes
So31*	PRNA	26-Jun-16	*(1)*	*(0)*	*(0)*		No
So34	PRNA	15-Jun-17	Unk.	3	3	71	Yes
So35	LSP	13-May-17	*(5)*	*(0)*	*(0)*		No
So36*	BRNA	5-Jun-17	7	4	*(0)*	40	Yes
So45*	DBOS	1-Jun-17	6	4	3	72	Yes
So46*	DBOS	26-May-17	5	3	2	70	Yes
So47	PRNA	9-Jun-17	6	3	3	59	Yes
Average		June 2nd	5.714	3.375	2.667	68	
St. Deviation:		13.4 days	1.1127	0.5175	0.5	16.05	
Sample		N = 12	N = 7	N = 8	N = 9	N = 7	

Note: BRNA: Bobcat Ridge Natural Area, Fort Collins, CO; DBOS: Open Space, Loveland, CO; LSP: Lory State Park, Bellevue, CO; PRNA: Pine Ridge Natural Area, Fort Collins, CO.
Unk: Unknown status either because could not see nest in cavity (So28) or nest found after listed breeding stage (LB19, So34)
(#): Not included in average measurements because abandoned (So35) or failed before fledging (So31, So36 – these were included in overall fledging success though).
*: Predation at nest either total (So31, So36) or partial (So45, So46).

Four nests of 12 suffered predation by snakes that were either confirmed by camera trap or suspected due to the observed presence of a snake in the vicinity of a depredated nest (Table 9). Two of these nests were completely depredated (So31 at Pine Ridge Natural Area and So36 at Bobcat Ridge Natural Area) but the other two nests had at least two fledglings old enough to escape the attack (So 45 and So46 at Devil's Backbone Open Space) (Table 9). Through camera trap pictures three of the nest predators were identified. Two of these predation events (So45 and So46) were perpetrated by bull snakes (*Pituophis catenifer*), and one (So36) was by a Prairie Rattlesnake (*Crotalus viridis*) (S. Mackessy, personal communication). One nest had a woodrat pass by on several different evenings but it was never seen entering or exiting the cavity or tampering with the nest, though they are a known nest predator of Rock Wrens. Other wildlife seen at nests included spotted towhees, American crows, rabbits, and deer.

Song Type Repertoire Size

I found that mated and unmated males did not have different repertoire sizes overall (P = 0.4962), with mated males having 68 ± 15 (SD) different song types on average and unmated males having 64 ± 23 (SD).

Song type repertoire size was not significantly correlated with any measures of female preference (First egg lay date, clutch size) or male quality (home range size, tarsus length, tail length, wing chord, weight, body condition (weight over tarsus), and fledge count) (Table 10). In fact, several relationships (clutch size, home range size, tail length, body condition) trended in the opposite direction from what would be predicted if larger song type repertoire sizes indicate male quality (Table 10).

Table 10: Linear regression correlation results of song type repertoire size in relation to multiple measures of mated male quality and female preference.

Repertoire Size By:	**R^2**	**N**	***t***	***P***
First Egg Lay Date	0.061 (+)	7	0.57	0.5940
Clutch Size	0. 542 (-)	6	-2.17	0.0954
Home Range (Ha)	0.032 (-)	5	-0.32	0.7720
Tarsus Length (mm)	0.173 (+)	7	1.02	0.3531
Tail Length (mm)	0.002 (-)	7	-0.10	0.9232
Wing Chord (mm)	0.272 (+)	7	1.37	0.2295
Weight (g)	0.114 (+)	7	0.80	0.4581
Body Condition	0.088 (-)	7	-0.69	0.5181
Fledge Count	0.194 (+)	7	1.10	0.3232

Parental Effort

A total of nine nests were monitored (four in 2016 and five in 2017) using Reconyx Hyperfire trail cameras and used for prey delivery analyses, as they successfully hatched chicks. These cameras ran for a total of 199 days, with each camera running an average of 20.3 ± 12.67

(SD) days (8 to 35 days). Combined, they collected over 92,000 photographs (Per camera average 9528.5 ± 10,148.7 (SD) (443 - 34895 photos). Both males and females removed fecal sacs from the nests. Both males and females entered the nest cavity with prey to deliver to chicks. Males sometimes passed food off to the female who then fed it to the chicks, but the reverse was never detected in camera footage. On two separate occasions, once in 2016 and once in 2017, a male was seen with a paving rock for the nest patio, a behavior that had previously only been attributed to females at our field sites (Warning and Benedict 2014).

Prey types were variable at different nests. Prey type was unknown in 46.21% of instances where a prey delivery event was certain (Table 11). Grasshoppers were the most commonly identified prey item at all nests at 37.01% (Table 11). Other prey types seen delivered at the nest included beetles, grubs, caterpillars, spiders, moths, dragonflies, flies, and snails (Table 11). Prey deliveries were made at all hours of the day, but most occurred between 5:30 AM and 8:30 PM with peaks from about 6:00 AM to 11:00 AM and 4:00 PM to 8:00 PM.

