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Acoustic signalling in Savannah Sparrows, *Passerculus sandwichensis*: Diel and seasonal variation, male-male vocal interactions, and responses to playback

By
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Acoustic signalling in Savannah Sparrows, *Passerculus sandwichensis*: Diel and seasonal variation, male-male vocal interactions, and responses to playback

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Declaration of co-authorship

I hereby declare that this thesis incorporates material that is the result of joint research, as follows: I am the sole the author of chapter 1 and 4 and the principal author of chapters 2 and 3. Both chapters 2 and 3 were conducted under the supervision of Daniel Mennill (University of Windsor) and Ryan Norris (University of Guelph), and the guidance of two additional co-authors, Stéphanie Doucet (University of Windsor) and Amy Newman (University of Guelph), who contributed input on experimental design, analyses, and writing as well as providing funding and logistical support for field research. In all cases, the key ideas, data analysis, interpretation, and writing were performed by myself, and my co-authors provided assistance with experimental design, refinement of ideas, and editing the manuscripts.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-author(s) to include the above material(s) in my thesis.

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Abstract

In this thesis, I examine the function of acoustic signals in Savannah Sparrows, *Passerculus sandwichensis*, by analyzing temporal variation in vocal activity, and by conducting a playback experiment. In my first data chapter, I analyze long-term acoustic recordings to study diel and seasonal variation in vocal activity of Savannah Sparrows. I show that singing activity of male Savannah Sparrows varies with time of day, time of year, and breeding stage. Males exhibit the highest level of song output in May, upon arrival on the breeding grounds, and the lowest level in August, before departure from the breeding grounds. Song output peaks in the early morning, consistent with dawn chorus behaviour, but this pattern is common only prior to pairing; after pairing the dawn chorus is reduced and male song output peaks in the evening, consistent with dusk chorus behaviour. These patterns suggest that dawn choruses serve a territorial function whereas dusk choruses serve a female-related function in Savannah Sparrows. In my second data chapter, I present the results of a playback experiment designed to test whether Savannah Sparrows signal their intention to attack a rival male. I simulated an intruder using song playback and a taxidermic model, and explored which behaviors were associated with physical attack. Savannah Sparrows produce soft songs and chip calls at significantly higher levels before attacking rivals, whereas three other measured behaviors (aggressive calls, wing waving, and passes over the model) do not predict attack. My research advances the field of animal communication and provides a foundation for future research on signal function and social interactions involving vocal signals.

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CHAPTER 1: General introduction

Introduction

Many animals produce vocal signals. These signals serve diverse functions, including mate attraction, territorial defense, alarm, identity recognition, and dominance signalling (Bradbury & Vehrencamp, 2011). Many animal signals vary seasonally, and this variation can inform our understanding of signal function and signalling behaviour (Slagsvold, 1977). Songbirds provide a compelling study system to investigate the function of acoustic signals because they have evolved complex and diverse vocal signals. In particular, songbirds use songs to defend territories and attract mates (Catchpole & Slater, 2008). My thesis focuses on investigating the functions of songs in Savannah Sparrows, *Passerculus sandwichensis*, with an emphasis on diel and seasonal variation of song output (Chapter 2), and the behaviour of birds during territorial interactions (Chapter 3). In this General Introduction, I provide a brief overview of the function of bird songs and seasonal variation in avian vocal behaviour and male vocal interactions, to provide background information for the two data chapters that follow.

Animal vocal signals: function and variation

Although animal vocal signals serve many functions including alarm, contact, and indication of food sources, two principal functions of vocal signals are territorial defense and mate attraction. For example, territorial male Galápagos Sea Lions, *Zalophus wollebaeki*, bark to attract females and to defend their harems from intruders (Kunc & Wolf, 2008). This dual function of animal vocal signals is well established, especially in birds (i.e. the “dual function theory” of vocal signals by Catchpole & Slater 2008). Indeed, male temperate songbirds are widely recognized to produce songs that are important both in territorial competition and mate attraction during the breeding season (reviewed in Catchpole & Slater, 2008).

Several lines of evidence reveal a strong association between vocal behaviour and mate attraction. Few experimental studies reveal that songs are important in mate choice but many observational studies show that unpaired males sing far more than paired males (e.g. Amrhein et al., 2004; Catchpole, 1973; Cuthill & Hindmarsh, 1985; Hennin et al., 2009). Once pairing or egg-laying has taken place, males also tend to reduce their song output (Amrhein et al., 2008; Ezaki, 1987). A common pattern observed during courtship behaviours is the increase in vocal activity or vocal complexity of male songs; in many temperate songbirds, song production peaks during the fertile period (Catchpole, 1973; Lampe & Espmark, 1987; Slagsvold, 1977). The investigation of the relationship between variation in singing activity and breeding stages provides us with a deeper understanding of the various functions of male songs.

In addition to the role that vocal signals play in mate attraction, a large body of evidence reveals that male vocal signals play a role in male-male interactions and territory defense. This was originally demonstrated through muting experiments, playback experiments, and speaker occupation experiments. Muting experiments demonstrated that muted males were unable to maintain their territories (e.g. Red-winged Blackbirds, *Agelaius phoeniceus*: Peek, 1972; Scott’s Seaside Sparrows, *Ammodramus maritimus peninsulae*: McDonald, 1989). Playback experiments established that the playback of male songs triggered aggression from nearby neighbours (e.g. Indigo Bunting, *Passerina cyanea*: Emlen, 1971, Song Sparrows, *Melospiza melodia*: Searcy et al. 2006); and speaker occupation experiments confirmed that playback of songs effectively repelled rival males from speaker-occupied territories (Great Tits, *Parus major*: Krebs et al., 1978; Song Sparrows: Nowicki et al., 1998; White-throated Sparrows: Falls, 1988). Songs are thus important signals for maintaining territories, and effective signals for deterring rivals. Exploring how males use their songs during male-male interactions provides insight into the role of songs in territory establishment and maintenance of boundaries.

Male-male interactions

Territorial interactions between rival males routinely involve aggression (Todt & Naguib, 2000). Given that aggressive encounters may involve costly physical attacks, many animals have evolved agonistic signals to facilitate communication without physical confrontation during aggressive contests (Johnstone, 1997). As contests escalate, animal signals can transition from low-level threat signals to high-level threat signals. For example, Sac-winged Bats, *Saccopteryx bilineata*, call at lower pitches and greater rates when territorial contests escalate (Behr et al. 2009) and Green Frogs, *Rana clamitans*, decrease the pitch of their calls when territorial contests escalate (Bee et al., 2000; Reichert & Gerhardt, 2013). In addition, songbirds often change their singing behaviour to escalate or de-escalate contests (Todt & Naguib, 2000a). Previous research on signal escalation has focused on song overlapping, song-type matching, song switching, or modulation of the fine structure of song elements (e.g. Burt et al., 2001; DuBois et al., 2009; Naguib, 2005; Searcy et al., 2006), and many of these signalling behaviours change as contests escalate or de-escalate.

The best-studied system of territorial contest escalation comes from Song Sparrows (Akçay et al., 2013; Beecher & Campbell, 2005; Searcy & Beecher, 2009). In a series of experiments designed to study aggressive signalling during Song Sparrow territorial disputes, Searcy and Beecher (2009) established a compelling model for contest escalation. Their hierarchical signalling model proposes that during a dyadic interaction, a male may escalate a contest by singing the same song as his rival's songs, a behaviour known as song matching, or de-escalate by switching to a non-matching song (Akçay et al. 2013). Similar investigations have been conducted in other taxa, which all point towards the possible existence of graded signals of contest escalation (e.g. Hof & Podos 2013). Songbirds can use other vocal communication strategies to escalate interactions such as song frequency matching (Baker et al., 2012), increases in song frequency and song rate (DuBois et al., 2009), decreases in song amplitude (Ballentine et al., 2008; Grieves et al., 2015; Hof & Hazlett, 2010; Reichard et al., 2011; Ritschard et al., 2012; Searcy et al., 2006; Xia et al., 2013), and production of non-song calls (Ballentine et al., 2008, Baker et al., 2012, Elie & Theunissen 2015). Remarkably few studies have explored the precise function of signals during aggressive interactions or described the graded signalling strategies that comprise a hierarchical signalling model. The second data chapter of my thesis explores these ideas.

Researchers today focus on expanding our understanding of vocal signals to include both songs (i.e. complex learned vocalizations, usually produced by males during the breeding season) as well as calls (i.e. simple, short non-learned vocalizations, sometimes produced by both males and females throughout the year; e.g. Gill et al., 2015: Elie & Theunissen, 2015). Past research focused on songs as the main signal that plays a territorial and attractive function, whereas the importance of calls during these interactions remained poorly studied. Like songs, calls are often produced during periods of territorial defence and mate attraction, and have been shown to be produced in diverse contexts (Marler, 2004) and in large numbers (Beckers & Gahr, 2010). The second chapter of my thesis explore calls as potential signalling strategies to escalate interactions.

Study site and study species

My thesis research took place at the Bowdoin Scientific Station on Kent Island, New Brunswick, Canada (44°35'N 66°46'W), a 200-acre island in the Bay of Fundy. This island is home to a population of Savannah Sparrows with strong philopatry (Wheelwright & Mauck, 1998). Savannah Sparrows migrate from their wintering grounds in the southern United States (Woodworth et al. 2015) to their breeding sites across the United States and Canada (Bédard & LaPointe, 1984; Tufts, 1986). The birds on Kent Island have been studied annually for many decades (Williams et al. 2013). Each year, more than one hundred Savannah Sparrows at the study site are marked with a unique combination of leg bands to facilitate recognition of individuals in the field. Male

Savannah Sparrows arrive from migration in mid-April and begin to defend breeding territories from rival males (Woodworth et al. 2015). This is an important moment in the annual cycle of males; if they are to attract females and breed successfully, they need to establish a breeding territory (Potter, 1972). During territorial establishment, male Savannah Sparrows interact with other males by singing a single song type (Figure 1.1). Males appear to learn their songs within the first eight months of life (Wheelwright et al., 2008) and songs remain constant throughout an individual's life (Williams et al., 2013).

During territory establishment, male Savannah Sparrows use an array of vocal and visual signals during aggressive interactions with rivals. Male song is a regular component of male behaviour during territory establishment (Wheelwright & Rising, 2008). In addition to their songs, males emit a variety of calls during territorial encounters, including aggressive flight calls and hostile notes (a.k.a. buzz calls) (Gobeil, 1970; Wheelwright & Rising, 2008). Males also engage in agonistic visual displays that include parallel walking, flutter flights, tail raising, bill gaping, ptiloerection of crest feathers, and bouts of physical chases (Potter, 1972; Wheelwright & Rising, 2008). The relative importance of these diverse vocalizations and physical displays are poorly studied; they may be critical for territorial signalling in Savannah Sparrows, and they may comprise a graded system of aggressive signalling, although this has never been studied previously.

