PREDICTORS OF REPRODUCTIVE SUCCESS IN DARK-EYED JUNCOS (*Junco hyemalis*)

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Abstract

According to the theory of sexual selection, sexual traits are linked with reproductive success. Bird song is a male sexual trait that can communicate aspects of the signaler's quality to prospective mates and potential rivals, and signal traits that are physically challenging to produce can enforce signal honesty. These phenotypic traits, along with nest habitat characteristics and weather variables, have been shown to influence the reproductive success of birds. In the current study, I tested whether these variables are associated with reproductive success in dark-eyed juncos (*Junco hyemalis*). I found no evidence that song traits are challenging to produce, and average reproductive success per nesting attempt was not related to any measure of song structure or body size. Hatching success was significantly higher when canopy cover and average daily precipitation were lower and tended to be higher in nests with better insulation and at sites with lower stem density. Fledging success was not significantly associated with any microhabitat or weather variables, but it tended to increase with low nest exposure. The findings of this study suggest that the reproductive success of these ground-nesting songbirds depends more on nest habitat characteristics and temperature during the nesting period than on male phenotypic traits.

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Table of contents

Chapter 3: Nest micro-habitat, weather, and reproductive success in dark-eyed juncos......54

List of Tables

List of Figures

List of Appendices

CHAPTER 1: A REVIEW OF THE PREDICTORS OF REPRODUCTIVE SUCCESS IN SONGBIRDS

Selection, both natural and sexual, is considered one of the primary mechanisms that drive the evolution of a species, as they act to select traits or behaviours that increase the survival, reproduction, or mating success of individuals (Darwin 1859). Natural selection operates on the principle of survival of the fittest, where individuals with advantageous traits are more likely to survive and reproduce and pass on those traits to their offspring, while individuals with unfavourable traits are less likely to survive and reproduce (Darwin 1859). This process is mainly driven by the interaction of three key factors: genetic variation, environmental conditions, and competition for resources such as food and mates. However, natural selection alone could not explain sexually dimorphic ornaments like the peacock's tail that can impair survival by imposing energetic costs or increasing predation risk (Andersson 1994). Therefore, in *The Descent of Man, and Selection in Relation to Sex,* Darwin (1871) introduces the idea of sexual selection, a type of natural selection that occurs as a result of differences in mating success among individuals.

Sexual selection is the differential reproductive success that arises from variations in traits or behaviours among individuals that affect success in competition over mates and fertilizations (Andersson 1994). Scrambles, contests, endurance rivalry, and mate choice are the main forms of premating sexual selection, though fertilization success is also influenced by sperm competition in many species (Andersson 1994). Even though both males and females contribute an equal amount of genetic material to their offspring, the energetic costs are often much higher for females due to anisogamy (Trivers 1972). In some taxonomic groups such as mammals, females spend greater effort than males on parental care, however more recent work suggests that the sex with stronger pre-copulatory sexual selection is selected to offer less parental care (Queller 1997;

Kokko and Jennions 2008). Due to this differential parental investment, competition for mates is generally stronger among males, and females tend to be more careful in their choice of mating partner (mate choice) (Andersson 1994). However, it is important to note that the dynamics of intersexual competition can be influenced by the mating systems of different species. For example, in long-lived monogamous species, where individuals form long term pair bonds, the intensity of intersexual competition may be tempered compared to species with more promiscuous mating systems (Andersson 1994). Female mating choices are influenced by either direct or indirect benefits that would shape their fitness. Direct benefits include any direct nongenetic material provided by the mated male to the female or her offspring that increases the lifetime reproductive success of the female. Direct benefits include nutritional gifts during copulation, parental care, higher fertilization rate, protection against harassment from other males, and access to better resources such as territories with plentiful food, few parasites, and low risk of predation (Andersson 1994; Barbosa and Magurran 2006). Indirect benefits are thought to increase the female's fitness because her offspring inherit the qualities of their father, which will, in turn, enhance their viability (good genes) and their mating potential by making sons more attractive as mates (Weatherhead and Robertson 1979; Hasselquist 1998; Barbosa and Magurran 2006).

Sexual selection has a direct impact on reproductive success by affecting an individual’s ability to compete for mates and produce offspring (Andersson 1994). Individuals with traits or behaviours that are attractive to mates are more likely to have high reproductive success (Zahavi 1975). Similarly, characteristics of a mate that affect their ability to compete for fertilizations (e.g., ornaments, weapons, armour) are considered critical determinants of reproductive success in many species. Both prospective mates and opponents use different cues to assess an individual in terms of their genetic quality, fighting ability, or resource provisioning ability (Hagelin 2002; Candolin 2003). These cues can be phenotypic traits (i.e. acoustic, morphological, olfactory, or behavioural traits such as dominance) or resources that are produced or defended by the signaller (i.e. nest, territory; Candolin 2003). Numerous studies suggest these traits are honest and reliably convey the condition or status of the signalling individual (Ligon et al. 1990; Hill 1991).

In several taxa, including insects, frogs, and birds, male song and other acoustic signals are commonly selected traits that are shown to influence mating success (Searcy and Andersson 1986; Marler and Slabbekoorn 2004). Songs are generally characterized as long, complex vocalizations that are mostly produced during the breeding season (Catchpole and Slater 2008). Although songs can be observed in several animal groups, the best-known singers are the oscine birds, a subset of the order Passeriformes (Searcy and Andersson 1986). They use song primarily to attract mates and to defend their territories and/or mates against rivals (Catchpole and Slater 2008). While most research has focused on male song, recent research has shown that female song is more widespread than previously thought and that females sang in the common ancestor of modern songbirds (Odom et al. 2014). Factors such as song length, song complexity, repertoire size, song delivery rate and continuity (sung continuously without long pauses), that are physiologically or developmentally difficult to produce have been shown to be correlated with traits such as fighting ability and the quality of resources that a male can provide (Gil and Gahr 2002; Darolová et al. 2012; Scordato 2018; Sung and Handford 2020). For example, a study on white-crowned sparrows (*Zonotrichia leucophrys*) by Wasserman and Cigliano (1991) showed that female birds exhibit a preference for long songs and high song rates when choosing their mates. Similarly, some studies have shown that females actively select males with large repertoires, and males with large repertoires tend to obtain more mating opportunities through extra-pair paternity and to produce offspring with higher post-fledging survival (Buchanan and Catchpole 1997; Hasselquist et al. 1966; Mountjoy and Lemon 1996; but see Byers and Kroodsma 2009). There is also an indication that females use song frequency as an honest indicator of male size, where larger birds produce songs with lower emphasized frequencies (Ryan and Brenowitz 1985; Sung and Handford 2020). Males may even use song fundamental frequency to assess the size of a rival in male-male competition (Gil and Gahr 2002).

Apart from song production, body size and visual ornaments are also common sexually selected traits (Andersson 1994). Males of many bird species have more elaborate and colourful plumage than females, and these colourful feathers can be a sign of good health, fighting ability, or the ability to provide critical resources; females often prefer males with more elaborate plumage (Hill 1991). For example, a study on male house finches (*Haemorhous mexicanus*) found that females preferred males with brighter red plumage, indicating that plumage colouration can influence female mate choice (Hill 1990). Furthermore, a study on blue tits (*Cyanistes caeruleus*) revealed that the yellowness of the breast plumage correlates positively with nestling provisioning rates in both males and females, and high provisioning rates enable higher nestling survival (García-Navas et al. 2012). Similar to plumage, male body size acts as an indicator of condition and motivation (for male contests) and is favoured by both male-male competition and female mate choice (Andersson 1994; Hagelin 2002). Moreover, because body size is typically related to dominance, larger individuals are more likely to win contests, secure the best and most abundant resources, or intrude onto other males' territories and thereby obtain extra-pair copulations (Andersson 1994; Burg and Croxall 2006; Hutchinson and Griffith 2008).

Along with behavioural and morphological factors that predict lifetime reproductive success, many studies have shown that habitat quality contributes to the reproductive success of birds (Newton 1989; Germain and Arcese 2014; Jones et al. 2014). In territorial animals, high-quality (phenotypically or genetically) individuals are predicted to monopolize territories with plentiful food, abundant nest locations, and low predator density, since these traits can lead to increased offspring survival and reproductive success (Mainwaring et al. 2014; Potvin et al. 2015). In many bird species, parents' ability to invest in their young (e.g., clutch size, clutch mass, offspring size, and the number of nesting attempts) is often determined by food availability, whereas nest predation risk affects the likelihood that each nesting attempt will be successful (Martin 1987; Martin 1995; Nagy and Holmes 2017). Birds often select nest sites and build their nests in a manner that helps to shelter from elements of weather and to maintain eggs and nestlings at an optimal temperature and therefore increase nest survival and reduce the parents' energy expenditure (Haftorn 1988; Akresh et al. 2017).

For several reasons, it is essential to investigate the variables that affect the reproductive success of birds. For example, understanding these factors is essential for developing effective conservation strategies because threats such as habitat loss and climate change can negatively impact the reproductive success of bird populations. Additionally, research on reproductive success can shed light on the mechanisms of sexual selection and the evolution of sexual dimorphism. Researchers can learn more about the intricate biological interactions that shape communities and ecosystems by studying traits like song performance, dominance, and plumage colours, and how these relate to metrics of fitness such as the number of offspring produced each year and offspring survival. Studying the variables that affect reproductive success can also provide valuable insight into the fundamental biology of birds, aiding in understanding their ecology, physiology, and behaviour.

Study Species

The dark-eyed junco (*Junco hyemalis*) is a small sparrow (Family Passerellidae) found throughout North America from sea level to the subalpine tree line (Nolan et al. 2020). There are five subspecies of dark-eyed junco, differentiated by their morphology, vocalizations, and behaviour; the most widespread subspecies is the slate-coloured junco, which has a grayish-blue back, white belly, and dark head (Figure 1.1). The pink-sided junco is found in the western United States and has a pinkish-brown back and white belly. The white-winged junco has white wing bars and a white belly, and the Oregon junco has a brown back and white belly. The gray-headed junco is found in the southwestern United States and has a gray head and back and a white belly (Nolan et al. 2020). This study is based on slate-coloured junco (hereafter 'junco' or 'dark-eyed junco'), which is the only junco subspecies present in Newfoundland and Labrador, Canada.

Juncos have a long-life span, and they can live to 11 years old (Broun 1942). Dark-eyed juncos are considered socially monogamous birds even though extra-pair offspring are common (Ketterson et al. 1998). Male dark-eyed juncos defend their territories and court the females throughout the breeding season, which usually begins in May and ends in late summer or early fall depending on the geographic location; females build the nests and incubate eggs, and both parents protect and feed the nestlings and fledglings (Ketterson et al. 1998; Nolan et al. 2020; Reichard et al. 2017). They primarily nest on the ground and typically nest in a depression on sloping ground, under a protruding rock, amid the roots of a toppled tree, in sloping road cuts (especially if overhung by grass or other vegetation), under fallen tree trunks, or at bases of bushes, trees, or ferns (Nolan et al. 2020). Dark-eyed juncos often have more than one clutch during a breeding season, and the number of clutches per breeding season varies geographically (Nolan et al. 2020).

Research on dark-eyed juncos has provided valuable insights into various aspects of mate choice and reproductive success, including the morphometric, plumage, and acoustic traits that predict reproductive success (Whittaker and Gerlach 2016). Female juncos prefer larger males and males with experimentally enhanced white in the tail, which are known to dominate in aggressive encounters over resources (Mcglothlin et al. 2005; Whittaker and Gerlach 2016). When it comes to acoustic cues, male dark-eyed juncos produce two classes of song that appear to function as long-range and short-range signals (Titus 1998) (Figure 1.1). The long-range song is used in male-male competition and mate attraction (Ketterson et al. 1992; Titus 1998; Cardoso and Reichard 2016). The song consists of a single syllable repeated several times to form a trill, and song bouts normally repeat one song type (determined by the structure of the syllable composing the song) before switching to another type (Titus 1998; Newman et al. 2008). Each male typically has a repertoire of 2–8 different song or syllable types (Williams and MacRoberts 1977; Newman et al. 2008). Some studies suggest that junco song shows a performance trade-off, because the production of trilled songs requires rapid and precise coordination of vocal tract movements, resulting in a trade-off between trill rate and frequency bandwidth (Podos 1997; Cardoso et al. 2007; but see Kroodsma 2017). Cardoso et al. (2009) suggest that juncos use song types with higher performances (songs that cover the widest possible frequency bandwidth for a given trill rate) during bouts of more motivated singing (as evaluated by the average length of songs), suggesting that different song types are used in different contexts depending on their performance. Both males and females respond to the playback of long-range song throughout the breeding season by approaching the speaker, calling, and, in the case of males, producing their own long-range songs; these responses suggest that long-range songs are used in defending the territory (Cardoso and Reichard 2016; Nolan et al. 2020). There also is evidence that long-range songs are used in mate attraction, since unmated males in the middle of the breeding season sing more long-range songs than mated males (Ketterson et al. 1992). The short-range song usually comprises a variety of non-trilled and sometimes trilled syllables; it is frequently performed at a low amplitude during courtship (Titus 1998) and elicits an aggressive response from males with fertile partners (Reichard et al. 2011). Short range songs are extremely variable, and it is yet unknown what attributes might be attractive to females (Cardoso and Reichard 2016).

Dark-eyed juncos are a convenient model to study predictors of reproductive success because they are resident birds throughout the year in Newfoundland. Descriptions of the Newfoundland population from the 1940s described dark-eyed juncos as migratory and as uncommon winter residents (Burleigh and Peters 1951), but the population appears to have become resident year-round throughout the island during the last 75 years (Nolan et al. 2020). This makes it easier to study dark-eyed juncos in the context of predictors of reproductive success and makes them an interesting population to study considering that they are fairly recently established as a year-round resident population. Studying this population may also provide unique insight into the factors affecting reproductive success in this species, since the population is located at the extreme northeast of the species' range (Nolan et al., 2020), where it presumably experiences the harshest climatic conditions.

Research Objectives

My thesis research investigates whether song performance, body size, and ecological characteristics (nest microhabitat characters, weather parameters) predict various measures of reproductive success, including the number of eggs laid, number of eggs hatched, and the number of nestlings fledged.

In Chapter 2, I investigate whether song performance or the traits that compose it (trill rate and frequency bandwidth) are associated with reproductive success in male dark-eyed juncos. I hypothesize that vocal performance is subject to sexual selection, and predict that dark-eyed juncos with better vocal performance will have higher reproductive success. I also measure the body size of each individual as a possible predictor of reproductive success because body size has been shown to influence mate choice (Mcglothlin et al. 2005) and territory defence in dark-eyed juncos (Tamburello et al. 2015; Niederhauser et al. 2021) and vocal performance in other species (Podos 2001).

In Chapter 3, I investigate whether nest microhabitat characteristics are associated with egg hatching success and fledging success in dark-eyed juncos. I also test whether egg hatching success and fledging success are associated with weather parameters such as temperature and precipitation. I hypothesize that juncos have been selected to place nests in sites with better cover from predators and elements of weather, and thus I predict that nests placed in such sites will have higher hatching and fledging success. Given that Newfoundland is typically cool, wet, and windy, I predict that hatching and fledging success would be impacted more by hypothermia than by hyperthermia, and that hatching and fledging success would therefore be better in warmer and drier conditions.

Chapter 4 will integrate the information gathered in Chapters 2 and 3 with existing literature and provide a general discussion and conclusion to the study.

Co-authorship Statement

I conducted this research independently but with the guidance of my supervisor, Dr. David Wilson, who contributed to all aspects of this research, including statistical analysis and editing of the thesis chapters. Any manuscripts derived from Chapters 2 and 3 will include Dr. Wilson as a co-author. Data for Chapters 2 and 3 were collected by myself and by research assistants under my direct supervision. My supervisory committee, Dr. Carolyn Walsh and Dr. Pierre-Paul Bitton, aided with statistical analysis and revising the chapters. I wrote the initial drafts of all chapters.

1.1 Figures

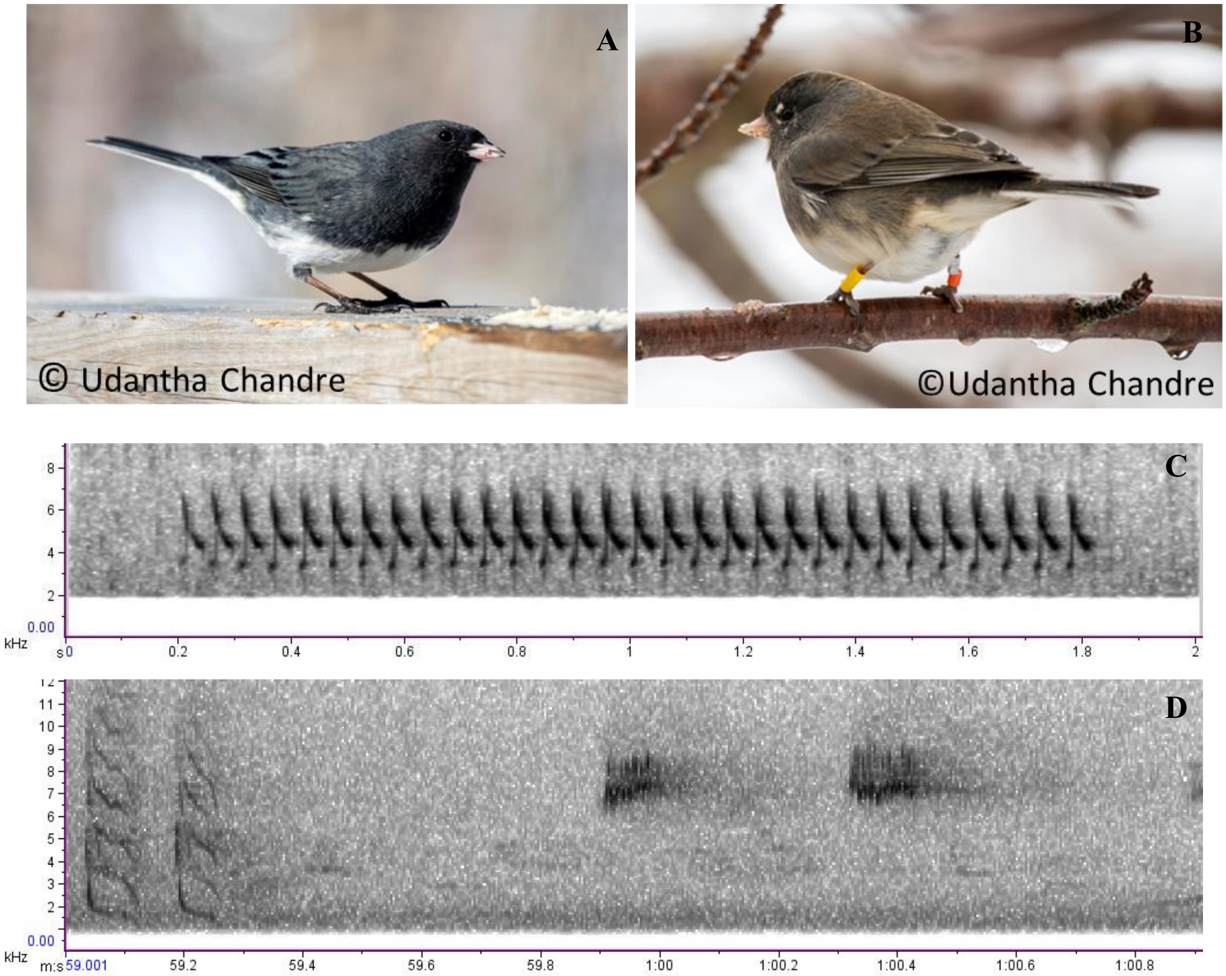

Figure 1.1: These images show a (A) male dark-eyed junco (B) a female dark-eyed junco (C) a spectrogram of a male dark-eyed junco long-range song, and (D) a spectrogram of a short-range song. Spectrograms were created using a 512-point fast Fourier transform, 87.5% overlap, and Hamming window.

