Cross-sectional and longitudinal analyses of three song parameters in an Atlantic

Canadian population of European Starlings, *Sturnus vulgaris* and whether they signal age

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ABSTRACT

Females from a range of taxa prefer to mate with older males as they may gain direct and/or indirect benefits that younger males are unable to provide. Male birds commonly sing to attract mates and older males are able to convey their superiority through song. European Starlings, *Sturnus vulgaris*, are a well-studied open-ended learner. Starling song has within-song type variations which are not well studied, and its function in Starlings is unknown. Repertoire size, within-song type variation and song bout length, as they relate to male age, were examined in an eastern Canadian population of Starlings. Surprisingly, repertoire size and song bout length did not increase with age in our analyses. However, younger males had more within-song type variation. These findings suggest that male repertoire and bout length do not signal age within my study population while within-song type variants may signal age, playing a potential role in mate attraction.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: General Introduction

Sexual selection

Sexual selection is a type of natural selection which acts on an individual's ability to mate. Two forms of sexual selection exist, which are intrasexual and intersexual selection. Intrasexual selection involves competition within the same sex to gain access to the opposite sex; a male competitor that defeats all other males gains access to females for mating, which leads to the evolution of certain male traits to be used as weaponry (Davies et al. 2012, Emlen and Zimmer 2020). For example, male Southern Elephant Seals, *Mirounga leonina*, with larger body sizes were dominant (high rank), were more successful in fights against other males and thus acquired the most mating events by defending a harem (McCann 1981). Similarly, the evolution of horns in ungulates is related to males defending their territory and maintaining dominance, which in turn determines their access to females (Janis 1982).

Intersexual selection involves interactions between sexes (males and females). Often the exaggeration of male ornamentation is used as a means to attract a female mate (Catchpole 1987). Females choose their sexual partner based on a male's phenotype – the exaggerated phenotype may indicate benefits that can help her indirectly, through genetic benefits passed on to the offspring, or help her directly through access to better territories or other material benefits (Kirkpatrick and Ryan 1991). For example, in Trinidadian guppies, *Poecilia reticulata*, females show strong selective preference for males with striking colours, specifically males that are bright orange or have orange areas (Auld et al. 2016). This orange colouration is obtained from a male's diet (Karino and Haijima 2004), so more orange colouration reflects the males' ability to forage for food rich in carotenoids. Therefore, orange colouration acts as an honest signal to females of a male's foraging ability. Karino et al. (2005) showed that foraging ability is heritable in these guppies. Thus a female choosing a male that is colourful, is indirectly choosing a male that will

potentially pass on effective foraging skills to their offspring (Karino et al. 2005). An example of a direct benefit is demonstrated in a study by Hill (1991). He experimentally manipulated the plumage colour of House Finches, *Carpodacus mexicanus*, and discovered that males with artificially brightened feathers (a trait typically dependent on the quantity of carotenoids in the diet) that were dyed for the experiment, paired earlier in comparison to individuals that had plumage artificially lightened with hair lightener, or that were unmanipulated controls. The males with naturally (not artificially dyed) colourful plumage provided more parental care, in terms of number of provisioning visits, demonstrating that male colouration is an honest signal to females of parental quality (Hill 1991). Similarly, brightly coloured male European Kestrels, *Falco tinnunculus,* hunted more and were preferred by females. Females that paired with brightly coloured males hunted less themselves and their nests had increased reproductive success (Palokangas et al. 1994).

Sexually selected traits such as weaponry used to compete, and elaborate plumage used to attract mates, are most commonly observed in males of a species because females are typically the limiting sex. The level of reproductive potential and parental care play a significant role in sexual behaviour. Females need to choose mates that would provide the optimum direct and indirect benefits for her to achieve maximum reproductive success (Davies et al. 2012, Emlen and Zimmer 2020).

In many birds and other taxa, females prefer older males (Trivers 1972, Manning 1985, Côté and Hunte 1993, Kokko and Lindstrom 1996, Kokko 1998) and those with more breeding experience (Brooks and Kemp 2001). It has been proposed that older males are preferred because they have demonstrated their ability to survive and therefore have higher quality viability genes which would be passed on to the offspring (Trivers 1972, Brooks and Kemp 2001). Older males also likely have more breeding experience and defend higher quality territories (Komdeur et al. 2005).

In species where bi-parental care exists, choosing older males may provide vital direct benefits. Generally, older males are more efficient foragers than younger males (MacLean 1986, Jansen 1989, Wunderle 1991, Desrochers 1992). Breeding experience plays a role in reproductive success (Nol and Smith 1987, Wooller et al. 1990). For example, older male Small Tree Finches, *Camarhynchus parvulus,* successfully fledged more nestlings compared to younger males in nests that had the parasitic fly, *Philornis downsi*. This fly is one of the leading causes of nestling death, surpassing predation in this population; the larvae hatch from eggs laid in the nests and feed on the nestlings. This finding supports the theory that older males are better at caring for offspring than younger males in unfavourable conditions (Wappl et al. 2020). In European Starlings, *Sturnus vulgaris,* (hereafter Starling)*,* sexually dimorphic iridescent hackle feathers are used in courtship displays. The length of the hackle feathers increases with age in both sexes (Komdeur et al. 2005, Barber and Wright 2017). Principal component one (PC1) in throat feather features (mainly a combination of length, iridescence and hue index) was higher in older males compared to females and younger individuals. Older females with a higher PC1 score mated earlier and also had larger clutch sizes; older males with higher PC1 scores had an increased probability of becoming polygynous and mating with higher quality females (Komdeur et al. 2005).

Parental care and birds

Socially monogamous avian species demonstrate more paternal care, to varying degrees, compared to other vertebrates (Ketterson and Nolan 1994). However, it is still common for the female to take on a larger role in the care provided compared to the male (Kessel 1957, Grundel 1987, Mountjoy and Lemon 1997). The extent of paternal care varies and can range from building nests (Soler et al. 1998, Friedl and Klump 1999, Walsh et al. 2011), to providing food to the female during egg laying (Silver et al. 1985), to a more direct role of incubating the eggs (Smith et al. 1995, Mountjoy and Lemon 1996, Auer et al. 2007) and feeding the nestlings (Pinkowski 1978, Breitwisch et al. 1986, Grundel 1987).

Sexual selection and birds

Avian males use a wide variety of tactics and innumerable sexually selected traits to attract mates (Petrie et al. 1991, Buchanan and Catchpole 1997, Siitari et al. 2002). For example, males have elaborate tails (Petrie et al. 1991), ultraviolet reflection of feathers (Siitari et al. 2002), and brightly coloured plumage (Hill 1991, Palokangas et al. 1994). Song is another trait commonly used by females to assess potential mates in birds, and is a sexually selected signal (Searcy 1984, Catchpole 1987, Mountjoy and Lemon 1996, Buchanan and Catchpole 2000), but it can also function in territorial defence (which is also a form of intrasexual selection) (Mountjoy and Lemon 1991, Lampe and Espmark 2003). Males have an array of song characteristics to attract mates. For example, males of many species sing complex songs (e.g. have a repertoire size which is defined as the total number of song types sung) (Eens et al. 1991a, Horn et al. 1993, Mountjoy and Lemon 1996, Buchanan and Catchpole 1997, 2000), long songs (Eens et al. 1991a, Gentner and Hulse 2000), flight songs (Buchanan and Catchpole 1997), or switch frequently between different song types (Horn and Falls 1991).

Starlings are a well-studied passerine species (Kessel 1951, 1957; Feare 1984) that sing long and complex songs (Eens et al. 1991b). Aspects of their song have been examined in the laboratory (Eens et al. 1992a, Gentner and Hulse 1998, 2000) and field (Mountjoy and Lemon 1991, 1995, 1996, 1997), providing extensive insights into the function of song in this species. Their song repertoire size and bout length have a positive correlation with age. Second year (SY: individuals that hatched the year before and are one year old) have a smaller repertoire size and sing shorter songs when compared to older males (Mountjoy and Lemon 1995). This increase was also found in Whinchats, *Saxicola rubetra* (Vaytina and Shitikov 2019)*,* Canaries, *Serinus canarius* (Nottebohm and Nottebohm 1978)*,* and Willow Warblers, *Phylloscopus trochilus* (Gil et al. 2001); older males had more complex songs compared to individuals breeding for the first time. Females may therefore use repertoire size and song bout length as a cue when selecting

4

mates, pairing with older males because these males are more experienced and may provide direct benefits, such as increased incubation and provisioning effort (Eens et al. 1991a).

Song production

Understanding song production is important in order to fully comprehend the role song plays in advertising male quality to females. The ability to learn and produce songs varies within oscine species (Marler 1990). The two overarching categories are close-ended learners and open-ended learners (Robinson et al. 2019). Close-ended learners only learn song within a strict time frame, known as a "critical period." This is the only time in which an individual has the ability to learn their song; after this critical period ends, they cannot acquire any additional songs. For example, the Song Sparrow, *Melospiza melodia*, has a critical period in which to learn the songs that will be used throughout the remainder of their life (Marler and Peters 1987). A Song Sparrow's learning period starts at 20 days after hatch and ends at approximately 60 days; peak learning occurs at the beginning and declines quickly as it approaches the end of the time frame (Marler and Peters 1987).

Song learning is a two-stage process; the first is the early sensory phase and the second is the sensorimotor phase (Peters et al. 1992, Peters and Nowicki 2017). In the sensory phase, individuals listen to songs produced in their surroundings. Song Sparrows can learn and produce an accurate conspecific song type after a song is played 30 times in one day. Nightingales, *Luscinia megarhynhos*, produced their conspecific song after being exposed to it 10 times. This shows that different species require a different number of song presentations to produce an accurate rendition of it (Marler 1990). During the second phase, individuals attempt to sing the song they have heard (Peters et al. 1992, Peters and Nowicki 2017). It is during the crystallization period that an individual establishes the stereotyped structure of the song, with the specific syllables sung in a pattern (Nottebohm 1968). The sensorimotor phase and crystallization phase

can happen simultaneously, or the crystallization of songs can happen later (Nottebohm 1968, Catchpole and Slater 1995).

Open-ended learners such as Pied Flycatchers, *Ficedula hypoleuca*, continue to expand their song repertoire throughout their life (Eriksen et al. 2011). Canaries are also able to add new song types to their repertoire beyond the first year of their life (Nottebohm and Nottebohm 1978, Nottebohm et al. 1986) with the repertoire of a second-year male being larger than that of a first-year male. However, they are only able to change their repertoire outside of the breeding season, throughout the summer and early fall; the repertoire remains unchanged during the breeding period (Nottebohm et al. 1986). European Starlings are also open-ended learners (Eens et al. 1992b, Mountjoy and Lemon 1995). Chaiken et al. (1994) was the first to demonstrate that Starlings learn songs in their adulthood. Male Starlings reared in a laboratory setting were exposed to recordings and live tutoring, and added new song types throughout the first 18 months of their life (Chaiken et al. 1994). Field studies have also shown increases in repertoire at four years of age in Starlings (Mountjoy and Lemon 1995). Starlings that were presented with new songs, memorized them and sang the newly acquired song types 18 months later (Chaiken et al. 1994). This demonstrated that there can be an extended period between the first and second phases of song production. Starlings learned most of the songs presented to them after they moulted for the first time, between 94 and 139 days after hatching. The time soon after fledging, between 38 and 81 days after hatch, was the poorest learning period. Starlings are able to learn and add new song types throughout the year, including during the breeding season (Chaiken et al. 1994). There is a significant gap in repertoire sizes between SY and ASY (ASY: males that are two years old or more) males (Eens 1997), demonstrating a clear relationship between age and song learning and production in Starlings.

Cross-sectional and longitudinal analyses with age-dependent traits

A common area for disagreement is whether sexually selected traits actually increase with age or if they reflect the ability of a male to survive (Forstmeier 2002, Forstmeier et al. 2006). Cross-sectional studies take a sample and assess individuals from varying age classes within the same year while longitudinal studies follow the same individual across years (Forstmeier 2002). With longitudinal studies, it is possible to assess the changes in a trait from one year to the next, and is therefore a reliable way to assess how it correlates with age. Conversely, cross-sectional studies are typically not as reliable as longitudinal studies, in this context, due to the trait not being independent of mortality (Forstmeier 2002).

Forstmeier (2002) describes, if song complexity is found to increase with age in a cross-sectional study, that may be because the trait in fact does increase with age, or instead, because younger males with smaller repertoire sizes are in poorer condition and so die relatively more often than older individuals with larger repertoire sizes that are in better condition. Therefore, the trait reflects male quality and viability (Forstmeier 2002, Forstmeier et al. 2006). A longitudinal study is more robust because song complexity is followed in the same individual, which avoids confounding variables such as mortality, which affect a purely cross-sectional study (Forstmeier 2002, Forstmeier et al. 2006).

One advantage of performing a cross-sectional study is the relatively short time frame (one year/field season). This is especially important when working in the field, as there can be numerous external factors that cannot be controlled. For example, banded individuals (e.g. birds) not returning to the study site the following year. A cross-sectional study is advantageous in this case as it is still possible to identify potential correlations from data collected in a short window of time, which can lay the foundation for long term studies (Levin 2006). As a result, it is important to observe these changes using both longitudinal and cross-sectional methodologies. For example, two separate populations of Great Reed Warblers, *Acrocephalus arundinaceus,* one

in Germany and another in Sweden were studied. In the German population, song repertoire did not increase with age in the longitudinal study while the cross-sectional study showed a positive relationship between song repertoire size and age. These findings suggest that repertoire size does not increase with male age, but does reflect male's longevity. In the Swedish population, the longitudinal study showed an increase in repertoire size with age, while the cross-sectional study in Sweden also showed a stronger positive correlation between age and repertoire size compared to that of the German population's. It is interesting to note that two populations of the same species showed different relationships between song and age (Forstmeier et al. 2006). In Starlings, both longitudinal (Mountjoy and Lemon 1995) and cross-sectional (Eens et al. 1992b) studies have demonstrated that individuals tend to add song types to their repertoire each year.

Multiple message, redundant-signal and unreliable-signal hypotheses

An individual can have more than one ornament signalling information on quality to the receiver. The multiple message hypothesis proposes that one trait provides honest information about one specific aspect of the individual while another trait honestly signals another aspect of quality (Moller and Pomiankowski 1993, Rivera-Gutierrez et al. 2010). Alternatively, the redundant-signal hypothesis proposes that these different ornaments all signal information on the same aspect of condition in the individual, reinforcing each other (Moller and Pomiankowski 1993, Rivera-Gutierrez et al. 2010). Finally, some traits do not convey any information on quality, which is known as the unreliable-signal hypothesis. These signals may still exist because they may not incur high levels of energy to produce (Moller and Pomiankowski 1993, Rivera-Gutierrez et al. 2010). These unreliable signals may also persist in the environment because the sender uses these signals to deceive the receiver to gain fitness advantages over the receiver (Mokkonen and Lindstedt 2016). Varying song characteristics in Great Tits, *Parus major,* supported both the multiple messages and the redundant signal hypotheses (Rivera-Gutierrez et al. 2010). Song consistency positively correlated with age while song repertoire size was positively

correlated with survival (individuals that were alive from one year to another either as determined by recapture or observation), demonstrating that two different characteristics of song provide information about two parameters of an individual. Two other song characteristics tested in this study examined the amount of time an individual sang (song performance) and the phrase and strophe rate known as song output, both of which correlated positively with song repertoire size. Therefore, redundant information is also conveyed through song. Some variables did not correlate with any trait (e.g. age was not correlated with body condition or survivorship) which supports the unreliable signal hypothesis; conversely, this lack of correlation could be due to not having tested the appropriate traits (Rivera-Gutierrez et al. 2010).

In Song Sparrows, both the redundant hypotheses and multiple message hypotheses were supported. Syllable repertoire size (syllables unique to an individual) was highly and positively correlated with syllable sharing (number of common syllables among individuals in the same local population). A correlation between these two measurements of song shows that they convey similar information, supporting the redundant hypothesis. This finding demonstrates not only an individual's ability to learn songs but also to learn songs of their local population. Song repertoire size was also highly correlated with song output. The two different characteristics of song complexity signalled two pieces of information, supporting the multiple message hypothesis (MacDougall-Shackleton et al. 2009). Often one ornament is exclusively explored with regard to one piece of information; but a single ornament can also be an indicator for multiple aspects of behaviour or provide no information.

Fine-scale vocal performance

Vocal performance is another way for females to assess male quality through song. For example, the ability for individuals to sing song types in a consistent fashion, as suggested by Byers et al. (2007), is a form of vocal performance, which can be further broken down to a fine-scale vocal

performance. Fine-scale vocal performance, for example, can be examined by assessing the consistency of timing and the pitch in song sung by males. Byers (2007) assessed the timing and pitch of song types of Chestnut-sided Warblers, *Dendroica pensylvanica*, and males that sang these parameters more consistently had more extra-pair young (Byers 2007). Trill consistency is an age-dependent trait in the Banded Wren, *Thryothorus pleurostictus*, which may be used to distinguish between younger and older males (De Kort et al. 2009). This suggests fine scale vocal performance such as how consistent song types, timing, pitch and trills are sung may also be used by females to assess male quality.

Many studies on Starling song have explored the role of song bout length and repertoire size but not fine-scale vocal performance. For example, Starling song bout length and repertoire size is shorter and smaller in SY males with 20.5 seconds and 26 song types, compared to ASY males with an average of 28.1 seconds with 51 song types. Observational studies on female preference in Starlings, provided evidence that females selectively paired with males that sang the longest bout with the largest repertoire size (Eens et al. 1991a). Song repertoire size also positively correlated with condition while it negatively correlated with the number of days until the first egg was laid (Mountjoy and Lemon 1996). It has been shown that there is song type variation within Starling song and that no song type is identical to itself, but its potential function has not been explored (Eens et al. 1989, 1991b). Song Sparrows also show considerable variation within a song type (Nowicki et al. 1994). The role of the within-song type variations has been explored further in Song Sparrows (Stoddard et al. 1988, Searcy et al. 2000) with respect to territoriality (Stoddard et al. 1988) and aggressive contexts (Searcy et al. 2000). Both forms of song, distinct song types and within-song type variations elicited responses in conspecifics or were used by Song Sparrows in aggressive situations, demonstrating that both forms of song complexity are important in this species. It is possible that many within-song type variations are simply transition errors (Nowicki et al. 1994), however it may not always be a plausible

10

explanation, as demonstrated by Song Sparrows that respond to both forms (Stoddard et al. 1988, Nowicki et al. 1994, Searcy et al. 2000). Additionally, Nightingales also have a large repertoire size, ranging from 160 to 280 song types which can be easily categorized as they have minimal variation within song types (Hultsch and Todt 1981). These studies support the idea that fine-scale features play a role in signalling male quality to females and it is therefore a form of vocal performance which should considered when studying male quality with respect to male song.

Study species

Starlings are a socially monogamous (Pinxten et al. 2007) and facultatively polygynous (Pinxten et al. 1989b) species with bi-parental care (Kessel 1951, Mountjoy and Lemon 1997). Males with larger song repertoires and long song bouts paired earlier and attracted more females (Eens et al. 1991a, Mountjoy and Lemon 1996). Both laboratory (Eens et al. 1993) and field studies (Mountjoy and Lemon 1996) on this species have shown that song plays a role in female mate choice. Males sang more complex and longer songs in the presence of females (Eens et al. 1991a, 1993). In an experimental study, females stayed at the nest boxes where longer song bouts, from prepared audio files, were played from a speaker. They also learned to distinguish which nest boxes had shorter songs playing and stopped responding to them. Interestingly, females did not listen to the entire song bout, suggesting they do not use the complete repertoire or the entire duration of song when preferentially remaining at nest boxes with the longer song bouts. However, the length of time that females listened to a song bout was positively related to its length and the number of motifs or song types (a motif or a song type are units that repeat structurally with minor variations and are composed of smaller acoustical elements) in the bout (Gentner and Hulse 2000).

Objectives

My study examines the relationship between age and song in an eastern Canadian population of Starlings. The second chapter focuses on two male song characteristics, song repertoire size and song bout length, and examines whether these two song characteristics increases with male age, using both cross-sectional and longitudinal analyses. This work adds to the body of research examining song characteristics in open-ended learners as they age. It will be interesting to determine if my focal population provides similar results to those found in other North American (Chaiken et al. 1994, Mountjoy and Lemon 1995) and European (Eens et al. 1992b) populations of Starlings.

The third chapter focuses on fine-scale vocal performance, variation within-song types and determines whether it changes with age. Within-song type variation and its role in mate selection has not yet been explored in this species. These results will add to our understanding of the variety of song characteristics used to signal information to conspecifics.

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CHAPTER 2: Analyses of song repertoire size and song bout length and whether they signal age

ABSTRACT

In many passerines, male song functions as an honest signal of age. Age can signal both direct (e.g. breeding experience) and indirect (e.g. viability and good genes) benefits to females choosing these males as potential mates. European Starlings, *Sturnus vulgaris*, sing long and complex songs, learn songs throughout their life, and are well studied with respect to their behaviour and physiology. My objective was to investigate whether repertoire size and song bout duration change with respect to age in an Atlantic Canadian population of Starlings. We recorded twelve males and obtained two years of song data for eight males and three years of song for one male. Older males did not have longer songs or larger repertoire sizes than younger males. Also, male song repertoire size and bout length does not significantly increase over a one-year period. Alternate song traits may be used to signal Starling male age to females.