Table 11: Prey Types delivered to all nests (N = 9) combined and proportion of the total by type of prey.

Prey Type	Proportion	Number of Prey
Unknown Prey	46.21	726
Grasshopper	37.01	581
Praying Mantis	3.18	50
Grub	2.99	47
Moth	2.93	46
Caterpillar	2.61	41
Beetle	2.42	38
Spider	1.53	24
Dragonfly	0.57	9
Fly	0.19	3
Butterfly	0.13	2
Snail	0.13	2
Centipede	0.06	1
Total	**100%**	**1570**

Only eight nests had prey deliveries captured in the first five days after chicks hatched in the nest, a critical period when the male brings food to young nestlings while the female continues incubating to keep chicks warm. In the five days post hatching, a total of 376 prey delivery events were detected at these eight monitored nests, with variable delivery rates among males and females at different nests. Overall, males were responsible for most prey deliveries (Table 12). Males delivered between 8 and 160 prey items in 5 days post hatching compared to females delivering between 2 and 13 prey items (Table 12). Only 8 prey items could not be attributed to the male or female parent (Table 12). The reported prey delivery numbers are the minimum amount of prey that were delivered to the nests, as it is possible that the cameras did not detect a bird's fast arrival in order to activate itself and could have not detected every delivery. Males made up the vast majority of prey deliveries, accounting for 50 to 100% of prey items delivered at a nest compared to females' 0 to 37.5% contribution (Table 12). Only one nest had a high proportion (23.1%) of prey deliveries that could not be attributed to the male or female parent (at So46's nest) (Table 12).

Table 12: Number of prey deliveries by males and females at each nest.

Bird ID	Repertoire Size (500)	Male Prey Delivery	Female Prey Delivery	Unknown Prey Delivery	Total Prey Delivery
LB17	81	17 (89.5 %)	2 (10.5 %)	0 (0 %)	19
So21	72	26 (100 %)	0 (0 %)	0 (0 %)	26
So28	54	8 (50 %)	6 (37.5 %)	2 (12.5 %)	16
So30	87	160 (92.5 %)	13 (7.5 %)	0 (0 %)	173
So34	71	24 (66.7 %)	12 (33.3 %)	0 (0 %)	36
So36	40	31 (86.1 %)	4 (11.1 %)	1 (2.8 %)	36
So45	70	46 (88.5 %)	4 (7.7 %)	2 (3.8 %)	52
So46	72	8 (61.5 %)	2 (15.4 %)	3 (23.1 %)	13

The total amount of prey delivered by males at each nest was not significantly correlated with female delivery rates, home range size, number of fledglings or repertoire size (Table 13).

Table 13: Linear regression of amount of prey delivered by male in relation to home range size, fledgling success, and repertoire size.

Male Prey Delivery Rate By:	R^2	N	*t*	*P*
Female Prey Delivery Rate	0.008 (-)	5	-0.16	0.8855
Home Range (Ha)	0.574 (+)	4	1.64	0.2426
Fledge Count	0.160 (-)	6	-	0.2010
Repertoire Size	0.125 (+)	8	0.92	0.3910

Discussion

None of my measures of male quality or reproductive success correlated strongly with song repertoire size in Rock Wrens. There was no difference in repertoire size between mated and unmated males in my study. In regards to body measurements, tarsus length, wing length, and weight increased with repertoire size, but not significantly so. Tail length showed no pattern, and body condition was (non-significantly) lower for birds with larger repertoires, which is contrary to the idea that large repertoires indicate better quality males. Males with large repertoires also tended (non-significantly) to have smaller clutch sizes, smaller home ranges, and later dates of nest initiation by females. All of these patterns trended in an opposite direction from what would be expected if females prefer males with large repertoires. This does not indicate a preference for small repertoires by females, as none of the patterns were significant, but it certainly supports the idea that females are not driving the elaboration of repertoires.

My results contrast with other studies that have found large song repertoire size to be positively related to some male quality measures (Hasselquist et al. 1996; Hesler et al. 2010; Horn et al. 1993; Krištofík et al. 2014; Potvin et al. 2015). For example, Darolová et al. (2012) found that in Marsh Warblers song complexity is positively correlated with early pairing dates, larger clutches, longer wings, shorter tails, and larger body sizes. All of these traits could be indications of better developmental conditions of potential mates (Hõrak 1994; Nowicki et al.

1998) signaled by a larger song repertoire size. However other studies have also found some measures of male quality not to be related to song repertoire size (Horn et al. 1993; Marshall et al .2007; Searcy et al. 1985), and Rock Wrens seem to be a species that fits this pattern. Further, some researchers have found positive correlations between female reproductive investment and male song repertoire size (Buchanan and Catchpole 1997; Catchpole 1980; Darolová et al. 2012; Gil and Slater 2000; Hiebert et al. 1989; Horn et al. 1993). I did not find reproductive investment by mates in earlier or larger clutches to be significantly related to song repertoire size in Rock Wrens.