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CHAPTER 2: SONG PERFORMANCE AND REPRODUCTIVE SUCCESS IN DARK-EYED JUNCOS

2.1 Abstract

According to the theory of sexual selection, sexual traits are linked with reproductive success. Bird song is a sexual trait that can communicate aspects of the signaler's quality to prospective mates and potential rivals, and signal traits that are physically challenging to produce can enforce signal honestly. In trilled bird songs, vocal tract mechanics impose a trade-off between frequency bandwidth and trill rate, with the upper limit of the bivariate distribution of these two traits indicating a performance limit. Trills closer to this performance limit are believed to be more difficult to generate, making them more appealing to females and more threatening to conspecific males. In this study, I tested whether song performance predicts male reproductive success in dark-eyed juncos (*Junco hyemalis*). Individual dark-eyed juncos have a small repertoire of trilled songs, but among members of the same population, song diversity is high and song type sharing is low. In my study, I did not find evidence of a performance trade-off, and average reproductive success per nesting attempt was not related to any measure of song structure (frequency bandwidth, trill rate, song duration) or body size. Discrepancies with previous studies of performance constraints in dark-eyed juncos and other species are discussed with reference to small sample size, methodological, and population differences.

2.2 Introduction

Songs are elaborate vocalizations used by many taxa for intraspecific communication (Searcy and Andersson 1986). Darwin (1871) suggested that songs evolved via sexual selection, and recent studies linking song production to reproductive success in the field and laboratory provide support for this theory (Searcy and Andersson 1986; Catchpole 1987). In birds, one of the primary functions of song is to defend a breeding territory and repel sexual competitors. In support of these functions, early naturalists noticed in temperate regions that song is produced primarily by males, and that the onset of singing coincides with territory acquisition and the onset of male aggression in spring (Catchpole and Slater 2008). Subsequent experiments showed that males that were surgically muted experienced more invasions into their territories, were involved in more fights, and were more likely to lose their territories, thus providing direct experimental evidence that song functions in territory defence (Catchpole and Slater 2008). Speaker replacement experiments similarly showed that males removed entirely from their territories could continue to repel territorial intrusions if they were replaced by loudspeakers broadcasting their songs (Catchpole 1987; Nowicki and Searcy 2005).

Apart from male contests and territorial defence, avian song functions in mate attraction, mate stimulation, and mate choice (Searcy and Andersson 1986; Catchpole 1987). For example, in cavity-nesting house wrens (*Troglodytes aedon*), females are significantly more likely to visit an unoccupied nest box containing a loudspeaker broadcasting male song than one containing a silent control loudspeaker (Johnson and Searcy 1996). Females stay for longer around the nest boxes broadcasting song and are more likely to show nest-building behaviour, which indicates that song prompts females to settle in a territory (Johnson and Searcy 1996). Females of several species similarly increase nest-building activity and courtship display when exposed to male songs, and they lay their clutches sooner when their mates sing more frequently (Nowicki and Searcy 2005). In Scott's seaside sparrows (*Ammodramus maritimus peninsulae*) and red-winged blackbirds (*Agelaius phoeniceus*), males that have been surgically muted also remain unmated and experience difficulty attracting and retaining mates, compared to normal unmuted males (Peek 1972; McDonald 1989).

Sexual selection can also influence variation in the production and structure of song among males. For example, males with larger song repertoires are more attractive to females in some species (Searcy 1984; Catchpole 1987; Nowicki et al. 2000), and in other species, males with larger song repertoires can acquire larger territories, retain those territories for longer, and achieve greater annual and lifetime reproductive success (Hiebert et al. 1989). Females of several species also prefer males that have larger repertoires, longer songs, or songs with higher syllable repetition rates, and such males consequently have better mating success (Eens et al. 1991; Wasserman and Cigliano 1991; Nowicki and Searcy 2005; Sung and Handford 2020). In other species, the frequency structure of song predicts female preference as well as the outcomes of male contests (Drăgănoiu et al. 2002; Gil and Gahr 2002; Nemeth et al. 2012).

Prospective mates and potential rivals can also consider a song's performance, which refers to how physiologically demanding the song is to produce (Cardoso et al. 2007). Factors such as body size, energy demands, aerobic capacity, and neuromuscular control of the vocal tract may all constrain song structure and song production (Podos 1997; but see Kroodsma 2017). One of the best-studied examples of a performance constraint in bird song is the trade-off between trill rate and frequency bandwidth in trilled vocalizations (Podos 1997). Producing a tonal syllable that spans a wide range of frequencies (i.e., frequency bandwidth) requires a bird to elongate its vocal tract and open its beak widely, but such dramatic reconfiguration of the vocal tract requires more time to complete and thus limits the rate at which multiple consecutive syllables can be produced (Hoese et al. 2000; Podos et al. 2004). Trills with low trill rates can therefore have either narrow or wide frequency bandwidth, but trills with high trill rates are constrained to have narrow frequency bandwidths. The resulting bivariate plot of frequency bandwidth on trill rate thus appears triangular, and the upper boundary of the triangular distribution is thought to reflect a performance constraint. Songs that are closer to the upper boundary of the distribution are thought to require greater neuromuscular control and are therefore considered to have greater vocal performance. Consistent with this idea, several studies have shown that high-performance trills are more attractive to females (Ballentine et al. 2004) and more threatening to rival males (Phillips and Derryberry 2017). For example, female swamp sparrows (*Melospiza georgiana*) exhibit more copulation solicitation displays in response to high-performance songs than in response to low-performance songs, which suggests that the females use vocal performance to assess males (Ballentine et al. 2004). Female canaries (*Serinus canaria*) alter their investment in eggs based on their mate's vocal performance (Garcia-Fernandez et al. 2013). In the context of male-male competition, the response to high versus low performance songs is variable (Phillips and Derryberry 2017). In red-winged blackbirds, males respond more strongly to lower performance songs, indicating that, when faced with an unfamiliar intruder, subjects might hesitate to approach an opponent with the ability and motivation to sing high performance songs (Cramer and Price 2007). In swamp sparrows, males with high-performance songs respond more strongly to intruders with high-performance songs, and males with low-performance songs respond less strongly to intruders with high-performance songs (Dubois et al. 2011; Moseley et al. 2013).

During the breeding season, male dark-eyed juncos (*Junco hyemalis*) produce simple trilled songs that appear to be subject to the same trade-off between frequency bandwidth and trill rate that has been observed in other species (Cardoso et al. 2007; Cardoso et al. 2009; Wilson et al. 2014). Since individual dark-eyed juncos have repertoires of two to eight song types, some studies suggest that the trade-off is created primarily by differences among song types (Cardoso et al. 2007). Consistent with this idea, the song performance of different individuals is similar when they all sing the same song type, but variable when they sing different song types (Cardoso et al. 2009). Regardless of how variation in song performance is created in dark-eyed juncos, few studies have tested for a relationship between song performance and reproductive success in field conditions (Ferreira et al. 2016). There is evidence, however, that dark-eyed juncos use song in territory defence, since males sing in response to intruding males and continue singing throughout the breeding season even after attracting and copulating with a mate (Cardoso and Reichard 2016; Titus 1998). Dark-eyed juncos also appear to use song for female attraction, since unmated males sing more than mated males (Ketterson et al. 1992). It is unclear whether song functions in stimulating females because males do not increase their singing rates when their mates are fertile (Titus et al. 1997).

In this study, I investigated whether song performance or the traits that compose it (trill rate and frequency bandwidth) are associated with reproductive success in male dark-eyed juncos. I did this by recording the songs of individually marked wild dark-eyed juncos, measuring the temporal and frequency attributes of their song, and testing for a trade-off between trill rate and frequency bandwidth. I also recorded the number of fledglings produced by each male as an estimate of their reproductive success. I hypothesized that vocal performance is subject to sexual selection, and that dark-eyed juncos with better vocal performance would have higher reproductive success. I also measured the body size of each individual as a possible predictor of reproductive success, since body size has been shown to influence mate choice in dark-eyed juncos (Mcglothlin et al. 2005), as well as territory defence (Tamburello et al. 2015; Niederhauser et al. 2021) and vocal performance in other species (Podos 2001).

2.3 Methods

Study site

Fieldwork was conducted at the Memorial University Botanical Garden (47º 34´N 52º 45´W), which is located within Pippy Park in St. John's, Newfoundland and Labrador, Canada. The Botanical Garden encompasses 0.44 km^2, which includes a 0.07 km^2 landscaped garden area and a 0.37 km^2 area containing natural ground vegetation, balsam fir (*Abies balsamea*) and black spruce (*Picea mariana*) as dominant tree species, and a trail system throughout.

Banding and morphometric measurements

Dark-eyed juncos were captured during the 2018 (N = 54) and 2019 (N = 53) winter seasons using treadle traps that were baited with sunflower seeds and placed on wooden platforms. Once a bird was captured, I measured tarsus length with a dial vernier calliper (+/- 0.5 mm). Wing chord and tail length were measured with a 15 cm wing and tail ruler (+/- 1 mm), and body mass with a Pesola 60 g spring scale (+/- 1 g). Age and sex were estimated based on plumage (Pyle 1997). A sample of ca. 50 µL blood was collected from each individual as part of another study by puncturing the brachial vein with a 26.5-gauge needle and collecting the blood in a microcapillary tube. Before releasing a bird, it was ringed with three plastic colour bands and one federal metal band for future identification. All captured birds were held in cloth bags when not being measured and were always banded and then released at their site of capture within ca. 45 minutes of capture.

Mapping territories

Although there are no previous studies of individually marked dark-eyed juncos in Newfoundland and Labrador, dark-eyed juncos are present at my study site throughout the year, and I therefore expected that birds banded in winter would establish their territories in the same general area during the breeding season. Contrary to this expectation, I observed high levels of dispersal out of the Botanical Garden and some new recruitment into the population during the breeding season. Consequently, even though I banded most of the dark-eyed juncos in the Botanical Garden in winter, the number of banded individuals remaining in the breeding season was low. Nevertheless, I mapped the territories of all previously banded male dark-eyed juncos that were present in the Botanical Garden between May and August 2018 (N=5) and 2019 (N=6). One male dark-eyed junco was studied in both 2018 and 2019, and I treated each year as a separate data point. I did not attempt to capture additional males during the breeding season because I needed to focus on mapping territories and recording songs; furthermore, males can be captured efficiently during the breeding season only by luring them into mist nets with playbacks simulating territorial intrusions, and I deemed this to be too disruptive.

I mapped the territory of each colour-banded male by following it for 2–3 hours and marking all of its singing perches with a handheld GPS (Garmin GPS map 78s; Kansas, USA; accuracy ca. ±3 m). To ensure that I had mapped the entire territory, I revisited the territory throughout the breeding season and marked the singing perches to ensure that I had captured its core territory and most of its singing perches. GPS coordinates of singing perches were plotted on a topographic map and territory maps were estimated and drawn using the minimum convex polygon method, implemented in ArcMap 10.3.1 (ESRI 2015).

Reproductive success

After mapping territories, I followed breeding pairs within their territories and searched for active nests using behavioural cues such as nest building, incubating, and nestling feeding. Once an active nest was located, I visited it once daily until the fledglings left the nest or the nest was depredated. The number of eggs, the number of eggs hatched (as evidenced by presence of nestlings in the nest), and the number of nestlings fledged (as evidenced by an empty nest following the 14-day nestling period or observing the fledglings with the banded male or female outside the nest) were recorded each day.

Dark-eyed juncos produce multiple nests in a given breeding season regardless of the success or failure of each nesting attempt (personal observation based on this study population). Nests are cryptic and therefore I was unable to locate all nests for a given breeding pair in a given breeding season. Therefore, it was not possible to measure annual reproductive success for each male, which is the total number of fledglings sired by the male in a breeding season. Instead, I measured average reproductive success among the nests that I did locate. I defined average reproductive success as the total number of fledglings sired by the male among all of his nests that I located in a given breeding season, divided by the number of nests that I located for that male during the same breeding season. This measure of reproductive success is not affected by the number of nests or fledglings that I was unable to locate for a given male. Note, however, that this measure of reproductive success could be influenced by when in the season nests were found, since clutch size has been shown to decrease by approximately one egg throughout the season in some populations (Smith and Anderson 1982).

Song recording

After locating a nest for a given male, I recorded his songs opportunistically between 05:00–19:00 h. Recordings were made when there was no precipitation, when the wind speed was below 30 km/h, and before the nestlings had left the nest. Although different males were recorded at different times of the year, there is no evidence that dark-eyed junco song structure changes throughout the year (Williams and MacRoberts 1977), though total song production does decline throughout the season (Titus 2002). During a recording session, I attempted to record at least 20 songs from one singing bout as the male patrolled his territory. If 20 songs were not produced during one singing bout, I revisited the territory later the same day or on a subsequent day to complete the recording. I chose to record 20 songs per male because it allowed me to balance the number of songs recorded per male and the number of males recorded. In dark-eyed juncos, males can repeat the same song type from one (that was observed once) to as many as 167 times within a one singing bout as explained by Newman et al. (2008). Therefore, it is unlikely to capture the full repertoire of each male from 20 songs, although it should allow to estimate inter-individual variation in the duration, frequency bandwidth, and vocal performance of songs. Songs were acquired with a Talinga 22” Parabolic reflector and an MKH20-P48 omnidirectional microphone connected to a Marantz professional PMD 661 MKII solid-state digital audio recorder (16-bit amplitude encoding; WAVE format; 44.1 kHz sampling rate; Marantz professional, Cumberland, USA).

Song selection and measurement

For each subject, I visualized its recordings as spectrograms (Hamming window, 512-point fast Fourier transform (FFT), 87.5% overlap) in Audacity software (Audacity recording and editing software, version 2.3.1; https://audacityteam.org/) and selected 15 high-quality songs with no overlapping background noise. Using the seewave and tuneR packages (Sueur et al. 2008; Ligges et al. 2018) in R (version 4.3.1; R Development Core Team, 2022), I quantified the signal-to-noise ratio of each selected song by comparing the mean amplitude of the song to the mean amplitude of the background noise from the 0.5 s immediately preceding the song. Signal-to-noise ratios of the unfiltered songs averaged 1.15 (SD ± 0.44). I then filtered the recordings with a 2-kHz high-pass filter and re-calculated the signal-to-noise ratios. The average signal-to-noise ratio after filtering was 13.90 (SD ± 18.83). Finally, each song, plus 0.2 s of background noise preceding and following the song, was normalized to a peak amplitude of 0 dB and exported as a standalone clip (WAVE format, 16-bit amplitude encoding, 44.1 kHz sampling rate) for further analysis.

For each exported song, I constructed a frequency spectrogram and mean power spectrum (Hamming window, 512-point FFT, 87% overlap) in Raven Pro (version 1.5; Bioacoustics Research Program, 2014). Following Zollinger et al. (2012), frequency bandwidth was calculated as the difference between the maximum and minimum frequencies occurring 20 dB below the peak amplitude on the mean power spectrum (Figure 2.1). I selected a -20 dB threshold because it captured most of the song's energy without being obscured by background noise. Trill rate (also known as syllable repetition rate in some studies) was calculated by dividing the number of syllables in the song, by song duration, which was measured on the spectrogram as the period from the start of the first note to the end of the last note. The number of syllables in the song was obtained by counting the number of discontinuous and nonoverlapping traces on the spectrogram.

The 15 songs selected from each male were grouped according to song type based on their syllable structure, as visualized on the spectrogram. The song types in my sample do not represent the complete song type repertoire of this population because a sample of 15 songs per male from 11 males is insufficient for characterizing individual-level or population-level repertoires. I present the song types for descriptive purposes and to provide a foundation for future research on this population.

