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**THE NOCTURNAL FLIGHT CALLS OF MIGRATORY SONGBIRDS: INTERSPECIFIC VARIATION IN  
THE "ZEEP" COMPLEX AND INTRASPECIFIC VARIATION ACROSS NORTH AMERICA**

By

BLAINE LANDBOROUGH

A Thesis

Submitted to the Faculty of Graduate Studies  
through the Department of Biological Sciences  
in Partial Fulfillment of the Requirements for  
the Degree of Master of Science  
at the University of Windsor

Windsor, Ontario, Canada

2017

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**The nocturnal flight calls of migratory songbirds: interspecific variation in the “zeep”  
complex and intraspecific variation across North America**

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4 October 2017

## **Declaration of Co-Authorship**

I hereby declare that I am the sole author of chapters 1 and 4. I am the principle author of chapters 2 and 3, which were conducted under the supervision of Dan Mennill (University of Windsor) and Jennifer Foote (Algoma University).

Chapters 2 and 3 were co-authored by my supervisors, Dan Mennill and Jennifer Foote. Dan and Jenn supported my research and contributed financial support and logistical input to the experimental design, analyses, and manuscript writing for each chapter. Chapter 3 was also co-authored by a fellow graduate student, Rachel Hasson, who provided input at the inception of this study, and assisted with data collection for this chapter as well as providing editorial feedback.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from my co--authors to include the above material in my thesis. I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

I declare that, to the best of my knowledge, my thesis does not infringe upon anyone's copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my thesis, published or otherwise, are fully acknowledged in accordance with the standard referencing practices.

I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis committee and the Graduate Studies office, and that this thesis has not been submitted for a higher degree to any other University or Institution.

## **Abstract**

The nocturnal flight calls of birds are short vocalizations, produced primarily during migration. Although these calls offer a unique opportunity for studying avian migration, there has been little research into many aspects of these calls, such as the species-specificity of the calls of closely related taxa, or variation in calls associated with age, sex, or geography. The objective of my thesis research was to investigate acoustic variation within the flight calls of songbirds to expand our understanding of these calls and their application in migration monitoring. I recorded the flight calls of birds held for banding as well as birds actively flying during their nocturnal movements. In my first data chapter, I investigated whether the nocturnal flight calls of nine warbler species (i.e. the “zeep” species-group) exhibited acoustic differences. Analysis of the acoustic properties of flight calls of these species revealed significant differences in call structure between species, including five species that were notably different from the others in one or more acoustic properties. My results revealed that flight calls could be assigned to the correct species more often (73%) than expected by chance (36%), although the classification was not perfect. Therefore, acoustic variation in the flight calls of the “zeep” complex can be used to identify more species than previously thought. In my second data chapter, I explored intraspecific variation in flight calls. I found no evidence of sex-based or age-based variation in three species, and no evidence of geographic variation in two species. Although I found geographic variation in the calls of Dark-eyed Juncos, there was no consistent pattern on an east-west axis. Together, these results provided very little evidence for variation in flight calls with sex or age and limited evidence for geographic variation. Consequently, flight calls may be used to identify species (or species-groups) but not to identify sex, age, or geographic origin. My research serves to enhance the capabilities of nocturnal flight call detections for monitoring migratory birds while improving our understanding of drivers of variation in these calls.

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## Table of Contents

Declaration of Co-Authorship.....	iii
Abstract.....	iv
Acknowledgements.....	v
List of Tables.....	ix
List of Figures.....	x
Chapter One.....	1
General Introduction .....	1
Bird Migration.....	2
Nocturnal Monitoring Approaches.....	3
Nocturnal Flight Calls.....	4
Limitations of Nocturnal Flight Call Detections.....	5
Acoustic Variation.....	7
Variation in Nocturnal Flight Calls.....	8
Thesis Objectives.....	9
Significance.....	10
Literature Cited.....	12
Chapter Two.....	16
Decoding the “Zeep” Complex: Quantitative Analysis of Interspecific Variation in the Nocturnal Flight Calls of Nine Wood Warbler Species ( <i>Parulidae</i> Spp.) .....	16
Chapter Summary.....	17
Introduction.....	18
Methods.....	21
General Field Methods.....	21
Spectrographic Analysis .....	23
Statistical Analysis.....	24
Results.....	25
Analysis of variance of flight call properties .....	25
Canonical discriminant analysis .....	27
Spectrographic Cross-Correlation .....	28
Discussion.....	28
Literature Cited.....	34
Chapter Three: .....	48



Intraspecific Variation in Nocturnal Flight Calls: Analysis of Variation due to Sex, Age, and Geography in Five Species of Migratory Songbirds .....	48
Chapter Summary.....	49
Introduction.....	50
Methods 1: Variation in Age or Sex.....	53
General Field Methods.....	53
Spectral Analysis .....	55
Statistical Analysis.....	56
Results 1: Variation in Age or Sex.....	58
American Redstart .....	58
Magnolia Warbler .....	59
Yellow Warbler.....	60
Methods 2: Geographic Variation.....	61
General Field Methods.....	61
Spectral Analysis .....	62
Statistical Analysis.....	63
Results 2: Geographic Variation.....	64
Discussion.....	66
Literature Cited.....	71
Chapter Four: .....	85
General Summary .....	85
Literature Cited.....	91
Vita Auctoris.....	92

**List of Tables**

**Table 2.1** Total number of individuals per species included in comparisons of flight call properties across species..... 37

**Table 2.2** Means  $\pm$  standard deviations of acoustic measurements of flight call properties for the nine “zeep” warbler species ..... 38

**Table 2.3** Mean similarity values produced from pairwise spectrographic cross-correlation of the nocturnal flight calls of nine warbler species (i.e. “zeep” complex”) ..... 39

**Table 3.1** Number of nocturnal flight calls recorded at 11 locations across North America ..... 74

## List of Figures

<b>Figure 2.1</b> Spectrographic visualization of 14 acoustic measurements of nocturnal flight calls produced by migratory songbirds.....	40
<b>Figure 2.2</b> Examples of nocturnal flight calls produced by the nine warbler species in the “zeep” species-group, a bioacoustic category of birds that produce similarly structured flight calls. ....	41
<b>Figure 2.3</b> Analysis of variance of mean duration of the nocturnal flight calls of nine Parulidae species.....	42
<b>Figure 2.4</b> Analysis of variance of mean distance to maximum amplitude of the nocturnal flight calls of nine migratory warblers .....	43
<b>Figure 2.5</b> Analysis of variance of mean inter-peak duration of the nocturnal flight calls of nine migratory warblers .....	44
<b>Figure 2.6</b> Analysis of variance of mean peak frequency at maximum amplitude of the nocturnal flight calls of nine migratory warblers .....	45
<b>Figure 2.7</b> Flow chart for assigning species identity to nocturnal flight calls of birds in the “zeep” flight call complex. ....	46
<b>Figure 2.8</b> Plot of first two canonical axes produced by canonical discriminant analysis using 14 acoustic measurements.....	47
<b>Figure 3.1</b> Examples of nocturnal flight calls produced by male, female, juvenile (i.e. Hatch Year and Second Year), and adult (i.e. After Hatch Year and After Second Year) American Redstarts.	75
<b>Figure 3.2</b> Examples of nocturnal flight calls produced by male, female, juvenile (i.e. Hatch Year and Second Year), and adult (i.e. After Hatch Year and After Second Year) Magnolia Warblers.	76
<b>Figure 3.3</b> Examples of nocturnal flight calls produced by juvenile (i.e. Hatch Year and Second Year) and adult (i.e. After Hatch Year and After Second Year) Yellow Warblers, which were held for banding.....	77
<b>Figure 3.4</b> Map of the 13 recorders deployed across North America to sample for geographic variation in the nocturnal flight calls of songbirds .....	78
<b>Figure 3.5</b> Examples of American Redstart flight calls recorded at two locations (Saskatoon, Saskatchewan and Bon Portage Island, Nova Scotia) .....	79
<b>Figure 3.6</b> A multivariate analysis of variance of American Redstart mean principal components revealed significant variation but no consistent geographic variation in call properties .....	80
<b>Figure 3.7</b> Examples of American Tree Sparrow flight calls recorded at two locations (Saskatoon, Saskatchewan and Long Point, Ontario).....	81
<b>Figure 3.8</b> A multivariate analysis of variance of American Tree Sparrow mean principal components revealed significant variation but no consistent geographic variation in call properties.....	82
<b>Figure 3.9</b> Examples of Dark-eyed Junco flight calls recorded at two locations (Prince George, British Columbia and Kent Island, New Brunswick). Flight calls were produced by different	

individuals and are representative of the magnitude of variation in fine structure within these vocalizations at these locations. .... 83

**Figure 3.10** A multivariate analysis of variance of Dark-eyed Junco flight call properties revealed significant differences between locations ..... 84

## **Chapter One**

### **General Introduction**

## **Bird Migration**

Bi-annually, billions of birds embark on seasonal journeys across North America between their breeding and winter habitats (Richardson 1990). While many birds migrate diurnally, a substantial proportion of avian migratory movements occur nocturnally under the cover of darkness (Evans and Mellinger 1999). Many birds migrate at night to take advantage of prevailing winds for more efficient flight, and because low light conditions minimize predation (Alerstam et al. 2011). Despite the advantages to nocturnal movements, migratory birds face dangers associated with long-distance nocturnal flights, including collisions with human-made structures (e.g. wind turbines, communication towers; Erickson et al. 2005) and risks associated with anthropogenic light (e.g. Gehring et al 2009; Watson et al. 2016). Further, many migratory populations are increasingly subjected to habitat loss and fragmentation throughout their northern breeding habitats and southern winter ranges (Donovan and Flather 2002). As a result, many migratory bird species have experienced significant population declines throughout their ranges in recent decades (Brennan and Kuvlesky 2005; Askins 1993), which heavily emphasizes the need for collecting demographic information on bird populations to facilitate the development and evaluation of conservation management plans (Bart 2005).

There are many challenges associated with research on population trends of migratory birds, particularly the remoteness of many species' breeding and winter grounds. Rather than surveying bird populations on their breeding grounds or wintering grounds, surveying animals during migration offers the opportunity to monitor migratory animals without the logistical challenges of sampling in remote locations (Dunn 2005). There is a suite of monitoring techniques and approaches used to track and study these large-scale population movements, and each approach presents unique limitations. A substantial proportion of the existing data on

population trends comes from a network of North American bird observatories that conduct intensive, site-specific migration counts during daylight hours, including daily censuses and mist-netting efforts (Dunn 2005; Bart 2005). While many raptors and cranes migrate diurnally to take advantage of thermal soaring, the majority of migratory passerines and small birds migrate at night when atmospheric conditions are more favourable to long-distance flight (Alerstam 2009). Many birds migrating past established monitoring sites, however, will not stop at that specific location, and diurnal sampling efforts may therefore miss these nocturnal movements (Dunn 2005). As a result, diurnal monitoring approaches may be unable to provide a comprehensive picture of migration through a region.

### **Nocturnal Monitoring Approaches**

Recognizing the need for sampling these nocturnal movements, biologists have developed and improved nocturnal monitoring approaches since the 19<sup>th</sup> century (e.g. Scott 1881). Moon watching, an approach involving aiming a telescope at the moon and counting the silhouettes of passing birds, was the first method developed to attempt to quantify the direction and magnitude of nocturnal migration (Lowery 1951). Moon watching is still used by biologists in areas of the world without access to more technologically advanced approaches (e.g. Zehtindjiev and Liechti 2003). In current times, however, biologists have a suite of tools at their disposal to study nocturnal movements of birds. To overcome the limitations of moon watching and to provide direct counts of migratory animals, ornithologists developed approaches to incorporate passive infrared cameras to detect the heat signals of passing birds (Gauthreaux and Livingston 2006). However, these infrared cameras are unable to provide accurate measures of distance or altitude of biological targets (Gauthreaux and Livingston 2006). Doppler radar, relying on North America's Weather Surveillance Radar system, is a powerful tool to detect the

density, location, and velocity of flocks of migratory animals within the atmosphere, including birds, bats, and insects (Larkin 1991; Gauthreaux and Livingston 2006; Horn and Kunz 2008). However, radar detection rates vary with an object's flight altitude (Dunn 2005). Further, radar and infrared camera technologies cannot differentiate the species of animals within migrating flocks and therefore these techniques cannot provide data on species-specific population trends (Horton et al. 2015; Dunn 2005). Currently, there is only one approach to migration monitoring that can discern the species-identity of nocturnally migrating birds: the recording of nocturnal flight calls (Farnsworth 2005; Horton et al. 2015).

### **Nocturnal Flight Calls**

Nocturnal flight calls are defined as the primary vocalizations produced by birds during long, sustained flights associated with migratory activity (Evans and O'Brien 2002). These calls are short, species-specific vocalizations typically high in frequency (2-11 kHz; Evans and Mellinger 1999). Although some species are not known to produce these calls (e.g. vireos and new world flycatchers; Farnsworth 2005), diverse species of nocturnal migrants produce these calls, including sparrows, thrushes, wood warblers, cuckoos, and woodpeckers (Farnsworth 2005; Evans and O'Brien 2002). Many species from other avian clades also produce nocturnal flight calls, including herons and rails (Evans and Rosenberg 2000); however, research on flight calls has primarily focused on passerines and their close relatives (Farnsworth 2005). To date, the biological function of these calls remains uncertain, and poorly studied (e.g. Hamilton 1962; Drost 1963; Thake 1981). Several studies have suggested that birds use nocturnal flight calls to maintain flock cohesion and to stimulate migratory restlessness in conspecific animals (Farnsworth 2005; Hamilton 1962). Despite our gaps in understanding the biological function of these calls, research in recent decades has focused on developing methods for using nocturnal



flight call detections as a powerful ornithological tool to study migratory bird populations (e.g. Larkin et al. 2002; Farnsworth et al. 2004; Farnsworth and Russell 2007).

Several studies have validated the applied use of nocturnal flight calls as a population-monitoring tool through comparisons of acoustic recordings with radar, infrared imaging, and bird-banding data (e.g. Horton et al. 2015; Sanders and Mennill 2014a). These studies found significant correlations in the magnitude and direction of bird movements between these monitoring approaches, substantiating the validity of nocturnal flight call monitoring (Farnsworth et al. 2004; Sanders and Mennill 2014a). Moreover, the detection of nocturnal flight calls offers distinct advantages over other nocturnal monitoring methods, including the species-specificity of detection, the capacity to conduct long-term monitoring with a broad geographic range, and the ability to save recordings as a permanent archive. The unique capacity to identify migrating flocks to the species level allows biologists to use these vocalizations to study behaviours associated with migration, including the effects of anthropogenic light on migratory behaviour (Watson et al. 2016). For these reasons, a small community of ornithologists strongly advocate for the incorporation of nocturnal flight call detections in monitoring programs for use in conjunction with other monitoring approaches to provide a more complete window into the nocturnal movements of migratory birds.

### **Limitations of Nocturnal Flight Call Detections**

While the detection of nocturnal flight calls offers several advantages for monitoring nocturnal bird migration, acoustic recording does possess substantial drawbacks as well. A notable disadvantage is the susceptibility of acoustic recording to disturbances from high wind or precipitation events, which can preclude sampling during poor weather conditions. Poor weather conditions, however, also affect traditional monitoring approaches, including bird

banding and radar. The time and resources required to process recordings from a large-scale acoustic study is another limitation of acoustic monitoring (Swiston and Mennill 2009). While automated techniques do exist to detect and identify animal vocalizations from acoustic recordings (e.g. Acevedo et al. 2009), these automated processes frequently experience issues concerning false-positive detections and the misidentification of vocalizations (Blumstein et al. 2011; Venier et al. 2017). Currently, nocturnal flight call studies routinely use trained experts to process recordings by annotating and identifying the detected calls (e.g. Sanders and Mennill 2014b). Although trained humans offer a higher accuracy rate for the detection and identification of vocalizations, this labour-intensive approach to sound analysis is time consuming, potentially limiting the amount of data capable of being processed and analyzed (Swiston and Mennill 2009).