An important caveat to these results is that sample size was small in this study, with repertoire size correlations to morphological measurements for just 7 males, home range size and nest initiation data for only 7 pairs, and clutch size correlations for 5 pairs. Nevertheless, though none of the above-mentioned relationships were significant, the trends overall did not support female choice as being a significant driver in the elaboration of song repertoires in the Rock Wren. My non-significant results could also be due to song presentation patterns as they relate to overall repertoire sizes. In a species with large repertoires that sings with randomized eventual variety, such as the Rock Wren, females would have to listen to approximately 500 songs in order to reliably discern repertoire size. Even with the most vigorous of male singers it would take over an hour in this species to sample that many songs. Neighboring males will have prolonged interactions that allow for repertoire assessment, and may dispute territory boundaries as they gather information about their neighbors, but females might benefit more from using other traits that are faster to assess than repertoire size when choosing mates (Byers and Kroodsma 2009; Hesler et al. 2010). For example, females could be assessing something else about males' singing, such as higher trill performance or use of more rapid, consistent, low-pitched songs (Byers et al. 2016; Hall et al. 2013; Kagawa and Soma 2013).

The amount of male prey deliveries did not predict the amount of food his female mate would deliver, wherein if males delivered more prey items this did not seem to mean the female adjusted her rate of prey delivery accordingly. Females may benefit from mating with males that are highly motivation to deliver prey items to the nest, but my results showed a non-significant trend that males delivering more food items ended up with fewer fledglings. This is contrary to the prediction that feeding more prey items would increase survival of chicks (Duckworth 1992). Males certainly contributed the larger part of prey items to the nest, and their contribution effort relative to the female appears to be important.

Paternal investment in the nest could still potentially be mediated by song type repertoire size in Rock Wrens, as my sample sizes might be considered too low for definitive conclusions. However, the observed trend was for the amount of prey delivered by the male to decrease with large repertoire size. These results match with those found in other species. Mountjoy and Lemon (1996) found no correlation between male repertoire size and parental effort in European Starlings, nor did Darolová et al. (2012) in Marsh Warblers, and Buchanan and Catchpole (2000) found a significant relationship between the two only in one of two field seasons in Sedge Warblers. Female choice for traits indicating male parental effort are expected to be important in species, such as Rock Wrens, where the female is reliant on males for reproductive success (Buchannan and Catchpole 2000). Male parental effort in many species contributes to fledgling weight and body condition, and decreases the amount of time nestling are vulnerable to predation (Magrath 1992: Buchanan and Catchpole 2000). Rock Wren females, therefore, are expected to assess male parental quality but may use cues other than repertoire size when doing so. One study found that higher song output from males in the early nesting period was related to more investment in chick feeding later in breeding (Ille and Hoi 2001) and could be a potential trait Rock Wren females assess to gauge male parental effort.

Though not significant, the number of young fledged and the total number of prey deliveries in the first five days post hatching were negatively related. However, all of the prey delivery metrics reported here do not account for quality or size of prey types delivered. The nutritional value of a large bodied invertebrate like a grasshopper compared to a smaller bodied insect such as a fly or an ant is likely to differ. Indeed, it would be energetically more efficient to find and bring back fewer higher quality prey items to chicks than to deliver many small morsels of food. Because I did not sample invertebrate sizes at my field sites by each prey type, and I could not reliably estimate size from photographs, I could not associate the amount of each prey type delivered with the nutritional value it confers to nestlings. Thus relationships between fledgling success and prey delivery rate are rudimentary in this study and a further in depth study of the relationship between prey type deliveries and fledging success would be informative. A more thorough investigation of prey availability on different territories in relation to repertoire size, nesting ecology, and fledgling success would also be immensely valuable (Bailey 1904; Merola 1995; Oppenheimer and Morton 2000; Warning and Benedict 2015b., 2016).

In addition to testing for intersexual selection on Rock Wren repertoire size, my research provided valuable data on Rock Wren breeding biology. I observed two different male Rock Wrens bring stones to the nest, which has never been recorded on our field sites (Warning and Benedict 2014), but observations of nests in the Sierra Nevada Mountains of California have also attributed rock paving to both males and females (Lowther et al. 2000; Oppenheimer and Morton 2000). I found average clutch sizes of 5.7 eggs ± 1.1 (N = 7), which are comparable to estimates in the literature, of about 5.6 eggs ± 0.8 (SD) for this species on average. Previous work across a wide geographic range has shown clutch sizes of 4 to 7 eggs in 10 nests in the Californian Sierra Nevada Mountains (Oppenheimer and Morton 2000), an average 5 eggs in 2 nests in New Mexico (Merola 1995), and 6 eggs found in one nest also in New Mexico (Bailey 1904). No estimates of average number of nestlings or fledglings per nest have been reported in the literature for Rock Wrens. There are also no estimates of fledging success reported per se, though 8 of 50 nests in West Kansas did suffer predations making for an overall fledging success of 84%, which is higher than my overall minimum fledging success of 42.86% (Matiasek 1998). These estimates are by default skewed by imperfect knowledge of nesting locations and affected by stochastic predation events. Analyzing additional ecological pressures that affect reproductive success, such as abundance of predators or prey, would be valuable in understanding the population fluctuations of Rock Wrens and their differential fledging success at different field sites (Salamacha 1992, Salamacha 1993, Salamacha 1994, Salamacha 1995, Salamacha 1996; Sauer et al. 1997).