Vocal performance

Most vocal performance studies have used the upper bound regression method to test for evidence of a vocal performance constraint (Blackburn et al. 1992). In this method, songs are plotted on a bivariate plot of frequency bandwidth versus trill rate. Trill rate is divided into bins of equal width and the maximum frequency bandwidth value from each bin is regressed against bin number (Blackburn et al. 1992; Podos 1997). A significant inverse relationship is considered evidence of a vocal performance constraint (Podos 1997). The orthogonal deviation of a song from the upper bound regression provides an estimate of the song's vocal performance, with smaller deviation values (i.e., songs closer to the upper boundary) reflecting better vocal performance (Ballentine et al. 2004). However, Wilson et al. (2014) showed that the upper bound regression method of detecting and estimating performance constraints is inaccurate and suggested that the method be superseded by 90% quantile regression. Cardoso (2019) then showed that coefficients derived from 90% quantile regression are influenced by the arbitrary assignment of trill rate and frequency bandwidth to the independent and dependent variables and proposed instead the double quantile regression method, which controls for this issue (Cardoso 2019). In the current study, I therefore use 90% double quantile regression to test for a trade-off near the upper boundary of the trill rate versus frequency bandwidth distribution of dark-eyed junco songs. Models were implemented with the lqmm package (Geraci 2014; Geraci and Matteo 2014) in R (version 4.3.1; R Development Core Team, 2022) and included bird identity and breeding year as random effects, as suggested by Kroodsma (2017). There was one male that was recorded in both 2018 and 2019, and I treated each year as a separate data point.

Contrary to previous studies that used different statistical methods and different subspecies of dark-eyed juncos, I did not find any evidence of a relationship between trill rate and bandwidth (see results, below) and therefore could not calculate vocal deviation as a measure of vocal performance.

Statistical Analysis

A principal components analysis was used to reduce four morphometric measurements (mass, wing chord length, tail length, and tarsus length) from 74 male dark-eyed juncos to a smaller number of factors representing ‘body size’. Using varimax rotation, only one factor with an eigenvalue greater than one was created, and this was used to represent body size. Factor scores were calculated for the 11 males for which nests were located and were used in a linear regression as a predictor of average reproductive success.

To assess whether average reproductive success is associated with song structure, I used three separate linear regression analyses, with average reproductive success per nesting attempt as the dependent variable in each. Average frequency bandwidth, average trill rate, and average song duration were included separately as single predictors in the three regressions because the small sample size precluded a multivariate analysis. The three predictor variables were not significantly correlated with each other or with body size (Table 2.1).

2.4 Results

I observed 10 breeding males in the 2018 and 2019 breeding seasons, including one male observed in both years. Based on the 15 songs analyzed for each male (or 30 songs for the male studied in both years), I identified 13 different song types based on distinctive syllable structures (Figure 2.*2*). Individual males produced between 1 and 3 song types, though this likely underrepresents the true repertoire size due to insufficient sampling of each male.

During the two breeding seasons, I observed 16 nests belonging to 10 males. Those nests contained an average of 3.44 eggs (SD = ±0.81, Min = 1, Max =4) per nest and produced an average of 2.63 nestlings (SD = ±1.36, Min = 0, Max = 4) and 1.94 fledglings (SD = ±1.57, Min = 0, Max = 4) per nest.

Principal component analysis reduced the four morphological variables (body mass, wing chord length, tarsus length, and tail length) from 74 male dark-eyed juncos banded in winter (one male included twice because its average reproductive success and body size were measured in both years) to one factor that accounted for 53.53% of the original variation. The correlation matrix and factor loading scores for body mass, wing chord length, tarsus length, and tail length are presented in Table 2.2 and Table 2.3, respectively. For those males that were studied during the breeding season, average reproductive success per male was not related to body size, as measured during the preceding winter ($R^2 = 0.219$, $F_{1,9} = 2.531$, $p = 0.146$; Figure 2.3).

Double quantile regression did not find any evidence of a performance limitation, as neither the 90% quantile regression of frequency bandwidth on trill rate (slope = 0.003, p =

0.956, N=180) nor the 90% quantile regression of trill rate on bandwidth (slope = 2.174, p = 0.178, N=180) were statistically significant (Figure 2.4). Consequently, measures of vocal deviation were not calculated and compared to average reproductive success. Average reproductive success was not significantly related to any of the measures of song structure, including song duration ($R^2 = 0.171$, $F_{1,9} = 1.860$, p = 0.206), frequency bandwidth ($R^2 = 0.051$, $F_{1,9} = 0.488$, p = 0.502), and trill rate ($R^2 = 0.002$, $F_{1,9} = 0.015$, p = 0.906; Figure 2.5).

2.5 Discussion

In this chapter, I assessed whether song structure and body size predict average reproductive success per nesting attempt in male dark-eyed juncos. The results indicate that neither body size nor song structure are associated with average reproductive success per nesting attempt. In fact, contrary to my prediction and previous research, I did not find evidence that dark-eyed junco songs are subject to a performance trade-off between trill rate and frequency bandwidth (Cardoso et al. 2007; Cardoso et al. 2009; Cardoso and Atwell 2016; Wilson et al. 2014).

A study on an alpine population of rock sparrows (*Petronia petronia*) in south-east France revealed that males with high reproductive success sang with higher maximum frequencies and at low song rates (Nemeth et al. 2012). In a wild zebra finch (*Taeniopygia guttata*) population, males with longer and more complex songs had greater reproductive success, and the song structure predicted hatching success and the number of genetic offspring surviving until they fledged (Woodgate et al. 2012). In male house wrens (*Troglodytes aedon*), however, trill consistency (how precisely syllables are repeated) and vocal deviation did not correlate with reproductive success, and the males with "better" songs did not have higher mating success or higher reproductive success (Cramer 2013). Therefore, some studies support the theory that male song structure predicts reproductive success and some studies do not. The differences in outcomes could be due to species-specific effects, differences in mating systems, and the different methodological approaches of each study (Soma and Garamszegi 2011).

Another possible explanation for why song structure did not predict reproductive success is that the function of song in dark-eyed juncos is unclear. If song structure functions to communicate male quality to prospective mates, then we might expect males singing high-quality songs to be paired to high-quality females that produce more offspring, or to females that invest more heavily in current reproduction, either of which would lead to a relationship between song structure and male reproductive success. Similarly, males singing songs that reflect their underlying quality might be expected to sire more extrapair offspring, which would further drive a relationship between song structure and male reproductive success. Indeed, 20-30% of offspring in other populations of dark-eyed juncos are sired by extra-pair males, though it remains unclear whether song structure differs between males with high extrapair paternity and those with low or no extrapair paternity (Ferree 2007; Gerlach et al. 2012; Atwell et al. 2014). In contrast, if songs function primarily to attract or stimulate a female, as suggested by some previous research (Ketterson et al. 1992; Cardoso and Reichard 2016), then we might expect variation in song structure to predict only whether a male obtains a mate that in turns builds a nest. Since my sample did not include unpaired males or males whose mates failed to produce a nest, it was not possible to test this hypothesis. Finally, it is possible that my small sample size or choice of song structure traits obscured a potential relationship between a male's song structure and reproductive success.

Contrary to previous studies, I did not find evidence of a trade-off between frequency bandwidth and trill rate in dark-eyed juncos (Cardoso et al. 2007; Cardoso et al. 2009). A possible explanation for this discrepancy is that each male dark-eyed junco sings a repertoire of two to eight song types (Cardoso and Reichard 2016). Within a population, song type sharing is low and song type diversity is high, with some song types being sung by only one male in the population (Cardoso et al. 2009, 2012; Cardoso and Reichard 2016; Newman et al. 2008). In my study, I recorded 13 different song types from 10 different males. It is possible that a trade-off between frequency bandwidth and trill rate only becomes apparent when the sample includes song types that are extreme in these respects, and that the probability of detecting such a pattern increases as more song types are recorded from each male; thus, the limited number of song types sampled from each male in my study, and the limited number of males sampled from the population, may have concealed a performance limit (Cardoso et al. 2012). According to Cardoso et al. (2009), the differences in performance between song types within the repertoires of dark-eyed junco males are higher than the differences in performance within a given song type among males. Song performance may therefore depend on which song type a male sings. Furthermore, previous research on dark-eyed juncos suggests that different song types might have different functions (Cardoso et al. 2012), and previous research on other species suggests that repertoire size itself might be used as a basis for opponent assessment or mate choice (Catchpole 1986; Hasselquist 1998). Future research on my study population therefore should use a larger sample of songs and males to capture complete repertoires and to test for evidence of performance limits within and among song types. In the current study, my sample of 15 songs per male was not sufficient to ensure that I had sampled a male's entire repertoire or to enable me to include song type as a factor in my analysis.

Several methodological differences between my study and previous studies could also explain why I did not find evidence of a performance limit. First, previous studies that found evidence of a performance limit in dark-eyed juncos were conducted in California, USA (Cardoso et al. 2007; Cardoso et al. 2009; Cardoso and Reichard 2016) on the *J. h. thurberi* subspecies, whereas my study was conducted in Newfoundland and Labrador, Canada on the *J. h. hyemalis* subspecies. Song structure may be subject to different selective pressures in these two geographically distant subspecies (Liu et al. 2008). Second, some of the songs analyzed in previous research were elicited using playback of a simulated intruder's songs (Cardoso et al. 2007), which has been shown to affect song structure in dark-eyed juncos and other species (Cardoso et al. 2009; Dubois et al. 2009, 2011). In contrast, all songs included in my study were recorded opportunistically from males that were already singing. Third, I measured frequency bandwidth using an amplitude-defined threshold on a power spectrum, as recommended by Podos (1997) and Zollinger et al. (2012). In contrast, previous studies that found evidence of a performance limit in dark-eyed juncos measured frequency bandwidth from a spectrogram (Cardoso et al. 2007; Cardoso et al. 2009), a method which has been criticized for being subjective (Zollinger et al. 2012). Fourth, previous studies on dark-eyed juncos tested for evidence of a performance limit using upper bound regression (Blackburn et al. 1992; Cardoso et al. 2007; Cardoso et al. 2009), which has since been criticized for being biased and for increasing the risk of false positives (Wilson et al. 2014). When data from those previous studies were re-analyzed, statistical support for a vocal performance constraint became inconsistent. For the smaller dataset (N=17) derived from Podos (1997), the regression describing the potential performance limit was non-significant when calculated using either upper bound regression or 90% quantile regression (Wilson et al. 2014). For the larger dataset (N=188) derived from Cardoso et al. (2007), the regression was statistically significant when calculated with upper bound regression and non-significant when calculated with 90% quantile regression (Wilson et al.

2014); when calculated with 90% double quantile regression (Cardoso 2019), the results were mixed, with the regression of bandwidth on trill rate being significant and the regression of trill rate on bandwidth being non-significant. Evidence for a vocal performance limit in dark-eyed juncos therefore is inconsistent across studies and statistical methods, and weak even when sample sizes are large. In my study, I used double quantile regression with individual bird identity and breeding year as random effects. This statistical approach is the current best-practice for testing for vocal performance constraints in trilled songs.

In conclusion, the results of this study suggest that dark-eyed junco songs are not subject to a performance limit that is manifested as a trade-off between frequency bandwidth and trill rate. Similar to previous studies, I observed high song diversity among males, which, when combined with my small sample size, may contribute to the lack of a significant relationship between frequency bandwidth and trill rate. Contrary to my hypothesis, song structure was not correlated with average reproductive success in male dark-eyed juncos. In Chapter 3, I therefore test whether male reproductive success is explained by territory quality.

2.6 Tables

Table 2.1: Pearson correlations among three song structure variables and body size. Correlation coefficients are given above the diagonal and p-values are given below the diagonal (2-tailed, N=11). (One male is included twice because its songs were recorded and its body size measured in two years.)

Variable	Frequency bandwidth	Trill rate	Song duration	Body size
Frequency bandwidth	-	-0.059	0.448	-0.095
Trill rate	0.863	-	0.011	-0.340
Song duration	0.167	0.974	-	-0.220
Body size	0.785	0.307	0.515	-

Table 2.2: Pearson correlations among four morphometric variables. Correlation coefficients are given above the diagonal and p-values below the diagonal (1-tailed, N = 75). One male is included twice because its body size was measured in two years.

Variable	Wing chord	Bird weight	Tarsus length	Tail length
Wing chord	-	0.283	0.387	0.525
Bird weight	0.007	-	0.345	0.295
Tarsus length	0.000	0.001	-	0.425
Tail length	0.000	0.005	0.000	-

Table 2.3: Factor loading scores from the principal components analysis of male dark-eyed junco morphometrics (N = 75 male dark-eyed juncos). Orthogonal rotation method: varimax.

Variable	Factor 1
Tail length	0.789
Wing chord	0.767
Tarsus length	0.741
Bird weight	0.618

2.7 Figures

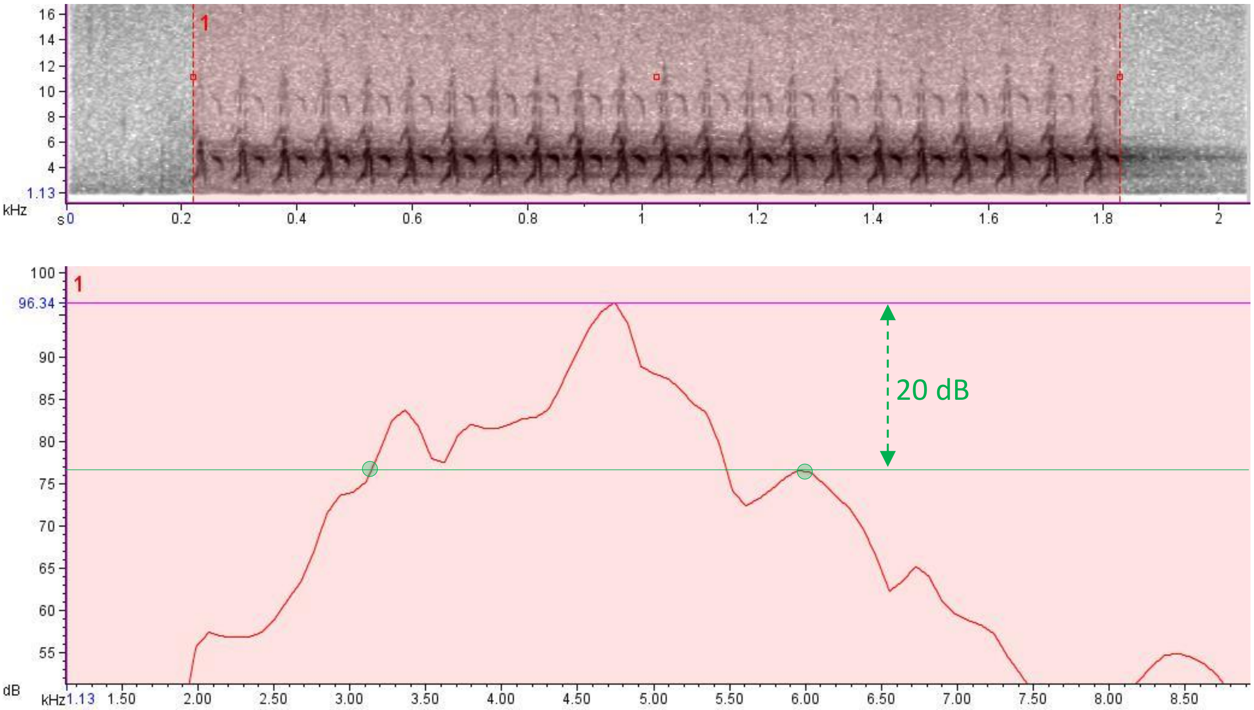
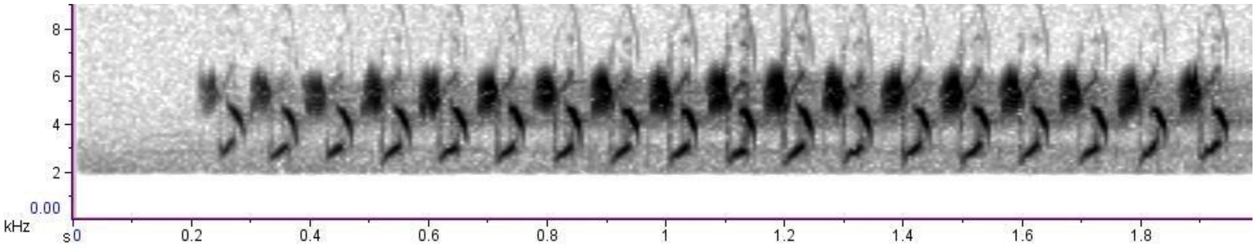
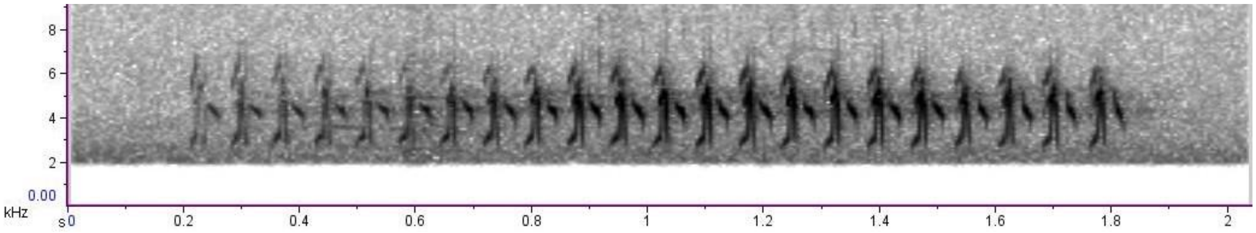


Figure 2.1: A spectrogram (top) and mean power spectrum (bottom; Hamming window, 512-point FFT, 87% overlap) of a dark-eyed junco song. The pink horizontal line indicates the peak amplitude of the mean power spectrum. Minimum and maximum frequencies were defined as the minimum and maximum frequencies of the mean power spectrum that were within 20 dB of the peak amplitude (the left and right green dots, respectively). Frequency bandwidth was the difference between the maximum and minimum frequencies, which for this song was 2.9 kHz. Trill rate was 13.56 syllables per second, which was calculated as the number of syllables in the song (22) divided by song duration (1.622 s).