Thesis goals

In this thesis, I explore the vocal behaviour of Savannah Sparrows with a focus on communication in the context of territorial aggression and mate attraction. My thesis has two components. Chapter 2 is an observational study based on detailed recordings of individually-marked Savannah Sparrows. I describe diel and seasonal variation in vocal output, evaluate how male singing behaviour changes with different breeding stages, and conduct a detailed analysis of recordings of territorial males. In Chapter 2, I ask the question: *what is the pattern of diel and seasonal variation in vocal activity of Savannah Sparrows, and what does this reveal about the functions of their vocal signals*? Chapter 3 is an experimental study based on a recently developed playback protocol that has been used to study aggressive signalling in other songbirds. I conducted a playback and model-presentation experiment to study the graded signals that are associated with contest escalation during Savannah Sparrow territorial encounters. In Chapter 3, I ask the question: *do Savannah Sparrows produce signals that predict physical attack during territorial interactions?* With this body of research, I hope to expand our understanding of vocal behaviour of Savannah Sparrows specifically, and, more broadly, to develop a better understanding of seasonal and diel variation in vocal signals and the dynamics of signalling behaviour during aggressive territorial encounters.

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Figures

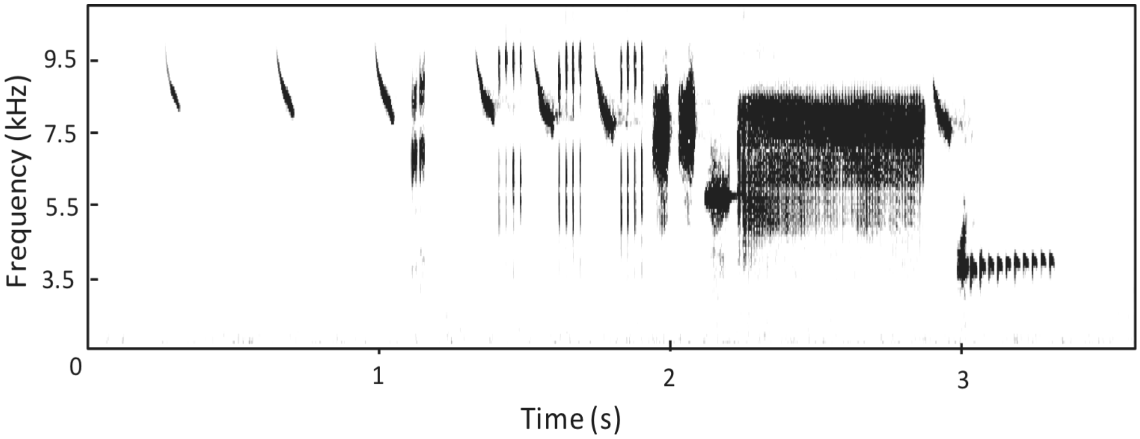

Figure 1.1. Sound spectrogram showing the song of a Savannah Sparrow recorded at Bowdoin Scientific Station on Kent Island, New Brunswick, Canada, 2015.

CHAPTER 2: Diel and seasonal patterns of acoustic signalling in Savannah Sparrows

Chapter summary

Animal vocal output can change daily and seasonally. Diel and seasonal variation in vocal output can be an indicator of signal function and studies of daily and seasonal rhythms can provide us with deeper insight into animal behaviour. In this study, we quantified diel and seasonal variation in song output in a breeding population of Savannah Sparrows, *Passerculus sandwichensis*. We used automated recorders to collect extensive recordings across the breeding season. We found significant diel and seasonal variation in vocal output with respect to male pairing and breeding status. In terms of diel variation, males showed a peak in the early morning, consistent with dawn chorus behaviour, but this pattern was common only prior to pairing. After pairing, males showed a peak in the evening, consistent with dusk chorus behaviour. In terms of seasonal variation, Savannah Sparrows showed the highest level of song output in May and the lowest level in August. Males showed the highest singing activity early in the year, when birds were first establishing territories and also when females were incubating. Unpaired territorial males had the most pronounced dawn chorus and higher levels of morning singing activity. A dusk chorus occurred later in the breeding season, during the fertile, incubation, hatchling, and fledgling periods, with paired males participating most heavily. These patterns suggest that dawn choruses have a territorial function whereas dusk choruses may have a female-related function in this species. We conclude that Savannah Sparrows show dynamic patterns of diel and seasonal variation, where vocal output varies with social context at different stages of their breeding season.

Introduction

Many territorial animals use vocal signals that vary daily and seasonally. Variation in signalling output is often linked with changes in seasons, when the levels of abiotic factors such as light and temperatures change. Several hypotheses have been proposed to explain why animals vary their vocalizations daily and seasonally and these fall under three main categories: temporal variation in vocal output can be related to mechanistic processes (e.g. the hormones that influence song output), social processes (e.g. the social contexts that affect vocal output), or environmental features (e.g. the environmental constraints on vocal output) (Burt & Vehrencamp, 2005; Cuthill & Macdonald, 1990; Kacelnik & Krebs, 1983; Mace, 1987; Staicer et al., 1996). The influence of social forces such as pairing status (Catchpole, 1973; Cuthill & Hindmarsh, 1985; Demko et al., 2013) and breeding status (Bruni & Foote, 2014; Foote & Barber, 2009; Hanski & Laurila, 1993; Zhang, Celis-Murillo, & Ward, 2016) have a profound influence on vocal output, and understanding the connection between social activities and changes in vocal behaviour has proven to be a revealing area of research. In this study, we investigate the social processes that influence the diel and seasonal vocal variation in a North American songbird species, Savannah Sparrows, *Passerculus sandwichensis*.

The singing activity of many animals varies with time of day, especially in species that exhibit dawn and dusk choruses (reviewed in Staicer et al. 1996). Dawn and dusk choruses are periods of heightened vocal activity that appear to be important for territorial defense, mate attraction, and extra-pair mate attraction (Burt & Vehrencamp, 2005; Catchpole, 1973; Kunc, Amrhein, & Naguib, 2005; Poesel et al., 2006; Slagsvold, Dale, & Sætre, 1994). Dawn choruses usually start before sunrise and conclude at sunrise, or shortly after sunrise, depending on the species or time of the year (reviewed in Staicer et al. 1996). Similarly, dusk choruses begin at sunset, and continue after sunset (e.g. Cuthill & Macdonald, 1990; Lein, 2007). Animals that show dawn and dusk choruses include primates (Schel & Zuberbühler 2011), lizards (Ord, 2008), and birds (Burt & Vehrencamp, 2005). Songbirds are particularly well-known for their dawn and dusk choruses, and they provide a model system in the field of acoustic communication to study diel variation in vocal output.

In addition to diel variation in vocal output, the singing activity of animals can also vary with pairing and breeding status. In many species of songbirds, for example, males sing extensively until they are paired and singing activity is greatly reduced after pairing (e.g. Sedge Warblers, *Acrocephalus schoenobaenus*: Catchpole, 1973), although other species continue singing even after they are paired (e.g. Reed Warblers, *Acrocephalus scirpaceus*: Catchpole, 1973). In some species, males use different types of signals when unpaired versus paired (Catchpole, 1973) or modulate their songs with different breeding stages (Zhang et al., 2016). The singing activity of songbirds can also vary with female breeding stages, often showing levels of high output during the fertile period (Amrhein et al., 2008; Foote & Barber, 2009; Slagsvold, 1977) or during both the fertile and incubation periods (e.g. Bruni & Foote, 2014). In other species, males increase their song rate during the nest-building stage (e.g. Northern Mockingbirds, *Mimus polyglottos*: Logan, 1983) or vary their song complexity during periods of female attraction (e.g. Blue Grosbeaks, *Passerina caerulea*: Ballentine et al., 2003). Singing activity can also vary during the egg-laying period. In Nightingales, *Luscinia megarhynchos*, for example, males lower their song output and reduce their song overlapping during the egg-laying period (Amrhein et al. 2004). Investigating variation in song output provides an opportunity for understanding the role of male songs with their association to female breeding status.

In this study, we collected long-term recordings to quantify seasonal and diel output in Savannah Sparrows, and we compare vocal output to breeding activities and observational data. We tested the hypotheses that vocal output in song varies with time of day and with time of year. We made *a priori* predictions about the relationship between variation in vocal output and breeding activities. We predicted that if the main function of dawn and morning chorus singing in male Savannah Sparrows is to defend and maintain their territory, then the song output should not vary with female breeding stages. Alternatively, if the main function of the dawn chorus is related to female breeding status, then song output should vary throughout the breeding cycle and serve different functions.

Material and methods

Study species and study site

Savannah Sparrows are small migratory birds that live in open grasslands throughout North America (Bédard & LaPointe, 1984). Males migrate from their wintering grounds in the southern United States to their breeding sites across the United States and Canada (Bédard & LaPointe, 1984; Tufts, 1986). We conducted our research at the Bowdoin Scientific Station on Kent Island, New Brunswick, Canada (44°35’N 66°46’W), a 200-acre island in the Bay of Fundy. This island is home to a population of Savannah Sparrows with strong philopatry (Wheelwright & Mauck, 1998). The birds in this population have been studied for more than three decades (Williams et al., 2013). Each year, more than one hundred Savannah Sparrows at the study site are marked with a unique combination of leg bands to facilitate recognition of individuals in the field. Male Savannah Sparrows breeding on Kent Island arrive from migration in mid-April, about one month before females’ arrival in mid-May, and begin to defend breeding territories from rival males (Woodworth et al., 2015). During territorial establishment, male Savannah Sparrows interact with other males by singing a single song type. This is an important period for males; if they can establish a breeding territory they will attract females more easily (Potter, 1972). Kent Island males’ territories size average is 0.21 ha and range in size between 0.03-0.60 ha (Wheelwright & Rising, 2008) which allow easy monitoring of multiple interactions between multiple males.

Recording technique

In 2014, we recorded 34 males using automated recorders, Wildlife Acoustics Song Meters (model: SM2; see Mennill et al. 2012). Given the small territories of Savannah Sparrows, these devices allowed us to record between one and seven males simultaneously with a single recorder. We recorded males for ten 24h-long recording sessions between mid-April and mid-September, with 14-day intervals between subsequent recording sessions at each site. In all recordings where multiple males were present, we distinguished individuals on the basis of their individually distinctive song (see Wheelwright et al., 2008).

Analysis of pairing and breeding status

To determine breeding stages of the 34 males in our diel and seasonal variation analysis, we collected behavioural observations and monitored nests every two days. Following Foote & Barber (2009), we divided the breeding season into five different periods from May to September: (1) males that were unpaired (the period starting from males' arrival around mid-April, up to 15 days later, start of May; females were absent during this period), (2) males paired with a fertile female (the period corresponding to 8 days prior to the laying of the penultimate egg), (3) males with a female incubating eggs (a period of 12 days), (4) males with a female provisioning nestlings (a period of 9 days), and (5) males with a female provisioning fledglings (a period starting from the end of the nestling stage to the start of the next brood or if no consecutive brood was attempted, a period of 7 days after the end of nestling period). We estimated the length of the fertile period based on the following logic: female Savannah Sparrows are thought to be fertile during the 1-3 days when nests are built and during the following period of 3-5 days when eggs are laid (1egg/day). We calculated the start of each breeding period based on the fertile period. But, in some cases, we estimated the first day of incubation by back-dating 12 days from known hatch dates because in those cases the nests were found later during the incubation stage thus the first day of egg-laying could not be determined (Dixon, 1978; Wheelwright & Rising, 2008).