My results provide a baseline from which to investigate the Rock Wren breeding success and the complex interactions influencing mate choice and reproductive productivity in this species. Ornithologists have a limited understanding of the Rock Wren’s life history and nesting ecology, as they are widespread, but often found in inaccessible habitats occupied by few other birds (Brewer 2001; Jones 1998). Population numbers are thought to be relatively stable overall, but some negative population trends have been detected in breeding bird surveys from the past 30 years and knowing more about the species’ patterns of reproductive success would help to reveal the factors behind these trends (Brewer 2001; Lowther et al. 2000). High nest predation has been proposed as a factor (Lowther et al. 2000). My results corroborate this conclusion, as snakes depredated three of the nine nests watched by trail cameras, although at least some of the nestlings managed to escape the predators. How populations in Larimer County are faring currently is unknown outside of the rudimentary work presented in this study, making my results valuable to land managers.

Overall, song repertoire size does not seem to be a signal that females use when evaluating males as potential mates in Rock Wrens. There was no difference in repertoire size between mated and unmated males. No morphology measurements typically associated with superior male quality were correlated with large repertoire sizes, including tarsus, tail, or wing length, weight, or body condition (weight over tarsus). Neither home range size nor number of fledglings produced were related to song repertoire size in any meaningful way. Measures of female reproductive effort including nest initiation date and clutch size also did not correlate. Male prey delivery did not predict how much prey their females would deliver, and was not related to his repertoire size, home range, or success at fledging chicks. Males did contribute more prey items to feed chicks in comparison to females at all nests, but male effort and female effort across pairs did not correlate with repertoire size and the relative amount contributed by each parent was variable at different nests. All in all, intersexual selection by female preference does not seem to be the most important driver of the elaboration of song type repertoire size in the Rock Wren.

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CHAPTER IV

SUMMARY AND FUTURE DIRECTIONS

Implications for the Function of Song Repertoire Size

In Northern Colorado's migratory populations of Rock Wren, it would seem that song type repertoire size is more important in territory defense contexts (Chapter 2) than in mate selection interactions (Chapter 3). However the subtle ways in which these birds are using song types in their repertoires and the importance of the characteristics of those song types still requires investigation. Those data would provide a more full understanding of the function of this communication signal. I showed that Rock Wren song type repertoires are relevant in modulating response intensity from a resident male when detecting an intruder. In contrast, Rock Wrens did not appear to use more song types in response to that intruder to demonstrate superior singing ability. In some instances birds would type match the songs in the playback track, but this was not quantified in this study because it seemed highly individually variable, where some individuals would type match on several instances through the playback experiment but in general this was not the norm.

Increased singing rate did seem to be driven by hearing more song types in the playback and is likely a real response to intruders detected on the territory, as detected in wood warblers (Szymkowiak and Kuczyński 2017). Intruders with larger song repertoires may well pose a larger threat to the resident male, if say larger repertoires indicate bigger males (Hesler et al.

2010), more aggressive opponents (Krippel et al. 2017), better territory defense (Hiebert et al. 1989), or some other aspect of better competitive ability (Kagawa and Soma 2013), and stands to be investigated further. Male Rock Wrens did respond to playback by singing wider bandwidth lower frequency songs after a simulated intruder and song is probably used as an honest signal of fighting ability and body size in males as has been seen in other avian species (Geberzahn and Aubin 2014; Hall et al. 2013). A very interesting study would be to evaluate each male's lowest frequency and frequency bandwidth averages in response to playback and see if it correlates with measurements of body size. This work could be done in the future with the current data set to investigate evidence for the motivation-structural hypothesis (Morton 1977) and the honest signaling hypothesis (Hall et al. 2013; Zahavi 1975; Darolová et al. 2012) as they relate to singing response. A similar singing response after playback was detected in Canyon Wrens (Benedict et al. 2012). Lower pitch is known to indicate body size in other bird species such that this could be a common response in songbirds (Geberzahn and Aubin 2014; Hall et al. 2013). In the *Acrocephalus* warblers, male songs with shorter, simpler structures are used in territory defense and longer more complex songs are used in mate attraction, but bandwidth was not measured (Catchpole and Slater 2003).