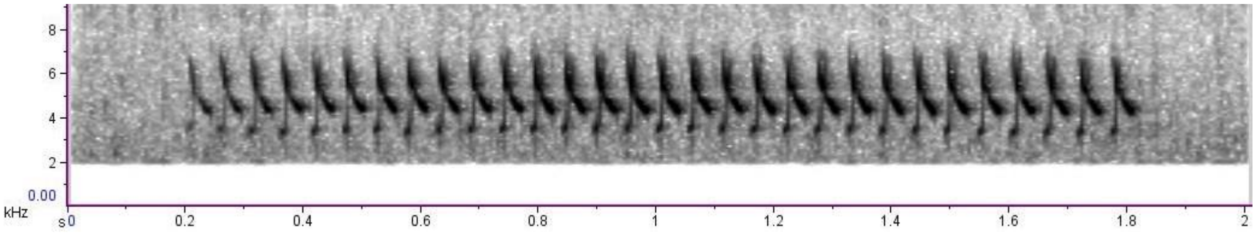
Song type 1



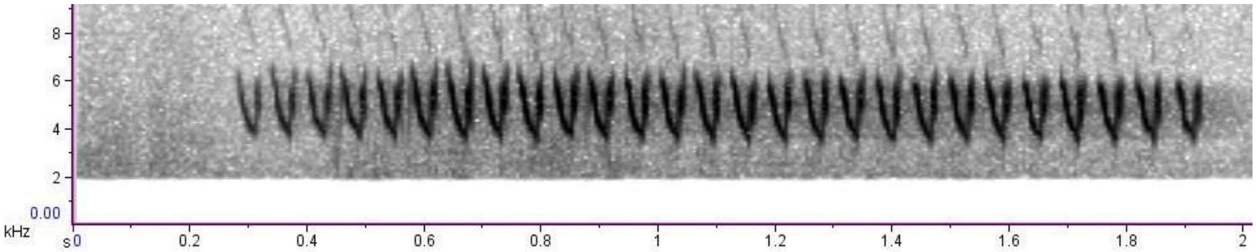
Song type 2



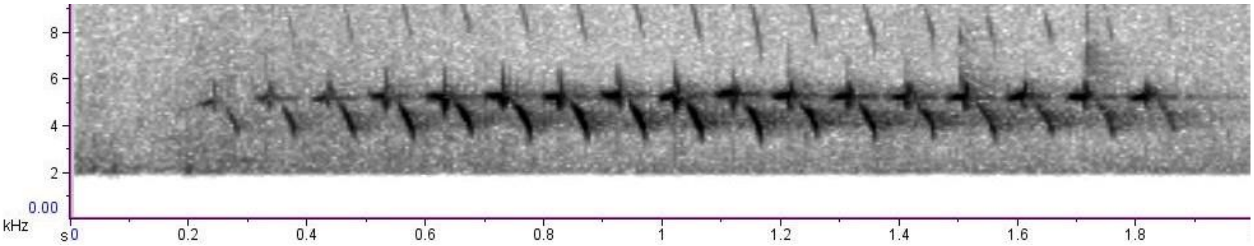
Song type 3



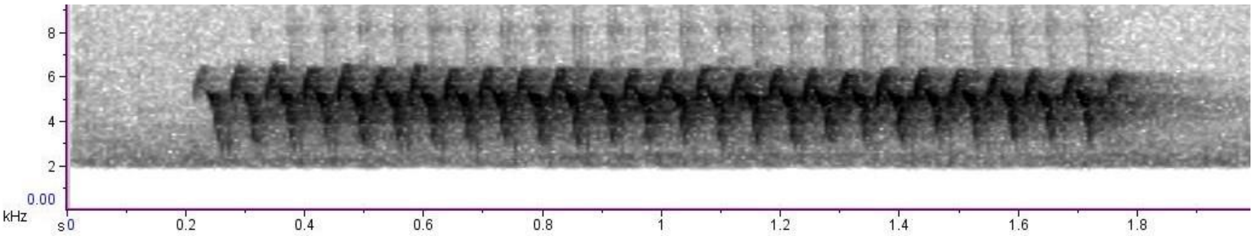
Song type 4



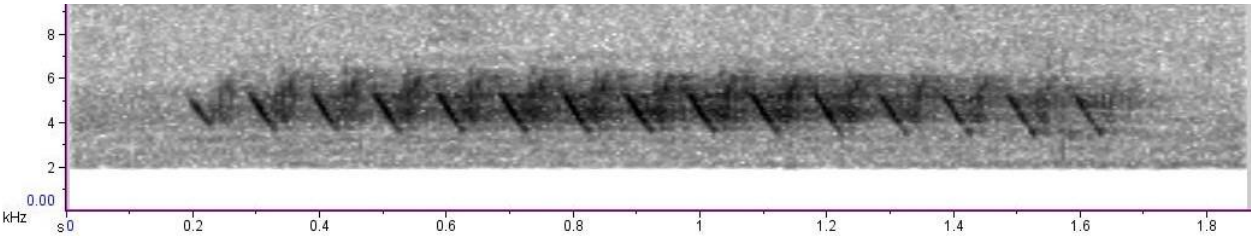
Song type 5



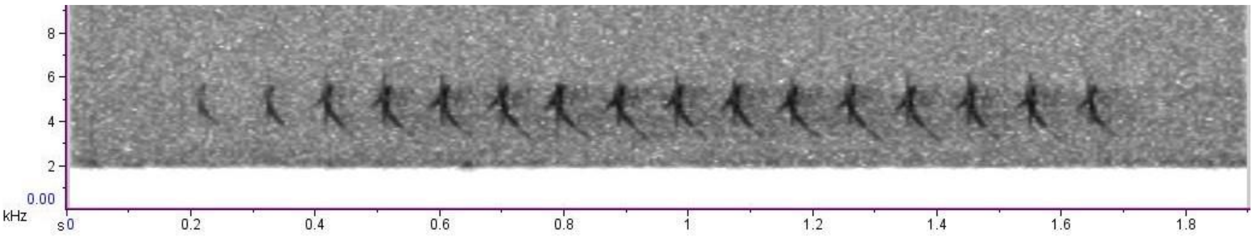
Song type 6



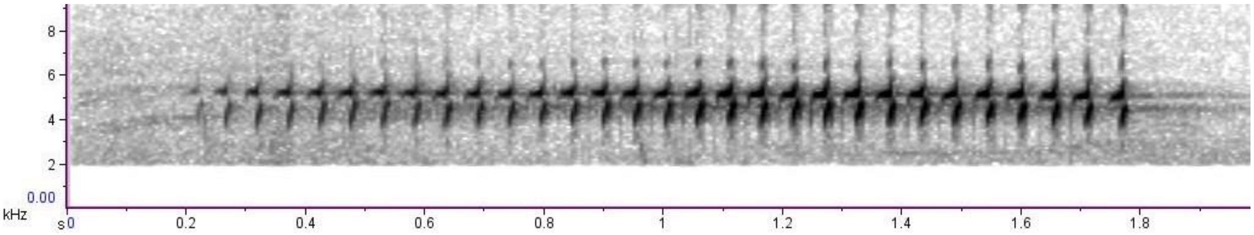
Song type 7



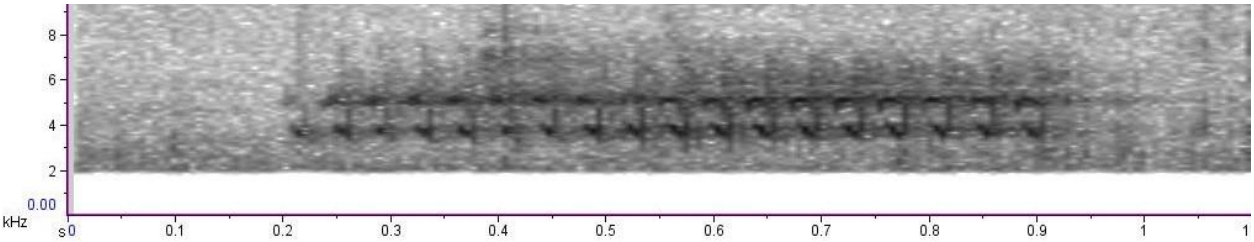
Song type 8



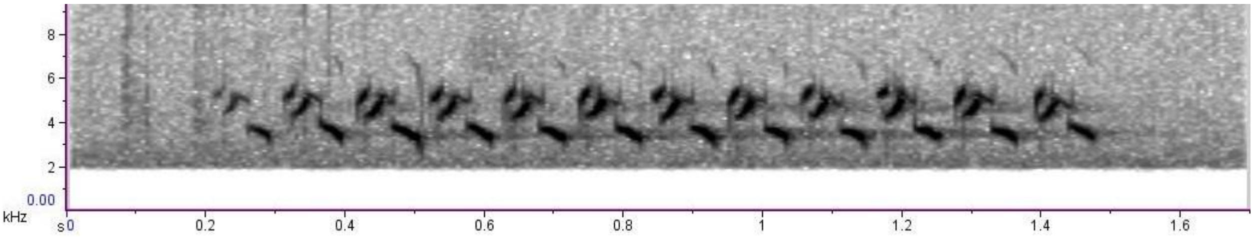
Song type 9



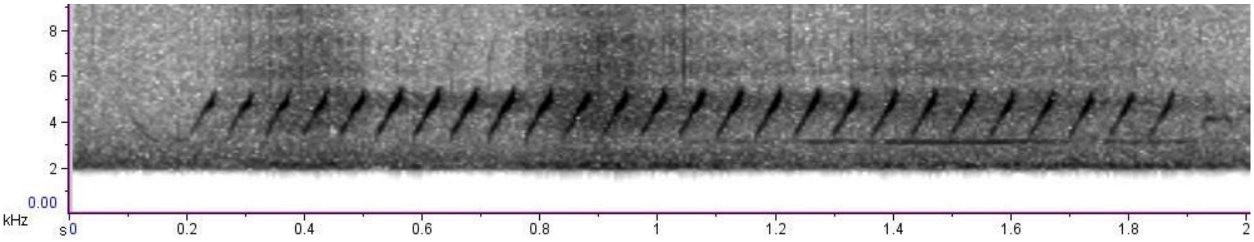
Song type 10



Song type 11



Song type 12



Song type 13

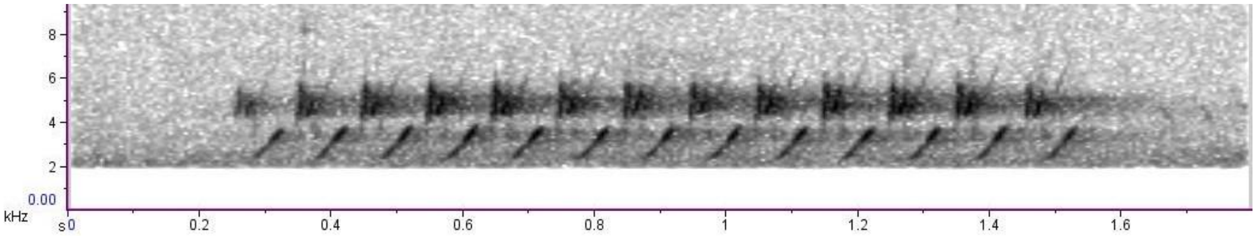


Figure 2.2: Song types of male dark-eyed juncos recorded during two breeding seasons in St. John's, Canada. Based on 15 songs per male and 10 males, a total of 13 different song types were identified.

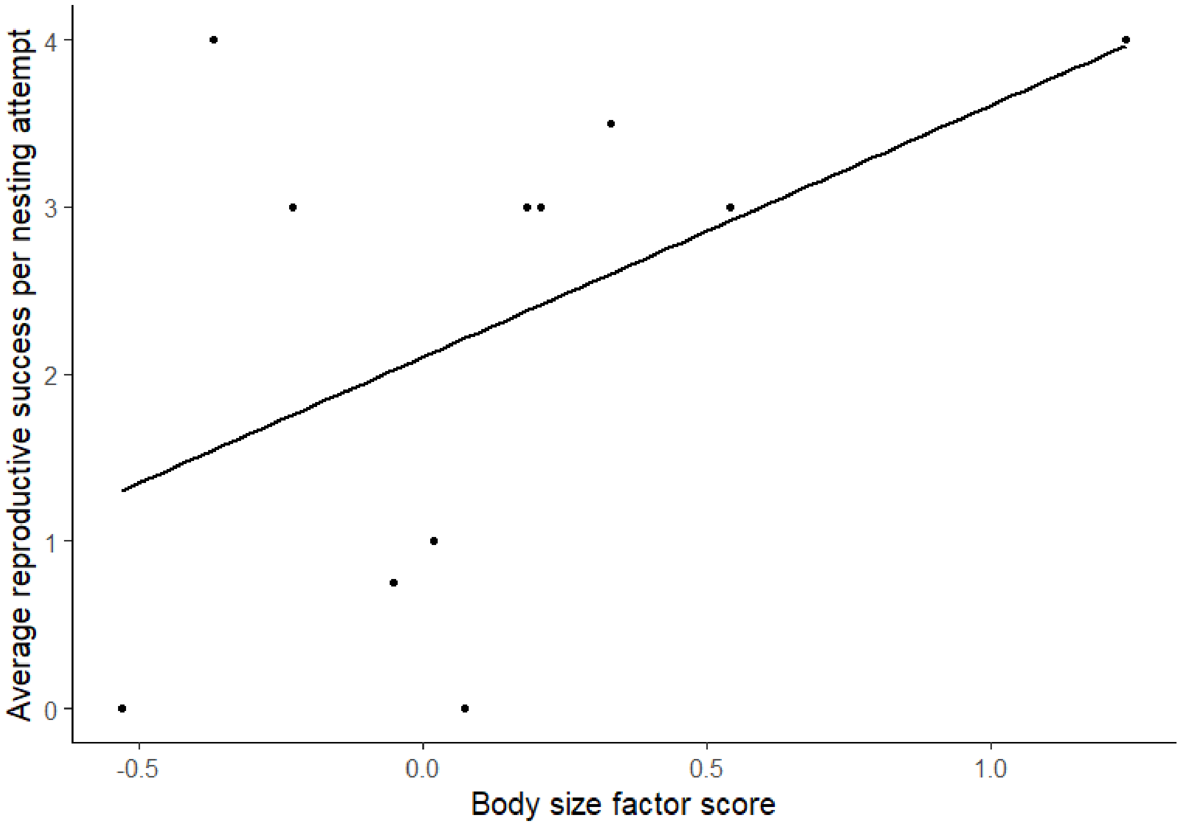


Figure 2.3: Scatter plot of average reproductive success per nesting attempt versus body size (obtained from principal component analysis) in 10 male dark-eyed juncos studied during the 2018 and 2019 breeding seasons in St. John's, Canada ($R^2 = 0.219$, $F_{1,9} = 2.531$, $p = 0.146$). One male is depicted twice because his average reproductive success and body size were measured in both years. A male's average reproductive success per nesting attempt is the average number of chicks that fledged per nest from among all his nests that I located that year.