Diel and seasonal variation

In order to compare song output between dawn chorus, daytime, and dusk chorus, we defined the dawn chorus as the period 30 minutes before sunrise, a time period that included some of the civil twilight and dawn (as in Liu, 2004; Naguib et al., 2016). Daytime was defined as the period starting from sunrise to sunset. Dusk chorus was defined as the period starting from sunset up to the last songs. Finally, for each of the five breeding periods, we investigated diel variation by calculating and comparing hourly values for the total song output during dawn chorus, daytime, and dusk chorus. Sunrise and sunset times for each day were obtained from the National Research Council, Herzberg Institute of Astrophysics sunrise/sunset calculator (www.nrc-cnrc.gc.ca). We excluded days where weather affected recordings (i.e. very rainy or windy days). On average, we included 7 ± 3 days of recording across the season for each individual.

Statistical analysis

We conducted an analysis of diel variation in song output using linear mixed models to compare diel song output between breeding stages. We included time of day (subdivided into 1-hr periods), breeding stage (unpaired, fertile, incubation, hatchling, or fledgling), and interaction between time and breeding stage as our fixed factors. We included individuals as a random effect to account for the fact that the same males were sampled repeatedly.

Finally, we compared the difference in song output between breeding stages for three periods: the dawn chorus, daytime, and the dusk chorus. Because the distribution of our data was non-normal, we compared song output across those time periods using a Wilcoxon test. To determine how song output varies between breeding stages, we then compared the average number of songs between breeding stages using a Wilcoxon test.

We analyzed the seasonal variation of the singing activity of Savannah Sparrows using linear mixed models to determine the period with highest and lowest song output per hour. For the analysis of seasonal variation, we included Julian day (83 days between 4 May and 26 July 2014), breeding stage (unpaired, fertile, incubation, hatchling, or fledgling) and the interaction between Julian day and breeding stage as our fixed factors. We included male identity as a random effect to account for the fact that the same males were sampled repeatedly.

We used JMP v12 (SAS Institute Inc. 2015) for all our statistical analyses. We considered results with $P<0.05$ significant and we represented values as means ± SE. Time of sunrise and sunset from the start of our recording on 4 May until the end of our recordings on 26 July 2014 varied respectively from 07:05 h to 06:06 h for sunrise and from 19:55 h to 21:00 h for sunset.

Results

Diel variation with respect to breeding status

We conducted an analysis of diel variation in song output using linear mixed models to compare diel song output between breeding stages. Savannah Sparrow song output varied significantly with respect to breeding stages ($F_{4,4687}=13.9$, $P<0.001$), with respect to time of day ($F_{1,4698}=22.5$, $P<0.001$), and interaction between breeding stage and time of day ($F_{4,4698}=52.4$, $P<0.001$), (n=34 males) (Fig. 2.1; Supplementary Table 2.1). Savannah Sparrows' diel song output showed significant variation between time of day in the unpaired period ($t_{83}= 8.9$, $P<0.001$), the fledgling period ($t_{4641}=6.2$, $P<0.001$), and incubation period ($t_{4670}=5.9$, $P<0.001$) (n=30 males). The diel song output during the fertile period ($t_{4724} = 0.42$, $P=0.67$) and the hatchling period ($t_{4720}= 0.84$, $P=0.39$) did not vary significantly across time of day (Fig. 2.1). We obtained a R^2 value of 0.12 and the distribution of values of random effect had a variance of 0.07.

One striking difference between unpaired males and males at other breeding stages (fertile, incubation, hatchling, fledgling) was the difference in song output in the dawn, daytime, and dusk chorus song activities. The song output of unpaired males peaked in the morning around 07:00 h and then males reduced their singing activity from late morning into the evening (between approximately 11:00 h to 22:00 h). Males at other breeding stages had a small increase in singing activity early morning (around 5:00 h), maintained their singing activity in morning (between 06:00 h to 12:00 h) and the afternoon evening (between approximately 12:00 h to 20:00 h) and increased their singing activity during dusk chorus (between approximately 20:00 h to 22:00 h).

Dawn chorus, dusk chorus, and daytime singing activity

We compared the difference in song output between breeding stages for three periods: the dawn chorus, daytime, and the dusk chorus. When comparing different breeding stages across three daytime periods (morning chorus, daytime, and dusk chorus), the total song output during the dawn chorus (30 min before sunrise until sunrise) was significantly higher when males were unpaired than all other breeding stages (Fig. 2.3), including the fertile period (Z=3.02, P=0.003), incubation period (Z=2.61, P=0.009), hatchling period (Z=2.33, P=0.02), and fledgling period (Z=2.20, p=0.03). Once males were paired, their singing activity during the dawn chorus decreased by about half compared to the unpaired stage and did not differ significantly between stages. During the day, male singing activity did not significantly differ between breeding stages except between the unpaired and fledgling stage (Z=2.26, P=0.02), where unpaired males sang more than males in the fledgling stage (Figure 2.4). During the dusk chorus period, unpaired males sang significantly less than any other breeding stage (Fig. 2.5). Unpaired males sang significantly less compared to the fertile period (Z=-4.24, P<0.0001), the incubation period (Z=-2.79, P=0.005), the hatchling period (Z=-2.04, P=0.04), and the fledgling period (Z=-2.57, P=0.01). The song output during the dusk chorus was also significantly smaller during the hatchling stage compared to the fertile stage (Z=-2.26, P=0.02).

Song output variation with respect to breeding status

Song output during the incubation stage was significantly higher than any other stage except the unpaired stage (Z=-6.7, P<0.001) and was not significantly different from the hatchling stage (Z=0.54, P=0.58). Song output per hour during the fledgling stage was significantly lower than the unpaired stage (Z=-2.67, P=0.008), and the hatchling stage (Z=2.02, P=0.04) but not the fertile stage (Z=-0.93, P=0.35).

Seasonal variation of song output

We analyzed the seasonal variation of the singing activity of Savannah Sparrows using linear mixed models to determine the period of the breeding year with highest and lowest song output per hour. The song output of Savannah Sparrows varied seasonally (Linear mixed model $F_{1,\,4372}$ =10.91, P=0.001), with respect to breeding stages ($F_{4,\,4627}$ =6.4, P<0.0001), and interaction between breeding stage and julian day ($F_{4,4680}$ =3.9, P=0.003) (Supplementary Table 2.2). Males decreased their song output per hour throughout the breeding season. Savannah Sparrows sang the most per hour early in the breeding season (May and June). We obtained a R^2 value of 0.08 and the distribution of values of random effect had a variance of 0.077

Discussion

Our study of diel and seasonal variation in song output of Savannah Sparrows highlights clear patterns of singing activity throughout the day and the breeding season and sheds light on the function of songs in species. Males showed diel variation in their song output with respect to pairing status of males and breeding status of females. Unpaired males sang most during the dawn chorus compared to paired males in all breeding stages, whereas unpaired males sang least during the dusk chorus compared to paired males in all other breeding stages. In addition, males showed vocal variation with respect to breeding stages. Males with fledglings sang the least compared to males during all other breeding stages, while singing activity was highest during the incubation stage and unpaired stage. Male singing activity also varied seasonally. Males sang with the highest song output per hour early in May, when they were unpaired and establishing territories. Later in the breeding season in June, the average male singing activity increased again although less than in the unpaired stage.

Social function of dawn chorus, daytime singing activity, and dusk choruses

Variation in vocal activity can reflect important aspects of an animal’s life history. In our study, the variation in the singing activity of Savannah Sparrows supports the idea that songs have a territorial function early in the breeding season and early in the morning. The high singing activity in the mornings of unpaired Savannah Sparrows, when females are absent from the island altogether, strongly suggests that the morning is a period of importance for territorial interactions to settle and defend territories in the month of April and May. Indeed, if the dawn chorus had an attractive function, unpaired males would not be expected to sing during this period. Defending territories with songs in Savannah Sparrows is a crucial activity that increases males' pairing success (Reid & Weatherhead, 1990). Particularly, pairing success in Savannah Sparrows appears to be influenced by male territory size or male song rate in this species (Reid & Weatherhead 1990). This may explain why males arrive at their breeding site about one month before females and sing and fight intensively for a month. This remarkable time difference between female and male migration arrival reveals the importance of establishing and maintaining a territory in this species.

Once paired, Savannah Sparrows' morning singing activity decreased by about half and remained constant throughout the breeding stages. This suggests that dawn chorus still plays an important social role even after territorial establishment. Morning choruses after paring in temperate birds have been suggested to act as a social period during which males maintain their territories (Amrhein & Erne, 2006; Erne & Amrhein, 2008; Foote et al., 2011), strengthen their pair bond (Erne & Amrhein, 2008) or solicit extra-pair copulations (Poesel et al. 2006). For example, the dawn chorus is an honest signal of male quality in Eastern Kingbirds, *Tyrannus tyrannus* (Murphy et al., 2008). When singing more and earlier, male Eastern Kingbirds have a higher chance of having extra-pair copulations. Similar to Savannah Sparrows, other bird species, like the

Collared Flycatcher, *Ficedula albicollis,* the Chiffchaff, *Phylloscopus collybita*, and the Nightingale, *Luscinia megarhynchos* (Kunc et al. 2005) maintain a dawn chorus throughout the breeding stages and show that dawn chorus is used for territory defence and maintenance only (Kunc et al., 2005; Pärt, 1991; Rodrigues, 1996; Staicer et al., 1996).

Our study provides quantitative evidence that Savannah Sparrows produce a dusk chorus. Dusk choruses showed striking patterns of seasonal variation, where males sang little or no dusk chorus in the absence of females, but once paired, males sang pronounced dusk choruses. This pattern suggests that in this species, the dusk chorus may function as a period of time during which males target females although males may also use this period for territorial purposes too. Dusk chorus has been hypothesized to function as a period during which males defend territories, obtain extra pair copulations or maintain their female-male pair bond in other species (Cuthill & Macdonald, 1990; Erne & Amrhein, 2008). Future observational studies and playback experiments on dusk chorus songs could shed light on the function of songs during the dusk chorus songs in Savannah Sparrows. Comparing the song output between unpaired males during the breeding season (i.e. from May till August) with paired males could answer whether dusk songs are sung only by paired males, in which case dusk chorus may function as a period of time during which paired males target females only.

Singing activity during breeding stages

In our study, we showed that singing activity changes as the breeding cycle progresses. When excluding the unpaired stage, Savannah Sparrows sang the highest when males were paired with an incubating female. Researchers have argued that an increase in song output during the incubation stage acts as a mate guarding, a female stimulant to incubate (Foote & Barber, 2009), or an all clear signal to indicate to females that the male is nearby and that no predators are around (Johnson & Kermott 1991). In the case of Savannah Sparrows, incubation may be an important period for males to obtain extra paired copulations. Similar to our study, Slagsvold's research (1977) on seasonal variation in the song behaviour of 20 species of songbirds showed that song activities are tightly related to breeding cycles and that singing activity peaks within few days of egg-laying. This finding was supported by other studies in the European Redwings, *Turdus iliacus* (Lampe & Espmark, 1987) and *Acrocephalus* warblers (Catchpole, 1973). Although, it is generally supported that singing activity peaks within the fertile period of the females (a period including egg-laying and nest-building) as suggested by the fertility announcement hypothesis (Møller, 1991), there are numerous cases of songbirds similar to Savannah Sparrows that do not follow this pattern and do not support this hypothesis. For example, Willow Warblers, *Phylloscopus trochilus*, do not sing during fertile periods (Gil, Graves, & Slater, 1999), and the songs of Song Sparrows, *Melospiza melodia*, peak during incubation (Foote & Barber, 2009). In addition, singing during the incubation near or on the nest is not uncommon and was shown to occur in about 160 species in North America (Leonard, 2008) and birds like the Common Chaffinch, *Fringilla coelebs* (Hanski & Laurila, 1993, Rodrigues 1996), and Willow Warbler, *Phylloscopus trochilus* (Gil et al. 1999).