INTRODUCTION

Females across a broad range of taxa prefer to mate with older males (Weatherhead 1984, Côté and Hunte 1993, López et al. 2003, Rodríguez-Muñoz et al. 2019). By choosing older males, females gain benefits that younger males are not able to provide. The benefits provided by age can be indirect, direct or a combination of both (Hasselquist 1998). In species where male contribution effort is simply to provide sperm, the female may be selectively choosing males that will provide superior genes for their offspring (Wetton et al. 1995). Older males have demonstrated the ability to survive, which signifies they have higher quality genes with the potential that these traits will be inherited by their offspring (Trivers 1972, Eens et al. 1991a, Brooks and Kemp 2001) and possibly their accrued experience is an essential to further survival (Komdeur et al. 2005).

In species where males play a large role in parental care, older males may provide a higher level or better quality of direct benefits (e.g. incubating, feeding of young, and defence against predators) to females than younger males due to their prior breeding experience, thereby optimizing their potential reproductive success. Generally, males in avian species demonstrate more paternal care as compared to other vertebrate species (Ketterson and Nolan 1994). However, it is still common for females to provide a larger percentage of parental care compared to the males (Kessel 1957, Grundel 1987, Mountjoy and Lemon 1997). The extent of paternal care varies among socially monogamous avian species. It ranges from indirect contributions such as nest construction (Moreno et al. 1994, Friedl and Klump 1999, Walsh et al. 2011) and provisioning for the female while she lays eggs (Silver et al. 1985), to direct contributions such as incubating the eggs (Smith et al. 1995, Mountjoy and Lemon 1997, Auer et al. 2007) and feeding the nestlings (Pinkowski 1978, Breitwisch et al. 1986, Grundel 1987).

Generally, older males are more efficient foragers than younger males (MacLean 1986, Jansen 1989, Wunderle 1991, Desrochers 1992). Breeding experience plays a role in reproductive success (Wooller et al. 1990) and older males likely have superior knowledge of relevant territory and may be better at defending the same territory (Komdeur et al. 2005). Increased breeding experience was preferred by Feral Pigeons, *Columba livia domestica* (Burley and Moran 1979) and Red-Winged Blackbirds, *Agelaius phoeniceus* (Yasukawa 1981a) which indirectly correlates with age. In Common Yellowthroats, *Geothylpis trichas,* social mating success correlated with mask size which increase with age (Freeman-Gallant et al. 2010). In unfavourable conditions such as parasitism of nestlings by a fly, *Philornis downsi*, older males were more successful in fledging young in parasitized nests, when compared against younger males. Older males differed in their search techniques for food which improves with age and were likely able to provide higher quality food items to the parasitized nestlings compared to younger males (Wappl et al. 2020).

Older males are recognized by females through traits that honestly signal their age. These sexually selected traits change or increase with male age in avian species (Kokko 1997). Often, younger males that are ready to mate for the first-time exhibit plumage traits that are similar to their female conspecifics, and maturation of these sexual traits will be developed after their first breeding attempt (Rohwer et al. 1980). In European Starlings (*Sturnus vulgaris,* hereafter Starling*)* sexually dimorphic iridescent hackle (throat) feathers are used in courtship displays (Komdeur et al. 2005). The length of the hackle feathers increases with age in both sexes (Komdeur et al. 2005, Barber and Wright 2017). Principal component one (PC1) in throat feather features (a combination of length, iridescence, and hue index) were higher in older males compared to younger males and females. Older females with a higher PC1 score mated earlier and had larger clutch sizes; older males with higher PC1 scores had an increased probability of becoming polygynous and mating with higher quality females (Komdeur et al. 2005).

In many birds, song is a sexually selected trait and females assess song as a signal of potential high-quality mates (Catchpole 1987, Mountjoy and Lemon 1996, Buchanan and Catchpole 2000). A wide variety of song characteristics have developed to attract mates. For example, males sing complex song (e.g. repertoire size which is defined as the number of unique song types an individual would sing) (Eens et al. 1991b, Horn et al. 1993, Mountjoy and Lemon 1996, Buchanan and Catchpole 1997, 2000), long song bouts (Eens et al. 1991a, Gentner and Hulse 2000), song rate (Hoi-Leitner et al. 1995), flight songs (Buchanan and Catchpole 1997), and switch between different song types (Horn and Falls 1991).

A widely studied form of song complexity is song repertoire, which increases with age in some species (Mountjoy and Lemon 1995, Forstmeier et al. 2006). Older males had more complex songs compared to first year breeding individuals in the Whinchat, *Saxicola rubetra* (Vaytina and Shitikov 2019)*,* Canary, *Serinus canarius* (Waser and Marler 1977)*,* Willow Warbler, *Phylloscopus trochilus* (Gil et al. 2001) and Starlings (Eens et al. 1992b). Individuals that are able to alter and add new song types to the song repertoire after the first calendar year are known as open-ended learners (Beecher and Brenowitz 2005). This can be further divided in two categories, where some individuals can only make alterations within the time period of their first calendar year (hatch year) to their second year of life (SY: individuals that hatched the year before and are one year old), while others continue to add new song types throughout their adult life (Benítez Saldívar et al. 2020). For example, Willow Warblers, *Phylloscopus trochilus,* are only able to add new song types between their first year and second year (SY) while Pied Flycatchers, *Ficedula hypoleuca* can add new song types throughout their adult life (Eriksen et al. 2011). Starlings can add new song types throughout their adult life (Eens et al. 1992b, Mountjoy and Lemon 1995).

In Starlings, a socially monogamous (Pinxten et al. 2007) and facultatively polygynous (Pinxten et al. 1989b) species with biparental care (Kessel 1957, Mountjoy and Lemon 1997), males with larger song repertoires and long song bouts paired earlier and attracted more females (Eens et al. 1991a). On average, repertoire size ranges from 21 to 68 song types (Eens et al. 1992b, Mountjoy and Lemon 1995) while song bout length, on average, varies from 18.5 seconds to 35 seconds. SY males sing shorter songs and have smaller repertoire sizes than after second year (ASY: males that are two years old or more) males (Eens et al. 1992b, a; Mountjoy and Lemon 1995). In older males in an eastern Canadian population, there were large within-individual increases in repertoire size from one year to the next (Mountjoy and Lemon 1995) while two European populations did not find evidence for this (Adret-Hausberger et al. 1990, Eens et al. 1992a).

Starling song repertoire size could also act as an honest signal of the males' condition which in turn may reflect the males' age to the female, because repertoire size also correlates with male condition in Starlings (Mountjoy and Lemon 1996). The positive relationship of condition and repertoire size suggests that males must be in good condition to produce complex songs and they incur energy to produce a large repertoire (Mountjoy and Lemon 1996). It could also mean that older males are in better condition than younger males, where young males are less skilled at foraging, which affects their food intake thus possibly condition (Marchetti and Price 1989, Wunderle 1991). In addition to condition, immunity correlated with song in Starlings. The amount of singing and song bout length positively correlated with cell-mediated immunity and humoral immunity, respectively (Duffy and Ball 2002). Immunity and condition could be benefits that females receive by choosing older males with larger song repertoires and longer song bouts than their younger counterparts.

Many studies have demonstrated the relationship between aspects of song in Starlings and female choice. For example, there was a negative correlation between repertoire size and the date

first egg was laid (Mountjoy and Lemon 1996) and negative correlation with pairing date (Eens et al. 1991a). Similarly, males with longer song bouts attracted more female mates at a faster rate, than males that sang shorter song bouts (Eens et al. 1991a). Song bout length correlated significantly with the number of fledglings, while repertoire had a nonsignificant correlation (Eens et al. 1991a). However, no correlation was found between repertoire size and either provisioning or incubation effort by males (Mountjoy and Lemon 1997).

There are several studies on Starling song characteristics and whether it reflects age (Eens et al. 1992a, b; Mountjoy and Lemon 1995), however sexual signalling of song characteristics can vary geographically even within the same species. For example, Forstmeier et al. (2006) showed that in Great Reed Warblers, *Acrocephalus arundinaceus*, the Swedish population demonstrated an increase in repertoire size with age, while the German population did not (Forstmeier et al. 2006). It is important to explore the function of these elaborate song displays in the same species in different geographical locations to observe whether song signalling is different at the population level.

The goal of this study was to examine two song parameters (song repertoire size and song bout length) with male age in a Starling population in Eastern Canada. Past studies have shown that both song repertoire size and bout length are significantly different between SY and ASY males (Eens et al. 1992b, a; Mountjoy and Lemon 1995). In another Eastern Canadian population, there were significant within-individual increases in repertoire size suggesting it is an age-dependent characteristic in this open-ended learner (Mountjoy and Lemon 1995). The aim is to investigate whether song is an honest signal for age in this population of Starlings using both cross sectional and longitudinal approaches. I predict that song repertoire size and song bout length increase with age, where older males will sing with larger repertoire sizes and longer song bouts than younger males.

METHODS

Study species

Starlings were introduced to North America in New York in 1890 and 1891 (Kessel 1957). Starlings nest in cavities and are a colonially breeding species. Starlings aggregate in groups during the winter but remain near their nests during breeding season (Feare 1984). They exhibit both social monogamy (Kessel 1957, Pinxten et al. 2007) and facultative polygyny (Pinxten et al. 1989a, b). Males breed outside their pair-bond; one study showed that 39% of males attempted to breed with other females to increase breeding success (Pinxten et al. 1989b). Males that are polygynous produced more fledglings than monogamous males (Pinxten et al. 1989b). Extra-pair paternity (EPP) exists, where offspring in the nest are not genetically related to the male parent attending to the nestlings. Studies show that 28.6% to 31.8% of nests contained eggs from EPP (Pinxten et al. 1993, Smith and Schantz 1993). Intraspecific brood parasitism (IBP) is also practiced, where a conspecific female lays an egg in the nest and removes one of the host's eggs. The amount of IBP in Starling's varies from 0% to 37% annually (Romagnano et al. 1990, Pinxten et al. 1993). Females are guarded by males after pair formation until the initiation of incubation, to prevent other males from copulating with them (Feare 1984, Pinxten and Eens 1997). Males only defend the areas around the nest; food resources are not defended (Feare 1984, Eens et al. 1991a, Mountjoy and Lemon 1997). Starlings have early and late clutches with some laying an intermediate clutch if the first clutch failed or if they were unable to procure a nesting cavity. The total number of eggs laid decreases in the intermediate and late clutches (Kessel 1957). Females lay one egg a day and on average, lay between four to five eggs (Feare 1984). Incubation starts after the second to last egg is laid. Monogamous males contribute more to incubation than do polygynous males; the total nest attendance in monogamous pairs was higher with 95% attendance, whereas polygynous pair's attendance was lower with 85% attendance (Pinxten et al. 1993). Eggs are incubated for an average of 12 days after the last egg is laid (Feare

1984). Parental care is provided by both parents, which includes feeding, faecal sac removal and replacing nest material that are covered in faeces (Feare 1984). Fledglings leave the nest on the 21st day after hatching and the parents continue to feed them for about 12 days (Feare 1984). Starlings primarily feed on invertebrates found on grassy environments and foliage (Feare 1984).

Study site

The study was completed at Saint Mary's University in Halifax, Nova Scotia, Canada (44˚ 37' 54.07" N, 63˚ 34' 47.09"). Starlings have been studied at this study site since 2007. There are a total of 45 nest boxes (length x width x depth, 35cm x 17cm x 18cm respectively) on campus, 40 were installed in 2007 and another were five added in 2009.

The field work was conducted over three years from mid-April to July in 2019, from February to March and June to July in 2020 (research stopped mid-way due to the global Covid-19 pandemic), and from February until April in 2021. In 2019, most of the males were already paired by late-April, and so most of the song recordings were obtained from the late brood in 2019. A total of five males from 2019, 11 males from 2020 and 6 males from 2021 were recorded.

Sexing and banding

Adults were caught using Mo traps. A Mo trap is a contraption where a flat wooden block is propped up with a stick inside the front end of the nest box. When an adult Starling enters the nest box and knocks over the stick, the wooden block falls over the nest box hole covering the entrance thus trapping the adult inside (Stutchbury and Robertson 1986). Adults were also caught by blocking the nest hole with a Swiffer. Adults were sexed from their bill and eye colour. The females have a pale pink colour under their bill while males have a dark black-bluish colour. This was also confirmed by examining their eye for the presence (female) or absence (male) of a yellow iris ring (Kessel 1951). Upon capture, a returning adult's (previously banded) Canadian

Wildlife Services (CWS) unique identification number was written down along with the unique colour combination (two plastic coloured bands which are only found on that specific bird for identification). For new adults, a CWS band was placed on their right tarsus. A plastic yellow band for males and a plastic pink band for females was placed above the CWS band. Two bands having unique colour combinations were placed on the left tarsus, to allow for individual identification.

A total of 14 adult males were banded during the 2019 field season to increase the sample size of banded males returning the following year. Individual identification of each male was vital for song recordings. In 2020, one unbanded male was recorded and this individual was banded when he was feeding his nestlings later in the field season.

Determining age

During the banding process, seven to eight hackle (throat) feathers were also collected from each male to determine their age category. I recorded a total of 22 males and the age of each male was determined. Age was determined by analysing male hackles (throat feathers). Starlings in their first year were distinguished from older adults from the length of the iridescent portion of the hackle. The iridescent portion of the feather is longest in ASY (after second-year) males, and shorter in SY (second year) males (minimum of 11.1 mm vs. less than 11.0 mm respectively) (Kessel 1951, Barber and Wright 2017).

A ruler was used to measure the total length of the hackle feathers and the iridescent portion of the feather under a dissecting microscope - to the nearest 0.1mm. Total visible portion of iridescence and the entire length from the tip to the rachis of the hackle were measured (Barber and Wright 2017). The total iridescence was used to assign males into SY (one-year old and first-time breeders) and ASY (ASY males have likely gained some past breeding experience) males. Age could be further estimated because all males were banded. For males banded in their SY,

their exact age could be inferred, while the age of males first banded as ASY could be approximated. Age and year of life (meaning in which of life a male is in) will be interchangeably used in this chapter.

Song recordings

Male Starling recordings started in late April 2019 and in mid-February 2020 and 2021. Most of the singing occurs during the breeding season and greater than 90% of their song is sung in bouts during the breeding season (Eens et al. 1991b). Males defend a small territory around the nest hole; this area is where they sing (Eens et al. 1991a, b; Bernard et al. 1996). After several days in the field recording song, it was determined that most of the singing was observed between 0700 to 1200PM and again in the afternoon from 1500 to 2000. Most recordings were made opportunistically during these time frames. A small percentage of recordings were made outside of these time frames.

A PMD Marantz professional recorder (Model number: 661MK111) and a shotgun Sennheiser microphone with a Sennheiser MZS 20-1 grip were used to record the song bouts. Occasionally, a Zoom H4n Pro Handy Recorder (Model number: ZH4NPRO) and a Sennheiser P48 Super-Cardioid Long Shotgun Condenser Microphone (Model Number: MKH-70-1) were also used. The recorder settings were PCM 44,100Hz, 16 Bit, Mono. Males were recorded daily until a minimum of 27 song bouts were obtained from each male but occasionally more recordings were obtained as a precautionary measure (Eens et al. 1991a, b, 1993). Recordings encompassed the time period prior to pairing to after pairing. One male was recorded while provisioning fledglings; he continued to sing at a nest box directly opposite his nest-box from the early brood. Batteries were charged overnight or between 1400 and 1600. All recordings were removed from the secure digital (SD) card daily, labelled and stored in an external hard drive for song analysis.

Song analysis

Starling song is composed of four sections: whistles, variable songs, rattles and high frequency

song types; these four sections make-up a full song bout (Eens et al. 1989, 1991b). This full song

bout does not include all the different types of whistles or variable song types a male would sing.

However, a full song bout does contain almost all the rattles and high frequency song types that

he would sing (Eens et al. 1989). When a bird's repertoire is made up of more than one song type,

these songs can be sung in two ways. Eventual variety is an individual repeating the same song

type multiple times before singing another song type. Immediate variety is where the song types

in a song bout change quickly. Starlings are immediate variety singers (Eens et al. 1989). A song

bout was defined as a song lasting a minimum of five seconds with no intervals that are longer

than 1.5 seconds, which is the same definition used in past studies (Eens et al. 1991b, 1992b;

Bernard et al. 1996). Song bouts used for my analysis did not need to contain all four sections

which can be found in a Starling bout.

Whistles (see Fig. 2.1) can be simple or complex; whistles often have integrated mimicry

of other species and anthropogenic sounds. Whistles are separated from each other by one or more

seconds (Adret-Hausberger and Jenkins 1988, Mountjoy and Lemon 1995), and are the most

easily identified of the four song types (Eens et al. 1989). Variable song (see Fig. 2.2) which has a

variety of song types with varying frequencies ranging between 1 to 8kHz, are sung over a short

period of time (Eens et al. 1989). They are also very low in amplitude and can only be heard near

the bird. Variable songs consist of notes that overlap because of double voicing. They also include

most of the heterospecific imitations and sounds such as clicks, buzzes and trills (Eens et al.

1989). Variable songs typically start with clicks (Eens et al. 1989) and are complex as the song

types can have within-song type variation, making it difficult to categorize into song types.

Variable song is followed by rattle song types, which sound similar to a rattle (hence the name)

(see Fig. 2.3). The bill is used to make the sound of a rattle. Rattle song types have clicks,

overlapping-notes and typically include sudden changes in amplitude (Eens et al. 1989, 1991b).

These songs are similar to variable songs but are easier to hear and to categorize. The fourth

section of song is composed of high frequency song types (terminal song types) (see Fig 2.4).

These song types are approximately between 7 and 12 kHz but are difficult to categorize into one

frequency range. This section has the highest amplitude and often sounds like a high-pitched

scream. For the most part, this section is where Starlings perform wing-waving. Song bouts rarely

end with high-frequency notes because a song bout can finish at any point in a song bout (Eens et

al. 1989).

Software Raven Pro Version 1.5 (Center for Conservation Bioacoustics. 2011) was used

to analyse songs. Only high-quality recordings where song types could be clearly distinguished

were used in the repertoire size analysis. However, if poorer-recording quality songs were

sufficient to obtain the song bout duration, they were included in the song bout analysis. For the

longitudinal study, eight males were analysed over two years. For the cross-sectional study, one

year of data was analysed for each of 12 males, (two males in 2019, and 10 males in 2020).

Classification of song types

A challenging part of the repertoire analysis is determining what is a song type and determining

what degree of change in a single song type is a different song type or the same song type.

Therefore, as a part of the repertoire analysis, criteria were created to make the determination of

song types as objective as possible. All song bouts used for the repertoire analysis were printed on

paper as this made it easier to see the entire song (it cannot be viewed in its entirety on the

computer) and to identify the different sections of the song. To be consistent, one individual

determined all the song types. With more complex and difficult song types, another individual

helped to determine song type classifications.

The following definitions were created for the song type classification.

Song Type: A unique acoustical structure that repeats in a song bout. A song type looks the same on a spectrogram and sounds acoustically identical or clearly similar to another one of the same type. The amplitude and pitch were not used to define song types.

Each section of a Starling song has its unique challenges when classifying the song types. The whistles are the least complex and can be classified as a song type without difficulty which has been described in other studies as well (Eens et al. 1989, 1991b). When classifying the variable song types, the song type definition made it easier to decide whether it was the same song type or a different song type. In my population, I found that a single song type is not identical when it is repeated within the same bout or across bouts and I call these differences within-song type variations. However, for this study these variations were not taken into consideration but were compared to other song types and was either grouped with a song type which it resembled the most or assigned a new song type label. These within-song type variations are assessed in Chapter 3. These within-song type variations have been observed in Starlings in other populations (Eens et al. 1989, 1991b). On some occasions the preceding or following variable song type was less complex but had similar components to the more complex song type. In this case, it is important to listen and visually analyse the two song types to determine whether they should be classified as the same song type or not.

If the simple song type had structures that were all seen in the more complex song type, these were considered the same song type. In this case, the more complex song type had added a few acoustical structures to the simple song type, but was still considered the same song type. In rattle song types, the amplitude of the song types changes (Eens et al. 1989), however it was not considered in our classification process. Many of the rattle song types changed minor acoustical elements but maintained the same base elements of the song type. When classifying rattle song

30

types, these base elements of the rattles were used to determine different song types; meaning if rattles had generally the same base elements with only minor variations in acoustical elements, they were considered the same song type. In some song bouts, the rattle song types were introduced in an alternating manner as opposed to the common style of singing one type once before introducing a new song type (see Fig. 2.6).

Generally, songs began with structurally simple chirps (see Fig. 2.5). When songs were initiated with these chirps, and were separated by less than 1.5 seconds from the first structurally complex song type (e.g. whistle or variable song type) in the song, then they were included in the song analysis. These chirps were not considered song types but as integrations of the first song type. When these chirps were also found in-between Starling song sections, they were randomly integrated as part of either the preceding or following song type which was structurally more complex than a simple chirp.