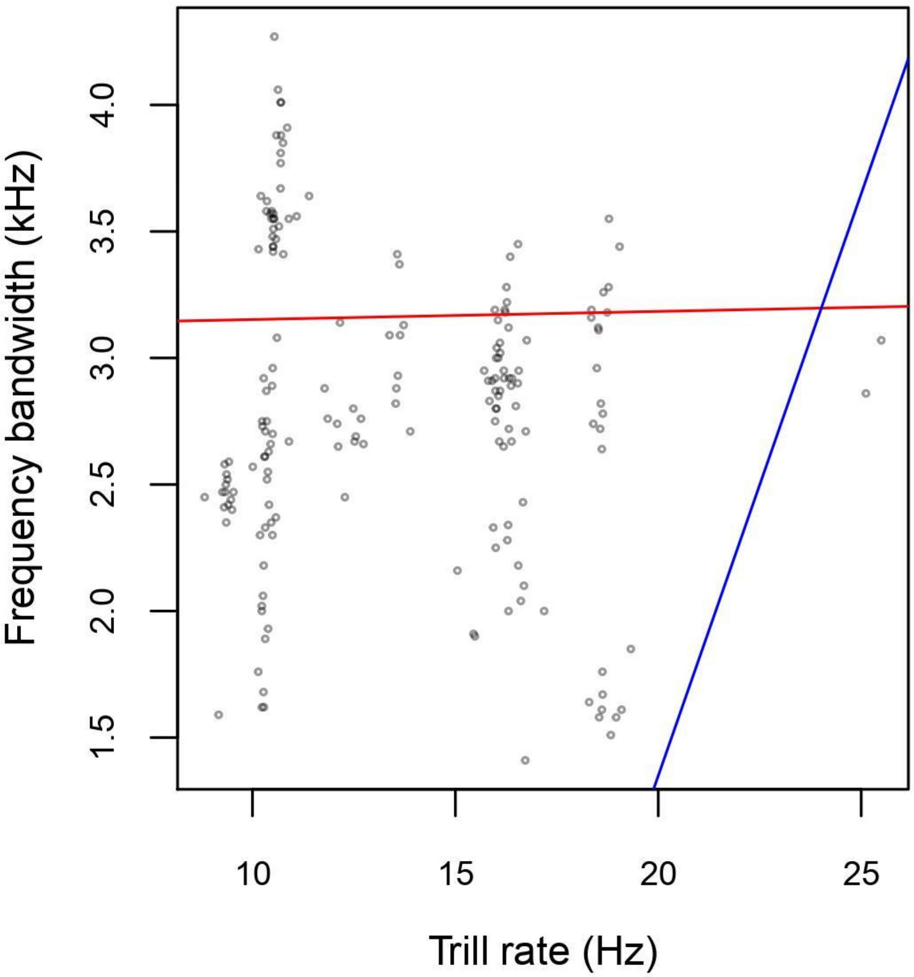
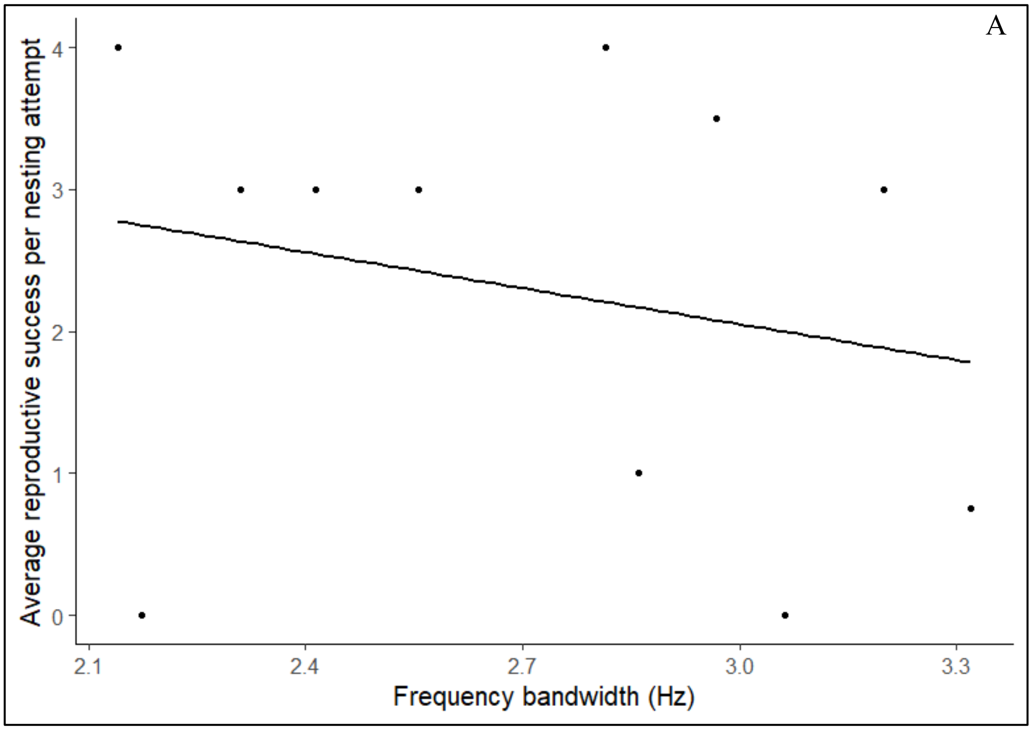
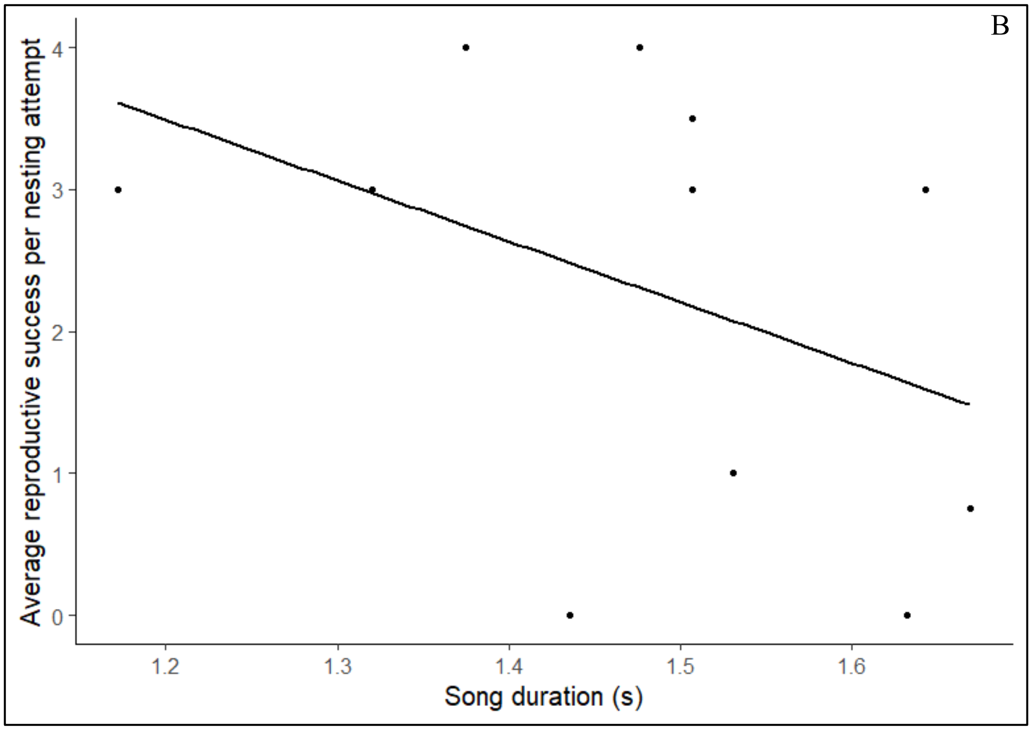


Figure 2.4: Scatterplot of frequency bandwidth versus trill rate in the songs of the dark-eyed juncos. The red line represents the 90% quantile regression of frequency bandwidth on trill rate, and the blue line represents the 90% quantile regression of trill rate on frequency bandwidth.


A
Average reproductive success per nesting attempt
0
1
2
3
4
Frequency bandwidth (Hz)
2.1
2.4
2.7
3.0
3.3


B
Average reproductive success per nesting attempt
0
1
2
3
4
Song duration (s)
1.2
1.3
1.4
1.5
1.6

B

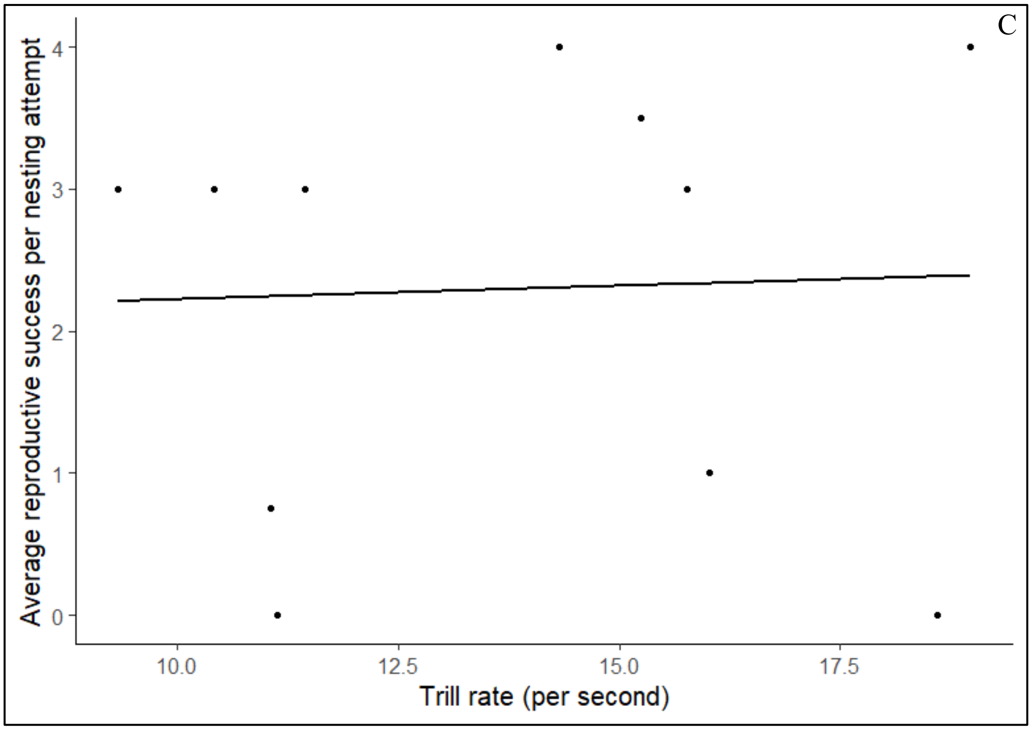


Figure 2.5: Scatter plots of average reproductive success per nesting attempt versus three measures of song structure, including (A) frequency bandwidth, (B) song duration, and (C) trill rate. A male's average reproductive success per nesting attempt is the average number of chicks that fledged per nest from among all his nests that I located that year.

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CHAPTER 3: NEST MICRO-HABITAT, WEATHER, AND REPRODUCTIVE SUCCESS IN DARK-EYED JUNCOS

3.1 Abstract

The selection of habitat by animals is influenced by the associated implications for fitness and the availability of critical resources. Nest site selection in birds is an example of habitat selection, with choices impacting the fitness of parents and young. Factors such as food availability, nest predation risk, and nest microclimate play crucial roles in reproductive success. However, selecting an ideal nest site that optimizes all these factors may not always be possible, leading to trade-offs. This chapter focuses on the reproductive success of breeding dark-eyed juncos (*Junco hyemalis*) in relation to nest microhabitat and weather parameters, with the general prediction that hatching and fledging success are higher for nests with better cover from predators and inclement weather, and for nests that experience warmer temperatures and less precipitation. The results from my study suggest that hatching success is significantly higher when canopy cover and average daily precipitation are lower, and that it tends to be higher in nests with better insulation and at sites with lower stem density. Fledging success was not significantly associated with any microhabitat or weather variables, but it tended to be higher for nests with lower exposure. Future research should focus on how predator density, specifically red squirrel density, affects the nesting success of ground-nesting passerines, and whether such relationships are mediated by red squirrel preferences for habitats with dense canopy cover and high stem density. Future research also should focus on how weather events like late spring cold snaps, snowfalls, and heavy rain affect the nesting success of ground-nesting passerines, as this would provide insight into the vulnerability of species to population declines arising from climate change.

3.2 Introduction

Given that habitats vary in their biological and physical qualities, animals are expected to select and occupy habitats that confer high fitness (Fretwell and Lucus 1969; Chalfoun and Schmidt 2012). In birds, the placement of a nest in dense vegetation is a common example of habitat selection. Nest location can influence the fitness of parents and young because it determines the availability of critical resources such as food, nesting material, and refuge from predators and harsh weather (Montevecchi 1978; Collias and Collias 1984; Walsberg 1985; Martin 1998). These factors can influence reproductive success, yet it might not be possible to select a site that optimizes all factors at once. For instance, certain habitats might have plentiful food but a high density of nest predators. Individuals that choose food-rich settings might have to compensate for heightened predation risk by renesting more frequently or sooner following nest depredation. By considering the combined effects of multiple environmental influences on offspring survival, it may be possible for parents to attain similar annual reproductive success in several different habitat types. Although nest site selection shapes annual reproductive success, it also can reduce body condition and life expectancy of the parents (Santos and Nakagawa 2012). Therefore, parents are expected to allocate resources and energy between themselves and their offspring in a manner that optimizes their overall lifetime reproductive success (Stearns 1989).

The availability of food influences nest site selection in birds because it has direct and indirect effects on reproductive outcome (Martin 1987). For example, food abundance can influence egg size, egg quality, and clutch size in some altricial species (Bryant 1975; Hussell and Quinney 1987; Martin 1987; Barrionuevo et al. 2014). In other species, it may not be possible for individuals to increase clutch size when food is abundant, but food abundance can allow those individuals to end a breeding attempt in better condition and to initiate an additional breeding attempt sooner (Martin 1987). In black-throated blue warblers (*Dendroica caerulescens*), for example, natural and experimental reductions in food abundance did not affect clutch size, but, instead, reduced the number of breeding pairs making multiple nesting attempts, which, in turn, reduced the breeding productivity of the population (Rodenhouse 1986).

Incubation and nest attentiveness are considered energetically demanding tasks for both parents, but especially for females in species where females provide the majority of care (Visser and Lessells 2001; Tinbergen and Williams 2002). By selecting a male with a territory containing abundant, high-quality food, the female can spend more time incubating and attending the nest (Amininasab et al. 2016), which can decrease the temperature fluctuations of her eggs, reduce the duration of the incubation period, and increase hatching success (Londoño et al. 2008). Increased nest attentiveness also enables faster food delivery rates, which accelerates nestling growth and thus shortens the nestling period, when offspring are most vulnerable to predation (Martin 1987). Studies suggest that, for species inhabiting shrublands, nests located in sites with higher shrub cover have enhanced nestling mass due to abundant insect prey associated with dense leafy foliage (Rotenberry and Wiens 1998; Morrison and Bolger 2002). Heavier offspring tend to have higher survival, which increases their parents' reproductive success (Chalfoun and Martin 2007).

Nest predation is the most common cause of reproductive failure in birds (Ricklefs 1969). The risk of nest predation can also affect life history traits such as clutch size and the number of broods per year, with birds experiencing higher nest predation risk favouring smaller clutch sizes that allow the parents to reserve more energy for renesting attempts following failure (Martin 1995; Lima 2009). This life history trade-off requires mechanisms that permit birds to evaluate and respond to the local risk of nest predation (Lima 2009). In dusky warblers (*Phylloscopus fuscatus*), parents place their nests in safer sites above from the ground and in more isolated bushes when predatory Siberian chipmunks (*Tamias sibiricus*) are locally abundant (Forstmeier and Weiss 2004). Similarly, birds may choose habitats with dense vegetation at nest height, which can reduce the likelihood of nest predation by concealing the nest (Martin 1988, 1993). Additionally, nest predation is believed to be greater in smaller habitat islands (a distinct patch of habitat surrounded by areas of degraded habitat such as agricultural lands or highways; Matthews 2021) because predators from surrounding areas can penetrate them more extensively and search them more efficiently (Martin 1988).

Apart from food availability and predation, a suitable ambient microclimate and shelter from the physical environment can improve offspring survival and increase the parents' reproductive success. Songbird embryos are ectothermic, and postembryonic offspring transition from ectothermic to endothermic as they grow in the nest (Visser 1998). The optimal temperature for embryo development is 36.5–38.5 °C, and development is suspended below 24–26 °C (Webb 1987; Durant et al. 2013). If embryos are exposed to temperatures above or below the optimal temperature range, the embryos can experience abnormal development and mortality (Durant et al. 2013). In the temperate zone, the ambient temperature is often below the optimal temperature required for developing embryos (Haftorn 1988). Parents therefore tend to locate and construct their nests in a manner that helps to maintain eggs and nestlings at an optimal temperature, thereby increasing nest survival and minimizing the parents' energetic cost (Haftorn 1988; Akresh et al. 2017). In some species, individuals that breed in colder environments build heavier nests with thicker walls, as compared to conspecifics nesting in warmer climates; this variation suggests that nest design is an adaptation to minimize convective heat loss and maintain eggs and nestlings at optimal temperature (Lundy 1969; Webb 1987; Rohwer and Law 2010; Crossman et al. 2011). Nest microhabitat characteristics such as foliage density, concealment, and composition of vegetation in the area immediately surrounding the nest also contribute to nest thermoregulation and survival by functioning as a windbreaker and providing radiative cover (With and Webb 1993; Schill and Yahner 2018). In lesser black-backed gulls (*Larus fuscus*), chicks grew faster when they were raised in nests that were near tall vegetation and therefore sheltered from cold winds than when they were raised in more exposed nests that faced cooler temperatures (Montevecchi 1978; Kim and Monaghan 2006). On the other hand, some species may benefit from cooler nest microclimates that buffer against hot temperatures experienced late in the season. Vegetation above a nest, such as canopy cover and understory vegetation, can cool the air around a nest by providing shade during the day (Jenerette et al. 2007; Becker and Weisberg 2015).

Many aspects of a species' ecological niche that I have discussed above, including habitat structure and biotic components such as food supply timing, composition, and abundance, as well as local predator foraging patterns, are influenced by weather and climate (Skagen and Adams 2012). The productivity, clutch size, and nest survival in the lark bunting (*Calamospiza melanocorys*) all positively correlated with seasonal precipitation, although intense daily precipitation events temporarily depressed the daily survival of nests. Also, the average temperature during the breeding season was positively correlated with nest survival in this species (Skagen and Adams 2012). These patterns in avian reproductive success may result from increased primary and secondary production (such as the biomass of invertebrate prey), which may be enhanced by increased precipitation (Rotenberry and Wiens 1998; Morrison and Bolger 2002). Rufous crowned sparrows (*Aimophila ruficeps*) were able to fledge 5.1 offspring per pair in 1998, a wet El Niño year, and 0.8 fledglings per pair in 1999, a dry La Niña year (Morrison and Bolger 2002). Cool, rainy El Niño conditions may have changed the activity levels of snakes, leading to low early-season nest predation and that combined with more favourable nesting conditions, may have allowed more pairs to have multiple broods (Ford and Burghardt 1993; Morrison and Bolger 2002). In contrast, in northern wheatears (*Oenanthe oenanthe*), parental nest visits decrease as daily rainfall increases, with this effect becoming more pronounced after consecutive rain days. Furthermore, rainfall during the nestling period reduced fledging success and male parental survival in northern wheatears (Öberg et al. 2015).

Dark-eyed juncos, *Junco hyemalis*, typically place their nests in a depression on sloping ground, under a protruding rock, amid the roots of a toppled tree, in a sloping road cut (especially if overhung by grass or other vegetation), under fallen tree trunks, or at the bases of bushes, trees or ferns, and occasionally they nest above the ground on horizontal branches (Nolan et al. 2020). Among 317 *J. h. carolinensis* nests that were found at the Mountain Lake Biological Station in Virginia, U. S. A. in 1999–2000, only 41 nests (12.9%) were elevated above the ground, and the highest nest was approximately 15 m (Nolan et al. 2020). In Arizona, U. S. A., *J. h. caniceps* preferred to nest in open sites with fewer total stems and more green vegetation cover on the ground, as compared to randomly selected sites in the same area (Martin, 1998). In this species, only females incubate, but both parents provision nestlings (Nolan et al. 2020).

In this chapter, I test whether hatching success and fledging success are associated with nest microhabitat characteristics and weather (temperature and precipitation) in slate-coloured dark-eyed juncos, which are the only subspecies of dark-eyed junco in Newfoundland, Canada. My study population is close to the northeastern limit of the species' breeding range and, therefore, nests are more likely to suffer hypothermia than hyperthermia. Nest predators such as American crows (*Corvus brachyrhynchos)*, Blue Jays (*Cyanocitta cristata*), introduced red squirrels (*Tamiasciurus hudsonicus*) and domestic cats (*Felis catus*) are also common across the island of Newfoundland. I therefore hypothesize that hatching and fledging success are higher for nests with better cover from predators and inclement weather, and for nests that experience warmer temperatures and less precipitation.

3.3 Methods

Study site

Fieldwork was conducted mainly at the Memorial University Botanical Garden (47º 34´N 52º 45´W), located within Pippy Park in St. John's, Newfoundland and Labrador, Canada. The Botanical Garden encompasses 0.44 km^2, which includes 0.07 km^2 of landscaped garden and a larger area (0.37 km^2) containing natural vegetation, balsam fir (*Abies balsamea*) and black spruce (*Picea mariana*) as dominant tree species, and a trail system throughout.