The applied use of nocturnal flight calls as a monitoring tool faces other significant drawbacks concerning our lack of understanding these vocalizations. In addition to the biological function of these calls being poorly understood, there remain substantial gaps in our knowledge of species' calling behaviour and the variation in call rates within and among species (Farnsworth 2005). The primary limitation to the applied use of nocturnal flight calls for studying bird migration is that the existing library of flight calls includes little information about the variation within these vocalizations, such as variation associated with age, sex, or geographic location of the calling animal (Lanzone et al. 2009; Farnsworth 2005). The current library of nocturnal flight calls is also limited by small sample sizes, species' nocturnal calls lacking diurnal confirmation of species identification, and the scarcity of information on intraspecific variation in these calls (Evans and O'Brien 2002; Lanzone et al. 2009). Further, many species produce calls that are highly similar in structure, which cannot be reliably identified to the species level (Evans and O'Brien 2002). As a result, previous nocturnal flight call studies have classified species with

similar calls into bioacoustic categories consisting of 2 to 9 species (e.g. Sanders and Mennill 2014a). It is vital that we quantify the interspecific and intraspecific variation in these signals in order to make the most of nocturnal flight call detections as an ornithological tool. This is a central motivation for the research I present in the two data chapters that comprise this thesis.

### **Acoustic Variation**

Acoustic signals play an important role in many aspects of animal behaviour, including mate attraction, territory defense, and species recognition (Bradbury and Vehrencamp 2011; Catchpole and Slater 2008). Acoustic signals often exhibit considerable variation within and among individuals (Ryan and Kime 2003; Podos and Warren 2007); however, variation in a signal can have direct consequences on animal fitness (Ripmeester et al. 2010). Acoustic divergence may be driven by several different evolutionary pressures including sexual selection (Gil and Gahr 2002), ecological selection (Slabbekoorn and Smith 2002), cultural drift (i.e. copying errors during learning; Wilkins et al. 2013), and genetic drift (Podos et al. 2004). Although multiple evolutionary pressures may operate on acoustic signals simultaneously, sexual selection is considered a primary driver behind the evolution of the high degrees of complexity and variation in song (i.e. vocalizations used in mate attraction and territory defense; Bradbury and Vehrencamp 2011). Outside of the context of sexually-selected songs, however, other selection pressures still act on non-song vocalizations (i.e. vocalizations that are not involved in the roles of territorial defense and mate attraction; Baker et al. 2000), which may promote intraspecific and interspecific divergence in these signals. Given that nocturnal flight calls are not understood to function in territorial defense or mate attraction, a separate set of processes likely influence their patterns of variation.

Research focus on bird songs has led to a deeper appreciation for the forces driving divergence in those vocalizations; however, our understanding of the intricacies and processes influencing the evolution of calls is much more limited (Marler 2004). Since the acoustic properties of the surrounding environment can affect signal transmission (Richards and Wiley 1980), birds may adjust the spectral and temporal properties of calls to maximize signal propagation (Boncoraglio and Saino 2006). Further, evolutionary pressures to facilitate species recognition between sympatric species may promote divergence in calls and vocalizations among species (Seddon 2005). Although there has been relatively little research focused on variation in the calls of birds, intraspecific variation has been described in calls for a limited number of species (e.g. the 'gargle' call of chickadees; Baker et al. 2000).

### **Variation in Nocturnal Flight Calls**

Although nocturnal flight calls of migratory passerines are superficially similar among taxa, these calls vary substantially in fine signal structure within and among species, at least in some groups of birds (Evans and O'Brien 2002). Scant attention has been given to the evolutionary factors responsible for this variation (Farnsworth and Lovette 2005). Previous studies have focused exclusively on interspecific variation within wood warblers (e.g. Farnsworth and Lovette 2005; Farnsworth and Lovette 2008). While the evolution of nocturnal flight calls in wood warblers is not subjected to morphological constraints (e.g. body mass and bill length; Farnsworth and Lovette 2005), a comparative analysis of 47 North American wood warblers revealed significant phylogenetic effects present among spectral and temporal properties of flight calls (Farnsworth and Lovette 2008). When controlled for phylogeny, flight call characteristics showed strong correlations with ecological properties (i.e. birds that occur in canopies that are more open were associated with flight calls of higher frequency; Farnsworth

and Lovette 2008). Differences in call structure may be the result of adaptation to the acoustic environment dictating the spectral properties of birds' flight calls (e.g. minimum and maximum frequency) for optimal signal transmission (Farnsworth and Lovette 2008; Wiley and Richards 1982).

Although some flight calls are species-specific, phylogenetic constraints or ecological constraints have resulted in many species producing flight calls with similar acoustic characteristics (Farnsworth and Lovette 2008). Currently, nocturnal flight call studies place acoustically similar flight calls into bioacoustic categories, which contain up to nine bird species that produce flight calls with remarkably similar spectro-temporal characteristics (e.g. Sanders and Mennill 2014a). The “zeep” species-group is one of the largest bioacoustic categories, comprising nine wood warbler species (Evans and O'Brien 2002). The inability to distinguish and reliably identify these calls to the species level inhibits nocturnal flight calls studies from providing information on the population trends of these species. Many warbler species in North America are experiencing substantial population declines and are of conservation concern (Askins 1993), including three species belonging to the “zeep” species-group (Worm-eating Warbler, *Helmitheros vermivorum*; Louisiana Waterthrush, *Parkesia motacilla*; and Cerulean Warbler, *Setophaga cerulea*); therefore, the ability to differentiate the “zeep” complex will help directly with conservation efforts.

### **Thesis Objectives**

The overall objective of my Master's Thesis research is to quantify interspecific and intraspecific variation in the nocturnal flight calls of migratory passerines in North America. My motivation is to improve the capabilities of acoustic recordings for monitoring bird migration by quantifying interspecific and intraspecific variation in these vocalizations. In my first data

chapter (Chapter 2), I investigate whether the fine structural characteristics of nocturnal flight calls produced by nine wood-warbler species (i.e. those species belonging to the “zeep” species-group) have diverged enough among species to allow reliable identification to the species level based on spectro-temporal characteristics of the call. I investigate differences in “zeep” calls using recordings of birds held for banding at two bird observatories in southern Ontario, between April-May 2016-2017 and August-October 2015-2016, and recordings collected from public databases of animal sounds. I explore the potential opportunities to discern these species based on the structural characteristics of their calls, which would have substantial benefits to acoustic recording through enabling the collection of species-specific population data for these nine warbler species.

For the second data chapter of my thesis (Chapter 3), I investigate intraspecific and geographic variation in the nocturnal flight calls of migratory passerines across North America. In part one of this chapter, I use calls produced by birds held for banding recorded at two bird observatories in southern Ontario during April-May 2016-2017 and August-October 2015-2016. I quantify intraspecific variation in nocturnal flight calls, and test the hypotheses that nocturnal flight calls encode information related to the individual’s age or sex. In part two of this chapter, I use calls collected from an array of automated recorders deployed across North America (15 August-15 November 2015-2016) to sample geography-based differences in these calls. I quantify geographic variation in nocturnal flight calls, and explore whether calls exhibit patterns of geographic variation along an east-to-west axis across North America.

### **Significance**

This research serves to enhance our understanding of the factors driving acoustic variation in nocturnal flight calls through a quantitative investigation of patterns of intraspecific

and interspecific variation. This research may help to discern superficially similar vocalizations produced by closely related species to improve the accuracy of acoustic recording for sampling nine wood-warbler species. By identifying species-specific features of these calls, this research may help to collect information on population trends of these birds and inform the conservation efforts of declining species. Further, this research is the first, to my knowledge, to investigate and quantify intraspecific variation in the nocturnal flight calls of migratory songbirds across a broad geographic range. This research will have significant applied implications through increasing our understanding of variation in these calls and improving the capabilities of nocturnal flight call detections for monitoring nocturnal migrants.

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## **Chapter Two**

### **Decoding the “Zeep” Complex: Quantitative Analysis of Interspecific Variation in the Nocturnal Flight Calls of Nine Wood Warbler Species (*Parulidae* Spp.)**

## Chapter Summary

Nocturnal flight calls are short vocalizations produced by birds during migratory activity. These calls offer a special opportunity for biologists to monitor migratory species on the wing. The flight calls of many birds exhibit species-distinctive characteristics, yet other species produce calls with very similar acoustic structure, forcing researchers to classify the calls of many species into catch-all bioacoustic categories. One notoriously challenging group of birds with similar calls is the “zeep” complex, consisting of nine warbler species. Our inability to distinguish species within this group inhibits the collection of information on population trends for these species. In this study, we test the hypothesis that the flight calls of nine warblers in the “zeep” species-group show sufficient acoustic differences to allow differentiation based on acoustic properties. We recorded birds held for banding at migration monitoring stations in southern Ontario, and we collected additional recordings from sound libraries to investigate divergence in these vocalizations. We used three bioacoustic approaches to compare the calls among species: analysis of variance in acoustic properties, discriminant analysis of acoustic properties, and spectrographic cross-correlation. The first approach revealed five species that were notably different in one or more acoustics properties. The second approach, using six warblers, revealed a level of assignment to the correct species (73%) that exceeded levels expected by chance (36%). The third approach, by contrast, did not reveal species-level acoustic differences, which we interpret as a limitation of spectrographic cross-correlation. Taken together, our results suggest the calls of at least some members of the “zeep” complex exhibit species-specific differences in structure, which may allow differentiation of up to five “zeep” species based on their calls alone. We advocate for the continued compilation of a comprehensive library of nocturnal flight calls to allow further resolution of calls with similar structure.

## Introduction

Acoustic recording technologies provide biologists with powerful tools to study and monitor animal populations across remote geographic areas and extended periods of time (Blumstein et al. 2011). Several studies have shown acoustic monitoring approaches to sample populations rapidly while providing reliable data on the occurrence and abundance of target species (Parker 1991; Celis-Murillo et al. 2009). To date, acoustic recordings have been used to collect information efficiently for populations of birds (Sanders and Mennill 2014a), anurans (Acevedo and Villanueva-Rivera 2006), mammals (Russo and Jones 2003; Payne et al. 2003; André et al. 2011), and insects (Penone et al. 2013). As bioacoustic technologies have improved and become more accessible, recent decades have seen a growth in the applied use of nocturnal flight call detections as a powerful tool for monitoring the populations and movements of migratory birds (Evans and Rosenberg 2000; Sanders and Mennill 2014b).

Nocturnal flight calls are short (50-300ms) species-specific vocalizations produced by diverse bird species, including warblers (*Parulidae*), sparrows (*Emberizidae*), thrushes (*Catharus*), and rails (*Rallidae*). They are produced by birds primarily in association with migratory behaviour (Evans and O'Brien 2002, Farnsworth 2005). These calls may be used to facilitate flock cohesion and to stimulate migratory restlessness (Hamilton 1962), although the biological function of nocturnal flight calls remains uncertain and little-studied (Farnsworth 2005). The utility of these calls has proven to be a compelling method for sampling the timing and magnitude of the nocturnal movements of migratory birds (Evans and Rosenberg 2000; Larkin et al. 2002; Farnsworth et al. 2004; Sanders and Mennill 2014b; Horton et al. 2015). While other approaches for monitoring migrants provide valuable information on movements of birds in flight (most notably, radar; Diehl et al. 2003), the detection of nocturnal flight calls is currently

the only approach capable of identifying the species composition of nocturnally migrating flocks (Horton et al. 2015). Nocturnal flight call detections can therefore provide important insight into migratory behaviours of birds during their nocturnal movements.

The ability to identify the species of nocturnally migrating birds is a major advantage of the detection of nocturnal flight calls, yet there are several challenges associated with this technique. The primary challenge of processing nocturnal flight call recordings is the dearth of information concerning the degree of acoustic variation in the birds' nocturnal flight calls (Lanzone et al. 2009). To date, there has been limited research conducted on acoustic variation within these calls, with only one study investigating intraspecific variation (Griffiths et al. 2016). Interspecific analyses of variation within the warbler family have revealed a lack of morphological constraints on the evolution of flight call properties but found evidence for phylogenetic effects acting on these vocalizations (Farnsworth and Lovette 2005; Farnsworth and Lovette 2008). Despite the calls of these species being similar, the nocturnal flight calls of many warblers exhibit species-specific differences in fine structure (Evans and O'Brien 2002; Farnsworth 2005). There have been no attempts, however, to quantify the degree of acoustic variation within many species or to quantify the acoustic divergence in calls among species that produce calls sharing similar spectro-temporal properties. Without accurate information on the amount of variation within and between these calls, it is difficult to develop reliable approaches to identify acoustically similar calls to the species level.

The scarcity of information concerning variation in these signals has limited the development of automated and human classification approaches for identifying structurally similar flight calls (e.g. Sanders and Mennill 2014b). As a result, previous studies have grouped species producing highly similar flight calls into bioacoustic categories of calls that cannot be distinguished between (e.g. Evans and O'Brien 2002; Sanders and Mennill 2014b). The "zeep"

category, a species-group consisting of nine migratory wood-warbler species (Worm-eating Warbler, *Helmitheros vermivorum*; Louisiana Waterthrush, *Parkesia motacilla*; Connecticut Warbler, *Oporornis agilis*; Cerulean Warbler, *Setophaga cerulea*; Magnolia Warbler, *Setophaga magnolia*; Bay-breasted Warbler, *Setophaga castanea*; Blackburnian Warbler, *Setophaga fusca*; Yellow Warbler, *Setophaga petechia*; and Blackpoll Warbler, *Setophaga striata*), is one of the largest bioacoustic categories (Evans and O'Brien 2002; Sanders and Mennill 2014b). Previous nocturnal flight call studies have been unable to collect accurate information on the populations of birds within these bioacoustic categories (e.g. Sanders and Mennill 2014b). The inability to identify calls within these bioacoustic categories to the species level reliably reduces the utility of nocturnal flight calls detections for population monitoring. The possession of a library of high-quality recordings of flight calls produced by known individuals of each species, however, may allow biologists to discern the calls of these species and identify future "zeep" recordings to the species level.

In this study, we use bioacoustic approaches to explore interspecific differences in the nocturnal flight calls of nine warbler species within the "zeep" bioacoustic category. We hypothesized that differences are exhibited in the fine structural details of nocturnal flight calls produced by these nine species, which may allow reliable differentiation of these nine species based on flight calls alone. To test this, we collected recordings of nocturnal flight calls produced by birds held for banding, and supplemented these with recordings from sound libraries. We used three spectrographic analysis techniques: analysis of variance in fine structural features of calls, discriminant analysis of fine structural measurements of calls, and spectrographic cross-correlation to quantify acoustic differences between species. This is the first study to investigate which bioacoustic properties may allow reliable discrimination between the nocturnal flight calls of the nine "zeep" species. The ability to differentiate the "zeep" flight calls of nocturnally



migrating birds based on the fine structure would considerably improve the capabilities of acoustic recording for studying the nocturnal behaviours of these nine warbler species, including multiple species of conservation concern (e.g. Cerulean Warbler, Louisiana Waterthrush, and Worm-eating Warbler).

## **Methods**

### *General Field Methods*

We collected recordings from birds held for banding at two bird observatories in southern Ontario, Canada: Long Point Bird Observatory (42.5829°N, -80.3984°W) and Holiday Beach Migration Observatory (42.0322°N, -83.0437°W). We recorded birds at Holiday Beach Migration Observatory between 15 August and 31 October 2015 and 2016, and Long Point Bird Observatory between 2 April and 3 June 2016 and 2017. Holiday Beach Migration Observatory is located within the Holiday Beach Conservation Area on the north shore of the western basin of Lake Erie. Holiday Beach, in combination with the adjacent Big Creek Conservation Area, is listed as an Important Bird Area due to high concentrations of diurnal raptors and migratory landbirds during fall migration. Long Point Bird Observatory is located at the base of the Long Point peninsula on the northern shore of Lake Erie. The peninsula is a 40 km sand spit hosting a wide array of habitats, including woodlands and marshes, and serves as an important migratory staging and stopover location for many passerine and waterfowl species.

Our focal species included nine wood warbler species in the “zeep” species-group: Bay-breasted Warbler, Blackburnian Warbler, Blackpoll Warbler, Cerulean Warbler, Connecticut Warbler, Louisiana Waterthrush, Magnolia Warbler, Worm-eating Warbler, and Yellow Warbler. We recorded birds of five of these species when they were held for banding: Bay-breasted Warbler, Blackburnian Warbler, Blackpoll Warbler, Magnolia Warbler, and Yellow Warbler (115

individuals). In order to increase our sample size of less-common and cryptic species (e.g. Worm-eating Warbler, Cerulean Warbler) and to facilitate inclusion of all nine species in our analysis, we collected recordings from public sources of flight call recordings: Xeno-Canto (<http://www.xeno-canto.org>; 25 individuals) and the *Flight Calls of Migratory Birds: Eastern North American Landbirds* CD-ROM (Evans and O'Brien 2002; 38 individuals; Table 2.1). All recordings taken from public sources indicated the birds were identified through visual confirmation.