Rock Wren song has previously been evaluated in regards to song characteristics for different social contexts, and this species favored songs types that were longer and lower in frequency, with narrow bandwidth and slow syllable trill rates during broadcast singing (Benedict and Warning 2017). Though syllable trill rates were not evaluated in this study, Rock Wrens in agonistic situations seem to be using wider bandwidth and shorter songs with low frequencies. This could represent selective use of different song types of the repertoire based on social context, and could be further examined using this data set by seeing if certain song types only come up after an intruder simulation. Song is certainly important in multiple different social contexts, particularly interactions with mates or potential mates and rivals or neighbors, but also with nestlings and fledglings, and the way male birds (and for some species the females) use this communication signal to convey different messages varies by species (Lattin and Ritchison 2009).

Bird song repertoire size could be conferring advantages to singers through the "dual function hypothesis" by means of both mechanisms of selection (Catchpole and Slater 2003). Both intra- and intersexual selection could be playing a role in the evolution of all repertoires but to different extents, making one type of force more important than the other depending on factors specific to each species. Different patterns may exist in different species such that intersexual selection forces may be primarily driving song repertoire size in some but for others, forces of intrasexual selection might have a stronger impact, if say the result of same sex interactions increases reproductive fitness more (i.e. securing larger or better quality territories). In Rock Wrens, intrasexual selection seems to be the more important selective pressure on song repertoire size.

Rock Wren Population Declines

Though a common species throughout the west of North America, Rock Wrens are understudied by scientists as they are found in habitats that can be challenging to access (high rocky outcrops, on rocky slopes, or in canyons) and that are occupied by only a few other bird species (Brewer 2001; Jones 1998). Only some information is known about Rock Wren life history and nesting ecology in general (Bailey 1904; Kroodsma 1975, 1977; Lowther et al. 2000; Merola 1995; Oppenheimer and Morton 2000) and even less is known about populations in Northern Colorado (Benedict and Warning 2017; Warning and Benedict 2015a., 2015b., 2016).

Rock Wrens are an iconic species of Western North America and as such are part of the Native American culture and folklore. They are recognized as their own entity in the Navajo language that distinguishes them from the Canyon Wren also common in the area (Brewer 2001). The species also comes up in the Lakota Sioux sweat lodge ceremonies and have otherwise influenced indigenous cultures through art, dances, and songs (Tidemann and Gosler 2010).

Though population numbers are reported as mostly stable, breeding bird surveys in the past 30 years have detected declines in density of 1.4% per year with no explanations (Lowther et al. 2000). Populations in Colorado have declined 2.7% from 2000 to 2011 (Sauer et al. 2011). Since there is very little historical data, whether this decline is of concern or not is unknown, though high predation rates are worrying (Brewer 2001; Lowther et al. 2000). Indeed, snakes visited four out of eleven confirmed nests (36.4%), two of which (18.2%) suffered total loss. This is a minimal estimation of the rate of predation on nests and does not consider the remaining pairs (9) that I suspected were attempting to breed but where I could not find the nest cavity and they were never seen with fledglings. These could very well all have suffered predations, which would make for at most 13 of 21 pairs (61.9%) suffering from predation in this population. Though merely a rough estimate, a minimum predation rate of 36.4% is quite significant and considering that only 3 banded birds overall were observed to return in multiple years (So21 and So24 in 2016, So28 in 2017) of the total 27 birds examined (11%), higher predation rates could be affecting population numbers in migrant populations.

Rock Wrens do relatively well with the presence of humans. They can be found near developed areas and in fact will use anthropogenic structures for nesting cavities (Bent 1964). Indeed, their sprightly singing and lively attitude is endearing to many visitors to natural areas of the Front Range including homeowners and recreationists alike. Their presence builds advocacy for the preservation and protection of these natural open spaces that are critical in offering refuge to species. However, with increased human population in the Front Range of Colorado and more encroachment into the foothills by housing development, this is increasing the fragmentation of the landscape and could be introducing more predators that fare well in fragmented environments and could be detrimental to some bird species' reproductive success (Brattstrom 1995).

Natural History Notes

Though not relevant to the questions investigated in my research, interesting natural history and nesting behavior were observed in the field. First, both male and female Rock Wrens responded to playback experiments. Males always responded to some degree if present and established on the territory, but their female mates would sometimes also be seen calling the characteristic "*tick-ear*" and investigating the speaker, such that at one site both members of the pair were caught (though the location of their nest could not be found despite continued efforts). Both males and females were also seen bringing rocks to the nest cavity for the rock patio structure as well as removing fecal sacs from the nest during nest rearing. Both of these activities are expected to be important to nest success in avoiding predator nest detection and access. Further, males and females were seen producing buzzing soft song notes when approaching each other or the nest, when the male would bring the female food early in breeding, or right before soliciting copulation. This intriguing vocalization was used in a number of different social contexts between mates and with fledglings, and is ripe for study. Researchers could examine the behavioral associations of soft song to see if there are subtle variations by social context that have insofar not been detected. It has been proposed that low amplitude songs can be used as contact calls between mated individuals of a pair (Smith 1988), communicate with young (Beecher 1990), or warn the chicks of an imminent food delivery so the parent can be more efficient and draw less attention to the nest (Huntsman and Ritchison 2002). The vocal behavior of this species is far from well understood and many avenues of interesting research exist to further our understanding of this iconic Western species' breeding habits and singing behavior.