Singing activity during the

Finally, in our study male Savannah Sparrows showed a high degree of variation in terms of singing activity throughout the breeding season. According to Amhrein et al (2008), this seasonal variation pattern in song output can partly be influenced by their reproductive strategies. Amrhein et al. (2008) compared both the singing activity pattern and reproductive strategy of two different tits, Blue Tits, *Cyanistes caeruleus*, and Great Tits, *Parus major*. Blue Tits, which are a facultatively polygamous and have a short breeding period and do not produce second broods; whereas Great Tits are a socially monogamous species and can have second broods (Amrhein et al. 2008). Great tits were found to increase their song output during egg laying and incubation periods and continued singing later in the breeding season, while Blue Tits decreased their song output after incubation of their first brood. Amrhein et al. (2008) explain that the multi-brooded reproductive strategy may constrain Great Tits to defend their territories over longer periods of time. Similar to Great Tits, Savannah Sparrows may be constrained to sing over longer periods of time (from April till July) due to their multi-brooded reproductive strategy that encourages males to sing (Wheelwright, & Rising, 2008).

In conclusion, we showed that singing activity varies throughout the day and breeding season, and with respect to breeding stages in this species. In terms of diel variation, unpaired territorial males had the most pronounced dawn chorus and higher levels of morning singing activity. A dusk chorus occurred later in the breeding season, during the fertile, incubation, hatchling, and fledgling periods. In addition, Savannah Sparrows singing activity is highest during the month of May and June. These patterns suggest that Savannah Sparrows show dynamic patterns of diel and seasonal variation, where vocal output varies with social context at different stages of their breeding season. Our study highlights the general singing patterns of Savannah Sparrows throughout the breeding season. Analysis of seasonal variation of singing like our study can provide insight into communication systems of song birds that heavily rely on singing interactions.

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Figures

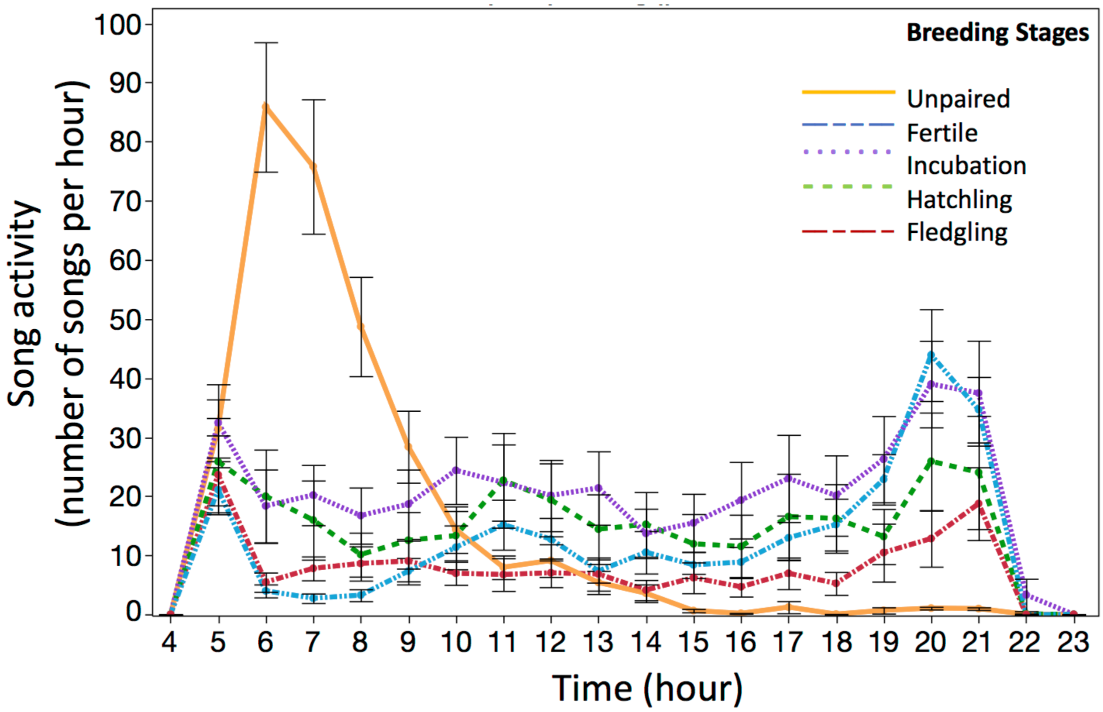

Figure 2.1. Diel variation of the singing activity of Savannah Sparrows with respect to breeding stages. Breeding stages included: unpaired stage, fertile stage, incubation stage, hatchling stage, and fledgling stage (n=34 males). Note the peak in singing activity in the morning around 7:00 and absence of dusk chorus for the unpaired males, and the presence of dusk chorus in paired males (the dots represent means for each hour of the day). Bars show mean ± SE.

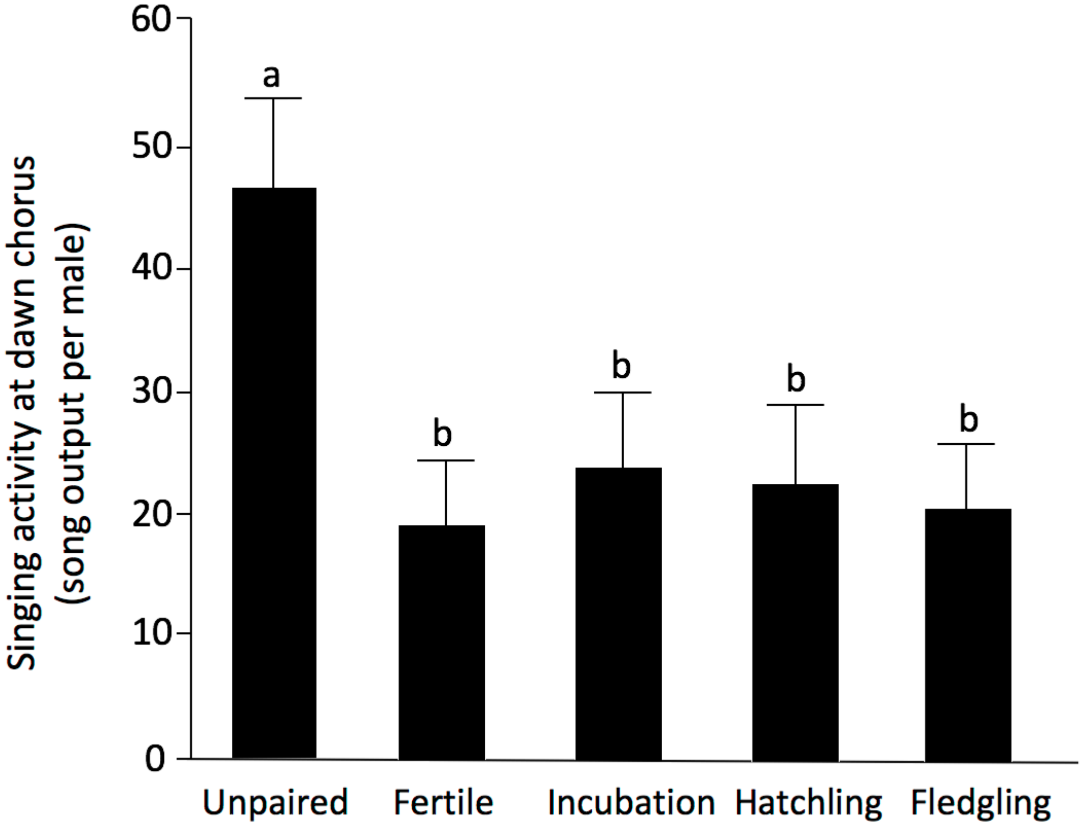

Figure 2.2. Singing activity during the dawn chorus (30 min before sunrise) with respect to breeding stage (unpaired, fertile, incubation, hatchling and fledgling) (n=34). Unpaired males sang in the absence of females significantly more than any other stages. Bars show mean ± SE, and levels that are not significantly different are represented with the same letter.

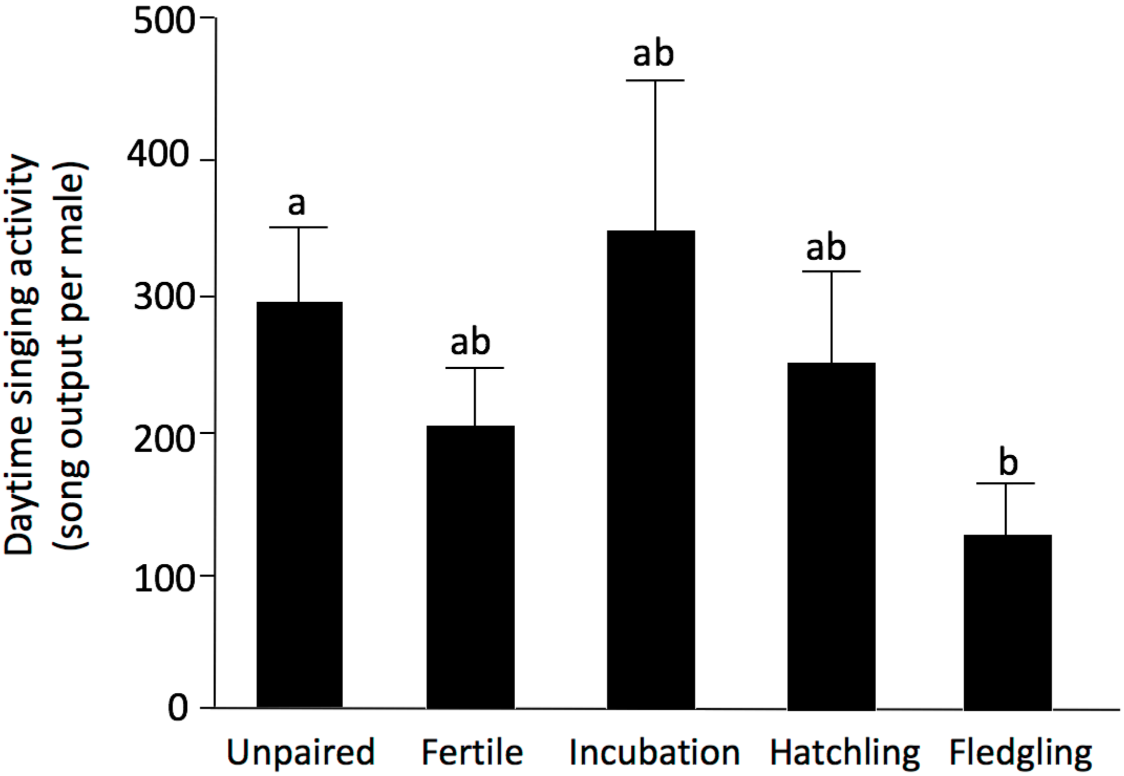

Figure 2.3. Singing activity during the daytime (time after sunrise and before sunset) with respect to five breeding stages (unpaired, fertile, incubation, hatchling and fledgling stages) (n=34). Unpaired sang significantly more than the fledgling stage. Bars show number ± SE, and levels that are not significantly different are represented with the same letter.