Birds were captured with mist nets as part of the standard migration monitoring procedure at the banding observatories. We banded birds with United States Geological Survey (USGS) bands marked with unique nine digit numbers, and we transferred birds to a separate building, on site, equipped with a darkened and sound-dampened recording booth (modified from Lanzone et al. 2009). We placed birds into a cylindrical, cloth chamber suspended within the booth for recording. We positioned an omni-directional microphone (Sennheiser ME62/K6 microphone) and digital recorder (Marantz PMD670; settings: WAV format, 44.1 kHz sampling frequency, 16-bit accuracy) 30 cm below the chamber to record the birds' vocalizations. Using a loudspeaker, we exposed birds to acoustic stimuli of recordings of nocturnal flight calls from congeneric individuals to induce calling. Acoustic stimuli consisted of predetermined sequences of calls taken from the *Flight Calls of Migratory Birds: Eastern North American Landbirds* CD-ROM (Evans and O'Brien 2002). Each stimulus set recording began with 45 seconds of silence to allow the bird to acclimate to surroundings. This period of silence was followed by a series of congeneric flight calls presented at five-second intervals for the remaining duration of the recording session. We recorded birds for up to five minutes and then read the birds' band number into the microphone. We immediately released birds back to the area they were captured. In addition to the five-minute recording session, we used a shotgun microphone

(Sennheiser ME66/K6 microphone) to record any vocalizations produced by birds upon release, which occurred in <5% of releases.

### *Spectrographic Analysis*

In the laboratory, we visualized recorded calls by generating spectrograms with Syrinx-PC (spectral settings: 4 ms line<sup>-1</sup>, 256 FFT size, Blackman window; J. Burt, Seattle, WA). We scanned through recordings and extracted all calls produced by each bird as a separate WAV file. The calls of live birds were easy to differentiate from our playback stimuli due to the five-second intervals between stimuli calls. To reduce background noise, we filtered the WAV files with a high pass filter at 4 kHz. We normalized the amplitude of the filtered calls to -1 dB in Adobe Audition (version 3.0; Adobe, San Jose, CA). We then measured the fine spectro-temporal parameters of each call using Avisoft-SAS (spectral settings: 0.73 ms line<sup>-1</sup>, 256 FFT size, Blackman window; Avisoft Bioacoustics, Berlin, Germany). We assigned a number to each call (ignoring calls where a portion of the signal was masked by background noise) and randomly selected one call from each individual, using a random number generator, for measurement thereby avoiding pseudo-replication in our analyses.

For each call, we measured a total of 14 fine structural properties (figure 2.1): bandwidth; minimum frequency; maximum frequency; duration; distance to maximum amplitude; number of frequency modulations; inter-peak duration; slope of frequency modulation from start to end of call; slope of frequency modulation from minimum to maximum frequencies; slope of frequency modulation from start to first peak of call; and the peak frequency at multiple locations including the start, maximum amplitude, center, and end.

We conducted spectrographic cross-correlation for 178 flight calls from the nine warbler species. We performed the cross-correlation in Avisoft SASLAB PRO correlator (Avisoft

Bioacoustics, Berlin, Germany; 500 Hz maximum frequency deviation). Spectrographic cross-correlation compares each pair of sounds by overlaying spectrograms to provide a value between zero (no similarity in the spectrograms) and one (perfect similarity in the two spectrograms; Cortopassi and Bradbury 2000).

### *Statistical Analysis*

We used three statistical approaches to investigate differences in nocturnal flight call properties between nine warbler species. First, we used analysis of variance on the means of the 14 fine structural measurements and post hoc Tukey's tests to infer differences in flight call properties across species.

Second, we conducted a forward stepwise canonical discriminant analysis, with leave-one-out cross-validation, using 14 acoustic properties to determine whether flight calls can be assigned to species correctly based on spectral-temporal properties. Our stepwise canonical discriminant analysis selected five variables for inclusion over five steps. At each step, we selected the variable that minimized the overall Wilks' Lambda score. Due to small sample sizes and to avoid violating the analytical assumptions of discriminant analyses, only six of the nine "zeep" species were included in the canonical discriminant analysis of acoustic characteristics: Bay-breasted Warbler, Blackburnian Warbler, Blackpoll Warbler, Magnolia Warbler, Worm-eating Warbler, and Yellow Warbler. Further, one Blackpoll Warbler call was unusually long and was classified as out-of-range and excluded, resulting in 159 flight calls for use in canonical discriminant analysis. Due to unequal sample sizes for each of the nine species, the number of calls included for each species influences the chance of any call being assigned to the correct species. As a result, we calculated the level of correct assignment by chance using the weighted

mean of the probability of each call being classified to the correct species (Fitzsimmons et al. 2011).

Third, we compared the mean spectrographic cross-correlation scores within and between species. If spectrographic cross-correlation revealed species-specificity in these calls, we expected mean similarity values to be higher when comparing calls within species versus between species.

If our hypothesis is correct, we predicted that one, two, or all three of these techniques would reveal differences between the species in the “zeep” complex. All statistical analyses were conducted in SPSS, version 23 (Armonk, NY; IBM corp.).

## **Results**

### *Analysis of variance of flight call properties*

The nocturnal flight calls of the nine “zeep” species all occupied a common acoustic space, sharing many spectral-temporal characteristics (Table 2.2; Figure 2.2), yet analysis of variance of each acoustic feature revealed significant test effects for all 14 acoustic measurements (Table 2.2). Three post hoc Tukey’s tests revealed one species that was different from all the others, whereas the remaining post hoc comparisons did not, as summarized below.

The mean duration of flight calls was significantly variable among the nine “zeep” species (ANOVA:  $F_8=10.53$ ;  $p\leq 0.001$ ). Louisiana Waterthrush flight calls were significantly longer in duration than all others (Figure 2.3; Tukey’s post hoc test:  $p\leq 0.001$ ), whereas all eight remaining species’ calls were similar in duration. The distance to maximum amplitude of calls showed similar significant variation between species (ANOVA:  $F_8=11.24$ ;  $p\leq 0.001$ ), where Louisiana Waterthrush calls had a significantly longer distance from the start of the call to the

point of maximum amplitude in comparison to the eight other species (Figure 2.4; Tukey's post hoc tests:  $p \leq 0.001$ ). The eight remaining species showed no significant difference in distance to maximum amplitude. The inter-peak duration of flight calls exhibited significant variation between species (ANOVA:  $F_8=34.11$ ;  $p \leq 0.001$ ), where the inter-peak duration of Bay-breasted Warbler flight calls was significantly longer than the other species (Figure 2.5;  $p \leq 0.001$ ). Further, the peak frequency at maximum amplitude was significantly variable among the nine species (ANOVA:  $F_8=10.96$ ;  $p \leq 0.001$ ), where Worm-eating Warbler calls were significantly higher in peak frequency at maximum amplitude than all other species except Louisiana Waterthrush (Figure 2.6;  $p < 0.05$ ).

The nine species exhibited notable differences for multiple frequency measurements: minimum frequency (ANOVA:  $F_8=8.07$ ;  $p \leq 0.001$ ), peak frequency at maximum amplitude, and peak frequency at end (ANOVA:  $F_8=9.69$ ;  $p \leq 0.001$ ). Both Cerulean Warbler and Yellow Warbler calls were significantly lower in frequency, for several measurements, than other species (e.g. Tukey's post hoc tests for peak frequency at maximum amplitude: Yellow Warbler vs. Magnolia Warbler  $p \leq 0.001$ ; Cerulean Warbler vs. Worm-eating Warbler  $p = 0.001$ ). There was no difference, however, in peak frequency at maximum amplitude between Yellow Warbler and Cerulean Warbler calls (Tukey's post hoc test:  $p = 0.98$ ) or between Cerulean Warbler and Magnolia Warbler calls (Tukey's post hoc test:  $p = 0.96$ ). Cerulean Warbler and Yellow Warbler calls were generally lower in frequency measurements than most species but were not significantly different from one another (Tukey's post hoc tests:  $p > 0.40$ ).

The nine species showed significant interspecific variation in the number of frequency modulations of calls (ANOVA:  $F_8=10.06$ ;  $p \leq 0.001$ ). Louisiana Waterthrush and Cerulean Warbler calls contained more modulations than most species. For example, Louisiana Waterthrush calls contained significantly more modulations than seven species: Bay-breasted Warbler, Magnolia

Warbler, Blackburnian Warbler, Connecticut Warbler, Worm-eating Warbler, Yellow Warbler, and Blackpoll Warbler (Tukey's post hoc tests:  $p \leq 0.002$ ). However, Louisiana Waterthrush and Cerulean Warbler flight calls did not contain different numbers of modulation (Tukey's post hoc test:  $p = 0.77$ ). Further, the rates of frequency modulation (e.g. slope of frequency modulation from minimum to maximum frequency and slope of frequency modulation from start to first frequency peak) did reveal significant variation between species (ANOVA:  $p \leq 0.001$ ) although there were no notable differences between species in the post-hoc analyses (Tukey's post hoc tests:  $p > 0.07$ ).

Five species (Worm-eating Warbler, Louisiana Waterthrush, Cerulean Warbler, Bay-breasted Warbler, and Yellow Warbler) were significantly different from others and able to be distinguished based on one or more acoustic properties in this dataset (Figure 2.7). Only calls produced by two species (Worm-eating Warbler and Louisiana Waterthrush) could be unambiguously assigned to species in all cases.

#### *Canonical discriminant analysis*

A forward stepwise canonical discriminant analysis of the flight calls of six species of warbler classified 73% of 159 calls to the correct species based on 14 fine structural properties (cross-validation: 68.6% assigned correctly; Figure 2.8). The five acoustic features selected by the model were inter-peak duration, maximum frequency, slope of frequency modulation from start to first peak, peak frequency at maximum amplitude, and number of modulations, respectively. In spite of the 27% misclassification rate, the 73% accuracy of the canonical discriminant analysis was significantly higher than the 36.1% level of correct assignment by chance (Binomial test:  $p \leq 0.001$ ). Spectral-temporal measurements with strongest loadings on

the first two canonical axes included the inter-peak duration, maximum frequency, and the peak frequency at maximum amplitude of the call.

Canonical discriminant analysis correctly identified 85% (51 out of 60) and 84% (21 out of 25) of Magnolia Warbler and Bay-breasted Warbler flight calls, respectively. Magnolia Warblers, however, were represented considerably more in the analysis (60 out of 159 calls). The analysis showed mixed levels of accuracy when classifying the four remaining species. Canonical discriminant analysis revealed the lowest accuracy when classifying Blackpoll Warbler flight calls (33% identified correctly), which were misidentified as Magnolia Warbler calls 44% of the time. Connecticut Warbler, Cerulean Warbler, and Louisiana Waterthrush were not included in the discriminant analysis due to small sample sizes.

#### *Spectrographic Cross-Correlation*

Spectrographic cross-correlation yielded no evidence of species differences in calls. In direct contrast to this idea, our pairwise spectrographic cross-correlations revealed the calls of eight of the nine “zeep” species to be more similar to heterospecific calls rather than calls produced by their own species (Table 2.3). The only species that showed a different pattern was the Louisiana Waterthrush, which showed remarkably similar spectrographic cross-correlation scores for within- and between-species comparisons (Table 2.3).

#### **Discussion**

Two of three bioacoustic comparison techniques revealed significant variation in the acoustic structure of nocturnal flight calls across the “zeep” species complex. Analysis of variance of fine structural details of flight calls showed significant variation between species in all 14 variables, although post-hoc analysis showed significant species-to-species differences for



a minority of variables and species. Further, a canonical discriminant analysis of fine structural properties assigned calls to the correct species at levels significantly higher than statistical chance, although not with perfect accuracy. The canonical loadings of our analysis suggest that inter-peak duration, peak frequency at maximum amplitude, and maximum frequency are important structural features for distinguishing these calls. There remains, however, a substantial level of misclassification for certain species (e.g. Blackpoll Warbler) and multiple misclassifications for every species. These results support our hypothesis that the nocturnal flight calls of some of the “zeep” warbler species exhibit species-specific differences in fine structural features, which may allow differentiation of these vocalizations. Whereas several species within this complex may be classified on the basis of their nocturnal flight calls, our results suggest that the majority can be classified only with moderate accuracy, with some classification error. Therefore, the use of nocturnal flight calls to differentiate species within the “zeep” complex must be done cautiously, and with recognition of this error.

Our analysis of variance of 14 acoustic measurements revealed notable differences in fine structure of these calls between species. Five species, Worm-eating Warbler, Louisiana Waterthrush, Cerulean Warbler, Bay-breasted Warbler, and Yellow Warbler, were significantly different from others and able to be distinguished based on one or more acoustic properties (Figure 2.7). Worm-eating Warbler and Louisiana Waterthrush flight calls were the only “zeep” calls able to be differentiated in all cases. In particular, Louisiana Waterthrush flight calls are significantly longer in duration and distance to maximum amplitude, and Bay-breasted Warbler flight calls have significantly longer inter-peak duration. As a result, “zeep” calls with a duration of  $>75\text{ms}$  and distance to maximum amplitude of  $\geq 47\text{ms}$  may be reliably identified as Louisiana Waterthrush, and calls with an inter-peak duration of  $>20\text{ms}$  can be reliably identified as Bay-breasted Warbler. Further, species exhibited other notable differences in structural details

between species. For example, Cerulean Warbler and Yellow Warbler flight calls were typically lower in frequency than other species but were not significantly different from one another, and therefore cannot reliably be distinguished on the basis of frequency characteristics alone.

Our results indicate there are discrete differences between these calls that allow the differentiation of some members of the “zeep” complex based on structural details. Past nocturnal flight calls studies have grouped these species together due to the similarities in fine structure and the limited information we have for these calls (e.g. Sanders and Mennill 2014b). This practice of classifying nocturnal flight calls into catch-all bioacoustic categories has inhibited the collection of species-specific population data on many bird species including the nine “zeep” warblers. Separation of the “zeep” flight calls would allow biologists to use passive acoustic monitoring to collect detailed information on the populations of these nine warblers, including multiple species of conservation concern (e.g. Louisiana Waterthrush, Cerulean Warbler, and Worm-eating Warbler).

Despite the differences in fine structure, there has been no research, to date, that has investigated whether these warblers are able to discern the flight calls within the “zeep” species-group. Many bird species, however, are able to communicate information about the caller in other types of calls, including species-identity in the alarm calls of many passerine birds (e.g. European Robin, *Erithacus rubecula*, Davies et al. 2004; Reed Warbler, *Acrocephalus scirpaceus*, Davies et al. 2004). Moreover, diverse bird species have demonstrated a high capacity for discerning discrete differences in the fine structure of calls (e.g. individual recognition of King Penguin parents’ calls, *Aptenodytes patagonicus*; Jouventin et al. 1999; kin recognition in contact calls of Long-tailed Tits, *Aegithalos caudatus*; Sharp et al. 2005). The ability of the migratory birds to discern their nocturnal flight calls could potentially provide fitness benefits. The nine “zeep” warblers exhibit differences in habitat preferences and

migration timing (Schieck and Song 2006; Francis and Cooke 1986), and discrete differences in call structure could serve a role in facilitating conspecific recognition during migration. Many warbler species participate in mixed species flocks during migration and may not have the necessity (or ability) to discern these calls (Rodewald and Brittingham 2002). The discrete differences in fine structure between species may be the result of different ecological pressures acting on the fine structure of flight calls for maximum propagation of sound within habitats that the birds are most strongly associated with (Morton 1975; Farnsworth and Lovette 2008).

Although two of three spectral comparison techniques showed promise in revealing interspecific differences in structure, our canonical discriminant analyses experienced a 27% level of misclassification. Several factors may have contributed to this level of misclassification. Notably, our analyses suffered from small and unequal sample sizes for uncommon and cryptic species that are not captured regularly by mist-netting efforts in our study area. Many of the birds that were captured and placed in our recording apparatus (>70%) did not produce flight calls during the five-minute recording period and therefore could not be included in our analysis. The limited sample sizes of three rarer species (Connecticut Warbler, Cerulean Warbler, and Louisiana Waterthrush) made it impossible to include all nine species in all three comparison analyses in order to avoid violating the assumptions of canonical discriminant analyses (Cohen et al. 2013).

A third spectral comparison approach, spectrographic cross-correlation, failed to differentiate the flight calls of any of the nine species. Spectrographic cross-correlation techniques have been suggested to produce misleading or confounding results when comparing acoustic signals containing overtones, which are often present in nocturnal flight call recordings (Khanna et al. 1997; Evans and O'Brien 2002). Nocturnal flight call recordings are often recorded with very low signal to noise ratio, which may introduce irrelevant background information into

the spectrograms and confound any similarity analysis. Further, attempts to compare sounds that are different in duration, using spectrographic cross-correlation, may provide inaccurate results (Khanna et al. 1997). As a result, spectrographic cross-correlation may not be appropriate for comparison of nocturnal flight calls, which are highly variable in duration within and among species (Farnsworth 2005; Farnsworth and Lovette 2008). Instead, our results suggest the analysis of fine structural details is an appropriate technique for the comparison on nocturnal flight calls.