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APPENDIX A

TABLE OF NEST OUTCOMES FOR ALL DISCOVERED ROCK WREN NESTS INCLUDING NEST CHECKING DETAILS BY DATE

Table A1. Nest final outcomes for all 12 discovered nests with nest check details for each date visited, including information on male repertoire size at 500 songs, estimated dates for first egg lay date, hatch date, and fledge date for each nest, as well as predation information if available.

	2016	**2016**	**2016**	**2016**	**2016**	**2016**
Date/Bird ID	**LB17**	**LB19**	**So21**	**So28**	**So30**	**So31**
Repertoire size (500)	81		72	54	87	
Estimated First Egg Lay:	8-Jun-16	14-May-16	10-Jun-16	17-May-16	30-May-16	26-Jun-16
Estimated Hatch date:	26-Jun-16	30-May-16	28-Jun-16	3-Jun-16	15-Jun-16	NA
Estimated Fledge date:	10-Jul-16	13-Jun-16	8-Jul-16	17-Jun-16	29-Jun-16	NA
2-Jun-16		nestlings*				
8-Jun-16				nestlings*		
10-Jun-16					4 eggs	
13-Jun-16		3 fledglings	4 eggs			
14-Jun-16	5 eggs			nestlings*		
16-Jun-16					2 chicks, 1 egg	
17-Jun-16				4 fledglings**		
21-Jun-16					3 chicks	
22-Jun-16	5 eggs	2 or 3 fledglings	7 eggs			
26-Jun-16				4 fledglings	3 chicks	1 egg
27-Jun-16	4 chicks	2 or 3 fledglings	7 eggs			
29-Jun-16					(1 failed egg)	no eggs
2-Jul-16				3 fledglings	3 fledglings	
4-Jul-16	4 chicks	3 fledglings	3 chicks			
6-Jul-16					(1 failed egg)	
8-Jul-16			2 fledglings***			
9-Jul-16				3 fledglings	(1 failed egg)	
11-Jul-16	nest empty		nest empty			
16-Jul-16				3 fledglings		
Final Outcome:	2 fledglings	3 fledglings	2 fledglings	3 fledglings	3 fledglings	0 fledglings
Predation?						Unconfirmed

Table A1. Continued.

	2017	**2017**	**2017**	**2017**	**2017**	**2017**
Date/Bird ID	**So34**	**So35**	**So36**	**So45**	**So46**	**So47**
Repertoire size (500)	71		40	72	70	
Estimated First Egg Lay:	15-Jun-17	13-May-17	5-Jun-17	1-Jun-17	26-May-17	9-Jun-17
Estimated Hatch date:	1-Jul-17	NA	25-Jun-17	20-Jun-17	13-Jun-17	28-Jun-17
Estimated Fledge date:	14-Jul-17	NA	9-Jul-17	1-Jul-17	26-Jun-17	12-Jul-17
17-May-17		5 eggs				
26-May-17		5 abandoned eggs				
13-Jun-17			7 eggs	6 eggs	5 eggs	
19-Jun-17				6 eggs	3 chicks at least	
20-Jun-17						6 eggs
21-Jun-17			7 eggs			
26-Jun-17			4 chicks, 2 eggs	4 chicks	Nothing in nest	
28-Jun-17	3 chicks					
29-Jun-17				3 or 4 chicks	3 fledglings	2 chicks, 2 eggs
30-Jun-17	3 chicks					3 chicks, 1 egg
5-Jul-17			nothing in nest	1 fledge** nothing in nest	3 fledglings	
7-Jul-17						3 chicks, 1 egg
14-Jul-17	Nothing in nest			3 fledglings	2 fledglings	1 failed egg
3-Aug-17	2 or 3 fledglings					3 fledglings
Final Outcome:	3 fledglings	Abandoned	0 fledglings	3 fledglings	2 fledglings	3 fledglings
Predation?			Snake Predation	Snake Predation	Snake Predation	

* Exact nest not found so # unknown, but parents with food deliveries

** Emergence on camera

APPENDIX B

PREY DELIVERY ITEMS AND NEST VISITS IN THE FIRST FIVE DAYS POST HATCHING BY MALES, FEMALES, AND AN UNKNOWN PARENT INCLUDING PROPORTIONS CONTRIBUTED BY EACH PARENT

Table B1. Prey delivery items and nests visits in the first five days post hatching by males, females, and an unknown parent, including proportions contributed by each at Rock Wren LB17's nest.