In the longitudinal analysis where the same males were followed over a two-year period, some males completely dropped song types and added new song types to their repertoire. One male sang different variations of the same song type analysed the previous year. The focus was on the number of different song types and so the repertoire turnover or the changes in variations from year to year were not analysed.
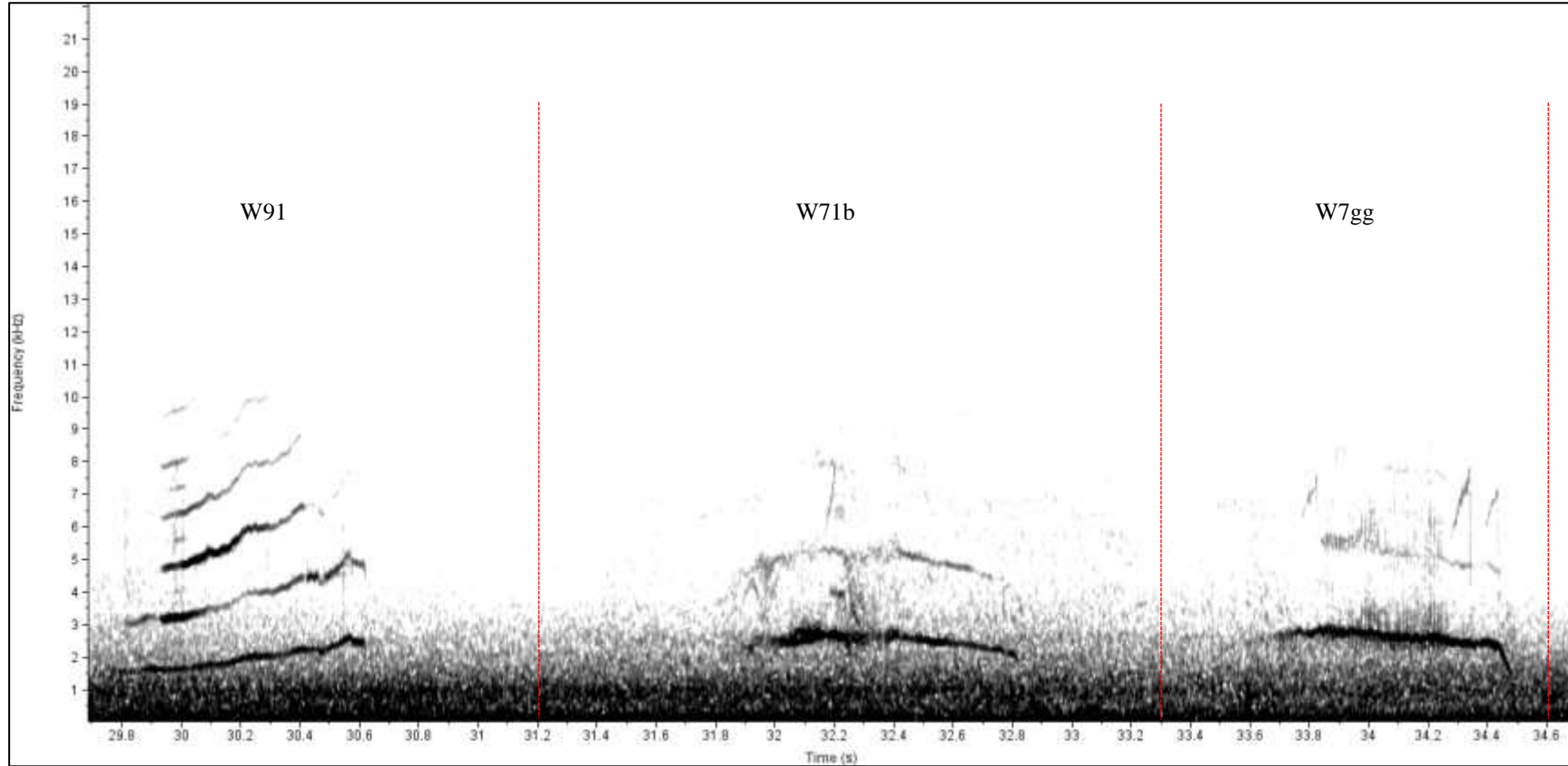
Figure 2.1. "W" denotes whistle song types. Whistles 91, 71b and 7gg were recorded from the male at nest box 22 in 2020. The red dashed line separates song types.
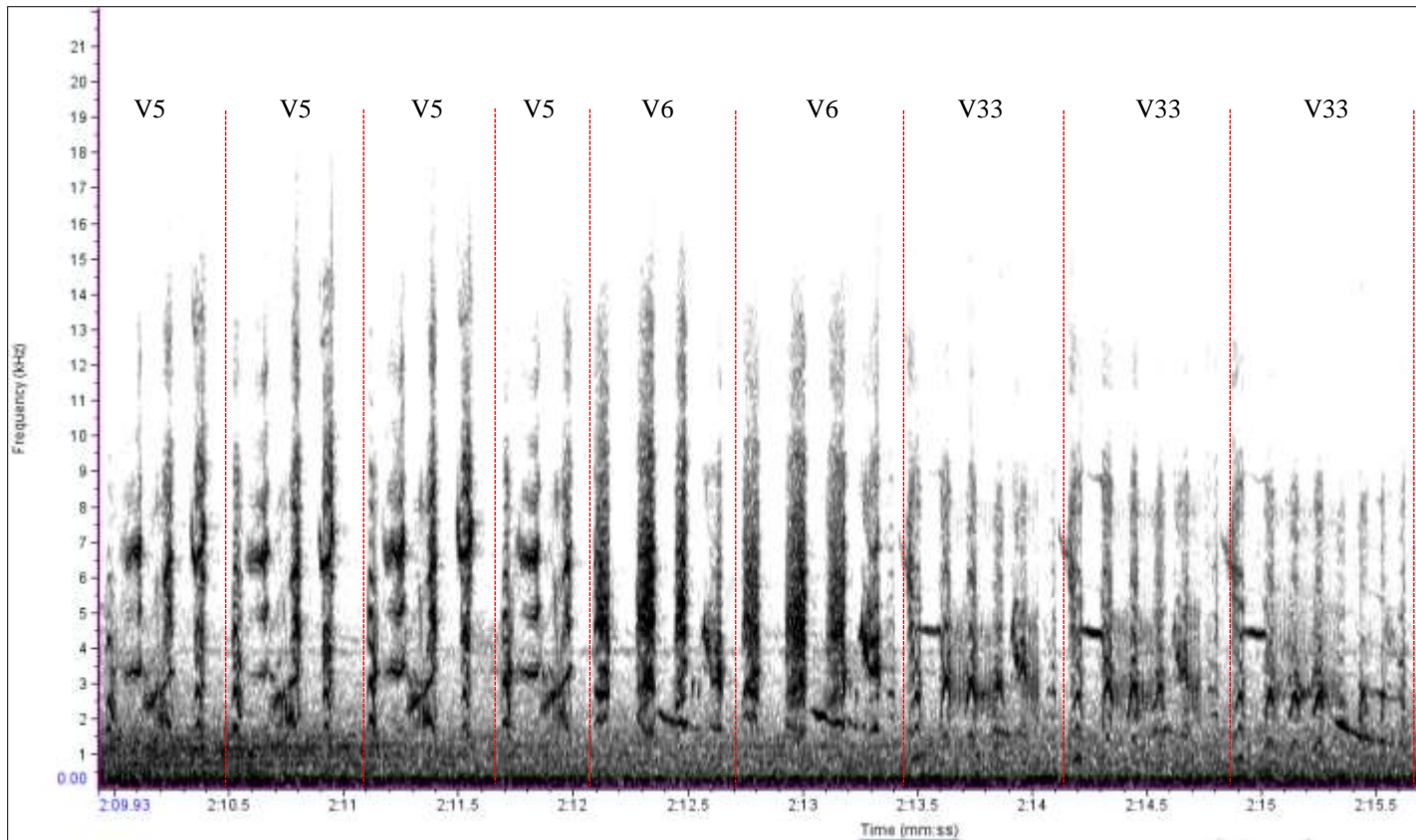
Figure 2.2. "V" denotes variable song types. Variable song types 5, 6 and 33 were recorded from the male at nest box 22 in 2019. The red dashed line separates song types.
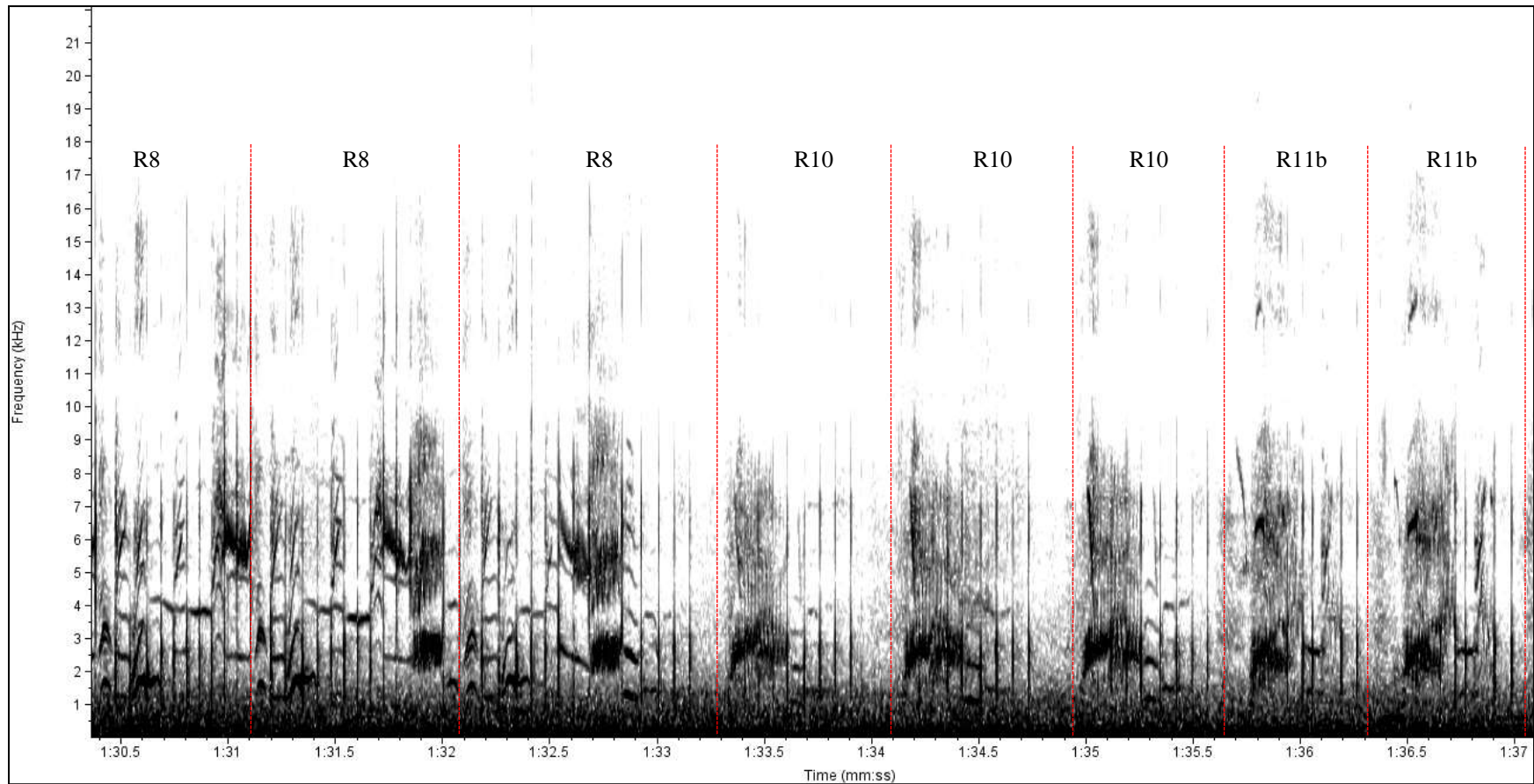
Figure 2.3. "R" denotes rattle song types. Rattles song types 8, 10 and 11b were recorded from the male at nest box 8 in 2020. The red dashed line separates song types.
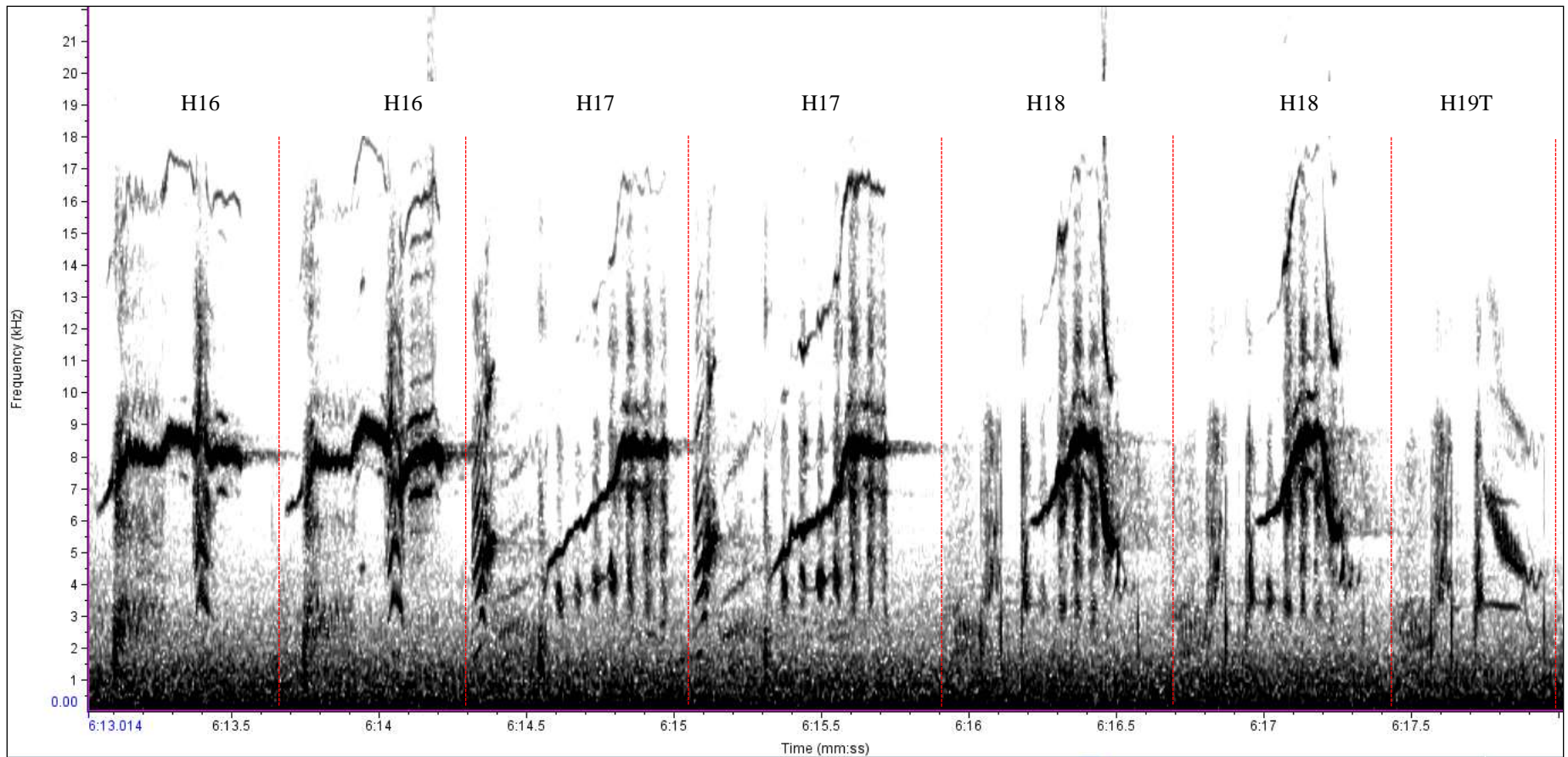
Figure 2.4. "H" denotes high frequency song types. High frequency song types 16, 17 and 18 are shown above, including an intermediate high frequency song type 19T recorded from the male at nest box 33 in 2020. The red dashed line separates song types.

35
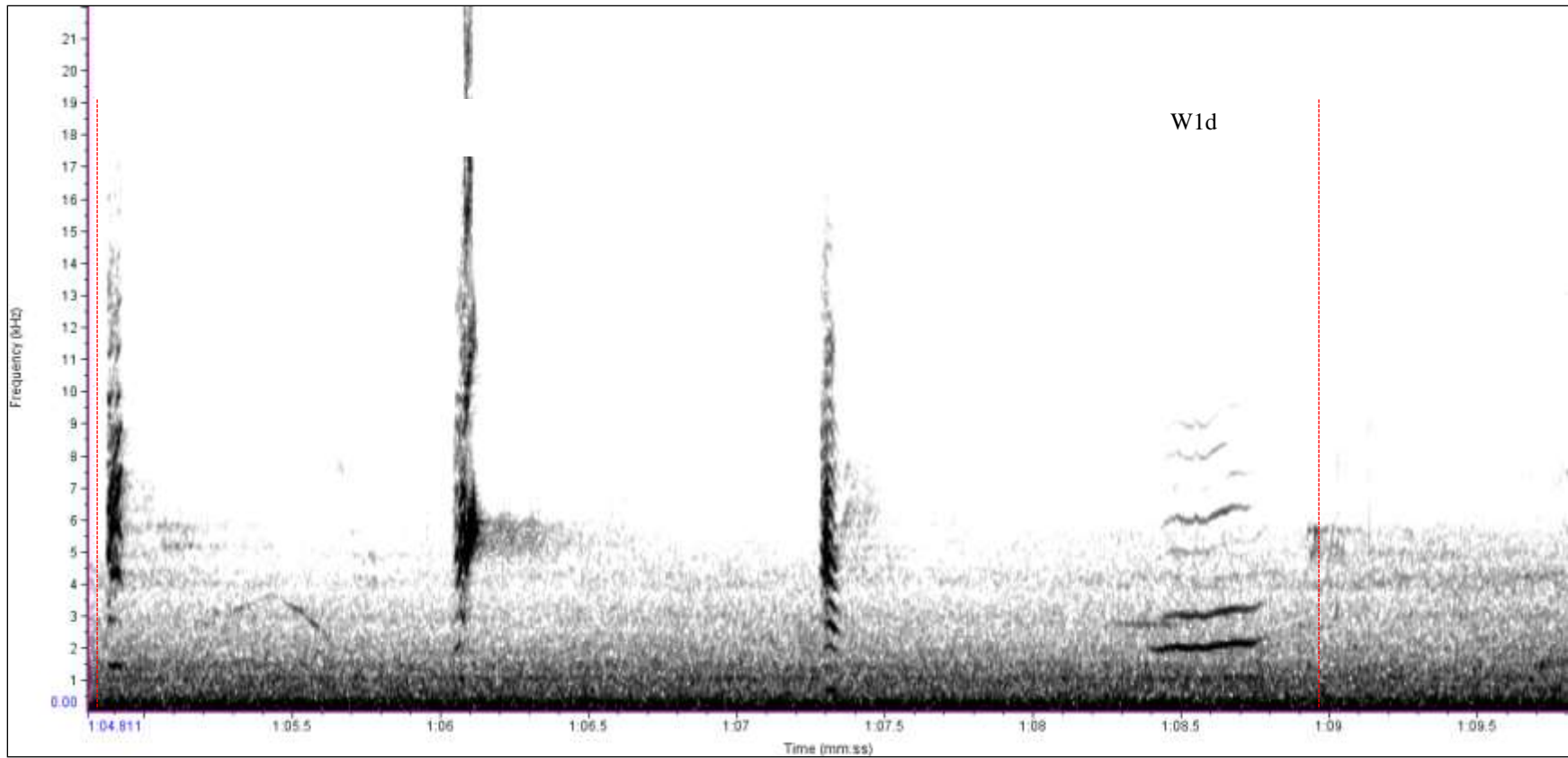
Figure 2.5. "W" denotes whistle song types. Whistle 1d is preceded by three chirps. These three chirps were considered part of the W1d. This

recording is from the male at NB 33 from 2020. The red dashed line separates song types.
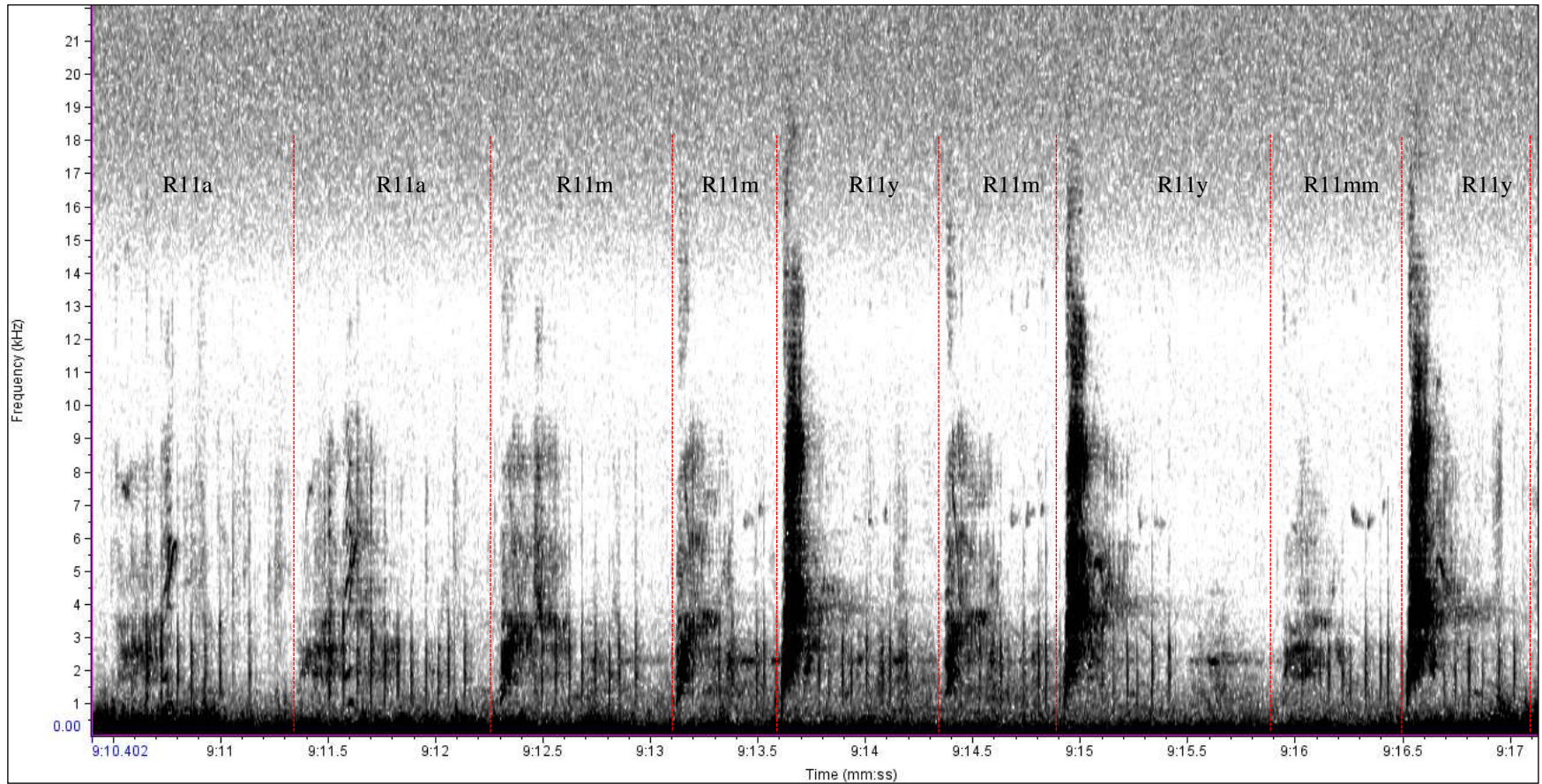
Figure 2.6. "R" denotes Rattle song types. Rattle song types are occasionally sung in an alternating manner as seen above where R11m song type alternates with R11y song type.