Some nests were found in the context of other research in an area that is outside the botanical garden but still within Pippy Park. Others were found on Memorial University's St. John's campus (Figure 3.1). These nests were found by coincidence and were included in the study to maximize sample size. Pippy Park contains natural vegetation similar to the Botanical Garden, whereas nests found at Memorial University were on landscaped lawns near buildings.

Banding, mapping territories, and locating nests

Dark-eyed juncos were captured in winter in 2018 (N = 54) and 2019 (N = 53) using treadle traps that were baited with sunflower seeds and placed on wooden platforms. Birds were ringed with three plastic colour bands and one federal aluminum band for identification purposes. Captured birds were held in cloth bags when not being measured and were released at their site of capture within 45 minutes of being trapped.

During the spring and summer of 2018 and 2019, I revisited the study site and located the banded dark-eyed juncos (N = 10 in 2018; N = 6 in 2019). Although many of the banded juncos had dispersed beyond the study site, I mapped the territories of each remaining colour-banded male by following it for 2–3 hours and marking all its singing perches with a handheld GPS (Garmin GPS map 78s; Kansas, USA; accuracy ca. ± 3 m). To ensure that I had mapped the entire territory, I revisited each territory at least once per week for 2–3 h throughout the breeding season and marked the singing perches to ensure that I had captured the core territory and most of the singing perches. GPS coordinates of singing perches based on all my visits were plotted on a topographic map, and territory maps were estimated and drawn using the minimum convex polygon method, implemented in ArcMap 10.3.1 (ESRI 2015).

After mapping territories, I followed breeding pairs within their territories and searched for active nests using behavioural cues such as nest building, incubating, and nestling feeding. Once an active nest was located, I visited it once daily until the fledglings left the nest or the nest was depredated. During these visits I observed the nest location from a safe distance through binoculars to avoid flushing incubating females or provisioning parents from the nest. Whenever possible, I determined if the nest was active by observing the behaviour of the adults or nestlings from afar (i.e., defending area, carrying food, begging calls of nestlings), thereby reducing the risk of attracting predators to the nest. Nestlings were banded with a federal metal band within 3–7 days of hatching. I only visited nests and banded the nestlings when the parents were not around and nests, and I always approached and vacated from different directions to avoid leaving dead-end trails (Martin & Geupel 1993).

Dark eyed juncos typically lay 3–5 eggs and rarely lay six eggs (Bent and Austin 1968; Smith and Andersen 1985). Unpublished data from Leslie Tuck suggest that Dark-eyed junco nests in Newfoundland consist of 3-6 eggs as well. After finishing the nest, females lay one egg each day, and incubation often begins on the day they lay their penultimate egg (Nolan et al. 2020). Incubation lasts 12–13 days, and eggs hatch in synchrony within a few hours, though in some cases eggs have been known to hatch over a 2-day period (Hostetter 1961; Nolan et al. 2020). Nestlings typically leave the nest 10–13 days after hatching, though nestlings may fledge 10–11 days after hatch if disturbed (Hostetter 1961; Bent and Austin 1968).

I determined the approximate age of all nests that I discovered and monitored during the 2018 and 2019 breeding seasons. For nests that were found after egg laying, nest age was calculated on the assumption that the female laid one egg per day until clutch completion (day 1 was considered the day that the female laid their penultimate egg). The age of these nests at discovery was estimated by subtracting the number of days between discovery and hatching from the typical incubation period of 13 days. For nests that were found after hatching, nest age at discovery was determined based on the size and appearance of nestlings (Jongsomjit et al. 2007), assuming the eggs had hatched in synchrony within a 24-h period.

For each nest, I determined the number of eggs laid, the incubation start date (the date that the female laid their penultimate egg), the hatch date, the number of eggs hatched (as evidenced by the presence of nestlings in the nest), the nesting end date (as evidenced by an empty nest following the 14-day nestling period, or by observing the banded fledglings outside the nest), and the number of nestlings fledged. If eggs remained un-hatched for 8 days past the normal incubation period, I considered the nest to have failed and noted the day that the female abandoned/stopped incubating the eggs. A nest was considered to be depredated if the eggs or nestlings disappeared before expected hatch date or before the earliest potential fledge date, or if there was evidence of the nest being depredated (e.g., eggshells, feathers, damaged nest). For nests that were depredated, I noted the last day that the female was seen incubating eggs or that nestlings were seen alive. Although some studies exclude eggs that were depredated or abandoned from the calculation of hatching success, I included all eggs because I was interested in the fate of all eggs in relation to microhabitat and weather, which could mitigate predation, hypothermia, and abandonment. If the nestlings were found dead inside the nest after a period of inclement weather (ambient temperatures below zero combined with precipitation), I assumed that they died of hypothermia. For nests where the eggs never hatched after a complete incubation stage, I assumed that the eggs were either infertile or that they died of hypothermia. Among 21 nests, three nests did not hatch any eggs.

Nest insulation measurement

One to two days after the nestlings fledged, I placed a temperature logger (RC-5 USB temperature data logger, Elitech Technology, San Jose, California, USA; accuracy: ± 0.55ºC between -20–40ºC) inside and outside the nest to determine whether nest site selection and nest construction could result in the inside of the nest being warmer than the outside, even when there were no nestlings or female present. The ambient temperature in St. John’s during the breeding season is often well below the optimum temperature for embryo development and nestling survival. Juncos who are unable to maintain their nest in an optimal temperature are more likely to fail. Therefore, a well insulated nest is important to reduce heat loss through the nest walls and maintain the nest at an optimum temperature when a parent is on the nest as well as during off-bouts. The data loggers were deployed only after nestlings left the nest to prevent disturbance. Since I only had four data loggers, I used each data logger on multiple nests. Loggers were assigned at random to either the inside or outside of the nest. The inside data logger was placed on a horizontal plane immediately over the nest lining. The outside data logger was placed as close as possible to the nest (always within a 1-m radius of the nest) at a site where it was not covered or sheltered by ground vegetation. Among 24 nests discovered, only one was found above ground on a tree. For this nest, I tied the outside data logger at the same height as the nest on a thin wooden stake that was stuck into the ground. The outside temperature logger was always placed in the same orientation as its corresponding inside logger to provide a contrasting measurement of the ambient temperature. Both temperature loggers were deployed at the same time at a given nest, and were then left to record the instantaneous temperatures inside and outside the nest every hour for 48 h. After 48 hours, both data loggers were collected, the data retrieved, and the loggers reset for use at the next nest. 'Nest insulation' was calculated by subtracting the minimum temperature of the outside data logger from the temperature recorded by the inside data logger at the same time. I predicted that the inside of the nest would always be warmer than the outside and that nest insulation values therefore would be positive.

Nest microhabitat and weather

Immediately after collecting nest insulation data, I measured several nest microhabitat variables, including canopy cover, stem density, nest exposure, ground foliage cover, and distance to the nearest trail or road. Mean canopy cover (%) was measured with a densiometer held in each of the four cardinal directions 1 m directly above the nest. Stem density was measured by holding a 1-m rod horizontally from my extended arm (1-m pole + 0.6-m arm length), rotating a full circle around the nest site, counting the number of dead or alive woody stems with minimum 1 cm diameter that the pole touched, and then calculating the number of stems per 1 m^2 (stems/m^2). Nest exposure was measured using a checkered circular disk that was 10 cm in diameter and contained 72 black and white squares. With the disk on top of the nest cup, I counted the number of squares visible from 1 m directly above the nest and from 1 m above and 1 m away from the nest in all four cardinal directions; nest exposure was the average of these five measures, divided by 72. The ground foliage cover was measured by placing a 1 m x 1 m square constructed of PVC pipe around the nest, with the nest at the centre of the square. I then photographed the ground vegetation inside the square from 1 m directly above the nest. The area covered (%) with green vegetation (i.e., moss, ferns, grass, herbaceous plants, seedlings, saplings) inside the square was quantified using Image J software (version 1.53m; W. Rasband, National Institutes of Health) using the method of Agehara (2020). All the green vegetation that I observed in the nest sites were closer to the ground, and therefore did not obstruct the view of the camera.

I acquired weather data from Environment and Climate Change Canada's online data repository (Environment and Climate Change Canada 2019), recorded at the permanent weather station at the St. John's International Airport. This weather station is located at 140.5 m elevation and within 4–5.5 km of all the nests that I observed in the study (Figure 3.1). The average daily temperature, average minimum temperature, and average daily precipitation were calculated separately for the incubation and nestling periods for each individual egg/nestling. Average daily temperature was the mean of the daily mean temperatures, average minimum temperature was the minimum of the daily minimum temperatures, and average daily precipitation was the mean of the daily total precipitation values recorded at the weather station. For eggs, the averages were calculated from incubation start date to hatching date or failed date. If an egg did not hatch, the failed date was considered as the day the female stopped incubating the egg or the hatching date of other eggs in the clutch. For nestlings, averages were calculated from hatching date to fledging date or failed date. Due to the proximity, I assume that the temperature and precipitation values recorded at the weather station were similar to those experienced at the nest sites. Descriptive statistics (average, SD, minimum, maximum) for the above measured variables were reported.

Statistical analysis

Statistical analyses were conducted in R (version 4.3.1; R Development Core Team, 2022). Generalized linear mixed effects models with logit-link function and binomial distribution (R package glm2 version 1.2.1; Marschner 2011) were used to test whether hatching success was related to nest microhabitat and weather variables, whereas Cox proportional hazards mixed models (R package coxme version 2.2-16; Therneau 2020) were used to test whether fledging success was related to microhabitat and weather variables. Logistic models were used for hatching success because nest age, which is required for Cox proportional hazards models, could not be ascertained for nests that were discovered midway through incubation and which failed before hatching. In all analyses, I considered individual eggs or nestlings rather than the nest as the unit of observation because each egg or nestling within a nest has a different probability of survival; I included nest identity as a random effect in all models to account for possible dependencies among eggs or nestlings from the same nest. Multiple nests belonging to the same parents were included in the analysis as separate data points because, during my study, none of the dark eyed juncos reused a previous nest. Rather, multiple nests from the same parents were always in different locations within the territory and thus experienced different microhabitats and weather that could be related to their differential reproductive success. There were two breeding pairs with 2 nests and one breeding pair with 3 nests in 2018 and one breeding pair with 2 nests in 2019. The pair that produced 2 nests in 2019 was also one of the pairs that produced 3 nests in 2018. However, I could not include the identity of the parents as a second random effect in the models because most parental pairs had only one nest in the data set and the models therefore would not converge.

During model development, Pearson correlations were used to test for possible collinearity between predictor variables. Ground foliage cover and nest exposure were significantly correlated with each other (Pearson's Correlation = -0.661, $P = 0.001$, $N=21$), as were the average and minimum temperatures during the egg stage (Pearson's Correlation = 0.987, $P < 0.001$, $N=21$) and the average and minimum temperatures during the nestling stage (Pearson's Correlation = 0.988, $P < 0.001$, $N=18$) (Table 3.1). Where collinearity was observed between two variables ($r > 0.6$), only the variable that I considered the most biologically relevant was retained in the models. Therefore, only average nest exposure, minimum temperature at egg stage, and minimum temperature at nestling stage were retained for subsequent analysis. I tried running the models with year as a fixed effect (since there is only two levels), however it was not possible with the limited sample size. Therefore, for all analyses I did not include year as a predictor variable due to the sampling limitations and also, because the year is not necessarily an important variable in my analysis and not directly related to my hypothesis. Whereas the weather variables have an important biological effect that can explain survival rather than the year. I did not include interaction terms when creating models to avoid overfitting models because of small sample size, and because I did not have a prior prediction to support the inclusion of interactions.

Canopy cover, stem density, nest exposure, distance to closest trail, nest insulation, average precipitation, and minimum temperature were included as predictor variables with fixed effects in preliminary models, with nest identity as a random variable. I used a modified backwards selection approach when developing candidate models. First, univariate models with just one predictor variable were constructed. Then, from those univariate models, I selected predictor variables with p-values less than 0.25 to create an initial multivariate model (Hosmer et al. 2013). Predictors with the highest p-values were eliminated from the multivariate model using backward stepwise elimination. At each step, the model fit was evaluated using Akaike's Information Criterion adjusted for small sample sizes (ΔAICc) (Lebreton et al. 1992). The final model was determined when dropping any of the remaining predictor variables increased the model AICc, as the model with the lowest AICc value is presumed to be the most parsimonious (Burnham and Anderson 2002). I considered variables in the final model to be statistically significant when $p \leq 0.05$, and to constitute a statistical trend when $0.05 \leq p \leq 0.1$.

3.4 Results

During the study, I noticed that each male junco sang from a small number of trees within their territory. Consistent with previous studies (Ketterson et al. 1992), I also observed male dark-eyed juncos using the same territories between consecutive years (Appendix A.1). I found a total of 21 nests during two breeding seasons, including 14 in 2018 and 7 in 2019 (Table 3.2). In 2018, nests were active between 10 May and 22 August, and the mean date of discovery (± SD) was 25 June (± 24 days). The incubation stage ranged from 10 May until 11 August, and the nestling stage ranged from 23 May until 22 August. Of the 14 nests found, 11 hatched at least one egg (79%), and six were successful in fledging at least one nestling (43%). In 2019, nests were active between 30 May and 4 August, and the mean (± SD) date of discovery was 22 June (± 15.95 days). The incubation stage ranged from 30 May until 20 July, and the nestling stage ranged from 11 June until 4 August. Of the seven nests found, all seven hatched at least one egg (100%) and fledged at least one nestling (100%). When the two years were combined, 18 of 21 nests (86 %) hatched at least one egg, and 13 of 21 (62%) fledged at least one nestling (Table 3.2).

The 21 nests contained a total of 75 eggs, including 49 in 2018 and 26 in 2019 (Table 3.2). In 2018, 17 of the 49 eggs failed, including six that failed due to predation and 11 that failed for unknown reasons, possibly due to low temperatures or being infertile. Of the 32 nestlings that hatched in 2018, 19 died, including 8 due to predation, 4 due to lawn mowing, and 4 that I assume died due to cold air temperature (found dead inside the nest immediately after a cold snap and snow fall). The causes of death for the remaining three nestlings were not identified. In 2019, only two of 26 eggs failed to hatch, possibly due to low temperatures or infertile eggs because the eggs remained in the nest after the others had hatched. Of the 24 nestlings that hatched in 2019, three died due to predation (Table 3.2).

In both years, the mean daily temperature and minimum daily temperature were colder at the beginning of the breeding season, but then warmed gradually throughout the season (Figure 3.2). In 2018, the average (± SD) mean daily temperature among 14 nests was 8.82 ± 4.86 ºC for the incubation period and 8.89 ± 5.59 ºC for the nestling period. In 2019, the average (± SD) mean daily temperature among 7 nests was 10.34 ± 2.06 ºC for the incubation period and 10.31 ±

2.07 ºC for the nestling period. Nests had an average insulation (± SD) of 1.2 ± 0.8 ºC (range: 0.1–3.1ºC; Table 3.3).

The final logistic regression model for hatching success included canopy cover, stem density, nest insulation, and average precipitation as predictors (Table 3.4). The odds of hatching were significantly higher when there was less canopy cover and less precipitation, and they tended to be higher when stem density was lower and nest insulation was higher (Table 3.5; Figure 3.3).

In my analysis of nestling survival, preliminary univariate models comparing fledging success to individual nest microhabitat and weather variables revealed that only nest exposure had a p-value less than 0.25 (Table 3.6). That model showed that fledging success tended to be higher when nest exposure was lower, but the relationship was not statistically significant (Table 3.6). Because none of the other predictor variables had p-values < 0.25, a multivariate model was not built.

3.5 Discussion

In this chapter, I assessed whether hatching success and fledging success in dark-eyed juncos were associated with nest microhabitat parameters and weather parameters. Nest failure and the death of individual offspring occurred most frequently during the nestling stage. During the egg stage, 68% of eggs that did not hatch appeared to fail due to low temperatures or infertility, as evidenced by the unhatched eggs remaining in the nest. In contrast, only 32% of the hatching failures were due to predation, as evidenced by the sudden disappearance of the eggs and/or the appearance of eggshell fragments. Canopy cover, stem density, average precipitation, and nest insulation were associated with hatching success. None of the microhabitat parameters or weather parameters showed statistically significant relationships with fledging success, although fledging success tended to be higher when nest exposure was lower.

Hatching success was higher when canopy cover was lower and tended to be higher when stem density was lower. These findings are consistent with previous research showing that dark-eyed juncos prefer to nest in areas with fewer trees and less canopy cover (Lance and Howell 2000). This result contrasts with some previous studies on other species that showed higher egg survival under denser canopy cover, presumably due to the canopy cover providing shade and protection from hyperthermia in the day, protection from radiative heat loss at night, and protection from predators (Segura et al. 2012; Becker and Weisberg 2015). Most of these studies, however, were conducted on species that nest above the ground and that experience predator pressure from corvids and raptors; a dense canopy may act as a visual barrier to such predators, which often rely on visual cues when searching for nests (Martin 1993; Segura et al. 2012; Becker and Weisberg 2015). Since dark-eyed juncos build nests primarily on the ground (Nolan et al. 2020), they may be less likely to be depredated by avian predators relying on visual cues (Söderström et al. 1998). Previous research showed that mammals were the primary predators of ground nests in deciduous-coniferous mixed forests in south-central Sweden (Söderström et al. 1998), and that red squirrels (*Tamiasciurus hudsonicus*) were the primary predators in northern conifer forests (Willson et al. 2003; DeGregorio et al. 2016). Red squirrels are introduced in Newfoundland but are still considered the dominant predator of nests on or close to the ground, including those of the Newfoundland gray-cheeked thrush (*Catharus minimus minimus*) (Lewis 2004; Whitaker et al. 2015). Red squirrels prefer territories with more trees and denser canopy cover, possibly due to the abundant cones and protection from avian predators (Posthumus et al. 2015). My finding that hatching success was lower in such habitats therefore might be explained by increased predation pressure from red squirrels. Another possibility is that greater canopy cover creates shade and results in lower temperatures closer to the ground (Vasconcelos and Sacht 2020; Aalto et al. 2022). Since temperatures recorded in my study at the nearby weather station were already quite low during the incubation and nestling stages, perhaps the shade created by habitats with greater stem density and canopy cover exacerbated temperatures near the nests, leading to hypothermia or suboptimal incubation temperatures during the daytime.