It is important to develop appropriate procedures for the identification and comparison of other bioacoustic categories of nocturnal flight calls. For example, the 'up' species-group consists of seven warblers (Ovenbird, *Seiurus aurocapilla*; Tennessee Warbler, *Oreothlypis peregrina*; Orange-crowned Warbler, *Oreothlypis celata*; Nashville Warbler, *Oreothlypis ruficapilla*; Mourning Warbler, *Geothlypis philadelphia*; Yellow-rumped Warbler, *Setophaga coronata* and Black-throated Green Warbler, *Setophaga virens*) and two sparrows (Vesper Sparrow, *Pooecetes gramineus*; and White-crowned Sparrow, *Zonotrichia leucophrys*) that produce flight calls described as a single modulated upsweep that currently cannot be identified to species level (Sanders and Mennill 2014b). In previous studies, biologists have attempted to identify unknown flight calls by narrowing down possibilities based on seasonality and local reports of sightings (Lanzone et al. 2009); however, this increases the risk of misidentification and error within a data set. Since the identification of nocturnally migrating birds represents a primary benefit of acoustic recording over other nocturnal monitoring approaches, it is critical to quantify the variation within and between the nocturnal flight calls of our migratory birds to allow discernment of these calls. Currently, nocturnal flight call monitoring studies cannot meaningfully inform population trends of many species within these bioacoustic categories, which contain two to nine species (Sanders and Mennill 2014b). Since species that produce

structurally similar calls all possess unique natural histories with distinct habitat preferences and migratory strategies, it is critical to determine which flight call characteristics, if any, can be used to discern species identity of other flight call bioacoustic categories in order to allow the collection of species-specific information on these birds.

Our results suggest that duration, inter-peak duration, peak frequency at maximum amplitude, and maximum frequency of nocturnal flight calls are useful acoustic properties for distinguishing certain species within the “zeep” complex. This is the first study, to our knowledge, to investigate if acoustic divergence in flight call properties of the “zeep” species-group may allow reliable discernment of these calls. We suggest that spectro-temporal differences in flight calls produced by these species allow the reliable identification of five “zeep” species based on acoustic properties. There remains, however, a moderate level of misclassification when identifying the flight calls of seven species. This level of misclassification highlights the need for the collection of a comprehensive library of nocturnal flight calls, which could provide further resolution and accurate classification of these calls. Further, it is imperative to continue collecting recordings from individuals of known age, sex, and species in order to elucidate the pressures driving acoustic variation within the nocturnal flight calls of migratory birds. This study serves to improve the capabilities of nocturnal flight call monitoring through allowing the collection of species-specific information on the populations of five migratory songbirds, including two species-at-risk (i.e. Louisiana Waterthrush and Cerulean Warbler).

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## Tables

Species	Total Number of Individuals	Recorded Birds	Recordings from Evans and O'Brien	Recordings from Xeno-Canto
Bay-Breasted Warbler	25	15	6	4
Blackburnian Warbler	17	7	7	3
Blackpoll Warbler	18	10	5	3
Cerulean Warbler	4	0	3	1
Connecticut Warbler	7	0	4	3
Louisiana Waterthrush	8	0	2	6
Magnolia Warbler	60	60	0	0
Worm-eating Warbler	11	0	6	5
Yellow Warbler	28	23	5	0
<b>Total</b>	<b>178</b>	<b>115</b>	<b>38</b>	<b>25</b>

**Table 2.1** Total number of individuals per species included in comparisons of flight call properties across species. We included one call per bird in analyses to avoid potential pseudo-replication. Flight calls included in our analyses were from our recordings or from public sources of animal sounds: Xeno-Canto ([www.Xeno-Canto.org](http://www.Xeno-Canto.org)) and the Flight calls of migratory birds: eastern North American landbirds (CD-ROM).

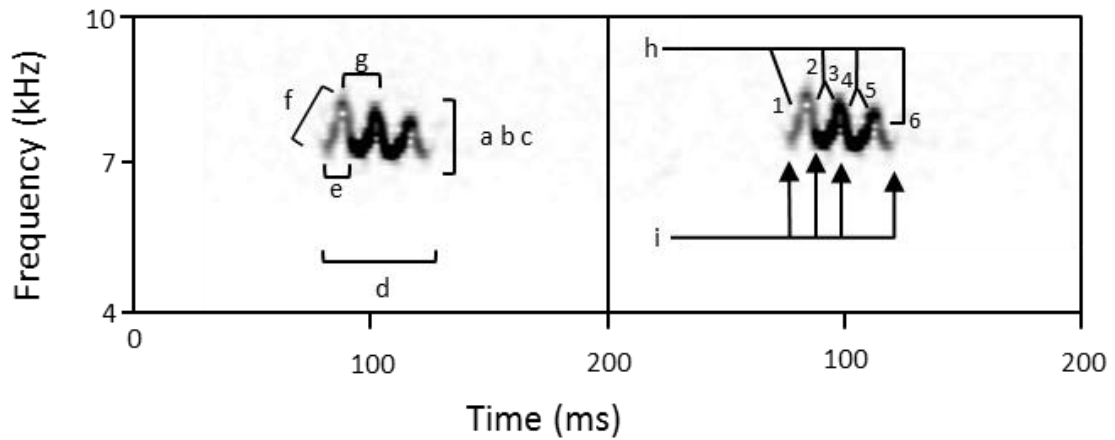
Species	Duration (ms)	Distance to Max. Amplitude (ms)	Min. Freq. (kHz)	Max. Freq. (kHz)	Bandwidth (kHz)	Peak Freq. at Start (kHz)	Peak Freq. at End (kHz)	Peak Freq. at Centre (kHz)	Peak Freq. at Max. Amplitude (kHz)	Number of Freq. Modulations	Inter-peak Duration (ms)	Freq. Modulation: Start to End	Freq. Modulation: Min. to Max.	Freq. Modulation: Start to Peak
Bay-breasted Warbler	46±8	24±9	6.7±0.6	8±0.6	1.2±0.4	6.9±0.5	7.2±0.6	7.2±0.6	7.2±0.6	3.96±0.9	23±3	-6.6±10.9	-29±14.7	-56.9±29.3
Blackburnian Warbler	53±20	25±13	6.7±0.5	8.7±0.6	2±0.6	6.8±0.6	7.5±0.9	7.7±0.9	7.1±0.6	5.35±1.7	17±2	-16.3±18.1	-42.6±14.3	-144±56.9
Blackpoll Warbler	53±7	25±6	6.7±0.4	8.1±0.4	1.4±0.4	6.9±0.5	7.2±0.3	7.3±0.5	7.2±0.5	6.56±1.3	15±1	-7.2±10.7	-27.1±7.2	-127.1±37.5
Cerulean Warbler	50±9	26±6	6.3±0.1	8±0.4	1.7±0.5	7.0±0.6	6.9±0.6	7.3±0.7	6.7±0.3	7.75±1.3	14±3	2±9.9	-35.1±6.7	-78.3±200.8
Connecticut Warbler	43±5	22±3	6.8±0.3	8.3±0.4	1.5±0.3	6.8±0.3	7.5±0.4	7.7±0.7	7.3±0.5	5.57±1.3	13±2	-14.5±8.1	-36.4±7.8	-89.5±79.4
Louisiana Waterthrush	77±6	50±2	7±0.4	8.8±0.3	1.8±0.3	7.3±0.1	7.3±0.6	7.7±0.6	7.6±0.1	8.88±2	15±2	-1±8.1	-23.6±4.6	-63.6±78
Magnolia Warbler	43±11	21±10	6.7±0.4	7.8±0.6	1.1±0.5	6.9±0.5	7.0±0.5	7.2±0.4	7.0±0.4	5.12±1.6	14±2	-5±13.6	-28±13.8	-56.2±46.6
Worm-eating Warbler	46±7	26±3	7±0.4	9±0.4	1.9±0.3	7.8±0.6	7.9±0.9	8.0±0.6	8.0±0.8	5.73±1.7	12±1	-1±18.2	-43.5±7.8	-113±187.9
Yellow Warbler	47±11	22±7	5.9±0.8	7.4±0.8	1.5±0.4	6.4±0.8	6.3±0.8	6.7±0.8	6.5±0.7	5.86±2.1	14±1	2.8±14.9	-34.1±13.4	-157.5±74.9
F value	10.53	11.25	8.07	13.44	10.67	7.01	9.69	8.03	10.96	10.06	34.11	3.43	4.71	6.99
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001

**Table 2.2** Means ± standard deviations of acoustic measurements of flight call properties for the nine “zeep” warbler species. Bottom two rows show the results from analysis of variance of acoustic measurements and the sample sizes for each species are given in Table 2.1

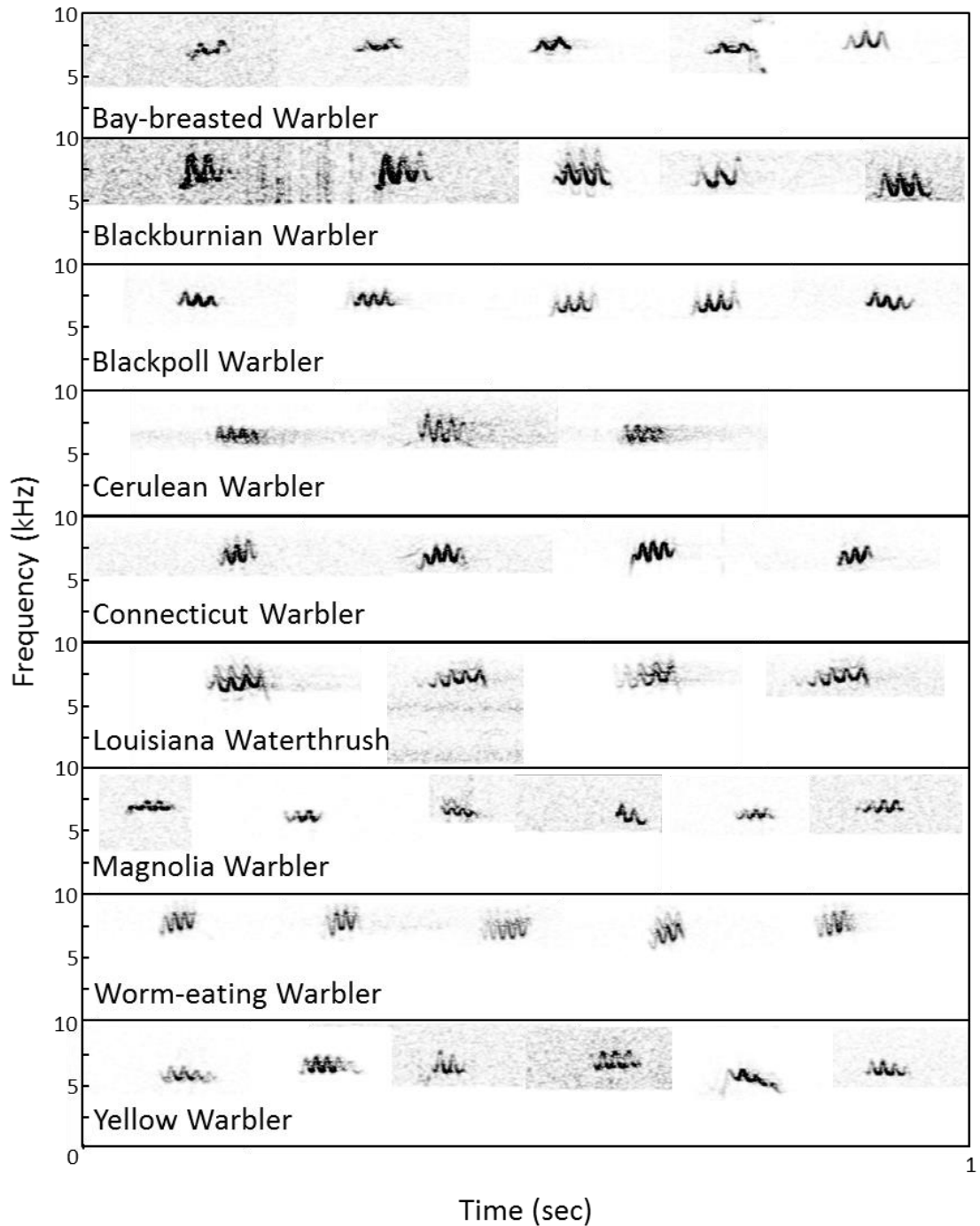
Species	Number of Comparisons (Within)	Number of Comparisons (Between)	Similarity Within Species (mean $\pm$ SD)	Similarity Between Species (mean $\pm$ SD)
Bay-breasted Warbler	300	3825	0.77 $\pm$ 0.13	0.78 $\pm$ 0.13
Blackburnian Warbler	136	2312	0.74 $\pm$ 0.11	0.78 $\pm$ 0.11
Blackpoll Warbler	153	2124	0.62 $\pm$ 0.19	0.79 $\pm$ 0.14
Cerulean Warbler	6	456	0.57 $\pm$ 0.06	0.78 $\pm$ 0.11
Connecticut Warbler	21	749	0.63 $\pm$ 0.09	0.79 $\pm$ 0.13
Louisiana Waterthrush	28	792	0.83 $\pm$ 0.2	0.83 $\pm$ 0.14
Magnolia Warbler	1770	2340	0.69 $\pm$ 0.14	0.81 $\pm$ 0.12
Worm-eating Warbler	55	308	0.63 $\pm$ 0.11	0.89 $\pm$ 0.1
Yellow Warbler	378	4200	0.81 $\pm$ 0.12	0.82 $\pm$ 0.14

**Table 2.3** Mean similarity values produced from pairwise spectrographic cross-correlation of the nocturnal flight calls of nine warbler species (i.e. “zeep” complex”). Spectrographic cross-correlation scores showed flight calls of eight species to be more similar to heterospecific calls than the calls produced by conspecific birds.