Intermediate Song Types: I defined these intermediate song types as follows: it comprises features of the song type that comes before and the song type that comes immediately after it. They are transitional song types, when a male is moving from one song type to another song type an amalgamated song type is sometimes created. These intermediate song types were also found in populations in Montreal (Mountjoy and Lemon 1995) and in those within New Zealand and Belgium (Eens et al. 1991b).

An intermediate song type must have:

1. A minimum of two different song features from the song type immediately before it
2. A minimum of two different song features from the song type immediately after it.
    Also,
3.   It cannot contain 100% of the song structure from either the preceding or following song as this would not be an intermediate song type but would be a within-song type variation of the song type (see Chapter 3), which is not assessed in this study. For this analysis they would be classified as the song type it resembles the most.
4. Some song types are simple and do not have many varying acoustic elements. If an intermediate song type is created from two structurally simple song types, then it does not need to have two different elements from the preceding and following song type to be considered an intermediate.
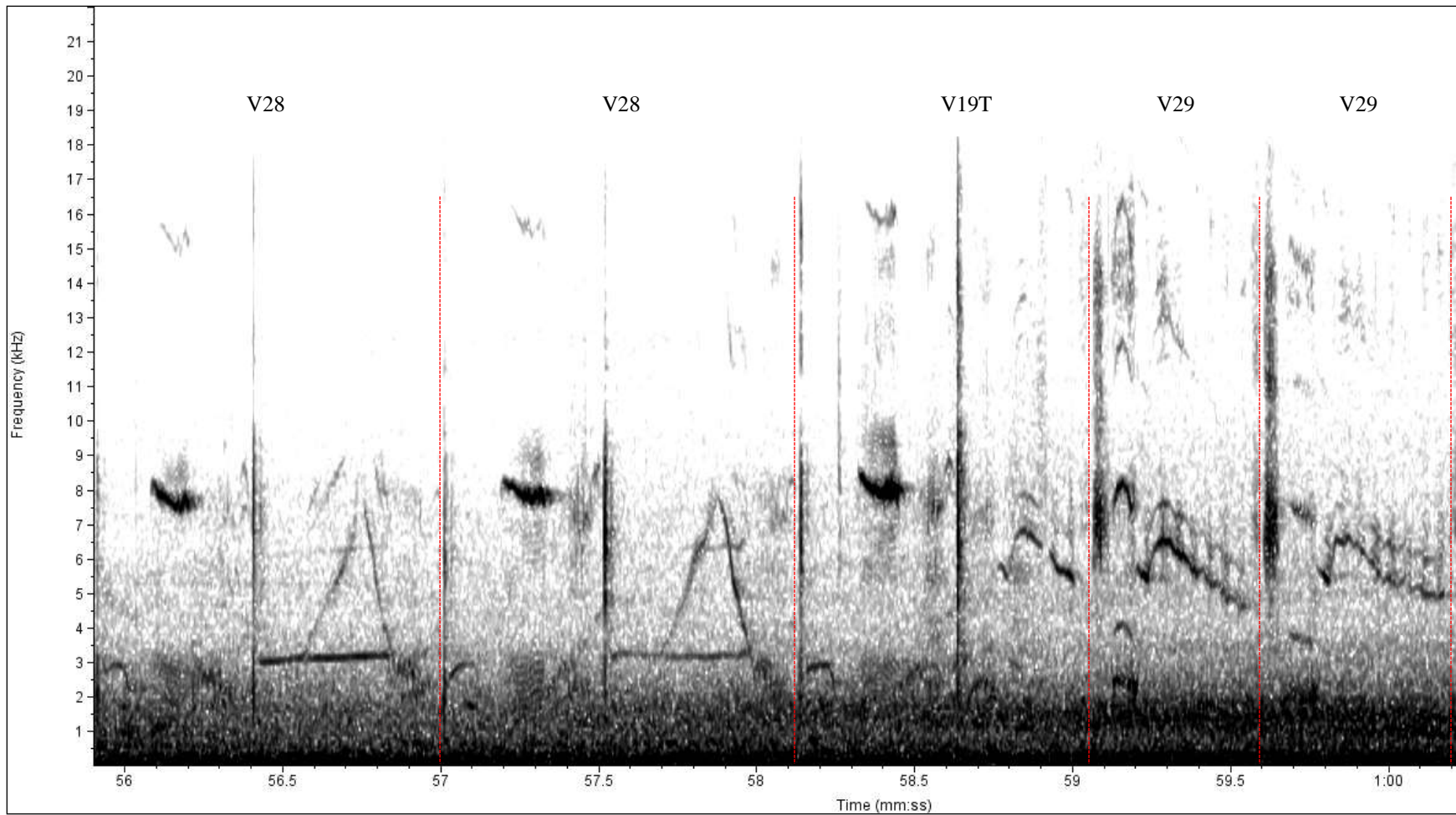
Figure 2.7. "V" denotes variable song types. Intermediate song type V19T is an amalgamation of variable song types 28 and 29. The red dashed line separates song types.

39
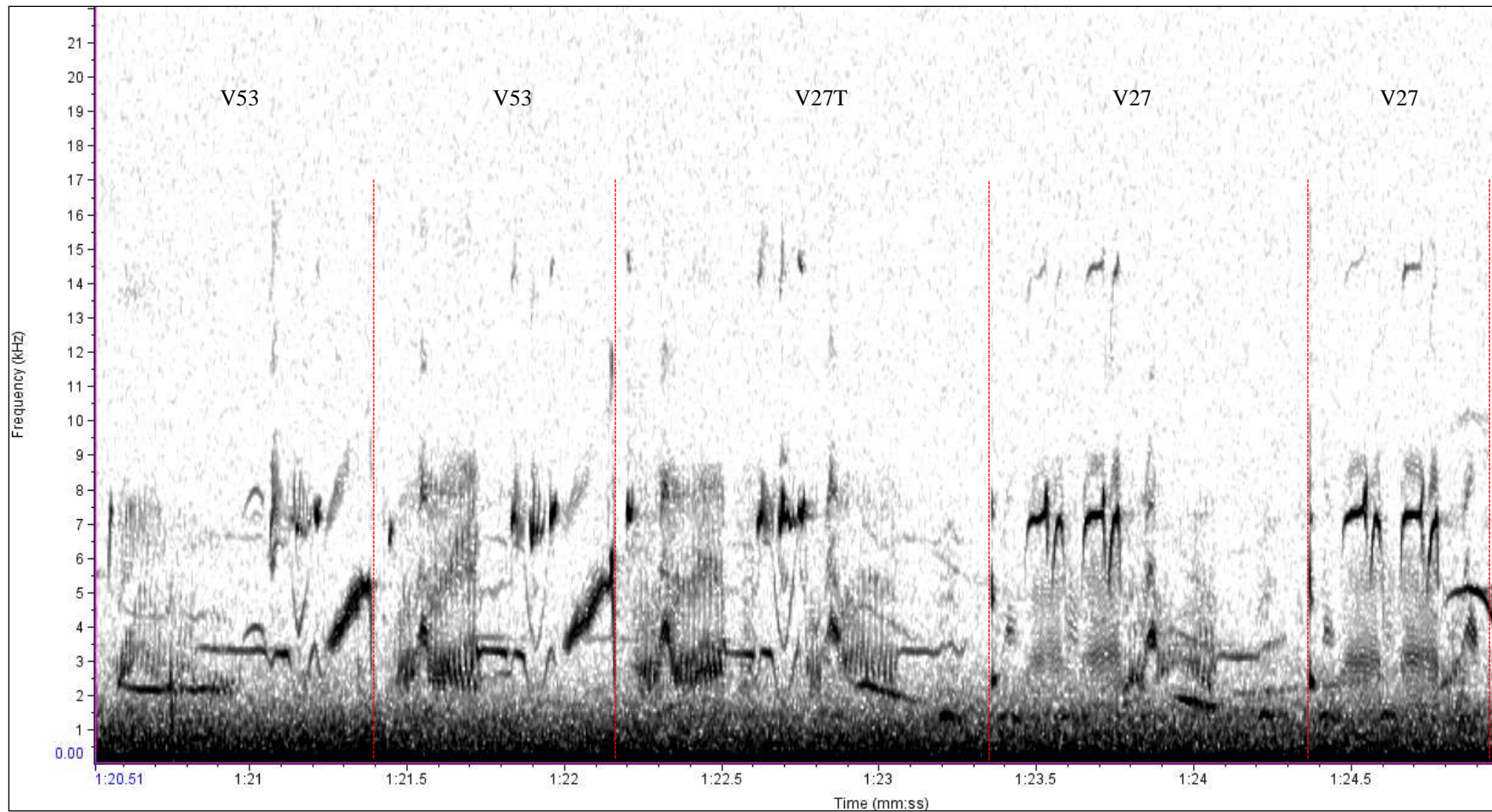
Figure 2.8. "V" denotes variable song types. Intermediate song type 27T is an amalgamation of songs 53 and 27. The red dashed line separates song types.

Determining song repertoire size

For repertoire size analysis, an average of 30.32 ± 2.12 (mean ± Standard Error (SE)) song bouts were recorded for each male (range: 11 to 50) (Table 2.1. and 2.2.). In past studies, an average of 22.9 to 26.8 song bouts (Eens et al. 1991b, a, 1992a) and 90 phrase groups (a group is composed of the same song type repeating one after another) where they indicate that an estimate of the repertoire can be estimated after sampling three or four songs (Mountjoy and Lemon 1995).

When examining the total number of song types, an asymptote for repertoire is reached after analysis of 300 to 400 similar or different song types (Eens et al. 1992b). However, there can still be new song types detected after analysing 550 songs (Eens et al. 1991a). Therefore, in my study, an average of 629.35 ± 19.53 song types (range: 335-716) comprising an average of 15.25 ± 0.91 (range: 10-28) song bouts were analysed. For 17 of the 20 males, a minimum of 635 song types were analysed. When the criterion of 635 song types was met in the middle of a song bout, then the rest of the song types within that bout were analysed so as to have complete song bouts.

For three males, 635 song types could not be analysed due to the lack of recordings obtained, however, there was sufficient data to derive a reliable estimate of song repertoire size for these three males even though their entire repertoire may not have been obtained. Mountjoy and Lemon (1995, 1996, 1997) analysed less song types (200 song types) than my lowest sample (335 song types), and they estimate they assessed at least 85% of the repertoires in their males. Therefore, I can conclude that I have obtained more than 85% of the male repertoires for these three males, and they were included in the analysis.

For this analysis, I likely did not obtain the entire repertoire of each male but the full repertoire of a male is not required for this analysis. A similar number of song types were analysed for each male and for the same male in different years to keep sampling effort

consistent. The criteria used was comparable to what was used in past studies allowing

comparisons between different studies (Eens et al. 1991a, b).

Table 2.1. Cross-sectional data for repertoire size and song bout length for the 12 males included in the analysis. Repertoire size and song bout length were determined for 12 males. Nest box (NB) is where each male was recorded and signifies the male identity. The year denotes when each male was recorded. Year of life denotes in which year of life the male was recorded (e.g., 3 means the male was in their third year of life). The number of bouts used to determine average song bout duration and repertoire is denoted by # SB and # Rep size, respectively.

NB	Year	Year of Life	Average song bout length (Seconds)	Repertoire Size	# SB	# Rep size
33	2020	3	44.67	81	25	12
39	2020	2	35.73	54	36	16
49	2020	3	30.16	53	21	18
8	2020	6	30.63	57	26	19
9	2020	6	38.96	68	27	17
26	2019	2	25.62	62	36	16
22	2020	8	46.53	68	27	12
37	2020	2	32.29	54	25	16
4	2020	3	43.87	59	22	12
2	2020	3	32.77	51	30	14
25	2019	5	31.73	41	24	16
3	2020	4	23.97	45	23	18

Table 2.2. Longitudinal repertoire size and song bout length data for eight and nine males included in the analysis, respectively. Nest Box (NB) is where each male was recorded and represents the males' ID. The year denotes when each male was recorded. Year of life denotes in which year of life the male was recorded (e.g., 3 means the male was in their third year of life). The number of bouts used to determine average song bout length and repertoire size are denoted by # SB and # Rep size, respectively.

NB	Year	Year of life	Average song bout length (Seconds)	Repertoire size	# SB	# Rep size
8	2019	5	28.35	56	30	18
8	2020	6	30.63	57	26	19
9	2019	5	24.82	62	22	12
9	2020	6	38.96	68	27	17
9	2021	7	24.47	N/A	50	N/A
26	2019	2	25.62	62	36	16
26	2020	3	32.63	67	11	10
22	2019	7	43.07	62	28	11
22	2020	8	46.53	68	27	12
33	2020	3	44.67	81	25	12
33	2021	4	33.04	81	29	13
4	2020	3	43.87	59	22	12
4	2021	4	33.49	61	48	11
3	2020	4	23.97	45	23	18
3	2021	5	19.19	57	44	28
49	2020	3	30.16	N/A	21	18
49	2021	4	27.29	N/A	29	N/A
39	2020	2	35.73	54	36	16
39	2021	3	25.24	44	50	16
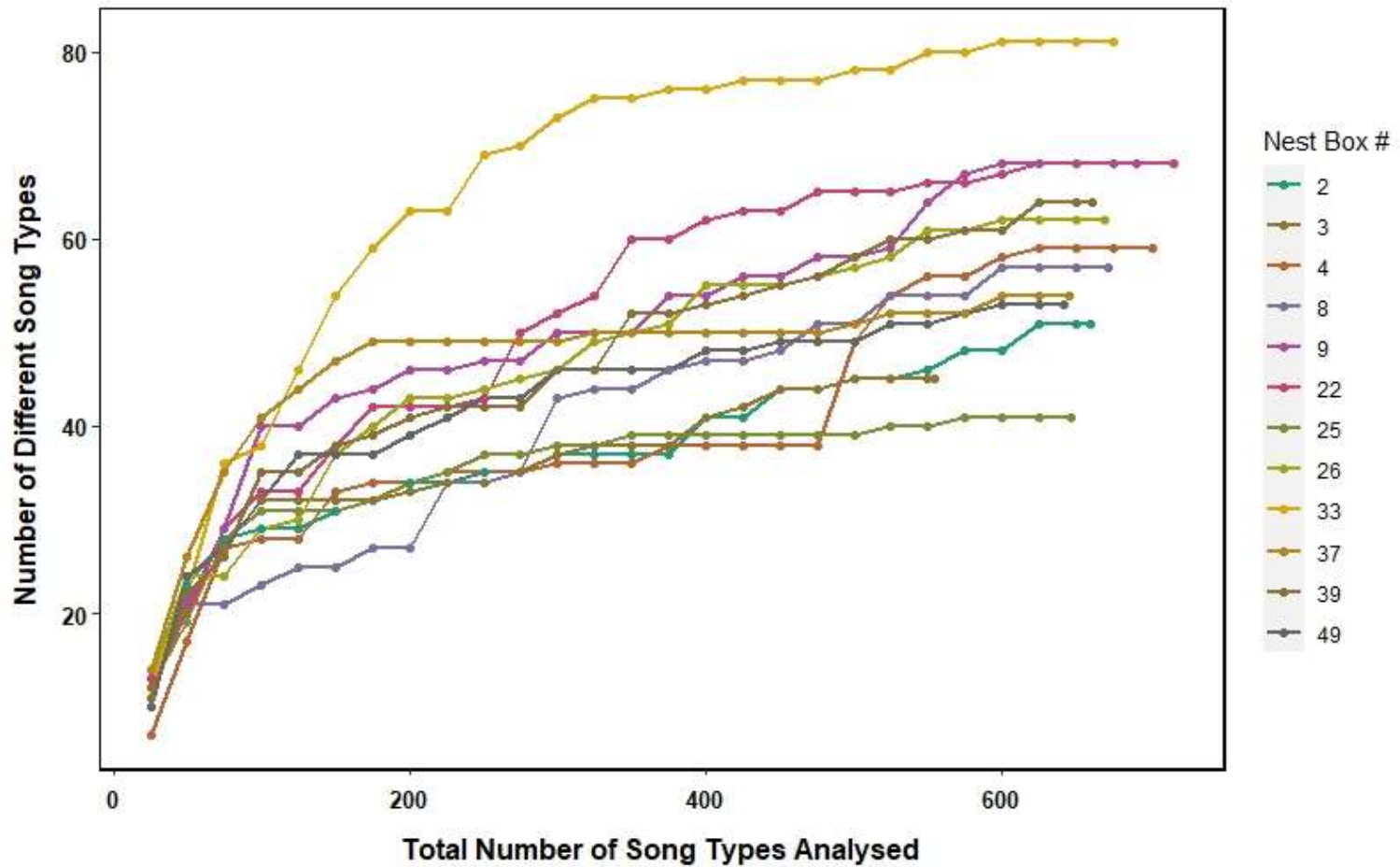
Figure 2.9. The cumulative plot of the number of different song types plotted against the total number of song types analysed for each male. This plot shows cross-sectional data of recordings of two males from 2019 and 10 males from 2020.
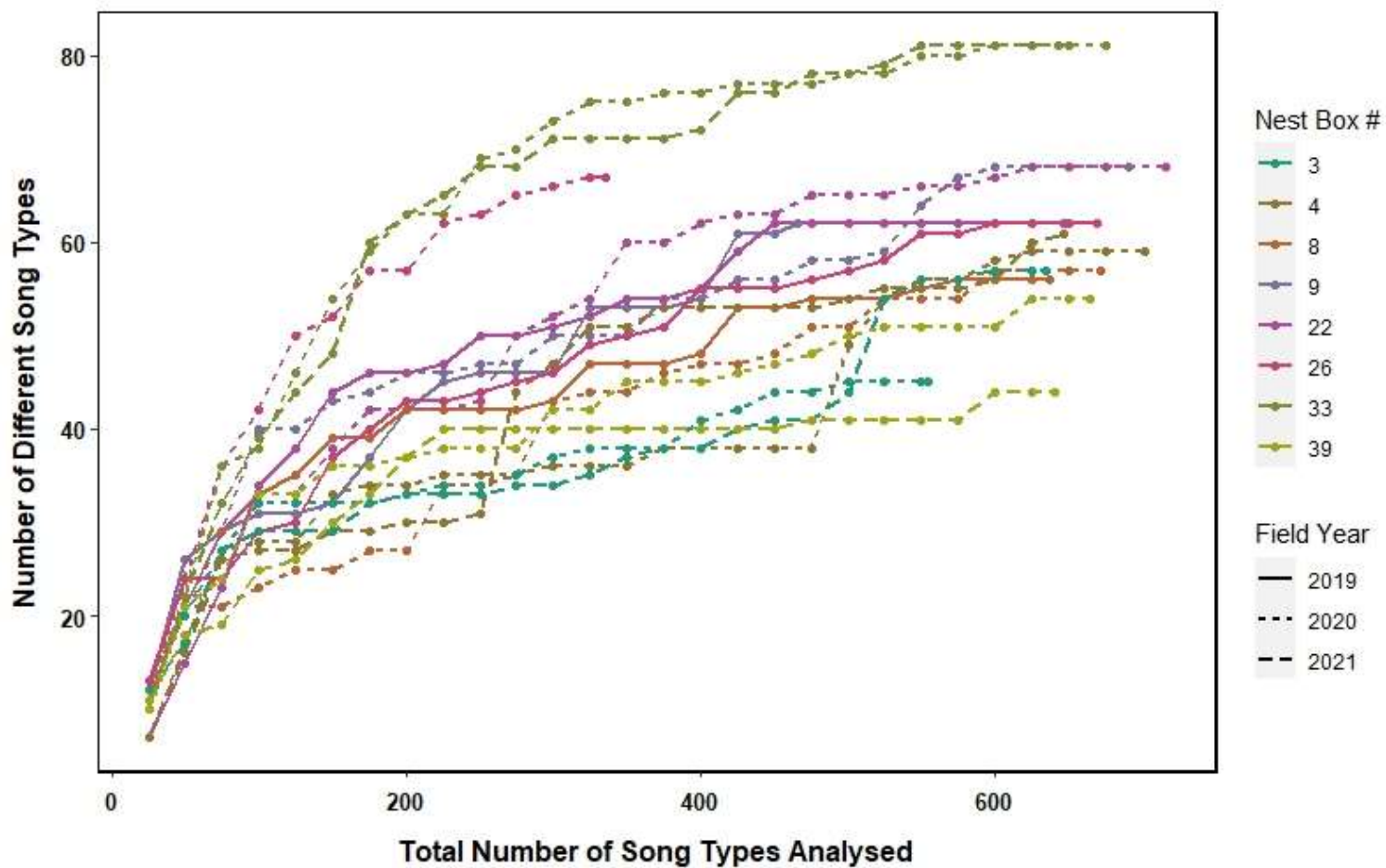
Figure 2.10. The cumulative plot of the number of different song types plotted against the total number of song types analysed for each male. This plot shows longitudinal data for eight males with song recordings obtained in 2019, 2020 and 2021.