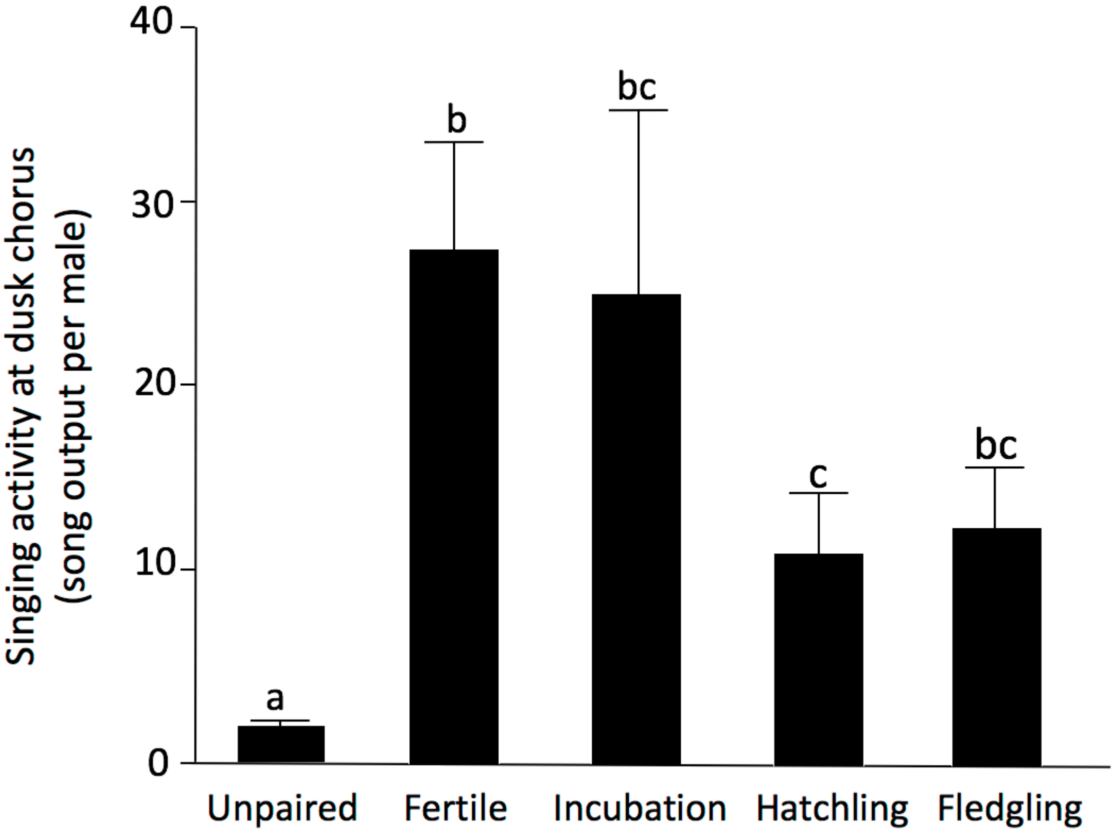

Figure 2.4. Singing activity during the dusk chorus (time after sunset) with respect to five breeding stages (unpaired, fertile, incubation, hatchling and fledgling stages) (n=34). Males who were unpaired sang significantly less than males at all of the other stages, suggesting that dusk songs have a female-related function. Bars show mean ± SE and levels that are not significantly different are represented with the same letter.

Supplementary Table 2.1. Results of diel singing activity LMM standard least square comparisons for all interactions.

	t Ratio	*P*
Treatment type		
Unpaired stage	8.95	<.0001*
Fertile stage	0.42	0.6765
Fledgling stage	-6.23	<.0001*
Hatchling stage	0.84	0.3993
Incubation stage	5.99	<.0001*
Hour		
Between hours	-4.75	<.0001*
Treatment type * hour		
Fertile stage*hour	6.38	<.0001*
Fledgling stage*hour	1.41	0.1584
Hatchling*hour	1.36	0.1737
Incubation*hour	3.29	0.0010*

Significant values shown with *

Supplementary Table 2.2. Results of seasonal singing activity LMM standard least square comparisons for all interactions.

	t Ratio	*P*
Treatment type		
Unpaired stage	5.09	<.0001*
Fertile stage	-2.37	0.0180*
Fledgling stage	0.93	0.3539
Hatchling stage	0.38	0.7018
Incubation stage	1.04	0.2988
Day		
Julian day	-3.30	0.0010*
Treatment type*Day		
Julian day*Fertile	1.66	0.0963
Julian day*Fledgling	-3.42	0.0006*
Julian day*Hatchling	-0.88	0.3803
Julian day*Incubation	0.07	0.9432

Significant values shown with *

CHAPTER 3: Quiet violence: Songbirds respond to playback simulated rivals using soft songs as aggressive signals of intention

Chapter summary

When animals compete over resources such as breeding territories, they often use signals to communicate their aggressive intentions. According to the hierarchical signalling hypothesis, animals progressively escalate interactions using specific signals to reveal their aggressive intentions towards conspecific rivals. In this study, we tested this hypothesis in male Savannah Sparrows, *Passerculus sandwichensis*, focusing on signals that precede physical attack against a territorial intruder. We simulated an intruder using song playback and a taxidermic model, and we determined which behaviors were associated with physical attack. Several previous studies have used this approach, and they provide evidence that many species produce songs of dramatically lower amplitude, or “soft songs”, as signals of aggressive intention. Savannah Sparrows, however, are not known to produce soft songs, and therefore they provide an interesting system for testing signals that predict attack. Out of 93 playback subjects, 23 males attacked the simulated intruder and 70 did not. To our surprise, Savannah Sparrows produced soft songs, a behaviour which had not previously been described for this well-studied songbird, and the number of soft songs was a significant predictor of attack on the simulated intruder. The number of “chip” calls also predicted attack on the simulated intruder, whereas three other measured behaviors (aggressive calls, wing waving and passes over the model) did not predict attack. Our study contributes to the growing body of research that supports the hierarchical signalling hypothesis (Searcy et

al., 2013; Searcy & Beecher, 2009) and corroborates with the idea that soft song is a widespread signal of aggression.

Introduction

Territorial interactions between rival animals routinely involve physical aggression. Given that aggressive encounters may involve costly physical attacks, or even death, many animals have evolved signals to facilitate communication during territorial interactions (Laidre & Johnstone, 2013; Vehrencamp, 2000). Some animals use graded signals to convey their intention to escalate or de-escalate encounters, including high-intensity signals of threat or low-intensity signals of submission (Searcy et al., 2013; Akçay et al., 2013; Hof & Podos, 2013). Although aggressive territorial signals have been documented in diverse animal taxa including mammals (August & Anderson, 1987; Behr et al., 2009; Clutton-Brock & Albon, 1979), birds (Searcy et al., 2006; Todt & Naguib, 2000), reptiles (Baird et al., 2013), frogs (Bee et al., 2000; Reichert & Gerhardt, 2013; Wagner, 1989), fish (Korzan & Fernald, 2007; Triefenbach & Zakon, 2008), mollusks (Schnell et al., 2016), and arthropods (Jonsson, Kravitz, & Heinrich, 2011; Tibbetts & Sheehan, 2011, Wagner, 1989), only recently have animal behaviourists come to understand the function of specific types of signals within a hierarchical signalling system of escalation and de-escalation. One particular experimental protocol helps to reveal the vocal and physical signals that predict attack; it involves the presentation of a simulated territorial rival (e.g. a taxidermic model, a mirror, or video playback) accompanied by acoustic stimuli (e.g. vocal playback; Searcy, et al., 2006). Under this experimental design, animals have the opportunity to attack the simulated rival, and we can gain insight into the function of specific signals by studying the behaviours that precede physical attack.

Hierarchical signalling models have been developed for animals across diverse taxa, including the signals associated with aggressive escalation in mammals (Poole, 1989), birds (Hof & Podos, 2013; Searcy & Beecher, 2009), fish (Triefenbach & Zakon, 2008), amphibians (Bortosky & Mathis, 2016), reptiles (Van Dyk & Evans, 2008), and arthropods (Schnell et al., 2016). For example, cuttlefish, *Sepia apama*, indicate their intention to attack a rival when they produce particular visual displays (specifically, shovel and lateral displays; Schnell et al., 2016), and knifefish, *Apteronotus leptorhynchus*, produce particular electrical signals as predictors of attack against rivals (specifically, with chirps and gradual frequency rises; Triefenbach & Zakon, 2008). Birds provide a model system for studying aggressive signals because they produce diverse acoustic signals, including elaborate and variable signals that are commonplace during territorial contests in many species (Todt & Naguib, 2000b). Acoustic signalling behaviours in birds include song overlapping (Helfer & Osiejuk, 2015; Naguib & Mennill, 2010), song matching (King & McGregor, 2016) variation in song rate (Baker et al., 2012), variation in trill rate and frequency bandwidth (Dubois et al., 2008), production of low-amplitude songs or soft songs (Ballentine et al., 2008; Grieves et al., 2015; Hof & Hazlett, 2010; Searcy et al., 2006; Xia et al., 2013), and production of non-song calls (Ballentine et al., 2008; Baker et al., 2012; Elie & Theunissen, 2015). A fine example of a model of hierarchical signalling comes from Little Blue Penguins, *Eudyptula minor* (Waas, 1991): Little Blue Penguins produce multiple graded signals (including growls and hisses), and when exposed to a taxidermic model of a conspecific rival, males produce growls to signal their intention to escalate an encounter, and hisses to signal their intention to attack (Waas, 1991). The most well-studied system of territorial contest escalation in birds comes from Song Sparrows, *Melospiza melodia* (Akçay et al., 2013; Searcy & Beecher, 2009; Searcy et al., 2006). Male Song Sparrows escalate interactions by matching the songs of their rivals, or de-escalate interactions by switching to non-matching songs (Akçay et al., 2013). They produce low-amplitude songs, also known as "soft songs", for their most-threatening signal, which precedes an attack on a rival (Searcy et al., 2006). Subsequent investigations in several bird taxa reveal that low-amplitude signals are often used as high-threat signals (reviewed in Searcy et al., 2014).

Low-amplitude signals are taxonomically widespread vocal behaviours found in birds (e.g. Dabelsteen et al., 1998; Reichard & Welklin, 2015), mammals (e.g. Gustison & Townsend, 2015), and invertebrates (e.g. Balenger, 2015). Historically overlooked in birds, soft vocalizations (soft songs and soft calls) are now known to be used by almost half of North American birds during territorial defence, courtship displays, and alarm, begging, or contact signalling (Reichard & Welklin, 2015). Thirty-one bird species are known to produce soft vocalizations as aggressive signals during displays of territorial defence in North America (Reichard & Welklin, 2015). Furthermore, males and females from six geographically widespread and distantly-related bird species produce soft songs (or soft calls) as a predictor of attack during territorial contests (Table 3.1), suggesting that these vocalizations are a taxonomically widespread, and possibly ancestral trait. Low-amplitude songs are interesting from a communication network perspective, because they may have evolved to limit the potential for eavesdropping; if low-amplitude songs are associated with the highest levels of aggression, animals may benefit by minimizing the broadcast of information about their occurrence to nearby animals (Dabelsteen et al., 1998).