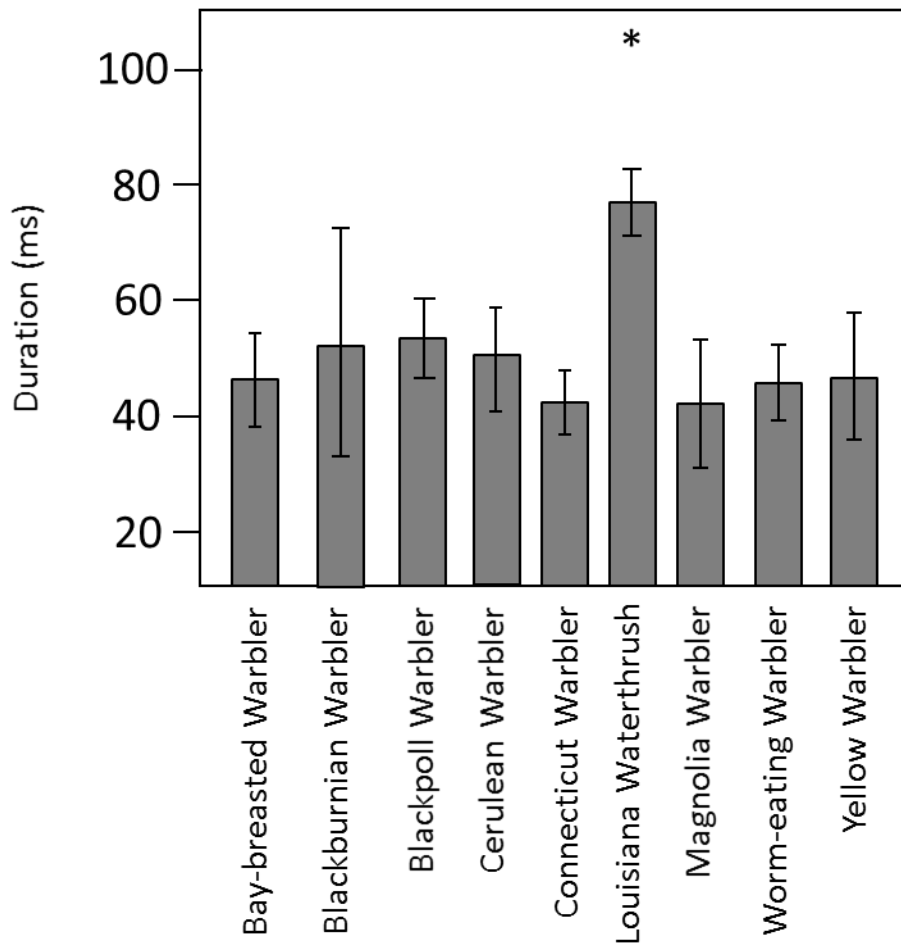
## Figures



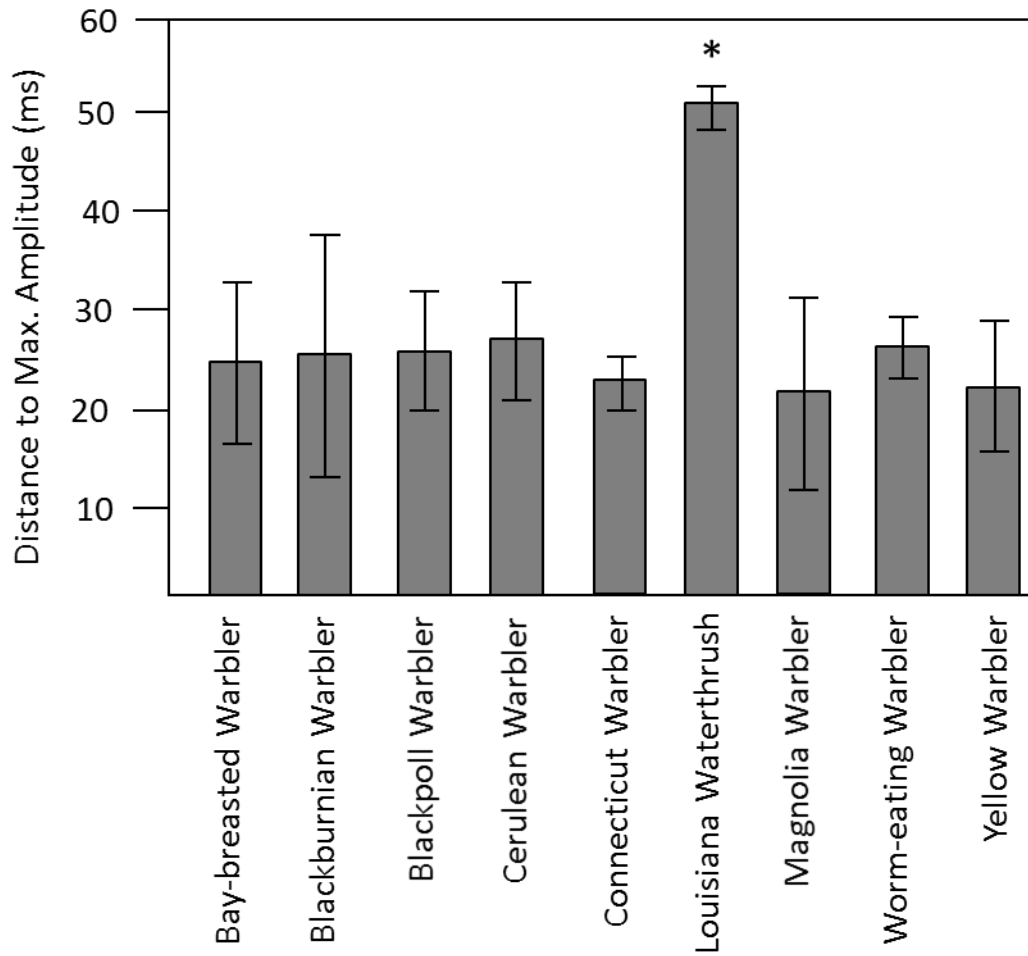
**Figure 2.1** Spectrographic visualization of 14 acoustic measurements of nocturnal flight calls produced by migratory songbirds: (a) call bandwidth; (b) minimum frequency; (c) maximum frequency; (d) duration; (e) distance to maximum amplitude; (f) slope calculations of frequency modulations over time (start to end, minimum to maximum, and start to first frequency peak of the call); (g) inter-peak duration; (h) number of frequency modulations; (i) peak frequency at the start, maximum amplitude, center, and end of the call.



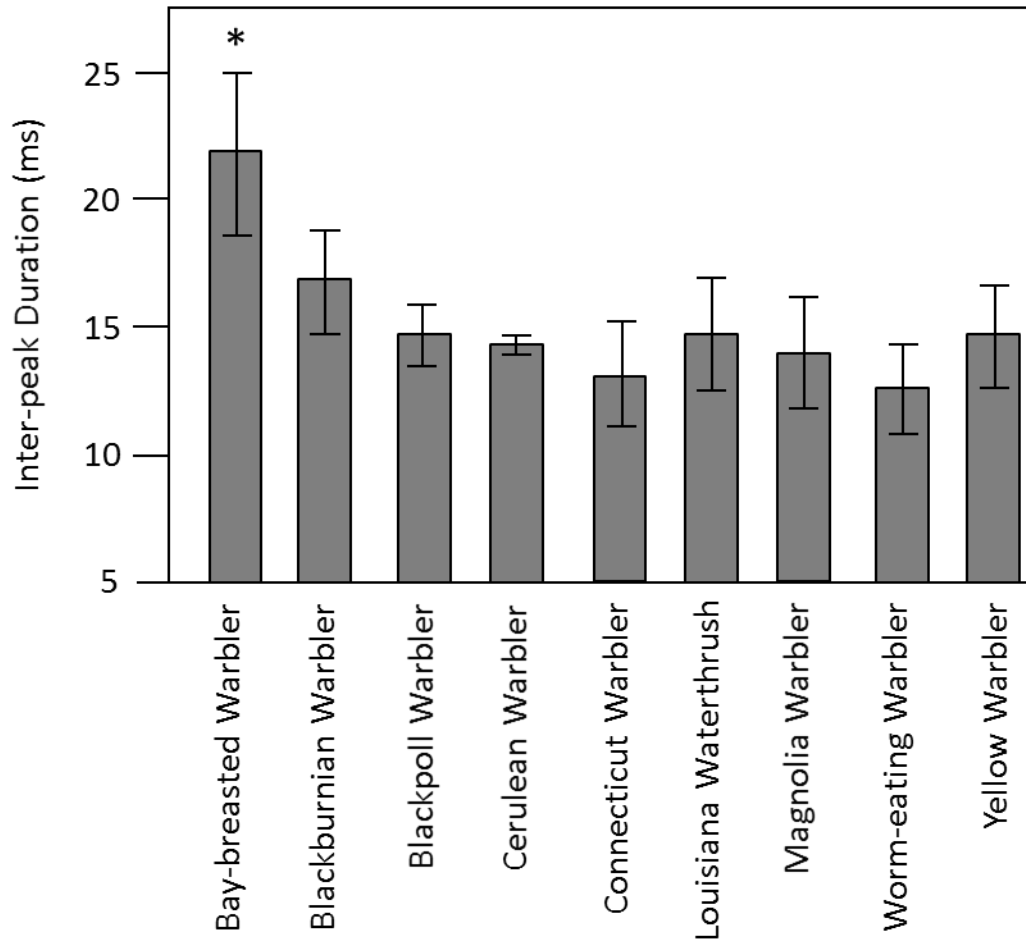
**Figure 2.2** Examples of nocturnal flight calls produced by the nine warbler species in the “zeep” species-group, a bioacoustic category of birds that produce similarly structured flight calls. Recordings of flight calls were recorded during spring and fall 2015-2017 migration periods and collected from public sources of animal sounds (e.g. Xeno-Canto and the Flight calls of migratory birds: eastern North American landbirds CD-ROM).



**Figure 2.3** Analysis of variance of mean duration of the nocturnal flight calls of nine Parulidae species ( $F_8=10.53$ ;  $p<0.001$ ). Tukey's Post Hoc tests revealed one species, Louisiana Waterthrush, was significantly longer in duration than the eight other species ( $77\pm 6$  ms;  $p<0.0001$ ). No significant differences were revealed between the calls of the eight remaining species.

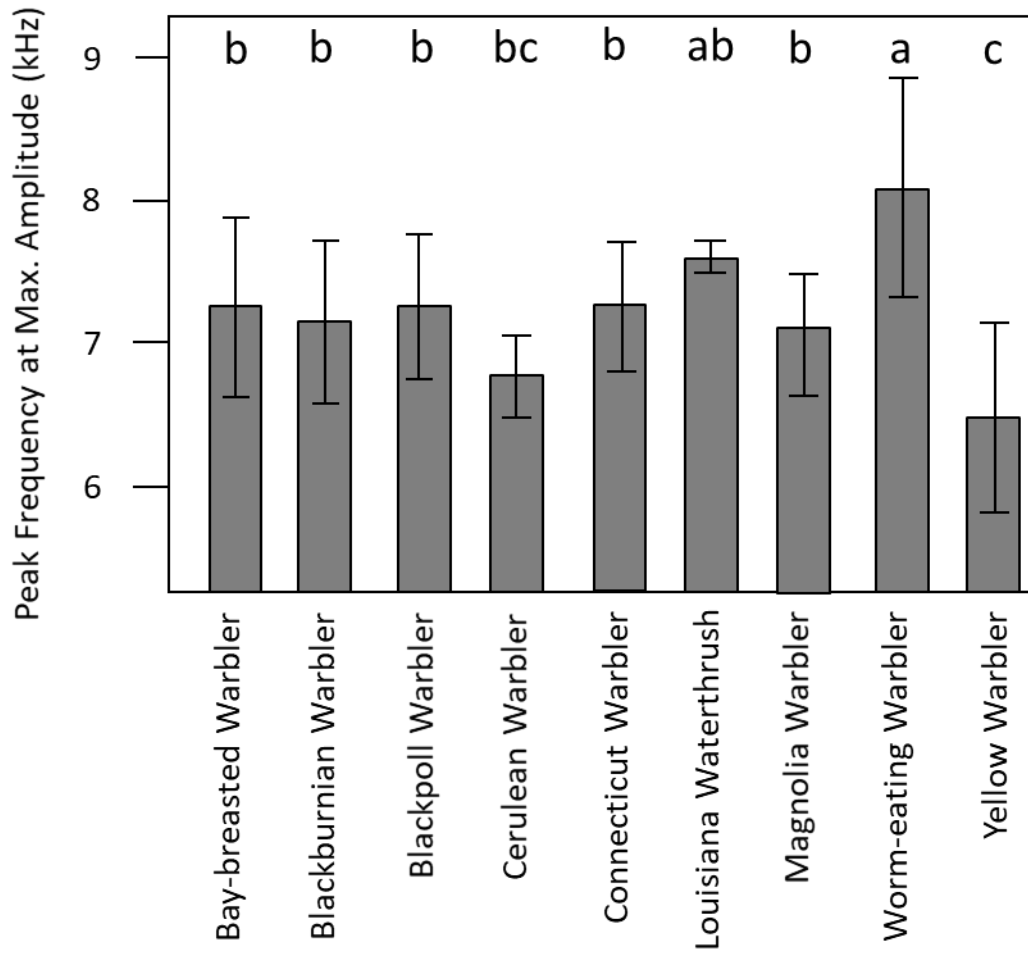


**Figure 2.4** Analysis of variance of mean distance to maximum amplitude of the nocturnal flight calls of nine migratory warblers ( $F_8=11.24$ ;  $p \leq 0.001$ ). Tukey's post hoc tests revealed only one species to be significantly different in distance to maximum amplitude from the eight remaining species ( $50 \pm 2$  ms;  $p < 0.001$ ). There were no significant differences between the flight calls of the eight remaining species.

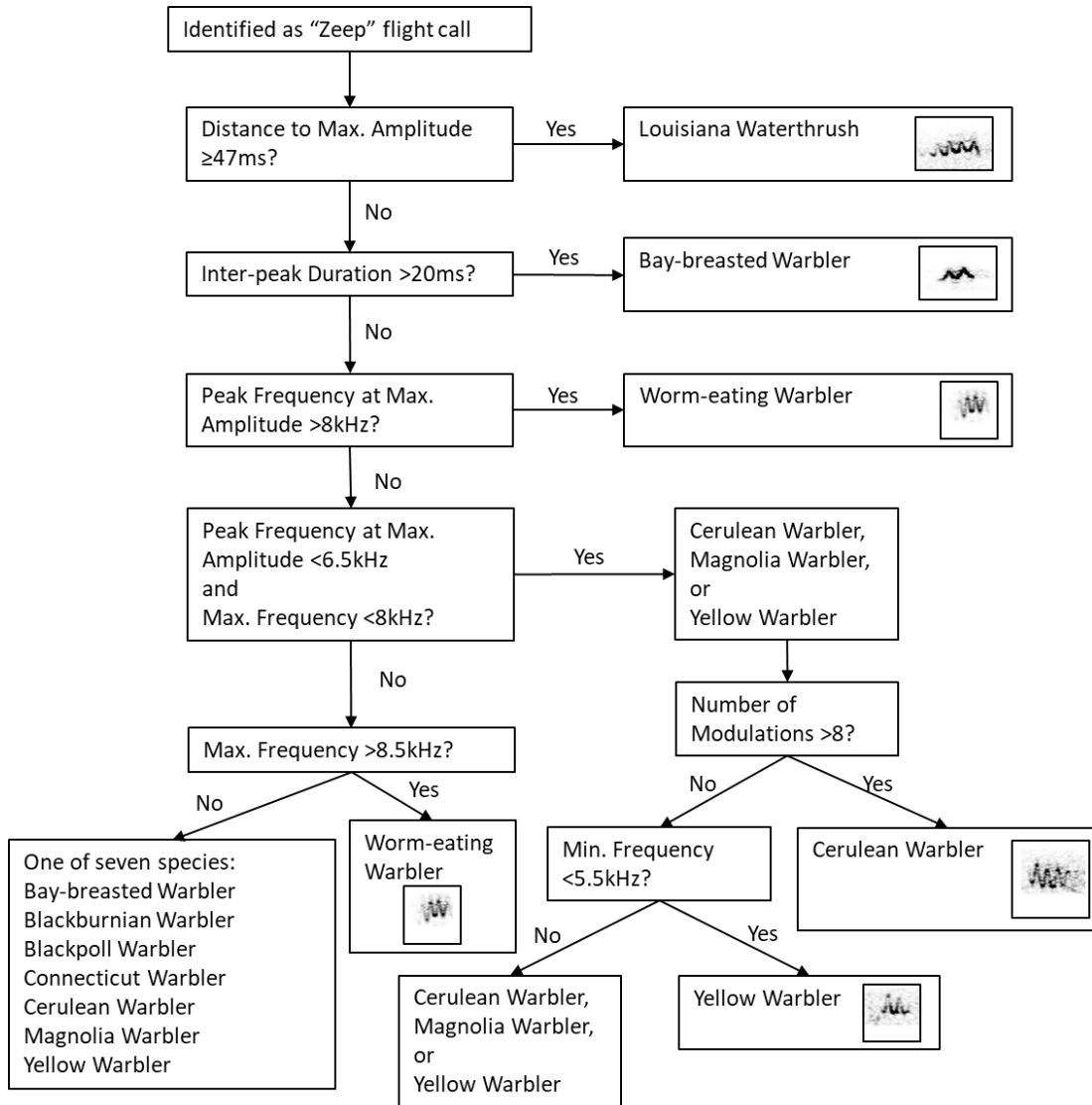


**Figure 2.5** Analysis of variance of mean inter-peak duration of the nocturnal flight calls of nine migratory warblers ( $F_8=34.11$ ;  $p \leq 0.001$ ). Tukey's post hoc tests revealed only Bay-breasted Warblers to be significantly different in inter-peak duration from the eight remaining species ( $22 \pm 3$  ms;  $p < 0.001$ ). There were no significant differences between the flight calls of the eight remaining species.