Determining song bout length

For the song bout analysis, data from 2019, 2020 and 2021 were used. The number of bouts used ranged from 11 to 50 bouts (Table 2.1 and Table 2.2). The number of bouts obtained for each male varied. One male had only 11 bouts recorded because the field season was paused due to Covid-19 pandemic. It was carefully considered whether to include that male in the analysis, and it was determined that he had a sufficient number of bouts to test for statistical significance (Eens et al. 1991a, Duffy and Ball 2002). All other males had a minimum of 22 bouts to obtain their average song bout length. A normality test and then a paired t-test were conducted to determine whether only 22 random bouts or all the song bouts recorded should be included in the song bout analysis. The average song bout length did not differ significantly between 22 randomly selected bouts versus all the bouts sung (mean $\pm$ SE, 32.35 $\pm$ 1.60 and 31.77 $\pm$ 1.57 respectively; paired t = 1.65, df = 22, P = 0.11). Therefore, all the bouts were used for each male to obtain the average song bout length.

Statistical analysis

To determine whether song repertoire size and song bout length change with age in an eastern Canadian population of European Starlings, cross sectional and longitudinal analyses were conducted. All statistical analyses were done in R (Version 1.4.1103). Generalized Linear Models (GLM) were used with the lme4 package (Bates et al. 2014). The MuMin package (Barton 2020) was then used to obtain the Akaike Information Criterion, corrected for small sample sizes (AICc) for each of the GLMs. The AICc values were used to observe the fit of the models and to determine if age (the year of life males were in) played a role in repertoire size and song bout length in Starlings. The best predictors for the data were determined by observing the models that had an Δ AICc < 2 (Guthery et al. 2003). Pearson correlations were conducted for cross-sectional analyses.

Cross-Sectional analysis

For the cross-sectional analysis, males recorded in 2019 and 2020 were included. If males were recorded in both 2019 and 2020, the song data from only one year was included, and the year was chosen randomly to avoid pseudoreplication. Twelve males were included in the cross-sectional analysis for song repertoire size and song bout length. Song repertoire size and song bout length were response variables, with age as a fixed effect and field year as a random effect. Song repertoire size is count data; therefore a Poisson distribution was used. When Poisson distribution could not be used because of over dispersed data, a negative binomial function was used (Crawley 2007). This analysis was also repeated for ASY males only.

Longitudinal analysis

Males recorded for two (or in one case three) years consecutively were used for the longitudinal analysis. Eight males were included for the longitudinal repertoire size analysis, whereas nine males were analysed for song bout length. My statistical analysis follows a similar methodology to that used by Forstmeier et al. (2006). The number of song types that were added or dropped was calculated to capture the within-individual changes for each male. The first year of recording was subtracted from the following year of recording for each male; a positive number indicates an increase in repertoire size while a negative value indicates a decrease (Table 2.3). By using the difference between years as the response variable instead of the direct measurements (e.g., number of song types per year), male ID does not need to be included as a random effect to account for pseudoreplication. A similar method was used for the song bout length, where the average song bout difference was obtained by subtracting the first year of recording from the following year of recording. One male was recorded for three consecutive years (Nest Box 9); meaning the first year of recording was subtracted from the second year while the second year was subtracted from the third year (Table 2.3). I therefore must take into account that we have two results from the same individual. The model for song bout length included male ID as a

random effect to account for pseudoreplication. Age is represented in the following manner; 0 represents a male transition from their 1st year of life to their second year of life; 1 represents a male transition from their second year of life to their third of year of life etc. (Table 2.4). Repertoire size and bout length were the response variables and age (the year of life the individual was in) was a fixed effect. This analysis was also conducted for ASY males only.

In addition to comparing the year of life and males repertoire size, I wanted to see if there was a general trend towards increasing repertoire size over time. The initial year of recording was assessed against the following year of recording. Comparing the final year of recording with the initial year captures whether repertoire increases with time, no matter the initial repertoire size. Repertoire size was a response variable, the year of recording (year 1 and year 2) was a fixed effect with male ID as a random effect. A Poisson distribution was used for this model.

Table 2.3. Below are differences obtained for repertoire size and song bout length differences (last year of recording – first year of recording) for the longitudinal analysis. Age was coded in a way to capture transition from year to the next. Data from male at Nest Box 9 is listed twice to show how the differences were calculated. Song bout length is in seconds.

Nest Box	Year of Recording	Year of life	Repertoire Size	Repertoire Difference	Song bout Length	Song bout length difference	Coded Age
8	2019	5	56	1	28.35	2.28	4
	2020	6	57		30.63		
9	2019	5	62	6	24.82	14.14	4
	2020	6	68		38.96		
9	2020	6	68	N/A	38.96	-14.49	5
	2021	7	N/A		24.47		
26	2019	2	62	5	25.62	7.01	1
	2020	3	67		32.63		
22	2019	7	62	6	43.07	3.46	6
	2020	8	68		46.53		
33	2020	3	81	0	44.67	-11.63	2
	2021	4	81		33.04		
4	2020	3	59	2	43.87	-10.38	2
	2021	4	61		33.49		
3	2020	4	45	12	23.97	-4.78	3
	2021	5	57		19.19		
49	2020	3	N/A	N/A	30.16	-2.87	2
	2021	4	N/A		27.29		
39	2020	2	54	-10	35.73	-10.49	1
	2021	3	44		25.24		

Table 2.4. Age assignments for the statistical models.

Age Assignments	
Year of life transition	**Coded Age**
1 to 2	0
2 to 3	1
3 to 4	2
4 to 5	3
5 to 6	4
6 to 7	5
7 to 8	6

RESULTS

Cross-sectional analyses

Repertoire size varied from 41 to 81 (mean ± SE, 57.75 ± 3.15) song types while song bout length varied from 23.67 to 46.53 (34.74 ± 2.12) seconds for the males included in the cross-sectional analysis. There was no relationship between male age and repertoire size and song bout length (Table 2.5., Fig. 2.11 and 2.12). Therefore, the two song parameters are not more elaborate in older males than younger males.

On average SY and ASY males had repertoire sizes of 56.57 ± 2.67 (N= 3) and 58.11 ± 4.19 (N= 9) respectively, while SY and ASY males had average song bout lengths of 31.21 ± 2.99 seconds (N=3) and 35.92 ± 2.62 seconds (N=9).

SY and ASY males pooled

For both repertoire size and song bout length, age was not a predictor (Table 2.5). There was no significant relationship between age and song repertoire size (Pearson correlation $r = 0.16$, $n = 12$, $p = 0.62$) nor was there a significant relationship between age and song bout length (Pearson correlation $r = 0.34$, $n = 12$, $p = 0.28$).

ASY males only

Age was not a predictor of song repertoire size and song bout length for ASY males (Table 2.6).

Longitudinal analyses

The repertoire size varied from 45 to 81 (60.13 ± 3.61, N= 8) song types in the first year of recording while the range was 44 to 81 (62.87 ± 3.84, N = 8) song types in the following year of recording. Song bout length ranged from 23.97 to 44.67 (33.36 ± 2.87, N = 9) seconds in the first year of recording and the following year it ranged from 19.19 to 46.53 (31.89 ± 2.62, N = 9) seconds. Only one male was recorded three years in a row (Table 2.2).

SY and ASY males pooled

Age was not a predictor of repertoire size and song bout length, where the models were not top-ranked. A trend was not observed between these two song parameters and age (Table 2.5, Figs. 2.13 and 2.14).

ASY males only

Age was not a predictor for song repertoire size and song bout length for only ASY males either (Table 2.6).

Initial year of recording versus the following year of recording

The repertoire changes observed from the first year of recording to the following year of recording was not a predictor but showed a trend with a Δ AICc of 2.59 and model weight of 21.5% (Table 2.7, Fig. 2.15).

Table 2.5. For all cross-sectional and longitudinal GLMMs for pooled SY and ASY males. Degrees of freedom (df), Akaike Information Criterion corrected for small sample sizes (AICc), the other models (Δ AICc), and the model weights (w_i) are provided. The bolded values are the models that have a Δ AICc < 2.

Cross-Sectional Models	df	AICc	Δ AICc	w_i	Coefficient ± Standard Error
Repertoire size ~ age + (1|year)					
Intercept only	2	98.2	**0.00**	0.830	-
Age (Year of life)	3	101.3	3.10	0.180	0.015 ± 0.02
Song bout length ~ age + (1|year)					
Intercept only	3	89.9	**0.00**	0.835	-
Age (Year of life)	4	93.2	3.24	0.165	1.30 ± 1.04
Longitudinal Models	df	AICc	Δ AICc	w_i	
Repertoire size ~ age					
Intercept only	2	57.7	**0.00**	0.862	-
Age (Year of life)	3	61.4	3.67	0.138	1.71 ± 1.34
Song bout length ~ age + (1|male ID)					
Intercept only	3	82.0	**0.00**	0.942	-
Age (Year of life)	4	87.5	5.56	0.058	1.14 ± 1.70

Table 2.6. The following cross-sectional and longitudinal GLMs only include ASY males. Degrees of freedom (df), Akaike Information Criterion corrected for small sample sizes (AICc), the other models (Δ AICc), and the model weights (w_i.) are provided. The bolded values are the models that have a Δ AICc < 2.

Cross-Sectional Models	df	AICc	Δ AICc	w_i	Coefficient ± Standard Error
Repertoire size ~ age + (1|year)					
Intercept only	3	80.5	**0.00**	0.970	-
Age (Year of life)	4	87.5	6.89	0.030	0.018 ± 0.038
Song bout length ~ age + (1|year)					
Intercept only	3	72.4	**0.00**	0.966	-
Age (Year of life)	4	79.1	6.72	0.034	0.99 ± 1.41
Longitudinal Models	df	AICc	Δ AICc	w_i	
Repertoire size ~ age					
Intercept only	2	41.9	**0.00**	0.991	-
Age (Year of life)	3	51.4	9.51	0.009	0.83 ± 1.41
Song bout length ~ age + (1|male ID)					
Intercept only	3	69.6	**0.00**	0.985	-
Age (Year of life)	4	77.9	8.30	0.015	2.18 ± 2.08
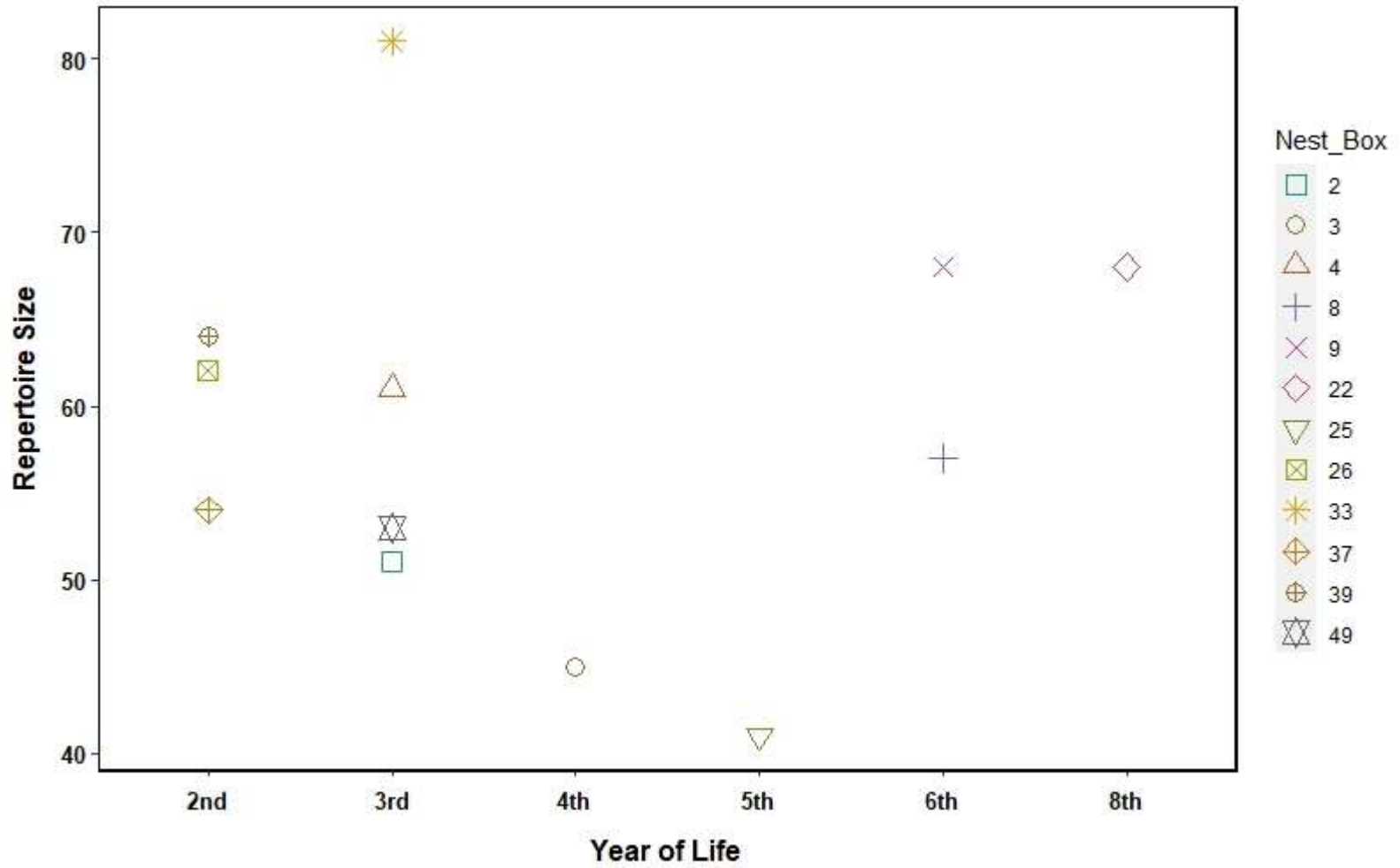
Figure 2.11. Scatterplot of the repertoire size of 12 males used in the cross-sectional analysis. Each male is represented only once.
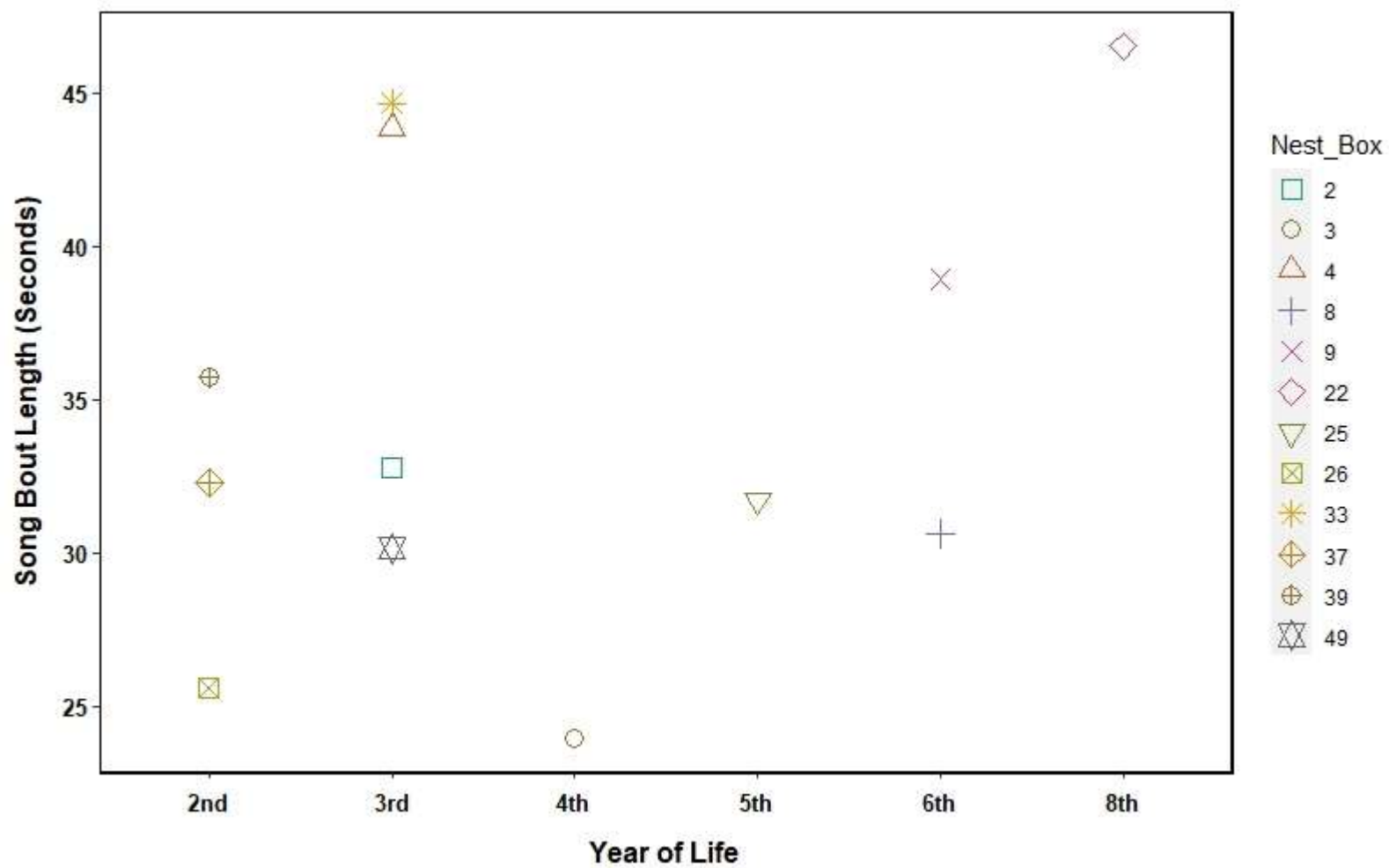
Figure 2.12. Scatterplot of song bout length of 12 males recorded for the cross-sectional study.
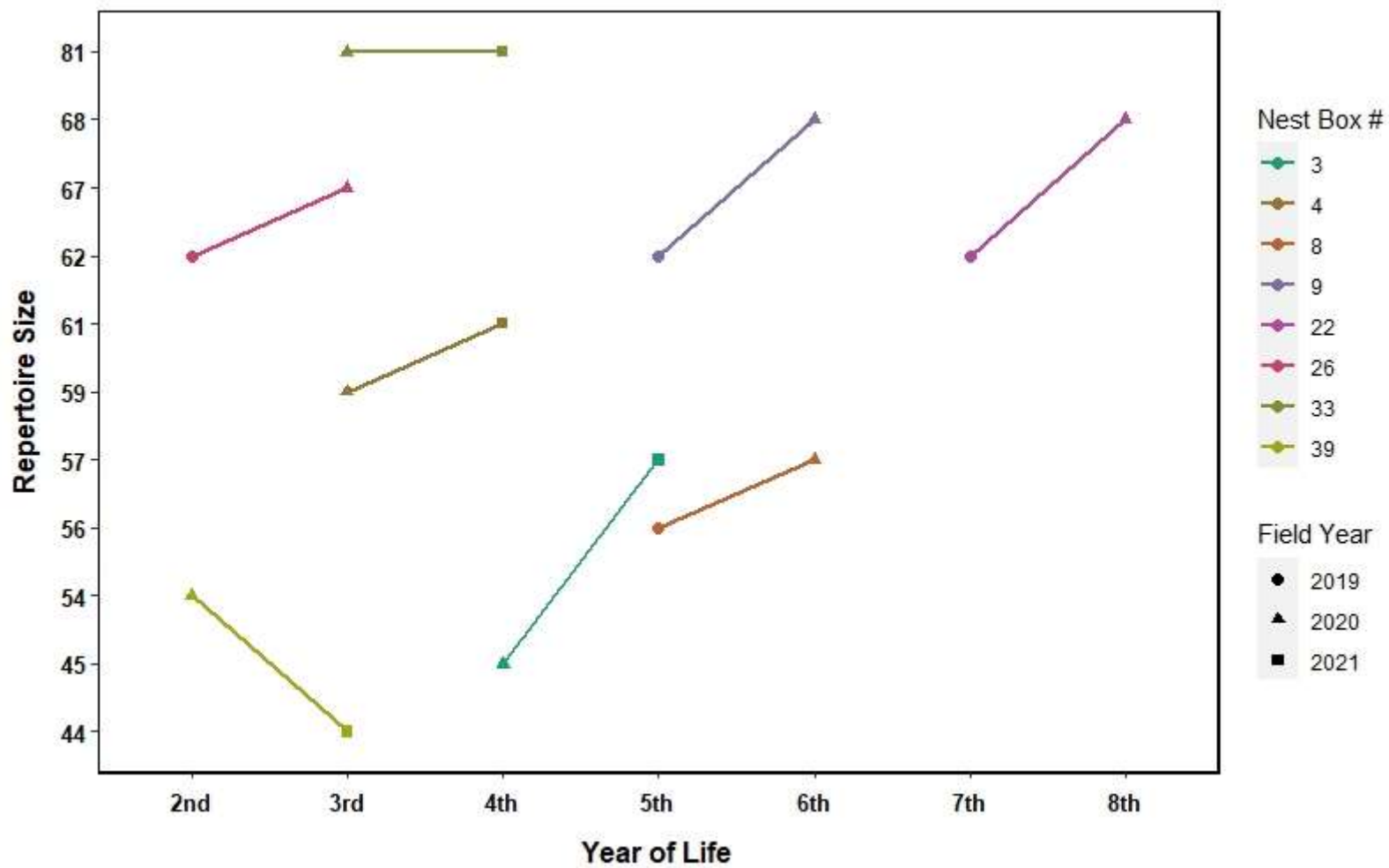
Figure 2.13. Scatterplot of the repertoire sizes of eight males over two consecutive years used in the longitudinal analysis.
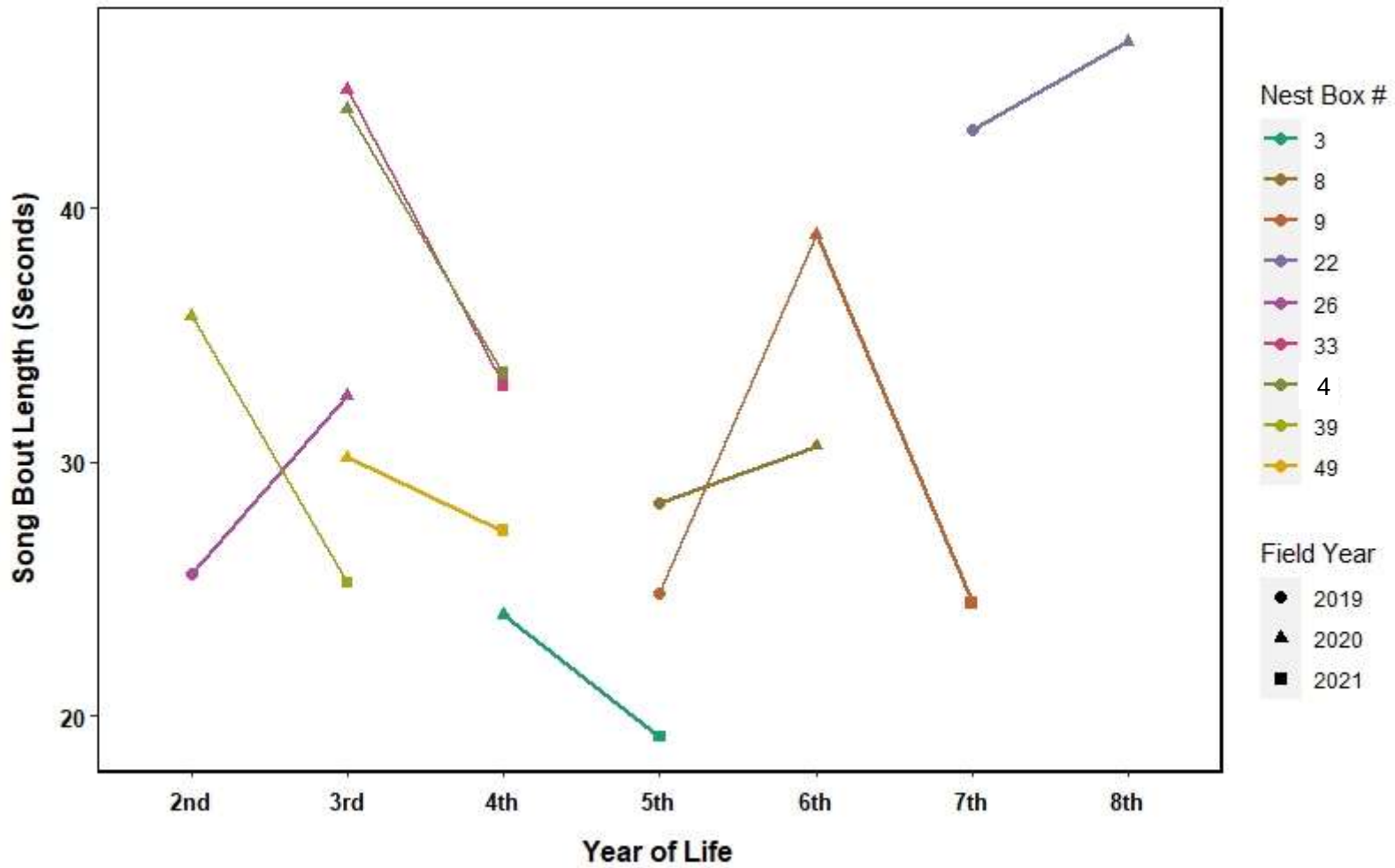
Figure 2.14. Scatterplot of song bout length for eight males that had song recorded over two consecutive years and one male recorded for three consecutive years used in the longitudinal analysis.