Songs are the main vocalization produced by songbirds during their daily activities on their breeding grounds, yet other vocalizations, such as non-song calls, are also produced during songbirds' daily activities. Calls, unlike songs, are simple and short non-learned vocalizations that serve various functions and can be gender-specific or used by both genders (Catchpole & Slater, 2008). In past studies that investigated vocal territorial strategies in birds, researchers mainly focused on song rate, song overlapping (a time-specific response to neighbouring songs), song-type matching (a pattern-specific response to neighbouring songs), song switching (a repertoire-related response in large song repertoire birds), or modulation of fine structural song elements as the primary aggressive signals in birds (Burt et al., 2001; DuBois et al., 2009; Naguib, 2005; Searcy et al. 2006). Surprisingly few studies have investigated calls as signalling strategies to escalate interactions (e.g. Ballentine et al., 2008), even though research on call signalling strategies can likely provide a deeper understanding of animal signalling behaviour.

In this study, we explore aggressive signalling during the territorial interactions in a temperate songbird, the Savannah Sparrow, *Passerculus sandwichensis*, by applying the experimental protocol developed by Searcy et al. (2006) involving playback and the presentation of a taxidermic model to test predictors of physical attack. We chose to study Savannah Sparrows using this approach because this species, in contrast to many other sparrows, is not known to produce soft songs (Searcy et al., 2006; Ballentine et al., 2008; Wheelwright & Rising, 2008; Reichard & Anderson, 2015). Therefore, Savannah Sparrows provide an interesting system for testing predictors of physical attack. The goal of our study is to explore which signals are threatening signals that best predict physical attacks in Savannah Sparrows. We predicted that Savannah Sparrows would reliably indicate their intention to attack a rival during territorial interactions using singing behaviours, calling behaviours, or physical behaviours during aggressive interactions.

Material and methods

Study species and study site

Savannah Sparrows are migratory songbirds that live in open grasslands across North America (Wheelwright & Rising, 2008). We conducted our research at Bowdoin Scientific Station on Kent Island, New Brunswick, Canada (44°35’N 66°46’W), a 200-acre island in the Bay of Fundy. This island is home to a strongly philopatric population of Savannah Sparrows (Wheelwright & Mauck, 1998). The birds in this population have been studied for many decades, including careful annual studies over the last thirty years (Williams et al., 2013; Woodworth et al., 2017). On an annual basis, we capture and band birds inhabiting our study site, giving each animal a unique combination of coloured leg bands to facilitate recognition of individuals in the field. Over half of our playback subjects were individually-marked animals from our study site (n= 52 individuals). The remaining playback subjects were unbanded males from outside our study site (n=41 individuals). We distinguished between different unbanded individuals on the basis of their territorial position and their individually distinctive song (as in Hick, Doucet, & Mennill, 2015; Moser-Purdy & Mennill, 2016).

Male Savannah Sparrows arrive from migration in mid-April (Woodworth et al., 2016) and begin to establish breeding territories using a variety of vocal signals (Potter, 1972). Males possess a single song type, which they are thought to learn within the first eight months of life (Wheelwright et al., 2008) and which remains constant throughout an individual's life (Williams et al., 2013). Males produce a variety of calls, including contact calls (the "chip" call), aggressive flight calls, as well as hostile notes (a.k.a. the "buzz" call; Gobeil, 1970; Wheelwright & Rising, 2008). In addition to their songs and calls, males also produce visual displays during territorial establishment including wing waving, flutter flights, tail raising, bill gaping, ptiloerection of crest feathers, parallel walking, and bouts of physical chases (Potter, 1972; Wheelwright & Rising, 2008). The relative importance of Savannah Sparrows' diverse vocalizations and physical displays remains poorly studied; these displays have the potential to comprise a graded system of aggressive signalling, although this has never been studied.

Playback experiment

We conducted our playback trials between mid-April and late-May 2016 between 6:00am and 12:00pm, when vocal interactions between territorial male Savannah Sparrows are commonplace. Prior to each playback trial, we positioned a taxidermic model of a Savannah Sparrow on top of a FoxPro Scorpion X1A loudspeaker, and we covered the model with a piece of camouflage-patterned cloth. We used four different taxidermic models of similar size and appearance, perched in a normal posture, and we alternated between these models across trials. We placed small flags on either side of the model, at distances of 1m, 2m, and 4 m, to help an observer estimate the distance of playback subjects from the model.

A schematic representation of our experimental protocol is provided in Figure 3.1, showing two phases of playback: the initial period and the experimental period. Our playback experiment follows the general procedure of Searcy et al. (2006) involving the presentation of looped playback of male song and presentation of a taxidermic model. During the initial period, we attracted birds to the playback area by playing songs while the taxidermic model was hidden under a cloth. We played songs for 1 min during the initial period, followed by a period of 5 min of silent observation. At 5 minutes and 45 seconds, we removed the cloth from the taxidermic model by pulling a string. At 6 minutes, the experimental period began. During the experimental period, with the taxidermic model exposed, we played songs for 2 min followed by up to 20 min of silent observation, or until the subject attacked the taxidermic model. If the subject attacked the model, we considered the trial complete and we removed the model; this minimized stress to the playback subject and protected the models for later trials. We considered an attack any physical contact between the subject and the taxidermic model. Attacks were unambiguous: subjects frequently flew at the taxidermic model, often landing on top of the model and pecking at it. We aborted any trials where males in neighbouring territories also responded, and any trials that did not attract a subject during the initial five-minute period.

During the initial period, we played songs at an amplitude of 87 dB, and during the experimental period, we played songs at a quieter amplitude of 78 dB. We chose these two amplitudes to match those used by Searcy et al. (2006) in their study of Song Sparrows, a species related to Savannah Sparrows, and we decreased the amplitude to 78 dB like in Searcy et al. (2006) because soft vocalizations have been shown to act as aggressive signals during territorial contexts in other species (Table 3.1). We confirmed the amplitude of sounds from the loudspeaker in the field with a Casella CEL-240 sound level meter (Casella CEL Inc., Buffalo, NY, USA; C-weighting; fast setting), placed 1.0 m away from the speaker.

To record birds' responses to playback, we used two microphones connected to the two channels of a Marantz PMD661 digital recorder. We placed a Sennheiser ME66/K6 microphone on the ground approximately 45 cm from the speaker and taxidermic model, to capture any low-amplitude sounds produced by the subject during playback. We mounted a Sennhseier ME66/K6 microphone on a tripod 15-20 m away from the speaker and taxidermic model, to capture broadcast songs produced by the subject. An observer (I.G.M.) narrated the behaviours of the subject while they responded to the playback, by whispering into the second microphone while seated on the ground next to it.

Playback stimuli

We created playback stimuli from recordings we collected from male Savannah Sparrows in 2013-2015. We collected these recordings during spontaneous bouts of singing by territorial birds, using a Marantz PMD661 digital recorder and a Sennheiser ME62/K6 microphone mounted in a Telinga parabola (sampling frequency: 44.1 kHz; sampling rate: 16 bit; WAVE format). Each playback subject received a different playback stimulus, and we chose stimuli that were recorded from a male at least 10 territories away from the subject (≥700m), ensuring that subjects were unfamiliar with the stimuli. From the original field recordings, we selected a single song with a high signal-to-noise ratio, and with little or no overlapping background sound. We then manipulated this song in Audition 3.0 software (Adobe, San Jose, CA, USA): we filtered out background noise with a high-pass filter of 1000 Hz and we normalized all sounds to the same amplitude (-1 dB). We then pasted each song into a longer sound file at a rate of six songs per minute, which is a typical song rate for Savannah Sparrows (based on natural song rate from focal recordings).

Analysis

We annotated our field recordings of the birds' playback responses in Syrinx-PC sound analysis software (Burt, Seattle, WA, USA), creating a second-by-second summary of the playback subjects' behaviour. We measured six aspects of each subject's behaviour: (1) number of broadcast songs, (2) number of soft songs, (3) number of "chip" calls, (4) number of aggressive calls (including both aggressive flight calls and buzzes (Wheelwright & Rising, 2008), (5) number of times the bird waved its wings, and (6) number of passes over the taxidermic model. We included wing waving for comparison purposes with other playback studies of sparrows (Ballentine et al., 2008;

Searcy, Anderson, & Nowicki, 2006) even though our ability to maintain visual contact with subjects throughout the trials was constrained by the thick grassy habitat at the study site; our estimates of wing waving are likely underestimates, because subjects sometimes disappeared from our view for brief periods during playback. This also explains why we did not include other physical behaviours like bill gaping or ptiloerection into our analysis.

Early in our playback trials we found that subjects often produced low-amplitude “soft songs” (see Results); to differentiate between broadcast songs and soft songs, the field observer indicated whether each song was a “broadcast song” or “soft song” while describing the subjects’ behaviour. We confirmed whether each song was a broadcast or a soft song by comparing the recording from the two microphones used to record subjects’ responses; soft songs were typically visible in the recording from the directional microphone closest the taxidermic model, but faint or even absent from the directional microphone positioned next to the field observer. In several cases, we detected soft songs that were not detected by the recordist in the field, but that were identified during the process of annotation with Syrinx-PC, only appearing in the channel closest to the taxidermic model. In addition, soft songs differed from broadcast songs in that they had unique soft notes placed in between their regular notes. Those unique notes were not present in broadcast songs.

We focused our analysis on the aforementioned six variables during two time periods: (1) during the first 5 minutes of the initial period, and (2) during one minute prior to the time that birds attacked the model during the experimental period. We were interested in comparing birds that attacked the taxidermic model versus birds that did not attack. To facilitate this comparison, we matched the timing of the one-minute-before-attack of birds that attacked the model to a corresponding one-minute period from randomly-chosen non-attackers, as in previous experiments that used this protocol (e.g. Searcy et al., 2006; Baker et al., 2012).

Statistical analysis

Our six response variables were not normally distributed, and could not be normalized through transformation due to a preponderance of zero values. We used a non-parametric Spearman's rank correlation to investigate the relationship between the six variables. We used non-parametric Mann-Whitney U tests to compare the behaviour of attackers versus non-attackers for each of the six response variables. In addition, we followed the approach used by Ballentine et al. (2008) to analyze similar playback-response data in Swamp Sparrows; we looked at the combinations of response variables that predicted attack, comparing attackers and non-attackers using a forward and backward stepwise discriminant function analysis.

Results

Of 93 male Savannah Sparrows that received playback accompanied by a taxidermic model simulating a conspecific rival, 23 males attacked the model and 70 did not. The playback subjects showed variation in their signalling behaviour, with most subjects producing broadcast songs, chip calls, aggressive calls, and some subjects producing wing waving displays and passing over the taxidermic model. To our surprise, a substantial number of birds produced soft songs during the trials one minute before attack (n=39), a behaviour that had not previously been described in this species (Wheelwright & Rising, 2008; Reichard & Welklin, 2015). Soft songs were structurally similar to broadcast songs, but were composed of additional notes not present in broadcast songs. Although not measured in this study, broadcast songs were observed to reduce in number once the playback experiment started. Once the playback started, males tended to sing more soft songs and calls.