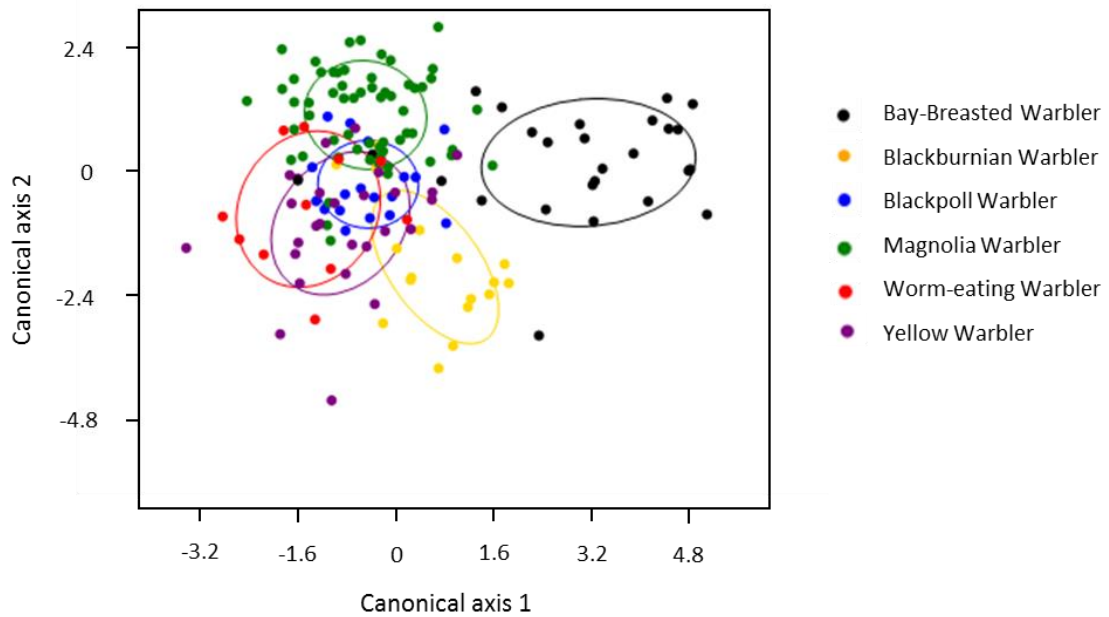




**Figure 2.6** Analysis of variance of mean peak frequency at maximum amplitude of the nocturnal flight calls of nine migratory warblers (ANOVA:  $F_8=10.96$ ;  $p \leq 0.001$ ). Tukey's post hoc tests revealed several significant differences among the nine species, including Worm-eating Warbler calls being significantly higher in peak frequency at maximum amplitude than seven of the eight remaining species ( $p < 0.05$ ). Worm-eating Warbler calls were not notably different in peak frequency at maximum amplitude from Louisiana Waterthrush calls ( $p=0.69$ ).



**Figure 2.7** Flow chart for assigning species identity to nocturnal flight calls of birds in the “zeep” flight call complex, based on recordings of 178 animals held for banding at banding stations in Ontario, and select calls from existing sound libraries. The calls of five species could be identified to the species level using one or more acoustic properties in this dataset. Only two species (Worm-eating Warbler and Louisiana Waterthrush) could be unambiguously assigned to species in all cases.



**Figure 2.8** Plot of first two canonical axes produced by canonical discriminant analysis using 14 acoustic measurements. Dots represent nocturnal flight calls from six warbler species. Circles represent 50% confidence ellipses. High values for canonical axis 1 were associated with inter-peak duration and slope of frequency modulation from start to first frequency peak. High values for canonical axis 2 were associated with maximum frequency and distance to maximum amplitude.

### **Chapter Three:**

## **Intraspecific Variation in Nocturnal Flight Calls: Analysis of Variation due to Sex, Age, and Geography in Five Species of Migratory Songbirds**

## Chapter Summary

Nocturnal flight calls are species-specific calls that many birds use primarily during migration. By recording these vocalizations, ornithologists can study the nocturnal behaviour and population trends of migratory birds, however, limited research has addressed variation within these vocalizations, such as variation due to age, sex, or geographic location. In this study, we used two recording and analytical approaches to investigate intraspecific variation in the flight call characteristics of migratory songbirds. We tested three hypotheses relating to variation in these calls: (1) that birds exhibit sexual dimorphism in flight call structure; (2) that birds exhibit age-based differences in flight call structure; and (3) that birds exhibit geographic variation in flight call structure. We focus on five species of migratory birds for which we collected a large dataset of acoustic recordings from birds held for banding (three warblers) and birds actively flying at night (one warbler and two sparrows). We present our findings in two parts: Part 1 focuses on sex- and age-based differences based on recordings of birds held for banding; Part 2 focuses on geographic variation based on recordings of nocturnally flying birds. We found no evidence for sexual dimorphism in calls of two warbler species. Similarly, we found no age-based differences in the flight calls of three warbler species. Further, we found no evidence for geographic variation in the flight calls of one warbler species and one sparrow species. While we found significant geographic variation in Dark-eyed Junco flight calls between locations, we found no consistent pattern of variation along an east-west axis. In conclusion, these analyses provide little evidence of sex-based, age-based, or geography-based variation in the nocturnal flight calls of songbirds. Our results suggest the monitoring of the nocturnal flight calls of songbirds cannot provide information beyond the species level. This research serves to improve our understanding of the pressures driving variation within these vocalizations.

## Introduction

The calls of birds are understudied within the field of avian communication. Biologists, instead, place emphasis on investigations into birdsong, which is considered one of the most structurally complex and variable types of animal vocalizations (Catchpole and Slater 2008). Whereas the songs of birds are typically long, complex signals associated with breeding and territoriality (Catchpole and Slater 2008), calls are short, simple vocalizations produced by both sexes (Marler 2004). Many bird species possess a repertoire of calls with each vocalization serving different biological functions (e.g. to communicate danger, Leavesley and Magrath 2005; or discovering a new food source, Smith 1997). Many songbirds possess repertoires of multiple call types, although some birds have substantially more (Marler 2004). Unlike the learned songs of many songbirds, the calls of most birds are understood to be innate (Benedict and Krakauer 2013); however, developmental plasticity in calls has been described in a few species (e.g. Red Crossbills, *Loxia curvirostra*, Sewall 2009; Budgerigars, *Melopsittacus undulatus*, Farabaugh et al. 1994).

Although bird calls are typically less complex than bird songs (Catchpole and Slater 2008), calls nevertheless show highly variability within and between conspecific individuals (Marler 2004). Many birds, for example, exhibit sex-specific differences in the fine structure of some of their calls (e.g. White-tailed Hawk, *Geranoaetus albicaudatus*; Farquhar 1993; Wilson's Storm Petrel, *Oceanites oceanicus*; Bretagnolle 1989; Bell Miner, *Manorina melanophrys*; McDonald et al. 2007). Further, the *chick-a-dee* calls of the chickadee (*Paridae*) family have been shown to contain information about a signaller's sex and flock identity (Smith 1997; Freeberg et al. 2003). While the differences in calls are often less pronounced than the sex-specific differences found in the fine structure and use of songs (Marler 2004), calls can exhibit notable

differences in frequency, duration, or other call properties that signal the sex of the bird (Taoka et al. 1989). For example, nocturnal flight calls produced by American Redstarts (*Setophaga ruticilla*) may contain information concerning sex where calls produced by males are significantly longer in duration than female calls (Griffiths et al. 2016). Yet most previous studies that investigated sex-specific variation in calls have focused on nonpasserine birds (e.g. Taoka et al. 1989; Bretagnolle 1989; Barbraud et al. 2000), highlighting a need for more thorough investigations of passerine calls.

Although there is strong evidence for sex-based variation in calls, there remain few examples of age-specific differences in calls. Age-based variation is well documented in mammals, such as Yellow-bellied Marmots (*Marmota flaviventris*) where individuals can differentiate alarm calls produced by adult and juvenile marmots (Blumstein and Munos 2005). Moreover, the calls of Red Crossbills and other *Carduelinae* species have been shown to exhibit open-ended imitation (Mundinger 1979; Sewall 2009), where calls may change in structure over a bird's life in response to social affiliation (Sewall 2009). In spite of this evidence for ontogenetic change in the subfamily *Carduelinae*, there is no evidence of age-specific differences in their calls (Mundinger 1979; Sewall 2009).

In addition to sex-based and age-based differences, many bird vocalizations exhibit considerable variation between geographic locations. There are multiple ways that variation in vocalizations may manifest (e.g. dialects, Marler and Tamura 1962; clinal variation, Mundinger 1982; or macrogeographic variation, Mundinger 1982). Although geographic variation is recognized to be less pronounced in calls versus songs for some species (e.g. Song Sparrow, *Melospiza melodia*; Peters et al. 2000), calls produced by some birds do exhibit geographic variation (e.g. discrete dialects in flight calls of Evening Grosbeaks, *Coccothraustes vespertinus*; Sewall et al. 2004). Outside of the *Carduelinae* subfamily, where calls exhibit limited vocal

plasticity (Groth 1993; Sewall 2009; Sewall 2011), there are limited examples of geographic variation in a passerine call (e.g. the gargle call of Black-capped Chickadees, *Poecile atricapillus*; Baker et al. 2000). Further, Evans and O'Brien (2002) suggested Palm Warblers (*Setophaga palmarum*) may exhibit patterns of discrete geographic variation in nocturnal flight calls between subspecies. Due to the scarcity of research on bird calls, however, it remains uncertain whether calls exhibit less sex-based, age-based, and geography-based variation than songs, or if this is just a product of a lack of careful study.

One call type that has attracted considerable interest in recent decades is the nocturnal flight calls of migratory songbirds. Nocturnal flight calls are species-specific vocalizations that birds produce primarily during migration (Farnsworth 2005). These calls are typically 2-11 kHz in frequency and 50-300ms in duration (Evans and O'Brien 2002). Many birds produce nocturnal flight calls during migratory activity including wood warblers (*Parulidae*), sparrows (*Emberizidae*), thrushes (*Turdidae*), and rails (*Rallidae*; Evans and O'Brien 2002; Farnsworth 2005). Despite gaps in knowledge concerning the function of these calls (Farnsworth 2005), the applied uses of nocturnal flight call detections have gained substantial attention as a powerful ornithological tool to study migration and monitor bird populations. The application of flight calls as a population-monitoring tool is restricted, however, by the scarcity of information concerning acoustic variation in these signals, both within and among individuals (Farnsworth 2005). If the nocturnal flight calls of songbirds exhibit sex-based, age-based, or geography-based differences, it is critical to investigate and quantify intraspecific variation in these calls to drastically increase the information collected from passively monitoring these calls.

Our goals of this research were to quantify intraspecific variation in the nocturnal flight calls of migratory songbirds and attempt to elucidate the drivers of variation within these calls. We tested three hypotheses regarding variation in these calls: (1) that birds exhibit sexual



dimorphism in the spectro-temporal properties of their nocturnal flight calls; (2) that birds exhibit age-based differences in the fine structural properties of their flight calls; (3) that birds exhibit geographic variation in the fine structure of their flight calls across wide geographic ranges. To test these hypotheses, we used two recording and analytical approaches where we recorded both birds held for banding and birds actively flying during their nocturnal movements. We predicted that birds would exhibit sexual dimorphism in nocturnal flight calls based on previous research on American Redstarts (Griffiths et al. 2016). We predicted that birds would exhibit no age-based differences on the paucity of evidence for this in other taxa (reviewed above). We predicted that nocturnal flight calls would exhibit geographic variation on an east-west axis, based on the evidence from Evening Grosbeaks and Palm Warblers (Sewall 2009; Evans and O'Brien 2002). We present the Methods and Results in two parts, focusing on sex- and age-based variation in Part 1 using recordings of birds held for banding, and geographic variation in Part 2 using recordings of actively flying birds. This is the first study, to our knowledge, to investigate geographic variation in nocturnal flight calls of a passerine across North America.

## **Methods 1: Variation in Age or Sex**

### *General Field Methods*

We recorded birds held for banding at two bird observatories across Southern Ontario: Long Point Bird Observatory (42.5829°N, -80.3984°W) and Holiday Beach Migration Observatory (42.0322°N, -83.0437°W). We recorded birds at the former location between 15 August-31 October 2015-2016 and the latter location during 2 April-3 June 2016-2017. Recordings were collected during a six-hour period each morning beginning 30 minutes before sunrise. Three species were particularly plentiful, making them amenable for analysis: American Redstart (33

birds; 21 male and 12 female; 11 adult and 21 juvenile), Magnolia Warbler (*Setophaga magnolia*; 65 birds; 25 adults and 35 juveniles; 40 males and 14 females), and Yellow Warbler (*Setophaga petechia*; 23 birds; 20 males and 3 females; 8 adults and 15 juveniles). We recorded many additional sparrow and warbler species in an attempt to quantify variation in the flight calls of common migrants; however, we did not include species with small sample sizes in our analysis.

We banded birds with United States Geological Survey (USGS) bands and recorded biometrics. Once banded, we transferred birds to a separate building with a darkened and sound-dampened recording booth to mimic nocturnal conditions (adapted from Lanzone et al. 2009). We placed birds into a cloth chamber suspended within the booth to record the birds' nocturnal flight calls. In order to stimulate the birds to call, we exposed the birds to five-minutes of acoustic stimuli of fixed sequences of congeneric flight calls separated by five-second intervals of silence. Each recording session began with 45 seconds of silence to allow birds to acclimatize to the darkened room. The recordings used to create acoustic stimuli were taken from the *Flight Calls of Migratory Birds: Eastern North American Landbirds* CD-ROM (Evans and O'Brien 2002).

We recorded the responses of birds using a Sennheiser Omni-directional microphone (ME62/K6) and digital recorder (Marantz PMD670) located 30 cm below the cloth chamber containing the bird. We recorded numerous birds of different migratory species; however, many species exhibited low response rates (<25%) during the five-minute recording sessions and were excluded from analysis. All calls were recorded in WAV format using 44.1 kHz sampling frequency with 16-bit accuracy. After recording, we dictated band numbers into the microphone to facilitate comparison among conspecific individuals. We labelled each WAV recording with the species, band number, and date.

## *Spectral Analysis*

Using Syrinx-PC (spectral settings: 4 ms line<sup>-1</sup>, 256 FFT size, Blackman window; J. Burt, Seattle, WA), we visualized sounds by generating spectrograms of flight call recordings. We visually scanned recordings, 20-seconds at a time, to locate and annotate calls produced by birds. Once located, we annotated the frequency and time of recording for each flight call. Bird responses were easy to discern from our acoustic stimuli since the stimuli was a predetermined sequence of calls with five-second intervals of silence. We clipped calls from recordings, leaving a 1-second buffer on each side of the call, and created WAV files containing a single nocturnal flight call. We resampled recordings to 22,050 Hz, 16-bit accuracy. We then filtered flight call recordings to reduce ambient noise levels (high-pass filter: 4 kHz) and we normalized call amplitude to -1dB in Audition (Adobe, version 3.0, San Jose, CA).

Many of the recorded birds only vocalized once during the five-minute recording session. For birds that called multiple times, we assigned numbers to each call (ignoring calls that were partially masked by background noise) and used a random number generator to select one call per individual. In case inclusion of multiple call types enhanced this analysis and to imitate the sampling design used by Griffiths et al. (2016), we randomly selected five calls from a subset of American Redstart and Magnolia Warbler individuals that produced multiple calls during the recording period.

We used pairwise spectrographic cross-correlation to compare flight calls to the calls from conspecific birds (one call per individual), using Avisoft correlator (Avisoft Bioacoustics, Berlin, Germany; 500 Hz maximum frequency deviation). Spectrographic cross-correlation compares the similarity of spectrograms for each pair of calls and assigns a value between zero

(spectrograms share no similarity) and one (spectrograms are identical; Cortopassi and Bradbury 2000).

Using the automated parameter measurement tool in Avisoft SASLab software (Avisoft Bioacoustics, Berlin, Germany), we measured 11 characteristics of each call: the minimum and maximum frequency; call bandwidth; duration; distance to maximum amplitude; slope of frequency modulation from start to end of call; slope of frequency modulation from minimum to maximum frequencies; and the peak frequency at multiple locations of the call including the start, center, maximum amplitude and end. We included two additional manual measurements for Magnolia Warbler and Yellow Warbler calls that are important for distinguishing these species' calls (Chapter 2): number of modulations and inter- frequency peak duration.

### *Statistical Analysis*

We used three bioacoustic comparison approaches to test for sex-based and age-based differences in the nocturnal flight calls:

(1) We conducted a permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) on spectro-temporal characteristics of calls. We used a multi-factor design to facilitate the inclusion of age and sex in the same analysis to ensure we would detect any Sex × Age interaction, if present. We entered age and sex as fixed factors, which allowed us to independently investigate sex-based and age-based differences in call structure. We conducted a second permutational multivariate analysis of variance on the structural details of five calls per bird using a subset of individuals from two species: American Redstart and Magnolia Warbler. Age and sex were entered as fixed factors while band number (i.e. the bird's identity) was nested to prevent calls from the same individual being compared during the analysis.

We conducted PERMANOVAs using 999 iterations in Primer, version 7 (Clarke, K. and Gorley R., Auckland, NZ). Where the overall test statistic indicated significant differences between groups within fixed factors (i.e. age or sex), we used post hoc tests to infer sex-based and age-based differences in the flight calls of birds.