Table 2.7. This linear mixed effects model assessed the change in repertoire size with year as a categorical variable (Year 1 vs Year 2). Degrees of freedom (df), Akaike Information Criterion corrected for small sample sizes (AICc), the other models (Δ AICc), and the model weights (w_i) are provided. The bolded values are the models that have a Δ AICc < 2.

Longitudinal Model	df	AICc	Δ AICc	w_i
Repertoire size ~ year of recording + (1|male ID)				
Intercept Only	2	119.4	**0.00**	0.785
Year of recording	3	122.0	2.59	0.215
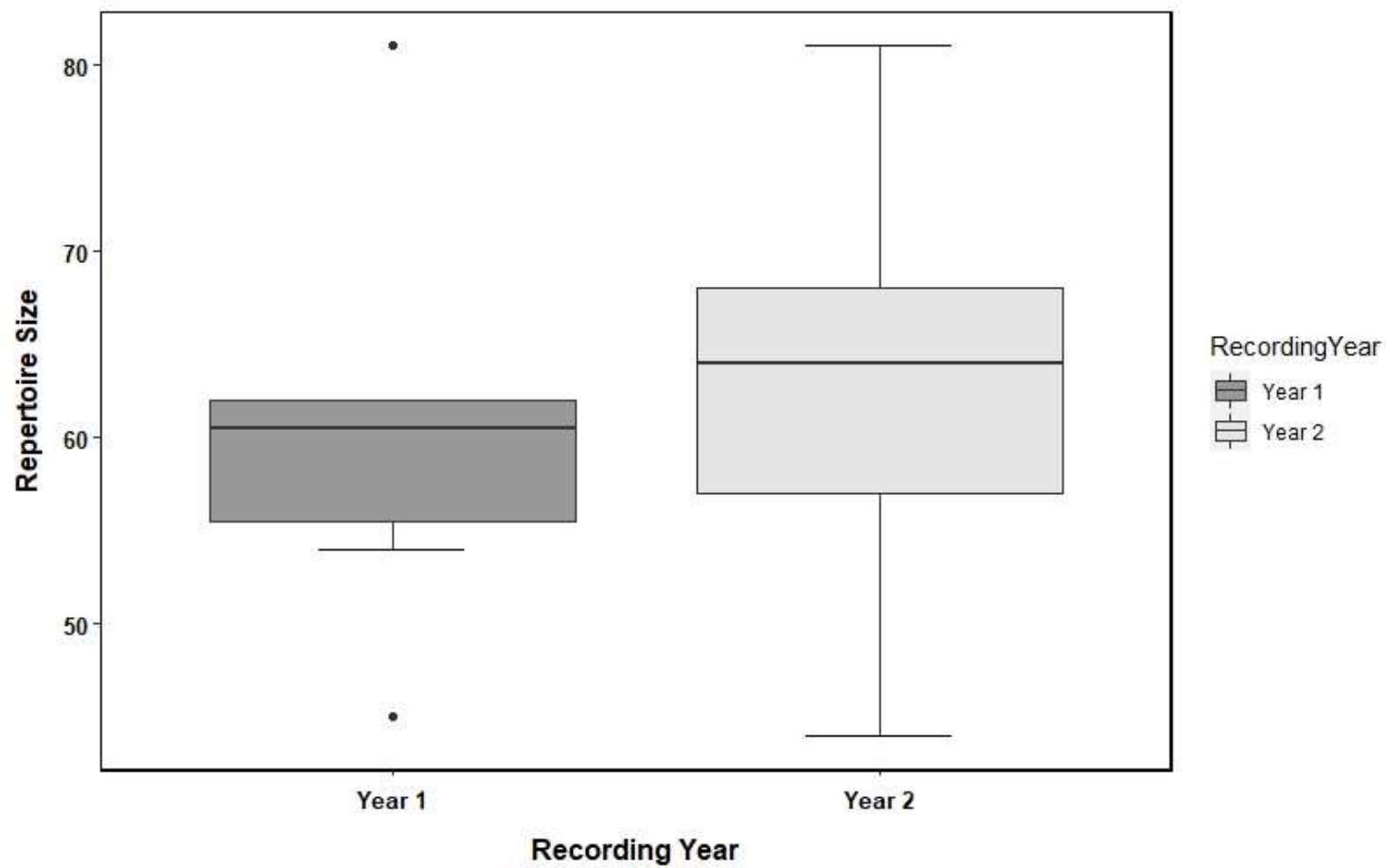
Figure 2.15. Year 1 denotes the first year a male was recorded (not age or year of life) and Year 2 is the following year the same male was recorded. The year of life is not considered here, all males are included. The two black points are outliers.

DISCUSSION

Song plays a role in signalling male quality to females, and age is one of these quality parameters (Kipper and Kiefer 2010). Starlings are one of the few passerine species that are able to drop and add new song types with age (Eens et al. 1992a, Chaiken et al. 1994, Mountjoy and Lemon 1995). Starling populations are found throughout North America (Kessel 1953), and in this study, I explored whether song repertoire size and song bout length increase with age in an eastern Canadian population of Starlings. The results of my study: **1)** the cross-sectional analysis did not support the prediction that age is a predictor for male song repertoire size (Table 2.5, Fig. 2.11); **2)** the longitudinal analysis also did not support the prediction that age is a predictor of male song repertoire size, though most males did increase their repertoire a small amount over the period of one year (Table 2.5, Fig. 2.13); **3)** the cross-sectional study did not support the prediction that age was a predictor of song bout length (Table 2.5, Fig. 2.12); **4)** and finally, the longitudinal study did not support the prediction that age was a predictor of song bout length, as males did not increase bout length over the course of one year (Table 2.5, 2.14).

Song repertoire size, song bout length and age

Both the cross-sectional and longitudinal results agree that age is not signalled through repertoire size in my focal population (Table 2.13, Fig. 2.11 and 2.13). Some SY males had larger repertoire sizes than males that were 4, 5 and 6 years of age (Fig. 2.11 and Fig. 2.13). Contrary to my results, other populations have found a significant difference in repertoire size (Eens et al. 1992a, b; Mountjoy and Lemon 1995) between SY and ASY Starlings, with SY males having smaller repertoire sizes than ASY males.

Similar to my results, no significant correlation was found between repertoire size and age in a cross-sectional analysis in a German population of Starlings and individuals of the same age had large differences in their repertoire sizes (Adret-Hausberger et al. 1990), which is similar

to what I found (Fig. 2.11 and Fig. 2.13). For example, males that were three years old had repertoire sizes that varied from 51 song types to 81 song types (Table 2.1, Fig. 2.11). At an individual level, three birds followed over two years increased their repertoire sizes (range: 3 to 4 song types) (Adret-Hausberger et al. 1990); this is also evident in my population where six of eight males increased the number of song types from one year to the next, but this increase was small (range: 1 to 12 song types) (Fig. 2.13). However, it is also possible that Adret-Hausberger's et al. (1990) study may not have captured the entire repertoire for each individual in both years, due to the low number of song types sampled. For the same male, the number of song types analysed between the first and second year (e.g. for one male in the first year 184 song types were analysed while the second year they analysed 761 song types) varied, which could affect the number of unique song types obtained for the repertoire; specifically, the repertoire size could be underestimated for one of the two years for some of the males (Adret-Hausberger et al. 1990).

Repertoire size potentially signalling age in ASY starlings seem to vary with different populations. In one population of captive Starlings, ASY males did not increase their repertoire with age, meaning males beyond their SY of life (3rd, 4th, 5th etc.) did not signal age with repertoire size (Eens et al. 1992a). ASY males in my population did not increase their song repertoire with age either (Table 2.6). My study was also conducted in the field, making it free of any drawbacks such as a physiological and behavioural stresses that individuals in captivity may experience (Bateson and Feenders 2010). Another constraint in captive studies which may play a role with respect to song learning, is the lack of available tutors and social interaction to learn songs (Mountjoy and Lemon 1995, García 2019).Taking these differences into consideration between captive studies and field studies, my study has provided similar results to Eens et al. (1992b) study. Another eastern Canadian population of Starlings found significant within-individual increases in repertoire size from one year to the next (longitudinal increase). The increases in repertoire sizes ranged from six to 14 song types (Mountjoy and Lemon 1995). The

differences observed in repertoire sizes increasing with age in Mountjoy and Lemon (1995) study could be due to different methodologies used. It is possible they did not obtain the entire repertoire of an individual as they sampled approximately 200 song types per male, where they expected 85% of the repertoire of each male to have been obtained. Whereas, in my study, I analysed an average of 627.30 songs (SD: 86.29 range: 335-716), therefore, I likely obtained the entire repertoire for all males except three. Eens et al. (1992) also sampled a similar number of song types for each male for his study (see Eens et al. 1992). But Mountjoy and Lemon (1995) followed a consistent criteria, where they sampled a similar number of song types for each male, therefore sampling effort did not differ for each male, meaning these differences could be due to differences at the population level.

My study also provided insight into differences at the individual level in repertoire size. Some males increased their repertoire while others decreased or maintained the same number of song types, behaviour which is seen in other populations (Eens et al. 1992a, Mountjoy and Lemon 1995). This adds to the body of literature that supports Starling males being open-ended learners. Furthermore, irrespective of the year in life the male is in, there is a tendency to increase their repertoire size from one year to the next (Table 2.7, Fig. 2.15). It seems in my population, males have varying starting sizes for repertoire. This is an area for further research, but it could be possible that this may signal past development stress in Starlings (Buchanan et al. 2003). Males that were in better condition may be able to learn more song types earlier in age as opposed to males of the same age that are in poorer condition which may be why there is so much variation at the same age.

Additionally, other open-ended learners do not always signal age through song repertoire size. For example, in another longitudinal study on an open-ended passerine, three of four Northern Mockingbirds, *Mimus polyglottos,* increased their song repertoire size from one year to the next, but it was not by a significant amount (Derrickson 1987). Pied Flycatchers, also an open-

ended learner (Eriksen et al. 2011), did not show any correlation with age and repertoire size in males that were at least two years old (Espmark and Lampe 1993).

The results from my study show that song bout length does not signal age (cross-sectional study) (Table 2.5, Fig. 2.11) in my focal population of Starlings and it does not increase with age (longitudinal study) (Table. 2.5, Fig. 2.13). Contrary to my predictions, some SY males had longer song bouts than males that were 4, 5 and 6 years of age (Table 2.1 and 2.2, Fig. 2.11 and 2.14). Other studies on song bout length in Starlings show that bout length positively correlated with age (Adret-Hausberger et al. 1990) and found significant differences between SY and ASY males in song bout length (Eens et al. 1992b, a). In the latter population, despite differences in SY and ASY males, song bout length did not increase in ASY males in the Zoersel colony in Belgium (Eens et al. 1992b). I found similar results, where ASY males did not increase their song bout length with age (Table 2.6). This provides evidence that each population of Starling may differ in how their song functions and what quality parameters it signals to females.

My study had a small sample of SY males therefore it was not possible to test the differences between SY and ASY males as done in other populations (Eens et al. 1991b, 1992b; Mountjoy and Lemon 1995). Future research should assess the differences in song parameters between SY and ASY males in my focal population.

The unreliable signal hypothesis

The unreliable signal hypothesis is supported in my study, where neither song repertoire size nor song bout length were correlated with age; though, the lack of correlation could also be due to the lack of male quality parameters tested such as a male condition or immunity and extent of paternal care (Rivera-Gutierrez et al. 2010). Repertoire size is time intensive to assess (Botero et al. 2008), and female Starlings do not listen to the entire song bout sung by a male (Gentner and Hulse 2000), therefore it is possible that other characteristics which are not as time intensive are

65

used to assess quality. Song parameters such as song rate (amount of songs produced in a given time) or song type consistency (see Chapter 3) may be used instead to signal age in my focal population.

There are variety of quality parameters that do correlate with song in Starlings. For example, song complexity correlated with condition (Mountjoy and Lemon 1997) while the amount of singing and song bout length positively correlated with cell mediated immunity and humoral immunity, respectively (Duffy and Ball 2002). Males that sang longer songs fledged more young and a similar trend was found with repertoire size (Eens et al. 1991a). It could be the case that, in our population, song repertoire size and song bout length convey quality parameters such as condition or immunity. In one population, the males that were polygynous had the largest repertoire sizes, suggesting the status of breeding could be related to repertoire size as well (Adret-Hausberger et al. 1990).

Song repertoire size could signal breeding experience not age, as observed in an open-ended learner and a long-distance migrant, Pied Flycatchers. Males that come to a breeding site for the first time could include males in their second year (one-year old) or third year (two-year old) of life. Song parameters of males in their second or third year of life did not show differences when arriving for their first breeding year. On the other hand, song repertoire size was different between first time and second time breeders, regardless of the age of the male. Males breeding for the second time had twice the repertoire size of first-time breeders. Males that do not return to the breeding site in their second year of life miss out on breeding experience. Males in their third year of life returning to the site for the first time, show the same breeding experience as second year birds returning to the site. Therefore, it is plausible that female's song parameters distinguish experienced versus non-experienced breeders (Motes-Rodrigo et al. 2017). This suggests that repertoire size may not be an indicator of age but rather breeding experience which was not a variable included in my study. Repertoire size and song bout length may be used to distinguish

66

between experienced versus unexperienced breeders, especially because not all SY Starlings

breed which could lead to differences in breeding experience in the same age.

Hackle feathers and age

A study conducted on the same Starling population as my study by Barber and Wright (2017)

found that both the length of the hackle and the iridescent portion were significantly longer in

ASY males than SY males. The largest change in length was observed in the transition year from

SY to ASY males and these hackle feathers continued to increase with age (Barber and Wright

2017). Hackle feathers may be a reliable indicator of age in our population and used by females to

distinguish SY males from ASY males. Similar to my results found with song bout length and

repertoire size, there was overlap in lengths of hackles and iridescence portion in ASY males,

therefore distinguishing ages in ASY males was not possible.

Delayed song maturation hypothesis

Delayed maturation is observed with respect to plumage and song characteristics in many bird

species (Cucco and Malacarne 2000). Delayed maturation may help females distinguish SY and

ASY males. It has been shown in several species that SY males have smaller repertoires than

older males in open-ended learners; for example, it is observed in Common Nightingales,

Luscinia megarhynchos (Kiefer et al. 2006), Eurasian Blackbirds, *Turdus merula* (Hesler et al.

2012)*,* Collared Flycatchers, *Ficedula albicollis* (Garamszegi et al. 2007), and the Canary

(Nottebohm and Nottebohm 1978)*.* Song bout length also increased with age in the Java sparrow

Lonchura oryzivora (Ota and Soma 2014), and older Barn Swallows, sang longer songs than SY

males (Galeotti et al. 2001).

Surprisingly, delayed song maturation hypothesis was not observed in my population,

where age did not predict song repertoire size or song bout length. Specifically, SY males did not

always have the smallest repertoire size or the shortest song bout length compared to ASY males.

67

This delayed maturation may not be consistent across all SY males. Repertoire turnover (changing the song composition by integrating and dropping song types) in a Russian Pied Flycatcher population was not consistent where some males showed large repertoire turnovers from their first to the second year while some did not. SY males that did not show large levels of repertoire turnover were indistinguishable from older males. This could be due to differing age-dependent strategies used by these Pied Flycatchers where some birds have large repertoire turnover while others sing a stable song which does not change from first to later years (Vabishchevich 2012). It could also be that SY males sampled from my population do not exhibit delayed maturation and sing similar repertoire sizes and song bout lengths to ASY males, whereas other SY males may have smaller repertoire sizes and shorter songs. In order to capture this, a larger subset of second year males need to be recorded in my focal population and compared to ASY males.