Although I did not detect a relationship between minimum temperature and either hatching or fledging success, hatching success was significantly lower when average precipitation was higher. Hatching failure therefore might be due to sudden cold snaps that were combined with increased rain or snow in the early breeding season, particularly in May 2018. Precipitation can affect the survival of nests both directly and indirectly. Although spring precipitation is important for the onset of vegetation growth and the production of food needed for nestlings, there is evidence that heavy precipitation events can depress nest survival (Skagen and Adams 2012; Shiao et al. 2015). During heavy rainfall or snowfall, nesting material gets damp and is likely to lose heat via increased conductance (Reid et al. 2002). Wet conditions can slow embryonic growth and prolong incubation, which, in turn, could reduce hatching success and increase exposure to predators (Higgott et al. 2020). Since dark-eyed juncos feed on seeds and arthropods found on the ground and in leaf litter (Nolan et al. 2020), it is also possible that snowfall during incubation reduced female foraging efficiency (Tinbergen and Dietz 1994), which may have forced females to spend more time off-nest and exposed eggs to hypothermia (Arlettaz et al. 2010; Whitehouse et al. 2013). Shiao et al. (2015) similarly observed that heavy rainfall led to nest mortality in cavity-nesting green-backed tits (*Parus monticolus*), with survival further decreasing when cold temperatures accompanied the rain.

Hatching success tended to be higher in nests with better nest insulation. Ground-nesting animals are known to select nest sites and build nests that minimize heat loss in cool environments or that prevent overheating in warm environments (Mainwaring et al. 2014). Loss of heat is a result of prolonged exposure to temperatures outside the optimal range, and this can lead to hatching failure (Lundy 1969). Heat is lost from the eggs by radiation and convection to the atmosphere and by conduction through the nest material (Turner 1991). Therefore, nesting material, nest structure, nest position, and habitat cover are essential for retaining heat. During my study, the average daily temperature was sometimes less than 5 ºC for consecutive days. Although hatching success was not related to the average minimum temperature experienced by a nest during incubation, it is possible that some eggs were protected from hypothermia through increased nest attentiveness by the female and better insulation provided by the nest.

Dark-eyed juncos prefer nest sites with more green vegetation cover on the ground (Martin 1998). In my study, most nests had high ground foliage cover, yet I did not find any evidence that this was associated with hatching or fledging success. One possible explanation for the lack of relationship is that I measured nest microhabitat after the nests were no longer active to avoid disturbing the nests. If ground vegetation changed rapidly throughout the nesting period, then my vegetation measurements may not have represented the ground vegetation that surrounded the nests during the egg or nestling stage (Gibson et al. 2016). Also, since dark-eyed juncos are known to occupy the same territories for consecutive years, it is possible that they choose territories/nest sites based on permanent structural characteristics or on other knowledge of the breeding area. These decisions might be based on the information gathered from previous years rather than habitat characteristics, as most deciduous vegetation may not emerge until after territories are established, nest are built, and eggs are laid (Doligez et al. 2002).

The measured microhabitat or weather variables were not associated with fledging success, though nests that were more exposed tended to have lower fledging success, which could be due to the exposure of nestlings to inclement weather and predators. The lack of relationship between fledging success and the other microhabitat and weather variables might have been due to the smaller sample size of nestlings versus eggs or to the two different statistical approaches used for analyzing hatching and fledging success.

In my study, hatching success at the nest level (i.e., nests in which at least one egg hatched) was 86% and fledging success at the nest level (i.e., nests that fledged at least one chick) was 62%. These values are comparable to previous research on dark-eyed juncos in other locations. For example, of 170 nests belonging to 93 males in the Appalachian Mountains of the USA (1989–1993), annual fledging success at the nest level varied between 25% and 80% over 9 years, with an overall hatching success of 71% and an overall fledging success of 55% (Ketterson et al. 1996). In northern Utah, 76% of *J. h. mearnsi* eggs that hatched produced fledglings, whereas in my study 61% of eggs that hatched produced fledglings (Smith and Andersen 1982). Fledging success at the nest level was 73% in an undisturbed boreal forest in the Yukon territory of Canada (Willson and Gende 2000), 77% in the coastal rainforest of southeastern Alaska, USA (Willson and Gende 2000), and 69% in the high-elevation temperate habitat in Arizona, USA (Martin 1993).

In conclusion, hatching success was significantly higher when canopy cover and average daily precipitation were lower. It also tended to be higher in nests with better insulation and at sites with lower stem density, though these relationships were not statistically significant. Fledging success was not significantly associated with any microhabitat or weather variables, though it tended to increase with lower nest exposure. Future research should focus on how predator density, specifically red squirrel density, affects the nesting success of ground-nesting passerines like dark-eyed juncos, and whether such relationships are mediated by red squirrel preferences for habitats with dense canopy cover and high stem density. Additionally, short-term and long-term climate projections forecast that heavy precipitation events will become more frequent and that daily ambient temperatures will become more extreme in North America (Masson-Delmotte 2021), including in Newfoundland (Finnis and Daraio 2018). Therefore, future research should focus on how extreme weather events like late spring cold snaps, snowfalls, and heavy rain affect the nesting success of ground-nesting passerines, since that would provide important insight into the vulnerability of species to population declines arising from climate change.

3.6 Tables

Table 3.1: Pearson correlations among nest microhabitat and weather variables. Correlation coefficients are given above the diagonal, and p-values are given below the diagonal [2-tailed, N=21 (egg stage), N=18 (nestling stage)]. Significant values are marked with an asterisk and the cells shaded with grey are irrelevant for the analysis.

Variable	Canopy cover	Average nest exposure	Ground foliage cover	Stem density	Distance to the closest trail	Nest insulation	Average temperature at egg stage	Minimum temperature at egg stage	Average precipitation at egg stage	Average temperature at nestling stage	Minimum temperature at nestling stage	Average precipitation at nestling stage
Canopy cover	-	-0.139	0.299	0.402	0.035	0.176	0.124	0.094	-0.209	0.192	0.174	0.053
Average nest exposure	0.548	-	-0.661*	-0.262	-0.172	0.116	-0.041	-0.061	0.005	-0.049	-0.083	-0.115
Ground foliage cover	0.188	0.001*	-	0.444	0.124	0.004	-0.149	-0.105	-0.089	-0.166	-0.117	0.231
Stem density	0.071	0.252	0.044	-	-0.035	0.003	0.009	-0.019	-0.211	0.059	0.053	-0.124
Distance to the closest trail	0.879	0.457	0.591	0.881	-	0.299	-0.280	-0.281	-0.153	-0.272	-0.277	-0.016
Nest insulation	0.447	0.617	0.986	0.989	0.187	-	0.117	0.077	-0.268	0.126	0.073	-0.177

Average temperature at egg stage	0.593	0.862	0.519	0.968	0.220	0.613	-	0.987*	-0.132	0.999*	0.990*	-0.182
Minimum temperature at egg stage	0.686	0.791	0.650	0.934	0.217	0.741	0.000*	-	-0.115	0.985*	1.000*	-0.117
Average precipitation at egg stage	0.429	0.958	0.728	0.387	0.488	0.236	0.719	0.873	-	-0.186	-0.113	0.998*
Average temperature at nestling stage	0.445	0.846	0.512	0.816	0.275	0.618	0.000*	0.000*	0.461	-	0.988*	-0.200
Minimum temperature at nestling stage	0.490	0.745	0.644	0.863	0.266	0.774	0.000*	0.000*	0.656	0.000*	-	-0.128
Average Precipitation at nestling stage	0.835	0.649	0.357	0.624	0.951	0.483	0.471	0.644	0.000*	0.426	0.614	-

Table 3.2: Hatching and fledging success of dark-eyed juncos in Newfoundland, Canada in 2018 and 2019.

	Number found	Number survived	Percentage success (%)
Hatching success based on nest level (hatched at least one egg)			
2018	14	11	79
2019	7	7	100
2018 and 2019	21	18	86
Fledging success based on nest level (fledged at least one nestling)			
2018	14	6	43
2019	7	7	100
2018 and 2019	21	13	62
Hatching success based on individual eggs			
2018	49	32	65
2019	26	24	92
2018 and 2019	75	56	75
Fledging success based on individual nestlings			
2018	32	13	41
2019	24	21	88
2018 and 2019	56	34	61

Table 3.3: Descriptive statistics for measured nest microhabitat and weather parameters for 21 nest sites in the 2018 and 2019 breeding seasons.

Variable	Mean	Standard Deviation	Minimum	Maximum
Nest exposure	0.27	0.24	0	0.99
Canopy cover (%)	48.91	40.85	0	98.7
Stem density (m^{-2})	0.64	0.62	0	2.23
Ground foliage cover (%)	41.20	30.26	0	100
Nest insulation (ºC)	1.18	0.85	0.1	3.1
Distance to the closest trail (m)	8.36	6.67	1.1	25.1
Average temperature at egg stage (ºC)	9.32	4.14	4.82	18.50
Minimum temperature at egg stage (ºC)	4.91	3.79	1.01	13.80
Average daily precipitation at egg stage (mm)	4.02	2.67	1.30	12.10
Average temperature at nestling stage (ºC)	9.44	4.51	4.42	18.50
Minimum temperature at nestling stage (ºC)	5.10	4.09	0.93	13.80
Average daily precipitation at nestling stage (mm)	3.75	2.13	1.23	8.36

Table 3.4: Comparison of competing models comparing hatching success in dark-eyed juncos to nest microhabitat and weather parameters during the 2018 and 2019 breeding seasons (N = 75). Models are ranked by Akaike's Information Criterion adjusted for small sample size (AICc); K is the number of parameters, ΔAICc is the difference of each model's AICc value from that of the highest ranked model (row 1), and w is the Akaike weight. Variables were only considered for these multivariate models if the p-value from their univariate model was < 0.25.

Model	K	AICc	ΔAICc	w
Canopy cover + Stem density + Nest insulation + Average precipitation	5	73.63	0.00	0.60
Canopy cover + Stem density + Average precipitation	4	75.70	2.07	0.21
Nest exposure + Canopy cover + Stem density + Nest insulation + Average precipitation	6	75.99	2.36	0.18
Intercept only	1	86.95	13.32	0

Table 3.5: Results of the top logistic regression model comparing hatching success in dark-eyed juncos to canopy cover, stem density, nest insulation, and average precipitation in St. john's, Newfoundland during the 2018 and 2019 breeding seasons. Significant variables ($p \leq 0.05$) are indicated with an ** and nonsignificant trends ($0.05 \leq p \leq 0.10$) are indicated with an *.

	Estimate	Std. Error	z value	p-value	Odds ratio	95% Confidence intervals
Intercept	3.434	1.410	2.436	0.015	30.998	1.044, 6.728
Canopy cover	-0.024	0.010	-2.454	0.014**	0.976	-0.046, -0.006
Stem density	-1.037	0.531	-1.953	0.051*	0.355	-2.166, -0.031
Nest insulation	1.113	0.591	1.884	0.060*	3.044	0.063, 2.387
Average precipitation	-0.310	0.138	-2.250	0.025**	0.734	-0.613, -0.060

Table 3.6: Results of univariate Cox proportional hazard models comparing nestling survival in dark-eyed juncos to nest microhabitat and weather variables in Newfoundland, Canada during the 2018 and 2019 breeding seasons. Any variable with a p-value less than 0.25 (indicated with an *) was selected for inclusion in the final model.

	Estimates	Std. Error	z value	p-value
Nest exposure	8.609	4.987	1.73	0.084*
Canopy cover	0.005	0.020	0.27	0.78
Stem density	-2.268	2.238	-1.01	0.31
Distance to closest trail	-0.029	0.104	-0.28	0.78
Nest insulation	0.051	0.729	0.07	0.94
Minimum temperature	-0.028	0.352	-0.08	0.94
Average precipitation	-0.152	0.351	-0.43	0.67

3.7 Figures

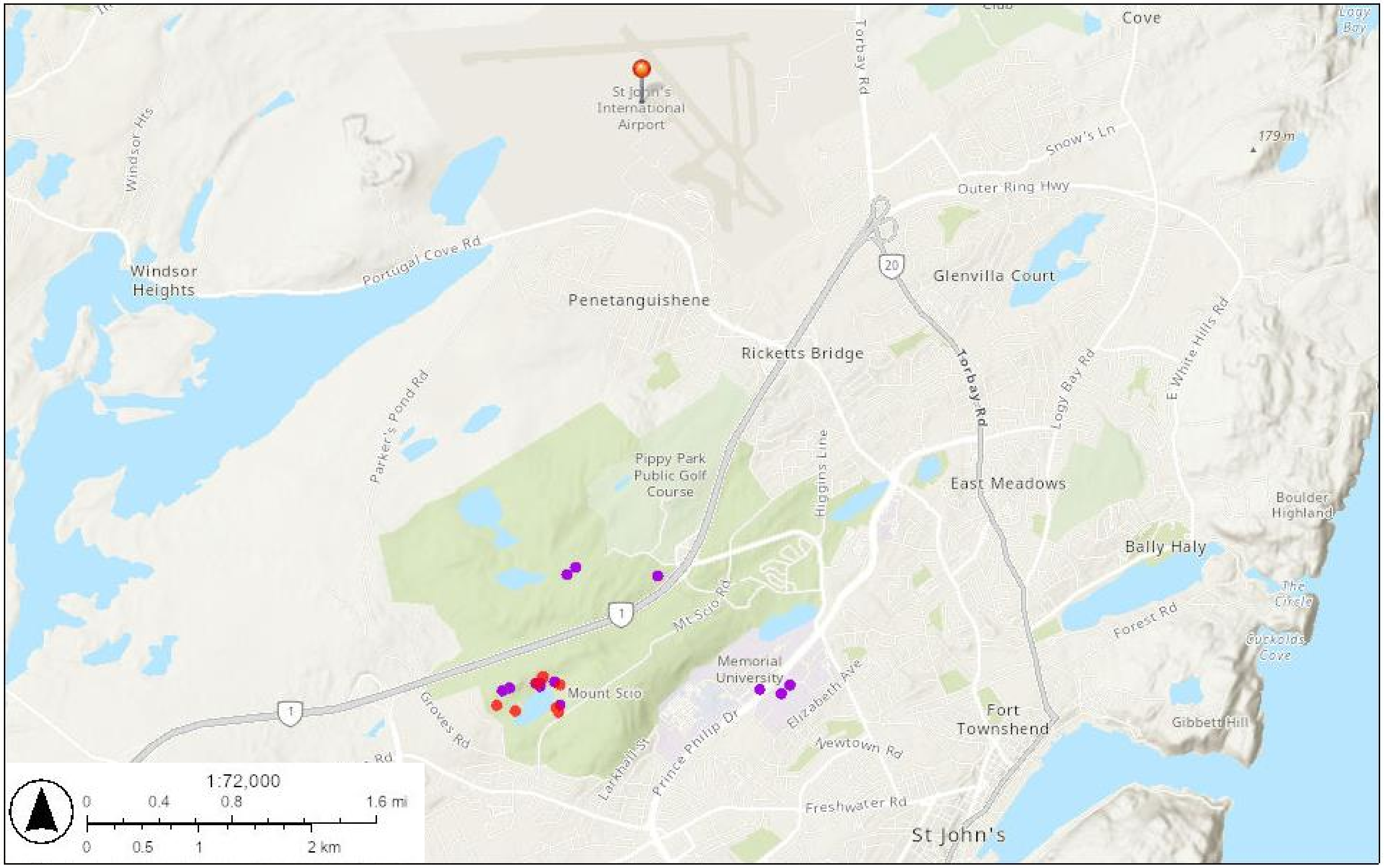

Figure 3.1**:** A map of nest sites at the Memorial University Botanical Garden, Pippy Park, and Memorial University St. John's campus (2018 nest sites are indicated in purple dots and red dots indicate 2019 nest sites). The St. John’s International Airport weather station is marked with a red pin.

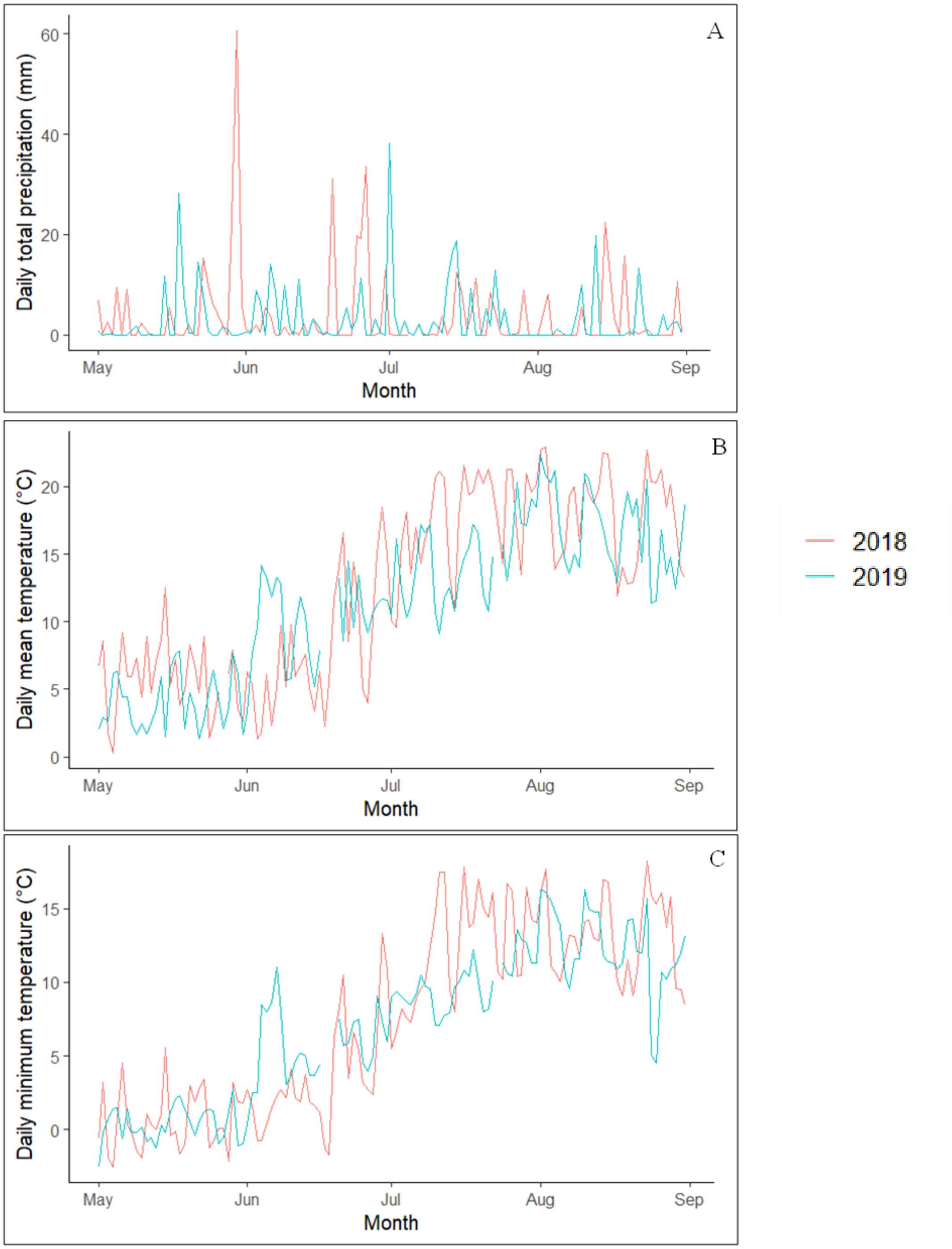

Figure 3.2**:** Graphs representing (A) daily total precipitation, (B) daily mean temperature, and (C) daily minimum temperature from May to August during the 2018 and 2019 breeding seasons. 2018 data are represented with a red line and 2019 data are represented with a blue line.