Initial period

In the initial period, we found no significant variation in the behaviour of birds that eventually attacked the model and those that did not (Fig. 3.2). Birds that eventually attacked the taxidermic model (hereafter “attackers”) and birds that did not (hereafter” non-attackers) did not differ in number of broadcast songs (U=0.22, P=0.83), soft songs (U=0.84, P=0.40), chips (U=0.87, P=0.38), aggressive calls (U=0.92, P=0.35), bouts of wing waving (U=1.0, P=0.32), and passes over the speaker (U=-0.20, P=0.84). In the forward stepwise discriminant function analysis of the six response variables, aggressive calls were the only variable that discriminated between attackers and non-attackers (Wilk's λ= 0.93, $F_{1,90}$= 6.92, P= 0.01). A backward stepwise discriminant function analysis with the same six variables converged on identical results, with aggressive calls as the last variable to exit the model (Wilk's λ= 0.93, $F_{1,90}$= 6.92, P= 0.01)

One minute before attack

We compared behaviours between attackers and non-attackers by comparing the minute before Savannah Sparrows attacked the taxidermic model, to a random comparison minute for birds that did not attack. Attackers sang significantly more soft songs (U=3.20, P=0.001), and produced significantly more chip calls (U=2.26, P=0.02) than the non-attackers (Fig. 3.3). Attackers and non-attackers did not differ in the number of broadcast songs (U=1.63, P=0.10), aggressive calls (U=0.26, P=0.79), wing waves (U=1.51, P= 0.13), or passes over the model (U=1.3, P=0.19) (Fig. 3.3). In the forward stepwise discriminant function analysis, amongst the six variables, soft songs entered the model first, followed by number of chips; this model discriminated between attackers and non-attackers (Wilk's λ= 0.92, $F_{2,90}$= 3.90, P= 0.02). A backward stepwise discriminant function analysis converged on identical results, with soft songs and number of chip calls representing the last variables to exit the model (Wilk's λ= 0.92, $F_{2,90}$= 3.90, P= 0.02).

Discussion

During simulated territorial intrusion, male Savannah Sparrows showed strong aggressive responses to simulated intruders. Twenty-five percent of males attacked a rival simulated with playback and a taxidermic model. Although soft songs have not previously been described in Savannah Sparrows, we found that territorial males regularly gave soft songs and that males that attacked sang significantly more soft songs prior to attack. In addition, attackers produced significantly more chip calls compared with non-attackers prior to an attack. Consistent with one prediction of the hierarchical signalling hypothesis (Searcy et al., 2013; Searcy & Beecher, 2009), we conclude that soft songs and chip calls are signals that predict attack in Savannah Sparrows.

Beginning with a study of Song Sparrows just over a decade ago by Searcy et al. (2006), ten additional studies have used a playback-and-model-presentation design to explore signals that predict attack during territorial encounters in birds, including both Oscines and non-Oscines (Table 3.1). Collectively, they point towards the conclusion that soft songs reliably predict attack across diverse species. Deterring rivals with low amplitude vocalizations, instead of broadcast vocalizations, is an intriguing behaviour. Why do so many different species lower the amplitude of their songs during aggressive territorial interactions? One compelling explanation is the eavesdropping avoidance hypothesis (Dabelsteen et al., 1998) which proposes that individuals sing softly to avoid social eavesdropping by conspecific males or females, or interceptive eavesdropping by predators (McGregor, 1993; Searcy & Yasukawa, 2016). Eavesdroppers can impose high costs on signalers, both in the case of social eavesdropping (e.g. Mennill et al. 2003), and interceptive eavesdropping (e.g. Randall & Matocq, 1997), hence signalers develop strategies to avoid eavesdroppers (Dabelsteen, 2005; Dabelsteen et al., 1998). Two studies have tested the influence of predators on the amplitude of male songs (Akçay et al. 2016; Searcy & Nowicki, 2006), and both showed that under higher risk of predation, birds do not increase their soft song output. Although more evidence is needed, these results suggest that eavesdropping predators do not pressure birds to sing softly. Similarly, one study tested whether eavesdropper males pressure fighting males to lower their songs during dyadic interactions (Searcy & Nowicki, 2006). In this study, more males intruded on territories when soft songs were sung compared to when songs were broadcast, which contradicts the eavesdropping hypothesis. Indeed, when males hear a foreign song and hear no responses from their neighbors, males or floaters may visit the intruded territory to either obtain parts of the territory, or to chase the intruder away to reduce competition. Finally, the last hypothesis that could explain the lowering in amplitude of male songs is the use of eavesdropping by conspecific females (Mennill et al., 2003); but presently, no studies have yet investigated the role of females in the evolution of soft songs.

A second compelling explanation for why males produce soft songs during aggressive territorial interactions comes from the readiness hypothesis (Akçay et al, 2011). When intruders invade another male's territory, territorial males may benefit by following the intruder at a close distance to visually track them. When singing loudly, however, males often angle their head backward, and this may not allow for visual tracking. Therefore, males may be physically constrained to sing softly in the presence of a rival, to keep track of his whereabouts. Savannah Sparrows are known to have preferred singing perches (Wheelwright & Rising, 2008) on which they have been observed to raise their bill when broadcasting their songs (Clark, 1976). During our playback experiment, males that sang soft songs seemed to maintain a closed bill and did not tilt their head back while on the ground near the taxidermic model. These observations are consistent with the idea that visual tracking of territorial intruders may constrain males to signal softly.

If soft songs are aggressive signals that predict an imminent attack against a rival animal, how do animals maintain an honest signalling system, when a territorial animal could easily “bluff” by producing soft songs even in the absence of their willingness to escalate a territorial interaction? Historically, the honesty of vocal signals was a topic of controversy because aggressive vocal signals were thought to be easy-to-bluff due to their low production cost (Smith, 1974; Smith, 1979; Smith, 1982). In fact, vocal signals can honestly signal aggressive intentions if receivers retaliate against the animals producing aggressive signals (Anderson et al., 2012; Enquist, 1985; Vehrencamp, 2000) or if receivers recognize their bluffing rivals based on past interactions (Laidre, 2005; Van Rhijn & Vodegel, 1980). Under both scenarios, receivers ensure the honesty of aggressive signals by imposing a receiver-retaliation cost on the signallers (Anderson et al., 2012; Enquist, 1985; Vehrencamp, 2000). Future research can develop a deeper understanding of receiver-retaliation costs by adopting a receiver perspective (sensu Searcy & Beecher, 2009) by using playback simulating a territorial rival producing broadcast song versus soft song; with soft songs predicted to incite more aggressive reactions.

In addition to songs, calls play a central role during social interactions in birds (Gill et al. 2015). Many avian studies that explore acoustic signals during the breeding season focus on songs, but rarely explore calls. Nevertheless, similar to our study, calls were found to be a good predictor of attack in Black-capped Chickadees (Baker et al. 2012) but calls were not good predictor of attack in other studies like Swamp Sparrows (Ballentine et al 2008) and Black-throated Blue Warblers (Hof & Hazlett 2010). Chip calls may be used in combination with soft songs as a signal of attack. However, because chip calls are also produced during other non-territorial interactions and are also produced by females (Wheelwright & Rising, 2008), chip calls may not be a reliable predictor of attack. Future research could investigate whether chip calls differ from soft songs in their functions by focusing on the rate of chip calls during territorial contexts.

In conclusion, our results show that Savannah Sparrows produce more low-amplitude soft songs before attacking a territorial rival, and produce more chip calls before attacking a rival. This pattern contributes to the growing body of work showing that many birds rely on low-amplitude aggressive signals to communicate their intension to escalate interactions. Future research should focus on testing the hypotheses that attempt to explain soft vocalizations, and should focus on elucidating the hierarchical signalling models of birds by investigating low-threat signals. In addition, other signals such as song rate, song frequency, song length, other song structural elements, and even behavioural or visual displays may act as predictors of attack and merit further investigations. Future studies should also focus on the structural differences between soft songs and broadcast songs, as some key elements of soft songs may indicate aggressiveness. Exploring the aggressive signals across a wide array of animals can expand our knowledge on animal communication and provide insight on how animals express aggression.

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Tables

Table 3.1. Summary of studies that experimentally investigated reliable predictors of attack in birds.

Measured behavioural responses ranged from soft vocalizations (soft songs or soft calls), visual displays (e.g. wing waving, throat inflation), aggressive calls (e.g. hoot), and increased signalling rate. Behavioural responses that were the most reliable predictor of attack are marked with “support”. If behavioural responses were not found to be the most reliable predictor of attack, they were marked with “no support”. Behavioural responses that were not tested, were marked as “not tested”. Studies that followed the playback methods or modified playback methods from Searcy et al. (2006) are indicated with an asterisk (*) and studies that used a taxidermic model are indicated with a dagger (†).

Species	Behavioural variables				Sample size	Number of attacks
	Soft vocalizations	Visual displays	Aggressive Calls	Signalling rate		
Little Blue Penguin (Waas 1991b) †	Not tested	Not tested	Support	Not tested	40	na
Black-capped Chickadee (Baker et al. 2012)* †	Not tested	Not tested	Support	Support	38	21
House Wren (Barnet et al. 2014) †	Not tested	Support	Not tested	Support	37	9
Smooth-billed Ani (Grieves & Logue 2015)†*	No support	Support	No support	Support	14	8
Song Sparrow (Searcy et al. 2006)* †	Support	No support	Not tested	Not tested	95	20
Swamp Sparrow (Ballentine et al. 2008)* †	Support	Support	No support	Not tested	31	9
Black-throated Blue Warbler (Hof & Hazlett 2010)* †	Support	Not tested	No support	No support	54	19
Corncrake (Ręk & Osiejuk 2011)	Support	Not tested	Not tested	No support	29	6
Brownish-Flanked Bush Warbler (Xia et al. 2013) *†	Support	Not tested	Not tested	Not tested	25	6
Savannah Sparrow (current study)* †	Support	No support	Support	Not tested	93	23

Figures

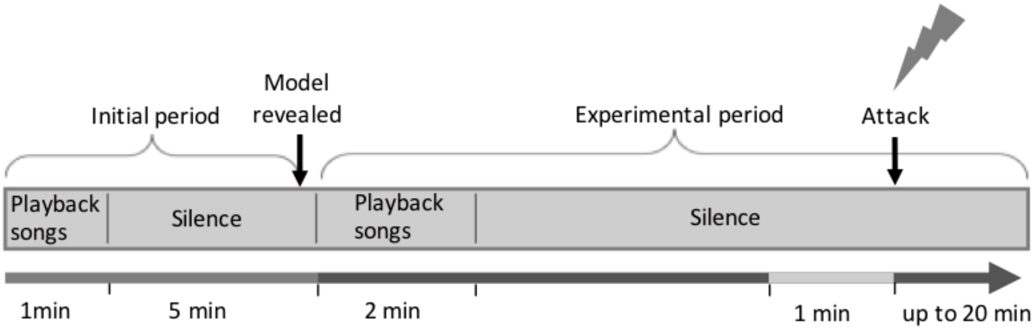

Figure 3.1. Diagram illustrating the playback experiment, based on the experimental design of Searcy et al. (2006). There are two periods: an initial period and an experimental period. The initial period consists of a playback phase (1min) followed by a silent phase (5min). The experimental period consists of a playback phase (2 min) followed by a silent phase that lasted up to 20 minutes or up to an attack with the taxidermic model. The taxidermic model was revealed at 5 minutes and 45 seconds. Vocalizations and behaviors were recorded from the time of the first song. We focused our analysis on two time periods: the initial period and the 1-minute prior to attack period.