Geography

Our population of Starlings did not reflect age through song repertoire size and song bout length. Other studies have shown that the same species in geographically different locations can vary in traits that are age-dependent. For example, Forstmeier et al. (2006) found that Great Reed Warblers in Germany did not show a longitudinal increase of song repertoire size while a Swedish population did. Additionally, the German population reflected longevity through song repertoire size and syllable switching. Both of these were correlated in cross-sectional studies but did not show longitudinal increases, therefore it is likely that males with larger repertoires and increased syllable switching reflect lifespan in this population (Forstmeier et al. 2006).

Behavioural differences in different geographical populations could lead to varying sexual signals. For example, males of two closely related subspecies, European Barn Swallows *Hirundo rustica rustic,* and Barn Swallows, *Hirundo rustica erythrogaster* differ in their incubation behaviour, where the former does not incubate their eggs (Smith and Montgomerie 1991, Neuman et al. 2007) while the latter does (Smith and Montgomerie 1991). In Europe,

varying characteristics in the tail play a strong role in mate attraction and signalling quality (Moller 1994, Saino et al. 1997). In North America the tail characteristics did not signal male quality and reproductive success to the same extent (Smith and Montgomerie 1991) but plumage colouration was positively correlated with pairing date and reproductive success (Safran and McGraw 2004, Neuman et al. 2007). The tail streamers are also shorter in North American males and it has been suggested that these males are more likely to experience broken tail streamers when they incubate (Smith and Montgomerie 1991, Neuman et al. 2007) which could affect the ability to attract a mate for the second brood. Incubation could also limit the time available for foraging to upkeep costly long streamers that are seen in their European counterparts (Møller 1989, Smith and Montgomerie 1991). This suggests that the same species, living in different geographic populations, often exhibit different behaviours (e.g. Eastern Canadian vs Belgium starlings) that can lead to sexual signals varying between populations. It is difficult to infer the exact cause of song repertoire size and song bout duration not signalling age within our population but it would have to be further investigated if it stems from different behaviours exhibited by our population than found in others. Starlings in our population overwinter close to the study site, as many are seen in the Fall and Winter, and at times are singing near the same nest boxes where they breed (pers. obs.). Starlings in Europe and other areas of North America also overwinter (Kessel 1957). During this time they do exhibit flocking and roosting behaviours including birds singing together in the Fall (Kessel 1957). Females may assess potential mates for the following breeding season, therefore song repertoire size and song bout length may not play a role in examining male quality via age when the breeding period arrives.

Signalling of sexual ornaments also differs between the open-ended learners Pied Flycatcher, *Ficedula hypoleuca,* found in Norway (Dale et al. 1999, Eriksen et al. 2011) and those in Spain (Potti and Montalvo 1991) as well as the Black-and-White collared Flycatcher *Ficedula albicollis,* found in Sweden (a species that is close in lineage) (see Dale et al. 1999). Females in

the Norwegian population use male plumage colouration to select a mate (Sætre et al. 1997, Dale et al. 1999). Conversely, the size of the forehead patch is used to assess males in Spain (Potti and Montalvo 1991) and in Sweden (Gustafsson and Qvarnstrom 1995). Sætre et al. (1997), proposed that the forehead patch does not play a role in mate choice in Norway, because of sympatric divergence. There are two similar species that occupy the same area, the Pied Flycatcher and the Black and White Collared Flycatcher. Females may be using different ornaments to distinguish between the species to avoid creating unfit hybrid offspring. Therefore, females may be using different sexual ornaments in order to recognize conspecifics (Sætre et al. 1997). However, similar to what Safran et al. (2004) indicates for Barn Swallows, the population of Starlings in my study population do not have closely related species occupying the same area and therefore are not subjected to the same selective pressures forcing them to distinguish between similar species (Safran and McGraw 2004).

Timing of recording and pairing status

Song recordings in my study were recorded opportunistically for the three years (2019 – 2021), where one year all recordings were from the later brood, and for the other two years song was recorded before the early brood. Starlings are able to change their song throughout the year (breeding and non-breeding seasons) (Chaiken et al. 1994). It is possible no correlation was found because the comparisons 2019 and 2020 were between songs that was recorded in the later brood versus the early brood. However, mixed results are found with respect to Starling song and its stability throughout the breeding season, as Adret-Hausberger (1990) found that song was consistent throughout the breeding season.

Many males were already paired when song recording started in February 2021 in comparison to February 2020. Males drop their singing activity with pairing (Cuthill and Hindmarsh 1985), therefore it is possible that song was recorded from some males with differing pairing status which may have affected song repertoire size and bout duration. However, males

Starlings can become polygynous (Kessel 1957, Pinxten et al. 1989b), and polygynous male are present in my focal population (pers. obs.), therefore it would expected for them to continue singing at the same levels in order to attract an extra-pair mate.

Statistical power

Finally, it cannot be dismissed that a trend may not have been detected due to low statistical power. The longitudinal study of ASY males conducted by Mountjoy and Lemon (1995) followed seven males which is similar to that in my study. However, for cross-sectional analysis, where SY males were compared to older males, Mountjoy and Lemon (1995) and Eens et al. (1992) had sample sizes of 19 and 25 respectively; which is larger than my sample size.

Conclusion

In conclusion, my prediction that male song repertoire size and song bout length would signal age was not supported from the song data collected from my study population. There is no difference between SY and ASY males in these song parameters either, although a larger sample size would be needed for statistical support. It is possible that alternate song parameters not included in this study may convey age to females in our population. For further research, additional song characteristics such as song rate and song consistency should be studied with respect to age. My study also shows that Starlings are open-ended learners as males in our population did add new song types to their repertoire but these additions are small, therefore open-ended learners do not always signal age through repertoire size. Finally, my results reinforce the idea of Forstmeier et al. (2006) that generalizations with respect to sexual signals cannot be made across the same species as they can vary even in populations that are relatively close in geography.

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CHAPTER 3: A cross-sectional analysis of within-song type variation and whether it signals male age

ABSTRACT

Male birds use a diverse array song characteristics to convey their quality to attract mates. Many females preferentially mate with older males that may offer benefits that younger males are unable to provide. For example, older males are experienced breeders, superior foragers and have demonstrated their ability to survive. Some song characteristics are age-dependent traits, and song repertoire size increases with age in many species, such as European Starlings, *Sturnus vulgaris,* who are open-ended learners. Repertoire size and song bout length are commonly studied song parameters in this species. Within-song type variation has been detected and studied in other species but its functions in Starlings are unknown. My objective was to investigate whether within-song type variation is an age-dependent trait. The cross-sectional analysis revealed that within-song type variation decreased with age. Song consistency signals male age and may therefore play an important role in mate attraction.

INTRODUCTION

Song has a dual role in birds; it is used for mate attraction (Catchpole 1980, Eens et al. 1991a, Mountjoy and Lemon 1996) as well as territorial defence (Yasukawa 1981b, Horn and Falls 1991). Song characteristics such as repertoire size (Eens et al. 1993), song bout length (Gentner and Hulse 2000), amount of song switching (Horn and Falls 1991), song rate (Collins et al. 1994), ability to sing complex song types (they are physically difficult to produce which is a form of vocal performance) (Ballentine et al. 2004) and when songs were sung with respect to sunrise (known as dawn chorus) (Poesel et al. 2006) have been studied in connection with female preference and mate attraction in many avian species. A commonly studied characteristic of male song is repertoire size; this form of song complexity has been shown to correlate with male condition (Mountjoy and Lemon 1996, Pfaff et al. 2007), immunity (Pfaff et al. 2007), earlier pairing (Eens et al. 1991a, Mountjoy and Lemon 1996), paternal care (Buchanan and Catchpole 2000) and greater reproductive success (Catchpole 1986, Hiebert et al. 1989, Gil and Slater 2000, Leitner et al. 2006). Song repertoire size increases with age in a very few species, examples include European Starlings, *Sturnus vulgaris* (hereafter Starling) (Eens et al. 1992b, Mountjoy and Lemon 1995), a Swedish population of Great Reed Warblers, *Acrocephalus arundinaceus* (Forstmeier et al. 2006), and Sedge Warblers, *Acrocephalus schoenobaenus* (Nicholson et al. 2007). Male Willow Warblers, *Phylloscopus trochilus* and Common Nightingales, *Luscinia megarhynchos* (Kiefer et al. 2006) showed an increase in repertoire between the first and second year of life (SY: an individual that hatched the previous year and is one-years old). However, longitudinal studies that follow the same individuals over time are required to determine whether repertoire size becomes larger with age; a study on Song Sparrows (*Melospiza melodia*) has shown that males with smaller repertoire sizes suffered higher attrition than males with larger repertoires (Hiebert et al. 1989) which could confound the findings of a cross-sectional study.

Song repertoire size is only one form of song complexity. Within-song type variation have been observed in European Starlings which are open-ended learners (Eens et al. 1989, 1991a, 1992b; Mountjoy and Lemon 1995) and in Song Sparrows, which are age-limited learners (Podos et al. 1992, Nowicki et al. 1994, Nordby et al. 2002). Variation within song types is described as slight modifications of a song type (Nowicki et al. 1994). Within-song type variants would have either minor differences with the integration of small acoustic units or have small elements removed in the song type, whereas differences between song types are starker (Nowicki et al. 1994). Within-song type variations have been studied in Song Sparrows with respect to song learning (Nowicki et al. 1999), territoriality (Stoddard et al. 1988) and aggression (Searcy et al. 2000). These studies demonstrate that within-song type variation should also be included in studies with respect to song in Song Sparrows as they showed a behavioural response and sang with song switching that included both distinct and within-song type variations (Stoddard et al. 1988, Searcy et al. 2000).

It has been suggested that these variations could be simply song types sung inaccurately (e.g. Nowicki et al. 1999; Searcy et al. 2000). However, it is difficult to characterize them as inaccuracies when Song Sparrows have similar responses to both within-song type variations and between-song type transitions. The ability to sing songs in a consistent manner has been described as a form of song performance. Song performance can be studied at a finer scale by investigating finer elements in songs such as trill rate (Byers 2007). Banded Wrens, *Thryothorus pleurostictus,* sing with increased trill consistency (fine-scale vocal performance) in their songs with age (De Kort et al. 2009) while Tropical Mockingbirds, *Mimus gilvus*, sang syllables more consistently with age (Botero et al. 2009). These two studies provide evidence that song consistency may play an important role in signalling age to conspecifics. Additionally, consistency may also signal male quality. For example, males that sang consistently in pitch and timing in Chestnut-sided Warbler, *Dendroica pensylvanica,* had higher reproductive success (Byers 2007). Within-song type

variation could potentially reflect song performance in Starlings, where individuals that sing with fewer within-song type variations are honestly signalling their age or other forms of quality to females.

Starlings have a repertoire size ranging from 21 to 67 song types (Eens et al. 1989, 1991a, b). Although within-song type variations have been identified and are known to occur, the potential function in this species has not yet been explored. The repertoire size of Starlings increases with age in some populations; older males had significantly larger repertoires in two different global populations (Eens et al. 1992b, Mountjoy and Lemon 1995), but not in mine (see Chapter 2). Females prefer males with larger song repertoires and pair earlier with males that have more complex song (Eens et al. 1991a, Mountjoy and Lemon 1996). The objective of my study is to examine within-song type variation as another form of song complexity and to determine whether it changes with male age in an eastern Canadian population of European Starlings. I predict that within-song type variation will decrease with age, with males singing more consistent song types throughout their life. This study is believed to be the first to focus on within-song type variation in this species.

METHODS

Study Site

The research was conducted at Saint Mary's University located in Halifax, Nova Scotia, Canada (44˚ 37' 54.07" N, 63˚ 34' 47.09") in 2019 and 2020. The field site has 45 nest boxes attached to trees around the university campus. Field work was conducted from mid-April until late July in 2019, and from February through March in 2020. Research was then paused due to the global Covid-19 pandemic and resumed in June until July in 2020.

Sexing and Banding

All males were banded at the field site. In 2019, the primary focus was to capture adults using Mo traps (Stutchbury and Robertson 1986) or by concealing the nest hole with a Swiffer once the male was inside the nest box. Eight males were banded in 2019 and one male was banded in 2020. Three males had been banded prior to the 2019 field season. The sex of the adults was determined using two criteria. One was the colour of the mandibular base; males have a dark blue colour while the females have a pale pink colour. The second criteria was the presence or absence of a yellow iris ring; females have a yellow iris ring and males do not (Kessel 1951).

Adults were banded with a Canadian Wildlife Services Band (CWS) on the bottom right tarsus. Males received a yellow plastic band while females received a pink plastic band on the top right tarsus, above the CWS band. On the left tarsus, adults were banded with two plastic bands having a unique colour combination so as to be able to identify each individual. The CWS number was recorded for all adults that had been banded in a previous year at the field site. The date on which they had been first banded was retrieved.

Determining Age

Seven to eight hackle (throat) feathers were also obtained from each male when they were banded and stored in labelled Ziploc bags. Starlings can be placed into one of two age categories SY vs. after second year (ASY: an individual that hatched a minimum two years ago) based on the length of iridescence in their hackles (Kessel 1951). Barber and Wright (2017) confirmed that hackle iridescence differs significantly between SY and ASY individuals and found that it continues to increase with age in male and female Starlings. Total hackle length and iridescence length of seven to eight feathers from each male were measured with a ruler to the nearest 0.1mm under a dissecting microscope (Barber and Wright 2017).

These measurements were averaged over the seven to eight feathers to obtain a single value for both the iridescent section and the entire length of the hackle (Barber and Wright 2017). The total iridescence was used to assign males into SY (one-year old and first-time breeders) and ASY (ASY males have likely gained some past breeding experience) males. For some males that had been captured in a prior year, their exact age could be inferred if they were first caught as a SY male, or further approximated if first caught as an ASY male. Analysis on SY and ASY males are not conducted in this study but rather age (the year of life each male is in) as a continuous variable.

Song Recording

Song recordings in 2019 were obtained starting mid-April. At this time, the majority of males were paired and were not singing at high rates (Cuthill and Hindmarsh 1985). Most of the recordings obtained were from intermediate or later broods in that year. In one instance in 2019, a male was recorded while he was provisioning his first brood while singing at another nest box in the vicinity. In 2020, song recording started in February and continued until the end of March, encompassing the period when males were unpaired until after pairing. Recordings were made opportunistically with the majority of singing taking place in the morning between the hours of 0700 to 1100, and occasionally between 1400 and 1600.

All song recordings were obtained with a PMD Marantz professional recorder (Model number: 661MK111) and a shotgun Sennheiser microphone with a Sennheiser MZS 20-1 grip. A Zoom H4n Pro Handy Recorder (Model number: ZH4NPRO) and a Sennheiser P48 Super-Cardioid Long Shotgun Condenser Microphone (Model Number: MKH-70-1) were also used. The recording settings were PCM 44,100Hz, 16 Bit, and Mono. Recordings were obtained daily until at least 27 song bouts were obtained from each male. This number of bouts was deemed sufficient for repertoire analysis (Eens et al. 1991b). The recordings were moved daily from the secure

digital (SD) cards to an external hard drive. They were labelled with male identity, date and time of recording for analysis. The batteries were charged between 1100 and 1400 or overnight.

Starling Song Structure

Starling song has four sections which consist of **1)** whistles, **2)** variable song, **3)** rattle song and **4)** high frequency (terminal) songs; these four sections comprise a complete song bout. However, not all song bouts are composed of all four sections nor will all their whistles and variable song be in each bout. In most cases, song bouts do include the rattles and higher- frequency song types (Eens et al. 1989). For more detail about the Starling song structure, please see Chapter 2. For this study a song bout was defined as a song lasting a minimum of five seconds with no intervals that are longer than 1.5 seconds, which is the same definition used in past studies (Eens et al. 1991a, b; Bernard et al. 1996).

Song Classification

Within-song types were identified for males that had clear recordings with sufficient detail to analyse the entire structure of each song type. Song from a total of 12 males was analysed and use for the cross-sectional analysis.

Most of the song types are repeated two to five times within a Starling song bout, but can infrequently be repeated more than five times (Eens et al. 1989). Some song types are only sung once. The repeating song types can be identical or can be almost identical, but with a modification which results in a slightly different version of the song type sung (Eens et al. 1989, 1991b). In this study, variation is defined as a process whereby an individual modifies a single song type with the addition or removal of acoustic elements. Doing so results in several variations of the same song type. To simplify the assignment of within-song type variations, the first occurrence of a distinct song type was assigned as the base song type, and all others that were similar to this base song

type were assigned as within-song type variations of it. Assigning the first distinct song type as the base made it possible to have consistent methodology throughout the song analysis.

The following criteria were developed to ensure that the process of assigning within-song type variations was as objective as possible:

1) A new song type has unique underlining structural characteristics that distinguish it from other songs. Conversely, a variation of a song type keeps the fundamental structure, but makes slight changes that can add variety to the song.

2) A song is considered a variation of a main song type if the song has the same structure, but one or more new acoustical elements is added.

3) A song is considered a variation of a main song type if the song has the same structure, but one or more acoustical elements is removed.

4) A whistle song type is considered a variation if components are added to whistles (e.g. a short whistle immediately preceding it or multiple chirps) before or after the main whistle.