(2) We conducted canonical discriminant analyses, using leave-one-out classification, to investigate whether discrete differences in call properties exist between sex or age groups of three species: American Redstart, Magnolia Warbler, and Yellow Warbler. To reduce our number of predictor variables and to control for multicollinearity, we conducted a principal components analysis on fine structural measurements and extracted all eigenvalues with a score above 1.0. Our first principal components analysis revealed call bandwidth to be weakly correlated with other measurements and was excluded from further analysis; however, the minimum and maximum frequencies of each call were still included. We conducted a second principal components analysis, without call bandwidth, and extracted the principal components, with an eigenvalue above 1.0, for all three species explaining most of the variation in the dataset (American Redstart: 78.1%; Magnolia Warbler: 81.7%; Yellow Warbler: 88.6%).

We conducted two separate discriminant analyses for American Redstart and Magnolia Warbler calls to determine if flight calls can be assigned to the correct age or sex on the basis of their bioacoustic properties. We conducted a single discriminant analysis on Yellow Warbler calls to investigate whether calls could be assigned to the correct age. Due to a small sample size of female Yellow Warblers, we could not investigate sex-based variation in Yellow Warbler calls.

(3) We compared the mean spectrographic cross-correlation scores within and between sex and age categories for each species. If these calls contain information related to sex or age, we expected spectrographic cross-correlation analysis to reveal mean similarity values that are

higher when comparing calls produced by individuals of the same age or sex (i.e. male calls should produce higher similarity values when compared to other male calls than female calls).

All statistical analyses were completed in Primer, version 7 (K. Clarke and R. Gorley, Auckland, NZ) and SPSS, version 23 (Armonk, NY; IBM Corp).

## **Results 1: Variation in Age or Sex**

### *American Redstart*

We found no significant differences in American Redstart flight calls produced by males or females when we compared one call per bird (Figure 3.1; PERMANOVA:  $F_{31}=0.6$ ;  $p=0.68$ ). A second analysis, using five calls per individual ( $n=105$  calls;  $n=21$  birds), also revealed no differences in call structure between the sexes (PERMANOVA:  $F_{104}=1.33$ ;  $p=0.23$ ).

We found no significant differences in call structure between age classes (Figure 3.1; PERMANOVA:  $F_{31}=1.0$ ;  $p=0.42$ ). Further, our second analysis, using five calls per bird, revealed no significant differences in calls produced by adults or juveniles (PERMANOVA:  $F_{104}=0.15$ ;  $p=0.84$ ). We did, however, find significant differences in call structure between individuals (PERMANOVA:  $F_{17}=8.4$ ;  $p=0.001$ ).

A canonical discriminant analysis of three principal components, derived from one call per individual, classified 72.7% of calls to the correct sex, but accuracy dropped to 60.6% during cross-validation. This 60.6% level of classification was not significantly higher than the 54% level of chance (Binomial test:  $p=0.35$ ). A second canonical discriminant analysis assigned 65.6% of calls to the correct age (65.6% cross-validated). The 65.6% level of assignment to correct age was not significantly higher than the 55.6% level expected by chance (Binomial test:  $p=0.29$ ).

Our pairwise spectrographic cross-correlations revealed male American Redstart flight calls to be similar to calls produced by other males or females (within-male=0.51 ±0.13; between-sex=0.47 ±0.13; Wilcoxon signed-rank test:  $W_{32}=0.91$ ;  $p=0.34$ ). Female calls were less similar to other female calls than when compared to the opposite sex (within-female=0.46±0.14). Flight calls produced by juveniles (i.e. hatch-year and second year birds) produced higher mean similarity values when compared to calls produced by the same age group (within-juvenile=0.52±0.13; between-age=0.49 ±0.12; Wilcoxon signed-rank test:  $W_{32}=13.2$ ;  $p=0.001$ ). Calls produced by adults were less similar when compared to other adults than between ages (within-adult=0.44±0.15).

#### *Magnolia Warbler*

We found no differences in Magnolia Warbler flight calls produced by male or females when we compared one call per individual (Figure 3.2; PERMANOVA:  $F_{54}=1.6$ ;  $p=0.15$ ). Our second analysis of five calls per bird ( $n=135$  calls;  $n=27$  birds) revealed no differences in call between males or females (PERMANOVA:  $F_{134}=0.8$ ;  $p=0.47$ ).

We found no differences in calls produced by Magnolia Warbler adults or juveniles when we compared one call per bird (PERMANOVA:  $F_{53}=0.77$ ;  $p=0.56$ ). A second analysis of five calls per bird found no differences in calls produced by adults versus juveniles (PERMANOVA:  $F_{134}=0.21$ ;  $p=0.79$ ).

A canonical discriminant analysis of one random call per bird assigned 77.8% of calls to correct sex and dropped to 74% accuracy during cross-validation. The 74% percent of calls assigned to sex correctly was not significantly higher than the 62% level of correct assignment by chance (Binomial test:  $p=0.07$ ). A second discriminant analysis classified 61.7% of Magnolia

Warbler calls to the correct age (55% cross-validation). The 55% of calls assigned to correct age was not significantly higher than the 51% level expected by chance (Binomial test:  $p=0.58$ ).

Our pairwise spectrographic cross-correlations showed female Magnolia Warbler calls were more similar to other female calls than when compared to the opposite sex (within-female= $0.61\pm 0.18$ ; between-sex= $0.52\pm 0.18$ ; Wilcoxon signed-rank test:  $W=15.6$   $n=54$ ;  $p\leq 0.001$ ). Male flight calls, however, were approximately similar to calls produced by both males and females (within-male= $0.51\pm 0.15$ ). Further, the flight calls produced by juveniles were equally similar when compared to calls within and between age categories (within-juvenile= $0.53\pm 0.16$  between-age= $0.53\pm 0.16$ ). Calls produced by adults were less similar to other adult calls than calls from different age groups (within-adult= $0.51\pm 0.18$ ).

#### *Yellow Warbler*

We found no structural differences in Yellow Warbler flight calls produced by adult versus juvenile birds (Figure 3.3; PERMANOVA:  $F_{2,2}=1.5$ ;  $p=0.18$ ). Due to the small sample size of female calls, we only investigated differences in nocturnal flight calls between age groups, and not between sexes.

A canonical discriminant analysis classified 65% of calls to the correct age based on spectro-temporal properties; however, accuracy reduced to 55% during cross-validation. The 55% level of classification revealed by cross-validation was equal to the 55% level expected by chance.

Our pairwise spectrographic cross-correlations revealed flight calls produced by adults and juveniles to have approximately equal similarity values (within-adult= $0.47\pm 0.22$ ; within-juvenile= $0.46\pm 0.18$ ; between-age= $0.46\pm 0.18$ ). The difference in similarities between within-adult and between-age groups was not significant (Wilcoxon signed-rank test:  $W_{2,2}=0.4$ ;  $p=0.53$ ).



## Methods 2: Geographic Variation

### *General Field Methods*

We deployed 11 automated acoustic recorders across North America during the 2014-2016 fall migration periods (15 August to 15 November). The digital recorders were deployed along an east-to-west axis extending across North America (Figure 3.4). We deployed five recorders across western Canada: Prince George, British Columbia (53.8928°N, -122.8163°W); Nanaimo, British Columbia (49.1565°N, -123.9691°W); Lethbridge, Alberta (49.6770°N, -112.8651°W); Edmonton, Alberta (53.4529°N, -113.5459°W); and Saskatoon, Saskatchewan (52.2269°N, -106.6928°W). We deployed units at four locations throughout the Great Lakes region: McKellar Island (48.3833°N, -89.2341°W), Batchewana Bay (46.9503°N, -84.5545°W), Pinery Provincial Park (43.2504°N, -81.8490°W), and Long Point Bird Observatory (42.5829°N, -80.3984°W). Further, we deployed recorders at four locations in eastern North America: Kent Island, New Brunswick (44.5821°N, -66.7559°W); Bon Portage Island, Nova Scotia (43.4572°N, -65.7443°W); Paradise, Newfoundland (47.5326°N, -52.8840°W); and Newark, Delaware (39.7012°N, -75.7591°W). Two locations, Nanaimo, British Columbia and Paradise, Newfoundland, detected low levels of traffic for target species and were excluded from subsequent analysis. We selected recording sites to sample across broad geographic regions across North America, with sites located within each of the four major migratory flyways (Boere and Stroud 2006).

To investigate geographic variation in nocturnal flight calls, we chose three birds with wide geographic ranges across North America: American Redstart, American Tree Sparrow (*Spizelloides arborea*), and Dark-eyed Junco (*Junco hyemalis*). We recorded the nocturnal flight calls of actively-flying birds: 500 American Redstart calls from 10 locations (Table 3.1; n = 50 calls

per location); 495 American Tree Sparrow flight calls recorded at nine locations (Table 3.1; n=55 calls per location); and 362 Dark-eyed Junco calls from nine locations (Table 3.1), ranging from 30-61 individuals per location. All three species produce flight calls that we can differentiate unambiguously on the basis of fine structure. To ensure calls collected were representative of all populations flying over each location, we sampled calls for each species from across the entire fall migration period (15 August-15 November). Moreover, these birds are common, widespread migrants that have separate migratory populations and produce nocturnal flight calls frequently during migration (Norris et al. 2006; Naugler et al. 2017; Evans and O'Brien 2002).

For automated recordings, we used a Wildlife Acoustics Song Meter SM2+, equipped with one omni-directional microphone (Wildlife Acoustics SMX-NFC), at all 13 sites. We collected recordings with 44,100-Hz sampling frequency and 16-bit accuracy in WAV format. We programmed automated recorders to record each night between astronomical dusk and astronomical dawn, as per Sanders and Mennill (2014). We deployed recorders at sites with an unobstructed view of the sky and minimal nearby tree cover to reduce ambient noise of insects and trees.

### *Spectral Analysis*

We generated spectrograms of audio recordings using Syrinx-PC (Spectral settings: 1 ms line<sup>-1</sup>, 256 FFT size, Blackman window; J. Burt, Seattle, WA) and visually scanned through recordings, 30-seconds at a time, to detect nocturnal flight calls of passing migrants. We used cursors in Syrinx-PC to annotate the time and frequency of each detected call. We compared each annotated sound to existing reference libraries of nocturnal flight calls (e.g. Sanders and Mennill 2014; Evans and O'Brien 2002) and identified the call to the species level. Once calls were identified to the species level, we clipped calls of target species creating short WAV files

containing a single call. In event of multiple conspecific calls occurring within a short period, we sampled 1 call/ target species for every 60 seconds of recording to ensure we did not sample the same individual twice (i.e. minimum number of individuals passing technique; Evans and Rosenberg 2000). WAV files containing single calls were then filtered using Adobe Audition (Adobe, San Jose, California) to remove background noise from recordings (High-pass frequency: 4 kHz; low-pass frequency: 11 kHz). We then normalized recordings to -1 dB in Audition.

To facilitate comparison of fine structure, we measured 11 fine-structural properties of each call using Avisoft-SAS (Avisoft Bioacoustics, Berlin, Germany): distance to maximum amplitude, duration, minimum frequency, maximum frequency, slope of frequency modulation from start to end, slope of frequency modulation from minimum to maximum frequency, bandwidth, and the peak frequency at the start, center, end, and maximum amplitude of each call.

### *Statistical Analysis*

We conducted a principal components analysis of all 11 flight call measurements to reduce the number of variables; however, one measurement, call bandwidth, did not show any strong correlations with any other measurement and was removed from analysis. We conducted a second principal components analysis with the 10 remaining variables. We extracted all principal components with a score above one for the three species: American Redstart, American Tree Sparrow, and Dark-eyed Junco. These principle components explained most of the variation in the original 10 measurements (American Redstart 83.6%; American Tree Sparrow 80.1%; Dark-eyed Junco 86.4%). We then conducted separate multivariate analyses of variance (MANOVA) using the principal components for each species, with location as the fixed factor. Box's M tests for homogeneity of covariance matrixes were significant ( $p < 0.001$ );

however, we used equal samples sizes of more than 30 individuals per location for two species (American Redstart and American Tree Sparrow) making MANOVA tests robust to significant Box's M tests results (Tabachnick and Fidell 2001). For Dark-eyed Junco calls, we used unequal samples sizes of at least 30 birds per location. Due to these unequal sample sizes, we present both Wilks Lambda and Pillai's Trace, which is more robust to violations of the Box's M test (Tabachnick and Fidell 2001).

We followed the suggestions of Nath and Pavur (1985) and Huberty (1994) in interpreting results of MANOVA. With MANOVA on multiple variables, a significant p-value is not sufficient to reject the null hypothesis. We use the Wilk's Lambda and Pillai's Trace as test statistics to test the MANOVA omnibus null hypothesis that the means of acoustic properties do not differ significantly between locations (Huberty and Petoskey 2000). We only reject the null hypothesis when the Wilk's Lambda statistic was close to 0 (or Pillai's Trace close or above to 1) in combination with a significant p-value (Todorov and Filzmoser 2010).

Where MANOVA indicated a significant overall test result and significant p-value, we conducted a second one-way MANOVA using the 11 acoustic measurements to facilitate comparison of specific call properties between locations. We then used Tukey's post-hoc tests in order to investigate significant differences in call between locations.

## **Results 2: Geographic Variation**

We found significant variation in American Redstart calls between the 10 recording sites (Figure 3.5; MANOVA: Wilk's Lambda=0.83;  $F_{36}=2.56$ ;  $p < 0.001$ ; partial eta squared=0.05; observation power=1). The high Wilk's Lambda test score, however, indicated a substantial amount of variance was not explained by differences between location (Todorov and Filzmoser

2010). Further, we found no consistent patterns of variation in American Redstart calls along an east-west axis (Figure 3.6).

We found significant variation in American Tree Sparrow calls between nine geographic locations (Figure 3.7; MANOVA: Wilk's Lambda=0.89;  $F_{24}=2.21$ ;  $p \leq 0.001$ ; partial eta squared=0.04; observation power=0.99). Again, the Wilk's Lambda score suggested a considerable amount of the variation cannot be explained by difference between location. We found no consistent patterns of geographic variation in these calls along an east-west axis (Figure 3.8).

We found significant variation in Dark-eye Junco calls between nine geographic locations (Figure 3.9; MANOVA: Wilk's Lambda=0.3;  $F_{24}=21.6$ ;  $p \leq 0.001$ ; partial eta squared=0.33; observation power=1; Pillai's Trace=0.842;  $F_{24}=17.22$ ;  $p \leq 0.001$ ; partial eta squared=0.28; observation power=1). Further analysis of the 11 structural properties revealed similar significant differences between recording locations (Figure 3.10; MANOVA: Wilk's Lambda=0.18;  $F_{80}=8.49$ ;  $p \leq 0.001$ ; partial eta squared=0.19; observation power=1; Pillai's Trace=1.2;  $F_{24}=6.6$ ;  $p \leq 0.001$ ; partial eta squared=0.16; observation power=1). The low Wilk's Lambda and high Pillai's Trace test statistics and significant p-value indicated a large degree of variance can be explained by differences between locations. Following an overall significant test result, we found a haphazard pattern of variation in the structural details of Dark-eyed Junco calls across North America (Figure 3.10). Our analysis revealed a complex pattern of differences in call properties across our study region. For example, maximum frequency of calls recorded at two eastern locations (Kent Island, New Brunswick and Long Point, Ontario) and one western location (Lethbridge, Alberta) were higher in frequency than all other locations (Tukey's post hoc tests: all:  $p \leq 0.01$ ). With the exception of one western location (Lethbridge, Alberta), several frequency properties exhibited patterns where calls recorded at eastern sites were higher in frequency

than calls recorded in locations that were further west. Further, calls recorded at Kent Island, New Brunswick were significantly higher in minimum frequency than all other locations (Tukey's post hoc tests: all:  $p \leq 0.01$ ).

## **Discussion**

We found little to no evidence of variation in the nocturnal flight calls of five species of songbirds on the basis of sex, age, or geographic location. Our analysis revealed no significant differences in fine structure of nocturnal flight calls produced by male and female American Redstarts and Magnolia Warblers. We found no differences in flight calls produced by adult or juvenile birds of three species: American Redstart, Magnolia Warbler, and Yellow Warbler. Although our third bioacoustic comparison technique, spectrographic cross-correlation, revealed inconsistent results for both age and sex, the overall results of our analysis did not provide evidence of sex-based or age-based variation in these signals. Further, we found no geographic variation in the nocturnal flight calls of two birds: American Redstart and American Tree Sparrow. Our analysis revealed significant variation in Dark-eyed Junco calls across our study area; however, we found a complex pattern of inconsistent differences in call structure along an east-west axis. While we did not find evidence for sex-based, or age-based variation in flight calls, we found evidence of geographic variation in the flight calls of one species and captured a substantial amount of variation in the spectro-temporal properties of all species. Overall, our results indicate that nocturnal flight calls are not used to communicate sex or age of the calling bird. Moreover, our results suggest that flight calls of some species (e.g. Dark-eyed Junco) might reveal the geographic origin of the calling bird, although further work is required to understand the geographic pattern.