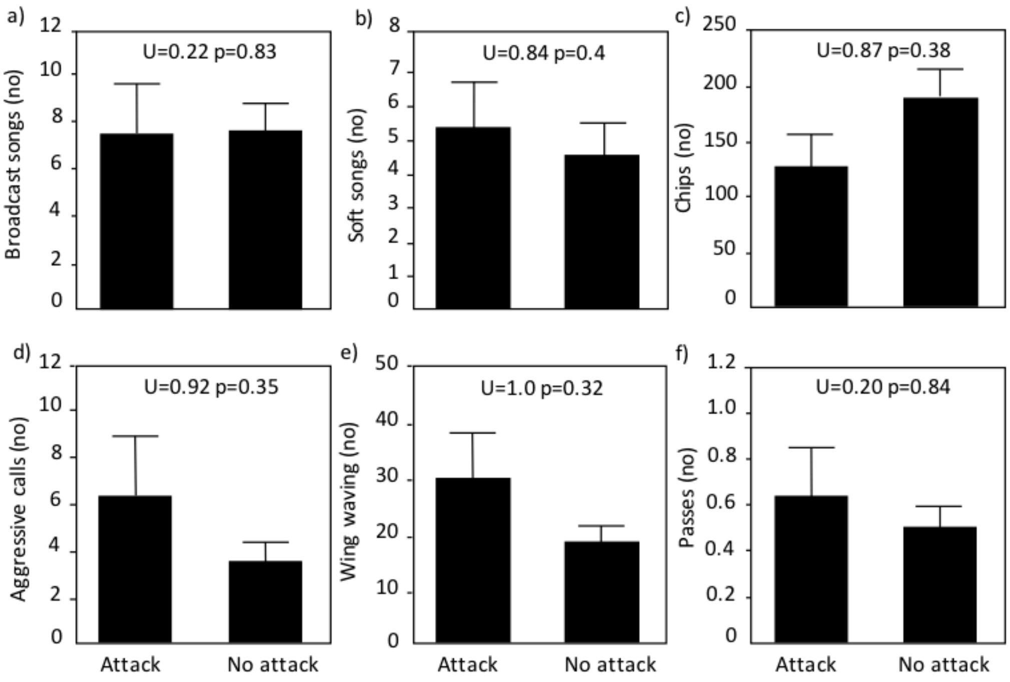

Figure 3.2. Behaviors between attackers and non-attackers during the five-minute initial period of the playback experiment. The behaviours, shown hear as the total number (mean ± SE) did not differ statistically between attackers and non-attackers during this initial period, for (a) broadcast songs, (b) soft songs, (c) chips, (d) aggressive calls (including both aggressive flight calls and buzzes), (e) wing waving, and (f) passes over the taxidermic model.

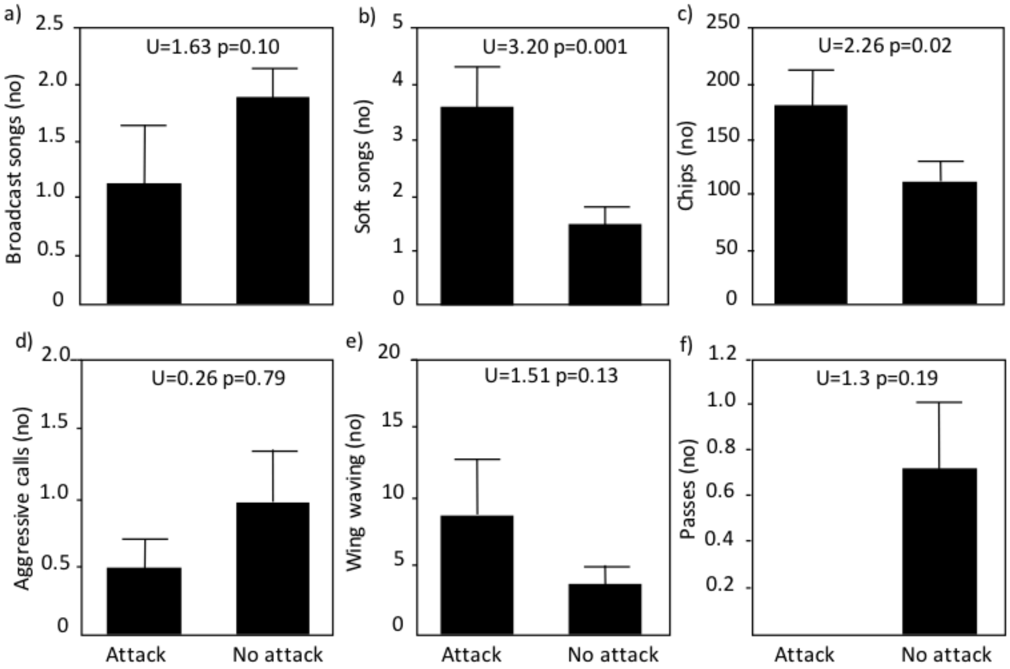

Figure 3.3. Behaviors between attackers and non-attackers during the one-minute prior to attack period of the playback experiment during taxidermy model presentation trials. For non-attackers, the timing of the one minute before the attack for attackers was matched to a corresponding one-minute period from randomly-chosen non-attackers. Behaviours (mean number ± SE) differed significantly between attackers and non-attackers with (b) soft songs, and (c) chips, but not with other behavioural measures, (a) broadcast songs, (d) aggressive calls (including both aggressive flight calls and buzzes), (e) wing waving, and (f) passes over the taxidermic model.

CHAPTER 4: General discussion

Bird songs are known to serve a dual function to both attract mates and defend territories (Catchpole & Slater, 2008), a pattern which is true across many other organisms across the animal kingdom (Bradbury & Vehrencamp, 1998). My thesis investigated those functions of bird song by focusing on variation in song output with time of the day, time of year, and breeding status (Chapter 2), and on the behaviour of animals during interactions with conspecific rivals (Chapter 3) in Savannah Sparrows, *Passerculus sandwichensis*.

In Chapter 2, I explored temporal variation in Savannah Sparrow song by analysing diel and seasonal variation in vocal activity. My analyses revealed that (1) male singing activity was highest in the month of May; (2) male singing activity was highest during the unpaired period and the incubation period; (3) territorial unpaired males had the strongest dawn chorus and morning singing activity, especially early in the year; and (4) territorial unpaired males did not sing, or sing very little, during the dusk chorus, but paired males sang significantly more during the dusk chorus during the fertile, incubation, hatchling, and fledgling periods. My results were consistent with other studies that show birds' singing activity varies with time of day and with time of year (Foote & Barber, 2009; Slagsvold, 1996; Topp & Mennill, 2008), and these patterns provide insight into the importance of heightened song output associated with breeding activities.

In Chapter 3, I investigated territorial signalling strategies in Savannah Sparrows by conducting a playback experiment that simulated territorial intrusion. Previous studies on songbirds and non-songbirds showed that soft song serves as a signal that predicts attack in birds (e.g. Barbara Ballentine et al., 2003; Hof & Hazlett, 2010; Rek & Osiejuk, 2011; Searcy et al., 2006), although not all birds use this behaviour to express their intention to attack (e.g. Black-capped Chickadees, *Poecile atricapillus,* Baker et al., 2012). I focused on the signals that predict attack during aggressive contests in Savannah Sparrows by following the playback protocol as in Searcy et al., 2006. My analysis of behavioural responses showed that soft songs were a reliable predictor of attack during aggressive interactions between males, consistent with studies of other species of sparrow.

Chapter 2 of my thesis provides evidence that time of day, time of year, and breeding stage influences singing activity of birds and the function of avian vocal signals, highlighting the necessity of carefully considering the time of year and time of day when ornithologists collect data on bird behaviour. For example, the results for my playback study in Chapter 3 might have been different if conducted later in the breeding season when females where present, given the dramatic reduction in song output shown in Chapter 2. Furthermore, in Chapter 2, I showed that breeding status can affect singing activity and this should also be taken into consideration when accessing singing activity. For example, during the playback experiment I presented in Chapter 3, all males had the same breeding status (i.e. territorial unpaired males) and and the observed behaviours may have changed once females arrived on the breeding grounds, especially if male propensity to produce soft songs varies with the presence of females. Consequently, when conducting playback experiments, controlling for time of day, time of year, and breeding status will allow researchers to carefully control for variation in vocal output.

Investigations into the function of low-amplitude soft songs are relatively new and many questions about soft songs remain unanswered (Reichard & Welklin, 2015). To further understand the function of soft songs, future research could consider using seasonal variation studies. Just as the results of Chapter 2 of this thesis provided insight into the function of normal song, investigating temporal variation of soft song output could reveal interesting singing patterns that would provide insight into the function of soft songs. Similarly, analysis of diel and seasonal variation could be used to investigate aggressive call output variation. Just as our understanding of soft song is limited, the specific function of many calls and their importance in social interactions remain unclear and worthy of further investigation.

Additional future research could explore signalling strategies that facilitate escalation or de-escalation early in aggressive interactions, and provide a more comprehensive understanding of the hierarchical signalling strategies of Savannah Sparrows and other species. It is interesting to note that soft songs are used as predictor of attack in related sparrow species (e.g. Swamp Sparrows and Song Sparrows; Ballentine et al., 2008; Searcy et al., 2006) as well as in distantly related species (e.g. Smooth-billed Anis and Corn Crakes; Grieves et al., 2015; Rek & Osiejuk, 2011), suggesting that this signalling strategy may have evolved from a common ancestor but was lost in some lineages (e.g. Baker et al. 2012). Investigating the taxonomic diversity of soft songs used during aggressive contexts would give further insight on how common this signalling strategy is used in birds.

Another question that remains answered is why soft songs evolved as reliable aggressive signals since they are so easily produced and easily cheatable? In order to answer this question, further future studies are needed to test several proposed hypotheses that explain why low amplitude songs are reliable aggressive signals, presumably through the cost of receiver retaliation (Akçay, et al. 2015).

In conclusion, my thesis provides strong evidence that the analysis of seasonal and diel variation in singing activity with respect to breeding stages is an important tool to understand how birds use their songs. My research showed that Savannah Sparrows have dynamic patterns of diel and seasonal vocal variation, where vocal output varies with the social context at different times of their breeding season. In addition, my research suggests that dawn choruses have a territorial function whereas dusk choruses may have a female-related function in this species. Furthermore, my thesis provides evidence that Savannah Sparrows use graded signalling strategies to interact during territorial interactions. Similar to past studies in other species, my research showed that Savannah Sparrows signal their intention to attack their rivals with soft songs. In addition to soft songs, Savannah Sparrows also produce chip calls that predicted attack. My research advances the field of animal communication and provides a basis for future research on signal function and on social interactions involving vocal signals.

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