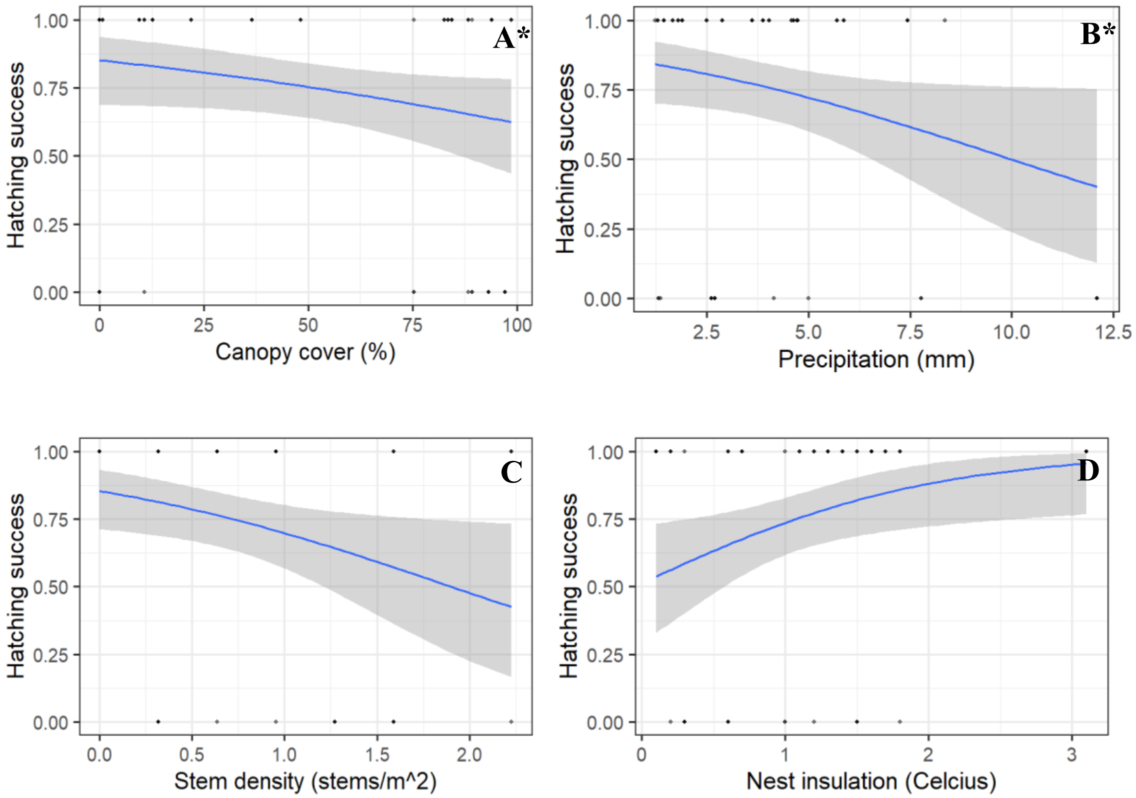

Figure 3.3: Scatter plots of hatching success versus (A) canopy cover, (B) precipitation, (C) stem density, and (D) nest insulation for 75 dark-eyed junco eggs during the 2018 and 2019 breeding seasons. Grey shade indicates the 95% confidence intervals and individual observations are shown as success and fail (1 or 0, respectively). Significant variables are indicated with an asterisk.

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CHAPTER 4: GENERAL DISCUSSION

Predictors of reproductive success in dark-eyed juncos in Newfoundland

According to the theory of sexual selection, sexual traits are associated with reproductive success (Andersson 1994). Among these traits, bird song is a male sexual characteristic that provides information about the signaler's qualities to potential mates and rivals (Catchpole and Slater 2008). Physically challenging signal traits can ensure signal honesty (Byers et al. 2010), and these, along with nest habitat characteristics and weather and climatic variables, have been shown to impact the reproductive success of birds (Newton 1989). My thesis research aimed to determine which of these predictors influence the reproductive success of dark-eyed juncos (*Junco hyemalis hyemalis*) in Newfoundland, Canada.

Dark-eyed juncos were historically considered a migratory population in Newfoundland, where they were recorded as an uncommon winter resident in the 1940s (Burleigh and Peters 1951). During the last 75 years, this population appears to have become resident year-round. During my thesis research, I studied whether song performance, body size, nest microhabitat, and weather affect the reproductive success of these year-round resident birds. I measured song structure, morphology, number of eggs hatched, and number of nestlings that survived and fledged from the nest of previously banded dark-eyed juncos during the 2018 and 2019 breeding seasons. I also obtained average air temperature, average minimum air temperature, and average daily precipitation for the nesting period. After the nestlings left the nest, I measured the nest microhabitat features.

In Chapter 2, I investigated whether song performance and body size of male dark-eyed juncos predict reproductive success. Based on previous studies, individual male dark-eyed juncos have a small repertoire of 2–8 different trilled song types (Williams and MacRoberts 1977; Newman et al. 2008); in my study population, I observed 13 different song types among 10 different males. Each individual male produced between 1 and 3 song types, although this number likely underrepresents the true repertoire size of each individual due to insufficient sampling of each male. I observed high song type diversity and low song type sharing in my study. I did not find evidence for a performance trade-off between trill rate and frequency bandwidth, as has been found for the trilled vocalizations of several other species (Wilson et al. 2014). The average reproductive success of male dark-eyed juncos was not significantly related to any song structure measures (frequency bandwidth, trill rate, song duration) or body size.

Chapter 3 showed that canopy cover and average precipitation were negatively associated with hatching success, and that nest insulation and stem density tended to be associated with hatching success. None of the variables showed a significant correlation with fledging success, although a statistically nonsignificant trend was observed between fledging success and nest exposure, where fledging success tended to be higher when nest exposure was lower. My finding that hatching success was higher when canopy cover was lower contradicts some previous studies that found higher nest survival under denser canopies that buffer against extreme temperatures and provide cover from aerial predators

(Segura et al. 2012; Becker and Weisberg 2015). However, my finding is consistent with the results of a study conducted in western Newfoundland, which showed that successful white-throated sparrow nests (also a ground-nesting species) had less canopy cover (Dalley et al. 2008). A possible explanation for finding higher hatching success under sparser canopies is the presence of introduced red squirrels, which tend to prefer territories with dense canopy cover, possibly due to the presence of large amounts of cones and protection from avian predators (Posthumus et al. 2015). Hence, at sites with dense canopy cover, there could be increased predator pressure from red squirrels that result in hatching failures among ground-nesting birds (Lewis and Montevecchi 1999; Lewis 2004). More research needs to be done on how introduced red squirrels affect the nesting success of ground-nesting passerines like dark-eyed juncos in Newfoundland, and how the impact might be mediated by this predator's habitat preferences and local population density. Hatching success was also lower when the incubation period coincided with greater precipitation. I suspect that heavier precipitation events (especially snowfalls) accompanied by colder temperatures after the eggs have been laid pose a significant threat to hatching success (Shiao et al. 2015). With climate change, precipitation events will become more frequent and more extreme (Masson-Delmotte 2021); therefore, it is essential to study the effects of heavy precipitation on the reproductive success of ground-nesting birds.

The fledging success of dark-eyed juncos at other geographical locations, as reported in the literature, was comparable to the fledging success that I observed during my study. It is important to note that dark-eyed juncos in Newfoundland possibly face colder and wetter spring conditions compared to most parts of their breeding range.

Based on observations of dark-eyed juncos at five locations (five on the Avalon Peninsula and one at Tompkins) in January and early February of 1947, Burleigh and Peters (1951) concluded that dark-eyed juncos were uncommon winter residents in Newfoundland. Burleigh and Peters (1951) also reported that juncos nest soon after their arrival and that young could be found by early June and July. These findings indicate that the junco population shifted from being migratory to being resident year-round sometime after the 1940s. This transition could be due to the effects of climate change, which have been associated with increased temperatures in winter, less precipitation in the form of snow, and a decrease in snow cover duration between October–January (Finnis and Daraio 2018; St . John's Climate Profile 2020). Dark-eyed juncos have difficulty nesting when there is still snow on the ground because the snow makes their nests conspicuous and food for nestlings difficult to find (Smith and Andersen 1985). Less snow on the ground, combined with increased temperatures in winter months, might have facilitated the transition to being resident year-round in Newfoundland. Being resident year-round may also allow juncos to begin breeding earlier and to have time for multiple nesting attempts in a single breeding season. During my study, I observed a breeding pair make at least four breeding attempts between May and August. However, there are no previous studies done on the migration, breeding biology, or degrees of dispersal and philopatry in this population, which is the most north-easterly population in the species' range. My study sheds light into the vocal behaviour, nesting behaviour, and reproductive success of this population, as well as the threats that it faces. I have also banded and collected morphometric data and blood samples from approximately 150 dark-eyed juncos at my study site (including nestlings) that might be useful for future studies of this population.

Scope and limitations

When considering the results of this study, it is important to note the limited sample size. It is also important to consider that the data were collected during only two consecutive years, in 2018 and 2019, in a spruce/fir-dominated forest with some human disturbance (i.e., Memorial University Botanical Garden) and in a heavily human-manipulated environment (i.e., Memorial University's St. John's campus).

I did not conduct paternity tests for the juncos in my research. Since dark-eyed juncos are known to have extra pair paternity (Nolan et al. 2020), it is possible that the observed reproductive success, as defined by the number of eggs laid and the number of nestlings fledged, is different from the actual reproductive success realized by males in my study. It is possible that observed reproductive success is over or under-represented because the paternity of the nestlings in a male's nest, and in his neighbours' nests, was not included in the study. Unfortunately, an extra-pair paternity analysis was not possible due to sampling limitations. Although I conducted winter banding at the Memorial University Botanical Garden and banded most of the birds present in the winter flocks, most of those birds were not found at the study site during the following breeding season due to dispersal. Furthermore, many unbanded birds moved into the study site in spring.

The high proportion of unsampled males at the study site during the breeding season made an extrapair paternity analysis not feasible.

Finally, it is also important to note that the nest microhabitat measurements were obtained immediately after fledging to avoid any disturbances to the nest. Therefore, the microhabitat parameters that I measured might not have been representative of those experienced at the nest during the incubation and nestling stages due to the growth of vegetation. Similarly, my weather variables were measured at a nearby weather station, where weather conditions might have differed from those experienced at the nest.

Future Directions and Recommendations

There are several avenues of future research that should be considered. First, installing autonomous audio recorders in each territory early in the breeding season would allow researchers to measure total song production and the variation in song production and song types throughout the breeding season. During my study, I observed that some males are highly vocal, whereas others only sing occasionally. This variation might reflect stable differences among males (i.e., personality) or variation within males that is related to their breeding stage (e.g., mating, incubation, feeding nestlings, feeding fledglings; Naguib et al. 2016; Mejías and Wilson 2023). Second, it would be helpful to continuously measure weather parameters such as ambient air temperature and precipitation using data loggers placed at the nest site throughout incubation and nestling stages, since temperature and precipitation can vary over short distances and among microhabitats. Third, if this type of study were to be repeated, it might be more powerful to compare microhabitat at nest sites to microhabitat at randomly selected sites within the males' own territory (Ethier and Wilson 2020). Future studies could also benefit from estimating dominance hierarchies established in winter flocks, and then testing whether dominance confers reproductive benefits to dark-eyed juncos in the following breeding season as it does in some other species (Mennill et al. 2004). Lastly, it would be valuable to gather data on the abundance and types of predators found near dark-eyed junco nests, and the impacts they have on reproductive success.

Conclusion

I did not find evidence that dark-eyed junco songs exhibit a performance trade-off, and none of the song structures or male body size correlated with male reproductive success. However, my data show that hatching success is associated with canopy cover, precipitation, nest insulation, and stem density at the nest site. These patterns suggest that some nests experience greater risk of hypothermia and predation by predatory red squirrels, though future studies on nest microclimate, extreme weather events, and the impacts of local predators on nesting success are required.

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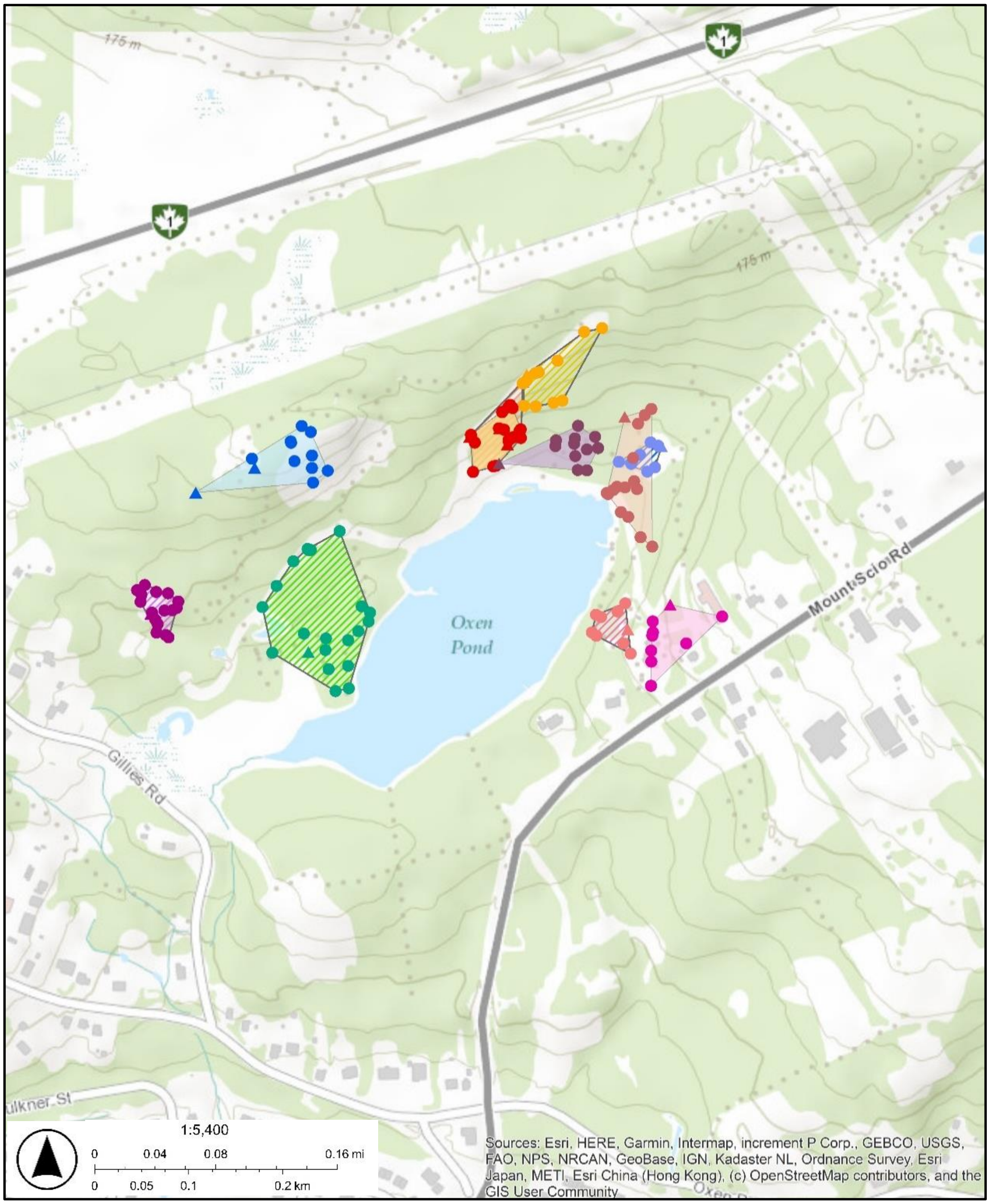
APPENDIX A



Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

Figure A.1: This map shows a subset of dark-eyed junco territories that were mapped during 2018 and 2019 breeding seasons at the Memorial University Botanical Garden. 2018 territories are marked with transparent colour and 2019 territories are marked with coloured streaks. Each color represents individual male. Each dot represents a singing perch of an individual dark-eyed junco and triangles represent their nest locations. Red transparent territory and red streaked territory was owned by the same male dark-eyed junco in two consecutive years.