5) Terminal song types are characterized as variations when the addition of acoustic elements (e.g. chirps, trills, whistles) are added or removed from either the start or end of the base terminal song.
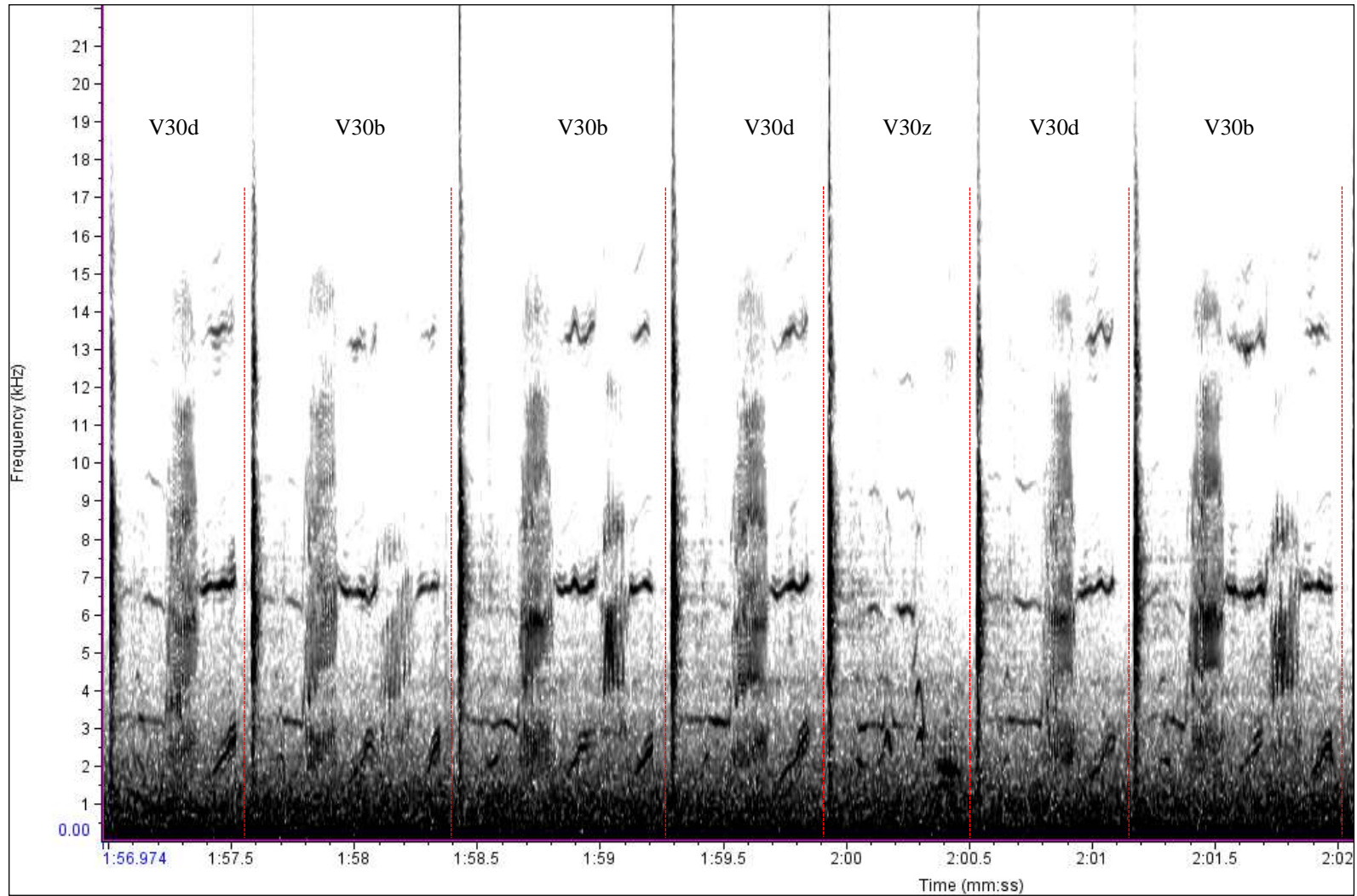
Figure 3.1. Song types recorded from Nest Box 33 in 2020. "V" stands for variable song type. V30d and V30b are within-song type variations of one song type (not shown) while V30z is within-song type variation of another song type (not shown). The red dashed line separates song types.
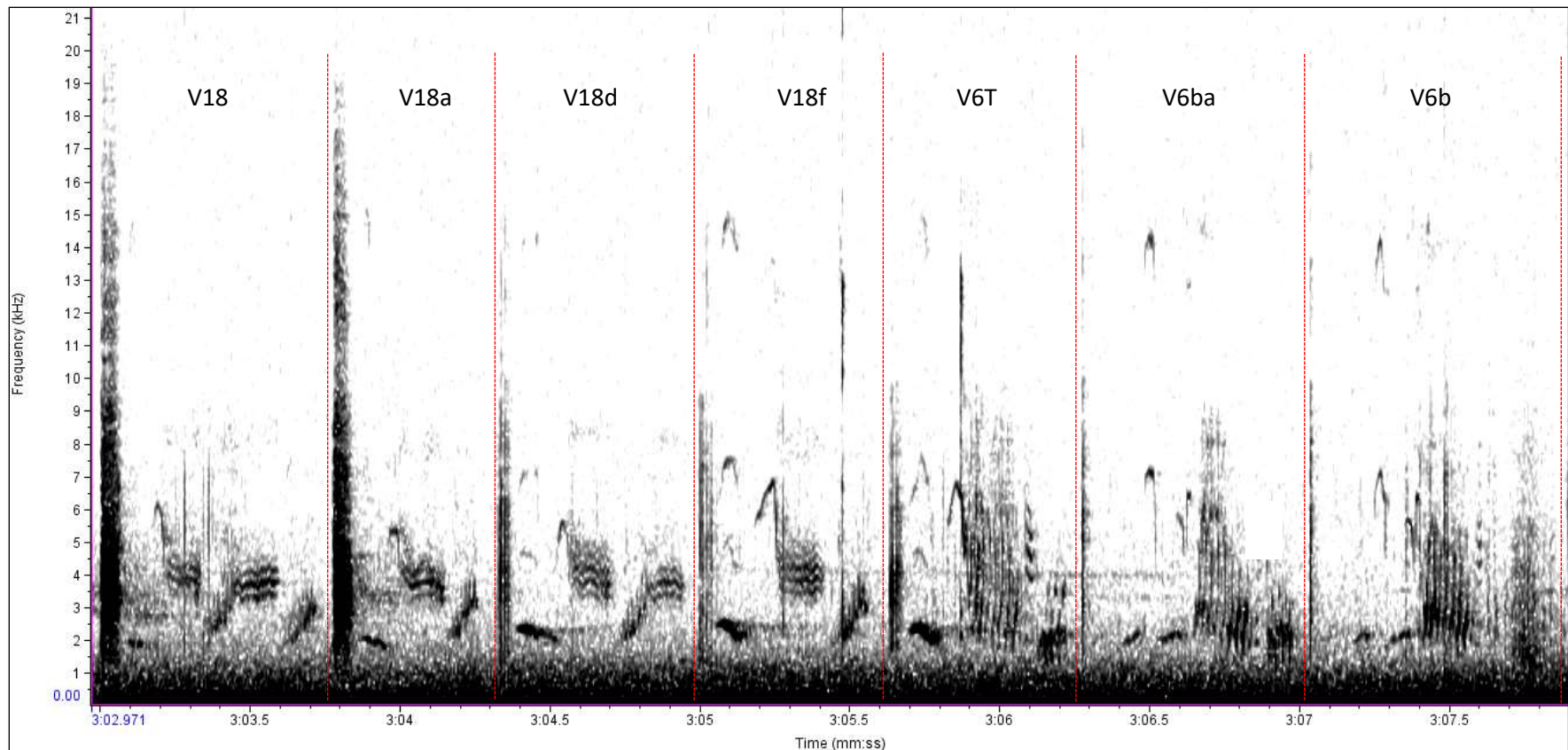
Figure 3.2. A song type and its variations recorded from Nest Box 37 in 2020. "V" stands for variable song type. V18, V18a, V18d, and V18f are within-song type variations of a single song type (base song type not shown). V6ba and V6b are within-song type variations of another song type (not shown). V6T is an intermediate song type, containing elements of two different song types that precede and follow it. The red dashed line separates song types.

Statistical Analysis

To determine whether within-song type variation changes with age in the focal population I conducted a cross-sectional analysis on 12 males (three SY males and nine ASY males) in R Version 1.4.1103. I also conducted a separate cross-sectional analysis on only the nine ASY males. A generalized linear mixed-model (GLMM) was used with lme4 package (Bates et al. 2014) with negative binomial distribution because of over dispersed count data (Crawley 2007). The response variable was within-song type variation while age was a fixed effect and year was a random effect. The Akaike Information Criterion corrected for small sample sizes (AICc) was obtained for the model to observe the best predictor for the data (Barton 2020). The best predictors were Δ AICc <2 (Guthery et al. 2003). A Pearson correlation was also conducted between age and within-song type variation, after testing for normality.

RESULTS

The within-song type variation ranged from 41 to 103 (mean $\pm$ standard error (SE), 67.42 $\pm$5.23, N = 12). SY males had an average of 90 $\pm$ 7 (N= 3) song types whereas ASY males had an average of 59.89 $\pm$ 4.20 (N = 9) song types.

For within-song type variation, age was the best predictor receiving 52.5% weight in the model (Table 3.1) when both SY and ASY males were pooled together. When only ASY males were included in the model, age was not the best predictor of within-song type variation, receiving 3.1% of the model weight (Table 3.1).

There was a non-significant negative correlation between age and within-song type variation (Pearson correlation $r = -0.55$, $n = 12$, $p = 0.066$; Fig. 2.3) when SY and ASY males were pooled. There was no correlation between age and within-song type variation (Pearson correlation $r = -0.20$, $n = 9$, $p = 0.61$; Fig. 2.3) in ASY males.

Table 3.1. Cross-sectional analysis of whether age is a predictor of within-song type variations with SY and ASY males pooled. Degrees of freedom (df), Akaike Information Criterion corrected for small sample sizes (AICc), the other models (Δ AICc), and the model weights (w_i) are provided. The bolded values are the models that have a Δ AICc < 2.

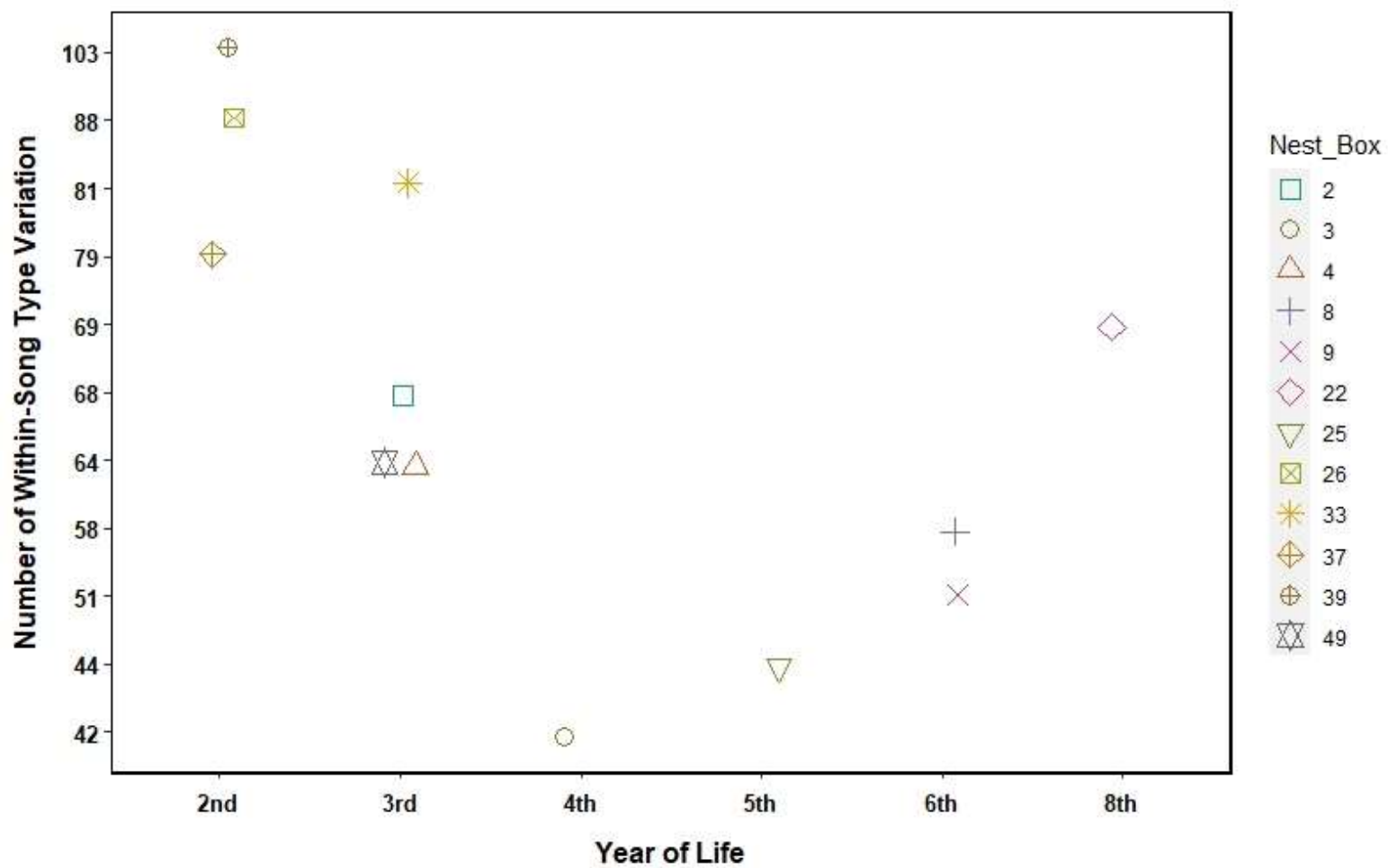|	df	AICc	Δ AICc	w_i	Coefficient ± Standard Error
SY and ASY males pooled					
Within-song type variation ~ age + (1|year)					
Intercept only	3	111.9	**0.20**	0.475	
Age (Year of life)	4	111.7	**0.00**	0.525	-0.073 ± 0.033
ASY males only					
Within-song type variation ~ age + (1|year)					
Intercept only	3	80.9	**0.00**	0.969	
Age (Year of life)	4	87.8	6.85	0.031	-0.022 ± 0.037

Figure 3.3. A cross-sectional analysis of number of within-song type variations of 12 ASY males. Each data point represents an individual male.

DISCUSSION

Age was a predictor of within-song type variation in this Atlantic Canadian study population of Starlings; within-song type variation decreased with age (or song consistency increased with age) supporting my prediction. However, age was no longer a predictor of within-song type variations when only ASY males were assessed. Two other studies have observed within-song type variability in Starling populations in Europe (Eens et al. 1989, 1991b), but its potential function was not explored. My study is the first to provide findings that suggest that within-song type variation is an important form of vocal performance and may potentially play a role in signalling age in Starlings. Within-song type variation function may be even more important in my focal population as song repertoire size and song bout length did not increase in my population. Therefore, it is also possible that within-song type variations is a sexually selected trait used to distinguish between younger and older males. Alternatively, it could also be that males with more within-song type variation die earlier, as a result only males with low levels of within-song type variation are represented in older age classes. Therefore, this will need to be further confirmed through a longitudinal study (Gil et al. 2001, Forstmeier et al. 2006).

Studies have shown that the ability to sing consistently is a vital form of vocal performance which could signal male quality. For example, Great Tits, *Parus major,* also showed a positive correlation with song consistency and age, where it increased in males in their second to fourth year of life. There was also a trend towards a decline in song consistency when males were in their fifth year (Rivera-Gutierrez et al. 2010). That study used a cross-sectional approach which is what I did. However, longitudinal studies will also need to be conducted to confirm if these increases in consistency are seen in the same individuals from one year to the next. Similar, trends were seen in Tropical Mockingbirds, *Mimus gilvus* (Botero et al. 2009) and Banded Wrens, *Thryothorus pleurostictus* (De Kort et al. 2009). In Tropical Mockingbirds, males sang syllables

in a more consistent fashion as they aged. There was a positive correlation between consistency and the amount of time between sampling, showing individuals improved with time (cross-sectional analysis). The males that also sang syllables in a more consistent fashion were higher ranked (alpha males) and experienced higher reproductive success in their social group (Botero et al. 2009). Male Banded Wrens from their second to fourth year of life, sang trills more consistently as they aged, suggesting that trill consistency is an age-dependent trait (De Kort et al. 2009). Singing in Banded Wrens is limited to their territory, therefore this consistency in singing would be closely related to territory tenure; where males that managed to defend territory would have had more opportunity to practice their singing compared to individuals of the same age that were not able to obtain territories. ASY Starling males may sing more consistently because they have had more time to practice their song therefore are able to vocally perform song types more consistently. This may be why age was no longer included as a predictor when SY males were removed from the model. Also, SY males would be breeding for the first time while ASY males would likely have had some past breeding experience (Kessel 1957, Barber and Wright 2017). Therefore, the within-song type variation patterns I observed with age in Starlings may play a role in allowing females to discern between SY and ASY males. Alternatively, it could also be that ASY males have likely developed knowledge of what within-song type variations work, as they have previously attracted a mate and bred. While SY males may still be exploring which versions of the song type is most likely going to attract a mate. This is a possibility because some song variations are frequently sung while others are rare.

Additionally, not all SY Starlings breed or get the opportunity to breed (Kessel 1957). Most of the males at our field site are ASY males (pers. obs.) and there is a high return rate to breed at this field site (pers. obs). Therefore, securing a nest box or cavity could be challenging for a SY males. However, some SY males do secure these nest boxes; it would be interesting to see if there is a correlation between song consistency and reproductive success compared to third

year males breeding for the first time. For example, male Chestnut-sided Warblers that sang with consistent pitch for their dawn song while consistency in pitch and in the intervals in between songs for their daytime songs, had higher reproductive success (more extra-pair nestlings) (Byers 2007). These studies provide evidence that not only song complexity but vocal performance (e.g. song consistency) are important in signalling male quality to females.

As established in Chapter 2, song repertoire size and song bout length did not reflect age in my study population of Starlings, unlike the findings from Starling populations in Europe and North America (Eens et al. 1992b, Mountjoy and Lemon 1995). However, I found that song consistency increased with Starling age which could signal age in males. It is possible that females in our population use within-song type variations to assess male quality as opposed to song repertoire size or length. Assessing song repertoire size as a signal would be time-consuming, especially in species with larger repertoire sizes (Botero et al. 2008, De Kort et al. 2009). Gentner & Hulse (2000) conducted an experimental study on Starlings in which they discovered that females preferred males that sang longer songs, but they also found that females did not listen to the entire song bout, therefore absolute length may not serve any significance. Listening to within-song type variation may be a quicker and more efficient way to evaluate male quality than assessing the number of song types (ranges from 41 to 81 in my study population) or the song bout length (ranges from 23.67 to 46.53 in this population). In Starling song, contrasting song types follow each other and their song is categorized as immediate variety (Eens et al. 1989). Males do repeat a song type on average 2.38 times before the next song type is sung, but the repetitions of the song types can vary anywhere from once to more than five times (Eens et al. 1989).

Unlike another open-ended species, the Canary (Nottebohm and Nottebohm 1978), Starlings keep most of their song types from the previous year (Eens et al. 1992a), and retaining these song types would allow them to practice the same song type over years. Tumer et al. (2007)

provided experimental evidence on a close-ended learner (in which song crystallizes at a certain stage of their life, and cannot be altered after crystallization), Bengalese Finches *Lonchura striata var. domestica,* that variation in these crystallized songs may be a source for individuals to refine their song through trial and error, and that these variations are not simply noise. Although Starlings are open-ended learners (Eens et al. 1992b, Mountjoy and Lemon 1995), it is possible that older males sing with more consistency because they have had the opportunity to refine their song through trial and error.

Producing song is a complex mechanism which involves the syrinx, trachea and numerous other muscles (see Suthers and Zollinger 2004). Male motor performance has been proposed to be a signal used by females when choosing mates. Motor performance consists of two categories: the first is vigour, which is described as repeatedly displaying energetically costly behaviours, while the second is skill, where challenging behaviours are accurately performed (Byers et al. 2010). Several studies have suggested that the ability to perform song types or elements of song in a consistent manner reflects their enhanced motor vocal performance (Christie et al. 2004, Byers 2007, Podos et al. 2009), and the ability for Starlings to sing song types with less variation may reflect their motor vocal performance.

In conclusion, this is the first study to my knowledge which has explored the function of within-song type variations in Starling song, which decreases with age. This also provides evidence that song characteristics beyond repertoire size and song bout length should be explored to further our understanding of song which is directed towards females in the breeding season. Finally, to fully understand the role of within-song type variation in Starlings, it will need to be confirmed longitudinally whether it is an age-dependent trait. A longitudinal study will provide data independent of mortality and delays in maturation of sexual traits.

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CHAPTER 4: General Discussion

In many avian species, females prefer to mate with older males (Kokko and Lindstrom 1996, Kokko 1998). In some species, older males are more sought after because they are higher quality mates (Eens et al. 1991a), have higher reproductive success (Lozano et al. 1996), defend superior territory (Hill 1988), and are better foragers (MacLean 1986, Jansen 1989). These males also have a greater ability to survive and their viability (good genes) may be inherited by their offspring (Trivers 1972, Brooks and Kemp 2001). If older males are preferred, then females need to be able to distinguish older males from younger males. Traits such as plumage, tail length or song are age-dependent in many species, potentially allowing females to discern the relative age of males to optimize their choice of mate (Eens et al. 1992b, Mountjoy and Lemon 1995, Siefferman et al. 2005, Lifjeld et al. 2011, Barber and Wright 2017).

In Starlings, a passerine known to be an open-ended learner, I found that age was not a predictor of song repertoire size and song bout length (Chapter 2). However, within-song type variation, a less studied vocal performance characteristic, decreased with age (their song became more consistent) (Chapter 3). Specifically, females may use song consistency to distinguish between SY and ASY males. Song consistency was a predictor of age when SY and ASY males were pooled, but was no longer a predictor of age when only ASY males were included in the analysis. My study also provides support for the idea that male Starlings are open-ended learners as they learned new song types from one year to the next, although the number of types added were not high.

Female Starlings prefer males with larger repertoire sizes (Eens et al. 1993, Mountjoy and Lemon 1996) and longer songs (Gentner and Hulse 2000). However, how information such as age is conveyed via repertoire size and song bout length vary across Starling populations. For example, another population of eastern (Quebec) Canadian Starlings, a longitudinal study showed

significant increases in repertoire size from one year to another (Mountjoy and Lemon 1995). While a longitudinal study on this species in Europe found no large differences in repertoire size from one year to the next (Adret-Hausberger et al. 1990, Eens et al. 1992a). Conversely, in a longitudinal study, song bout length showed a positive correlation with age (Adret-Hausberger et al. 1990). Additionally, past cross-sectional analyses have shown that SY males have smaller repertoire sizes and shorter bout lengths than ASY males (Eens et al. 1992b, Mountjoy and Lemon 1995). These results show age is signalled differently across Starling populations. Some increase their repertoire size significantly as they get older (Mountjoy and Lemon 1995), while in other populations females may use song repertoire size and song bout length to discern between SY and ASY males (Adret-Hausberger et al. 1990, Eens et al. 1992b, Mountjoy and Lemon 1995), while song repertoire size and bout length may not signal age at all, as established in our population.

I suggest that an alternate song parameter to song repertoire size and song bout length may be used in our population to discern age. My focal study population is largely composed of ASY males that return each year to breed, therefore it may be more difficult for females to distinguish the ages of ASY males through song repertoire size and song bout length. It would also be incredibly time-intensive for a female to listen to each male's repertoire size and song bout length (Botero et al. 2008, De Kort et al. 2009). The highest repertoire size detected in my focal population was 81 song types (range 41 – 81, 57.75 ± 3.15), the longest song bout recorded was 69.61 seconds in duration, and the longest average song bout duration was 46.53 seconds (the average bout length for the sampled males was 34.74 ± 2.12 seconds). According to my findings though, it is possible that within-song type variation plays a role in mate attraction in this population, as older males sang their song types more consistently than did younger males, resulting in fewer within-song type variants. Females would not need to spend as much time assessing this song parameter to accurately assess a male's age, and therefore his quality as a

mate. However, a longitudinal approach would need to be undertaken to confirm that males do sing with more consistency with age. Otherwise, the findings could be confounded by males with less consistent song experiencing higher mortality (which is why song consistency would increase with age). This study adds to the body of literature in which song characteristics other than repertoire size and song bout length are explored. Finally, this study also supports the research that finds that traits used by females to assess males can differ between populations of the same species or in species with close lineages (Dale et al. 1999, Safran and McGraw 2004, Forstmeier et al. 2006). Therefore, generalizations on the types of sexual signalling cues used cannot be made within the same species. The song data obtained from my study population did not support the predictions that song repertoire size and song bout length signal age. Age was a predictor of song consistency, where the ability to sing these song types consistently increased with age in males supporting my prediction. However, it is unknown whether Starlings respond to within-song type variation, therefore their response to these within-song type variation will require further study.

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