Bird ID: LB17

Camera Days: 5

		Male	Male to Female	Male Overall	Female	Unknown	Prey Total
Visits:	59	37	0	37	7	15	
Proportion		62.7118644	0	62.71186441	11.8644068	42.3312883	
	Nesting Material						
	Fecal Sac				1		
Prey Total:	19	17	0	17	2	0	19
Proportion		47.2222222	0	89.47368421	10.5263158	0	
	Unknown Prey	8					8
	Grasshopper	4			2		6
	Beetle						0
	Caterpillar	2					2
	Grub						0
	Spider	2					2
	Dragonfly	1					1
	Fly						0
	Moth						0
	Snail						0
	Rock in bill						
	Unknown in bill						

Table B2. Prey delivery items and nests visits in the first five days post hatching by males, females, and an unknown parent, including proportions contributed by each at Rock Wren So21's nest.

Bird ID: So21

Camera Days: 5

		Male	Male to Female	Male Overall	Female	Unknown	Prey Total
Visits:	52	14	19	33	19	0	
Proportion		42.4242424	57.57575758	63.4615385	57.5757576	0	
	Nesting Material						
	Fecal Sac						
Prey Total:	26	7	19	26	0	0	26
Proportion		26.9230769	73.07692308	100	0	0	
	Unknown Prey	1	15				16
	Grasshopper	3					3
	Beetle		1				1
	Caterpillar	2	1				3
	Grub		1				1
	Spider	1	1				2
	Dragonfly						0
	Fly						0
	Moth						0
	Snail						0
	Rock in bill						
	Unknown in bill						

Table B3. Prey delivery items and nests visits in the first five days post hatching by males, females, and an unknown parent, including proportions contributed by each at Rock Wren So28's nest.

Bird ID: So28

Camera Days: 5

		Male	Male to Female	Male Overall	Female	Unknown	Prey Total
Visits:	64	16	2	18	41	5	
Proportion		25	3.125	28.125	64.0625	7.8125	
	Nesting Material						
	Fecal Sac				4		
Prey Total:	16	6	2	8	6	2	16
Proportion		37.5	12.5	50	37.5	12.5	
	Unknown Prey	2	1		2	2	7
	Grasshopper	3	1		3		7
	Beetle						0
	Caterpillar	1			1		2
	Grub						0
	Spider						0
	Dragonfly						0
	Fly						0
	Moth						0
	Snail						0
	Rock in bill						
	Unknown in bill						

Table B4. Prey delivery items and nests visits in the first five days post hatching by males, females, and an unknown parent, including proportions contributed by each at Rock Wren So30's nest.

Bird ID: So30

Camera Days: 5

		Male	Male to Female	Male Overall	Female	Unknown	Prey Total
Visits:	387	137	121	258	123	6	
Proportion		35.4005168	31.26614987	66.66666667	31.7829457	1.55038760	35.400517
	Nesting Material						
	Fecal Sac				2		
	On nest				75		
Total Prey:	173	39	121	160	13	0	173
Proportion		22.5433526	69.94219653	92.48554913	7.51445087	0	
	Unknown Prey	23	82		6		111
	Grasshopper	15	28		3		46
	Beetle		2				2
	Caterpillar		3		2		5
	Grub		2		2		4
	Spider	1	2				3
	Dragonfly						0
	Fly		1				1
	Moth		1				1
	Snail						0
	Rock in bill						
	Unknown in bill						

Table B5. Prey delivery items and nests visits in the first five days post hatching by males, females, and an unknown parent, including proportions contributed by each at Rock Wren So34's nest.

Bird ID: So34

Camera Days: 5

		Male	Male to Female	Male Overall	Female	Unknown	Prey Total
Visits:	549	229	9	238	209	102	
Proportion		41.7122040	1.639344262	43.35154827	38.0692168	18.5792350	
	Nesting Material				3		
	Fecal Sac	31			27	1	
Prey Total:	36	15	9	24	12	0	36
Proportion		41.6666667	25	66.66666667	33.3333333	0	
	Unknown Prey	7	5		6		18
	Grasshopper	3	4		2		9
	Beetle	2					2
	Caterpillar	2			2		4
	Grub				1		1
	Spider						0
	Dragonfly						0
	Fly						0
	Moth	1			1		2
	Snail						0
	Rock in bill						
	Unknown in bill	9			9	4	

Table B6. Prey delivery items and nests visits in the first five days post hatching by males, females, and an unknown parent, including proportions contributed by each at Rock Wren So36's nest.

Bird ID: So36

Camera Days: 5

		Male	Male to Female	Male Overall	Female	Unknown	Prey Total
Visits:	185	127	1	128	48	9	
Proportion		61.6279070	0.387596899	62.01550388	32.1705426	5.42635659	
	Nesting Material						
	Fecal Sac						
Prey Total:	41	35	1	36	4	1	41
Proportion		83.3333333	2.777777778	86.11111111	11.1111111	2.77777778	
	Unknown Prey	9			3		12
	Grasshopper	22	1		1		24
	Beetle						0
	Caterpillar						0
	Grub	4				1	5
	Spider						0
	Dragonfly						0
	Fly						0
	Moth						0
	Snail						0
	Rock in bill						
	Unknown in bill						

Table B7. Prey delivery items and nests visits in the first five days post hatching by males, females, and an unknown parent, including proportions contributed by each at Rock Wren So45's nest.

Bird ID: So45

Camera Days: 5

		Male	Male to Female	Male Overall	Female	Unknown	Prey Total
Visits:	401	311	0	311	36	54	
Proportion		77.5561097	0	77.55610973	8.97755611	13.4663342	
	Nesting Material						
	Fecal Sac						
Prey Total:	52	46	0	46	4	2	52
Proportion		88.4615385	0	88.46153846	7.69230769	3.84615385	
	Unknown Prey	19			1		20
	Grasshopper	10			2	1	13
	Beetle	1			1		2
	Caterpillar	2					2
	Grub	7					7
	Spider	0					0
	Dragonfly	2					2
	Fly	0					0
	Moth	5				1	6
	Snail	0					0
	Rock in bill						
	Unknown in bill						

Table B8. Prey delivery items and nests visits in the first five days post hatching by males, females, and an unknown parent, including proportions contributed by each at Rock Wren So46’s nest.

Bird ID: So46

Camera Days: 5

		Male	Male to Female	Male Overall	Female	Unknown	Prey Total
Visits:	125	100	0	100	4	21	
Proportion		80	0	80	3.2	16.8	
	Nesting Material						
	Fecal Sac						
Prey Total:	13	8	0	8	2	3	13
Proportion		61.5384615	0	61.53846154	15.3846154	23.07692308	
	Unknown Prey	6			1	2	9
	Grasshopper	1				1	2
	Beetle						0
	Caterpillar	1					1
	Grub						0
	Spider				1		1
	Dragonfly						0
	Fly						0
	Moth						0
	Snail						0
	Rock in bill						
	Unknown in bill						

APPENDIX C

PLAYBACK EXPERIMENT LOCATIONS BY FIELD SITE AND ROCK WREN HOME RANGE SIZE IN HECTARES

Table C1. Playback experiment locations by field site and Rock Wren home range size in hectares.

Bird ID	Field Site	Year	Home Range Size (Ha)	Number GPS Points
LB17	BRNA	2016	1.4656	25
SP1	BRNA	2016		
So36	BRNA	2017	2.8777	29
So37	BRNA	2017	3.5319	13
So41	BRNA	2017	1.0231	12
SP3	DBB	2016		
So42	DBB	2017	1.3012	7
So43	DBB	2017		
So44	DBB	2017		
So45	DBB	2017		
So46	DBB	2017		
LB19	LSP	2016	1.7806	18
So21	LSP	2016	4.8970	25
So35	LSP	2017		
So39	LSP	2017		
SP08	LSP	2017	0.5103	8
SP15	LSP	2017	2.7895	9
SP2	PRNA	2016		
So24	PRNA	2016	0.8911	10
So28	PRNA	2016	0.8455	13
So29	PRNA	2016	0.3466	9
So30	PRNA	2016	0.8652	27
So31	PRNA	2016		
So32	PRNA	2016		
So34	PRNA	2017		
So40	PRNA	2017		
So47	PRNA	2017		
Average			1.7789	15.7692
Standard Deviation:			1.3564	8.0016

Note: BRNA: Bobcat Ridge Natural Area, Fort Collins, CO; DBOS: Open Space, Loveland, CO; LSP: Lory State Park, Bellevue, CO; PRNA: Pine Ridge Natural Area, Fort Collins, CO.

APPENDIX D

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL

UNIVERSITY of
NORTHERN COLORADO

IACUC Memorandum

To: Dr. Lauryn Benedict
From: Laura Martin, Director of Compliance and Operations
CC: IACUC Files
Date: February 16, 2016
Re: IACUC Protocol 1506C-LB-Birds-18 Amendment and Annual Approval

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The UNC IACUC has reviewed your request for an annual renewal and amendment request to animal use protocol 1506C-LB-Birds-18.

The committee's review was based on the requirements of the Government Principles, the Public Health Policy, the USDA Animal Welfare Act and Regulations, and the Guide for the Care and Use of Laboratory Animals, as well as university policies and procedures related to the care and use of live vertebrate animals at the University of Northern Colorado.

Based on the review, the IACUC has determined that all review criteria have been adequately addressed. The PI/PD is approved to perform the experiments or procedures as described in the identified modification as submitted to the committee on January 29, 2016. The PI/PD is approved to perform the experiments or procedures as described in the identified protocol for an additional year. The next annual renewal is due for approval prior to April 29, 2017.