Our results suggest the nocturnal flight calls of migratory songbirds do not exhibit sex-based variation in fine structure. Sexual dimorphism in the fine structure and use of other types of calls has been described in many taxa of birds (e.g. aerial alarm calls of male Red Junglefowl, *Gallus gallus*; Kokolakis et al. 2010; contact calls of male and female Zebra Finch, *Taeniopygia guttata*; Zann 1985). If migratory birds were found to exhibit sexual dimorphism or geographic variation in their nocturnal flight calls, it would have considerable implications for the applications of these calls by allowing biologists to collect more detailed information through passively recording these calls. The only previous study investigating potential sexual dimorphism in nocturnal flight calls found a result that stands in contrast with ours: Griffiths et al. (2016) found that the flight calls of male American Redstarts were significantly longer in duration than females (Griffiths et al. 2016). In our analysis involving a similar sample size (n=36 in Griffiths et al. 2016; n=33 in the current study), we found the opposite pattern, where calls produced by females were slightly longer in duration than males, although this difference was not significant. These contradictory findings suggest that sex-based differences in calls may vary geographically, and certainly indicate that larger sample sizes are required before sex differentiation on the basis of calls is warranted.

We found no evidence for age-based variation in flight calls produced by American Redstarts, Magnolia Warblers, and Yellow Warblers. The findings for American Redstarts are consistent with previous research, which found no differences in flight call properties across age groups or seasons (Griffiths et al. 2016; Farnsworth 2007). While it appears evident that nocturnal flight calls do not change over a bird's lifespan, there remains conflicting evidence whether the flight calls of passerines contain information relating to sex.

Although we found no evidence for sex-based or age-based variation in the nocturnal flight calls of songbirds, patterns of geographic variation in flight calls have been described in at

least one species, the Evening Grosbeak (Sewall et al. 2004). The flight calls of Evening Grosbeaks and their relatives within the *Carduelinae* subfamily are not primarily associated with migration and often serve other biological functions (Groth 1993). Evidence for vocal plasticity and call imitation has been well documented within the calls of the *Carduelinae* subfamily (Mundinger 1970; Mundinger 1979; Marler 2004), which may have played a role in facilitating the development of dialects in the flight calls of Evening Grosbeaks (Sewall et al. 2004).

With respect to the nocturnal flight calls of migratory birds more generally, Evans and O'Brien (2002) suggest that discrete differences in frequency range may exist between the subspecies of some birds (e.g. Palm Warbler; Evans and O'Brien 2002). We found significant variation in flight calls between certain locations, yet our analysis did not reveal any clear east-west patterns of geographic variation in two migratory birds (e.g. clinal, Mundinger 1982; dialects, Marler and Tamura 1962). However, we found a complex pattern of geographic variation in the flight calls of Dark-eyed Juncos, where calls tended to exhibit higher frequency properties at more eastern sites and calls recorded at our most eastern site was significantly higher in minimum frequency than all other locations. Adaptation to local environments could lead to structural differences in vocalizations between locations (Morton 1975). Our recording sites were located in different habitat types, but the microphones were oriented towards the sky and all birds were recorded while flying and experiencing presumably similar acoustic habitats. Further, the differences in call properties between locations are not consistent between habitat types or along an east-west axis. In our recordings of birds held for banding, we found significant differences in calls produced by different conspecific individuals. Moreover, the degree of within-individual variation in flight calls is poorly studied. We suggest high vocal variability between individuals may be the cause of the detected differences in American Redstart and American Tree Sparrow calls between locations. Dark-eyed Juncos, however,



exhibited significant differences between sites across our study area that are not explained by high between-individual variability in call structure or differences in local habitat.

Due to the logistical constraints associated with capturing the same migratory birds at a single location over multiple days, we only had a single five minute recording session with all birds held for banding. As a result, we were unable to investigate individual variation in the nocturnal flight calls of these species. While both our analysis and Griffiths et al. (2016) showed American Redstart flight calls are more similar within than among individuals, the comparison of calls from the same recording session makes it impossible to rule out whether the within-individual similarities were confounded by motivational state or other factors influencing the birds' behaviour (e.g. Vehrencamp et al. 2013). In order to investigate individual variation in flight calls, it is critical to quantify the within-individual variation in these calls through sampling birds over multiple days. This is an important avenue for further investigation in this field. Furthermore, there are many important aspects of these calls to be examined. While we found no evidence for geographic variation in nocturnal flight calls of two species, we found an inconsistent pattern of geographic variation in flight calls produced by Dark-eyed Juncos, a widespread species with distinct subspecies. In light of these results, investigations into potential geographic variation in the flight calls of other widespread migratory birds may be worthwhile. It is important to recognize, however, nocturnal flight calls may not be able to provide information beyond the species-identity of nocturnally migrating birds. Thus, it may be most advantageous to focus on describing the calls of other species (e.g. birds of western North America and birds from different taxonomic orders).

In conclusion, this study provides evidence that the fine structural characteristics of nocturnal flight calls produced by migratory songbirds do not exhibit sex-based variation or age-based variation. Across North America, we found high levels of variation in the flight calls of

three common, widespread migratory birds. We found no evidence of geographic variation in the calls produced by two species; however, our analysis revealed a complex pattern of geographic variation in the flight calls of Dark-eyed Juncos along an east-west axis across North America. It remains unclear what pressures are influencing intraspecific variation in the nocturnal flight calls of many species. It is critical to investigate the forces driving variation within these signals in order to both benefit the applied uses of nocturnal flight call detections and to improve our understanding of the evolutionary forces shaping these vocalizations in birds.

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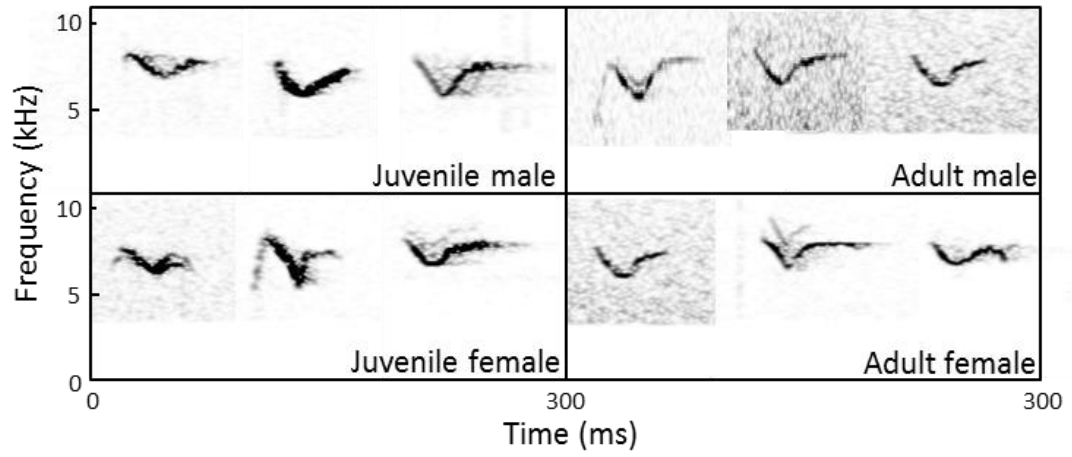
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Tables

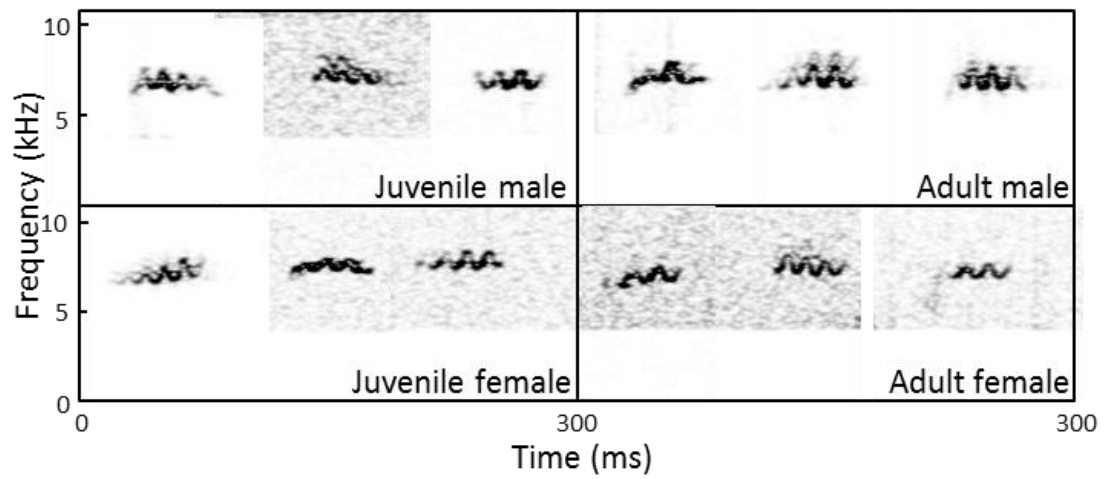
	Prince George, British Columbia	Lethbridge, Alberta	Edmonton, Alberta	Saskatoon, Saskatchewan	Thunder Bay, Ontario	Batchewana, Ontario	Pinery Provincial Park, Ontario	Long Point, Ontario	Newark, Delaware	Kent Island, NB	Bon Portage Island, NS	Total
American Redstart	50	0	50	50	50	50	50	50	50	50	50	500
American Tree Sparrow	55	55	55	55	55	55	55	55	55	0	0	495
Dark-eyed Junco	40	30	30	61	31	31	38	54	0	47	0	362

**Table 3.1** Number of nocturnal flight calls recorded at 11 locations across North America. Two recording sites, Nanaimo, British Columbia and Paradise, Newfoundland, detected low traffic rates of target birds and were excluded from analysis.

Figures

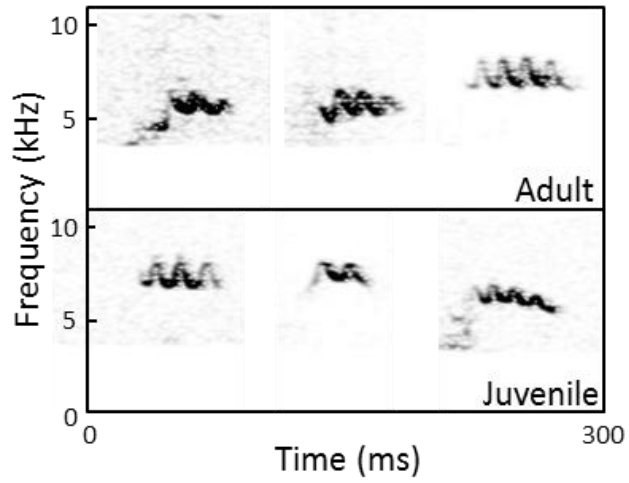


**Figure 3.1** Examples of nocturnal flight calls produced by male, female, juvenile (i.e. Hatch Year and Second Year), and adult (i.e. After Hatch Year and After Second Year) American Redstarts.



**Figure 3.2** Examples of nocturnal flight calls produced by male, female, juvenile (i.e. Hatch Year and Second Year), and adult (i.e. After Hatch Year and After Second Year) Magnolia Warblers.

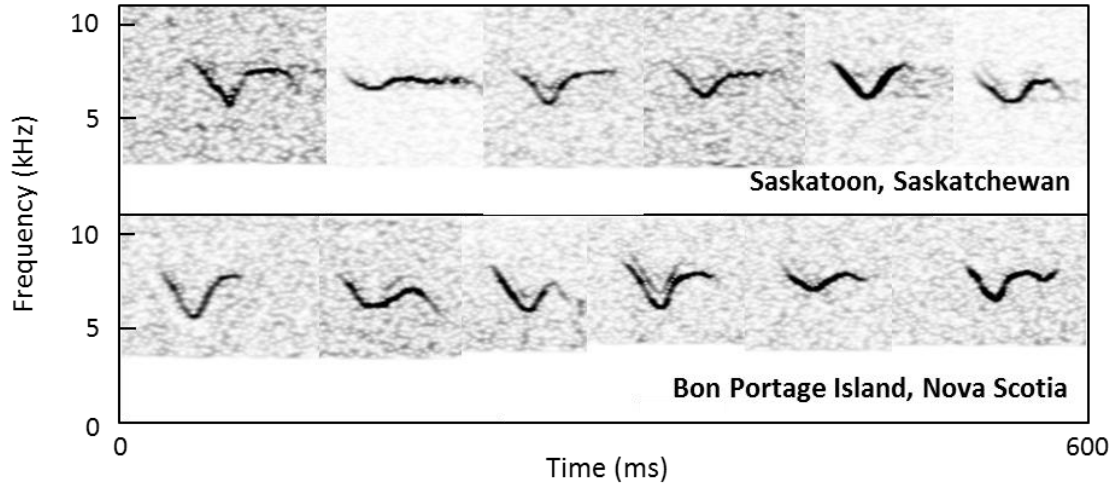




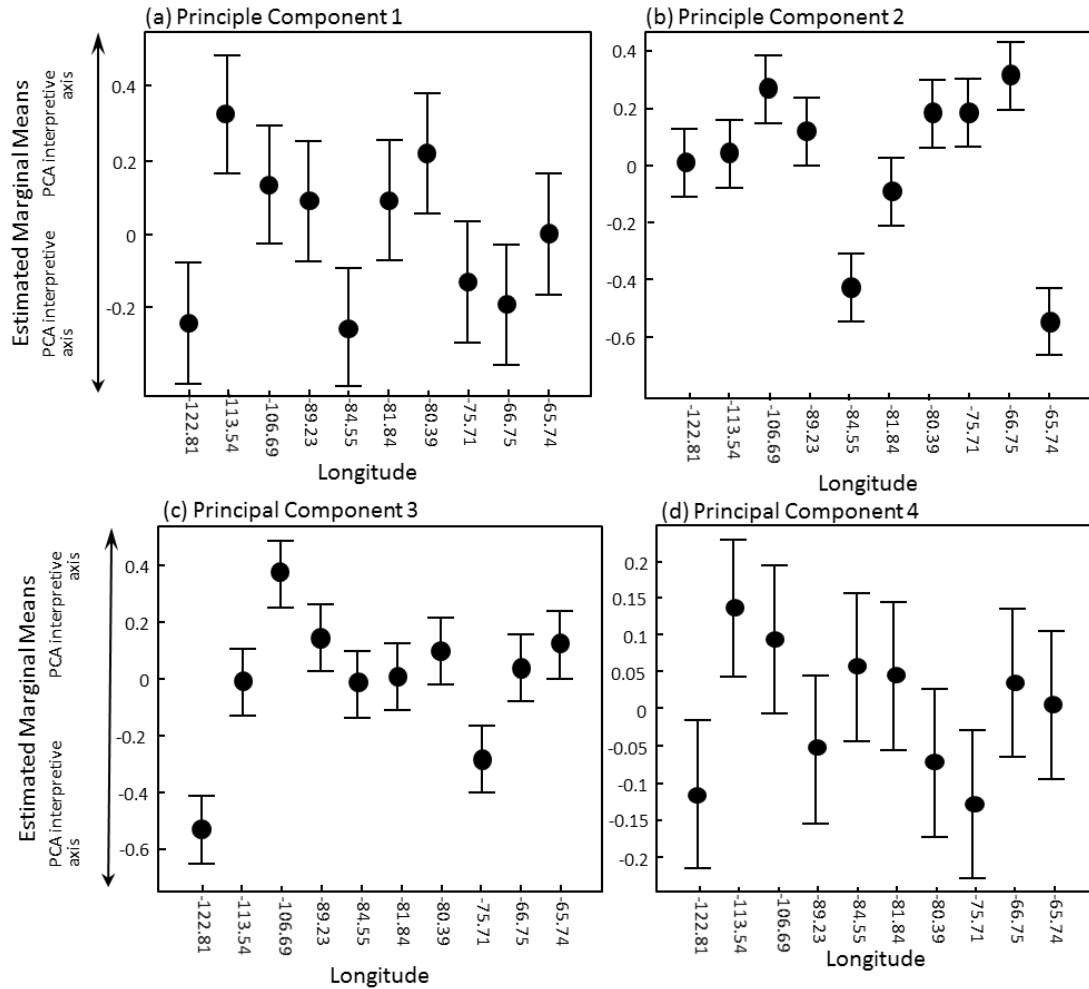
**Figure 3.3** Examples of nocturnal flight calls produced by juvenile (i.e. Hatch Year and Second Year) and adult (i.e. After Hatch Year and After Second Year) Yellow Warblers, which were held for banding.



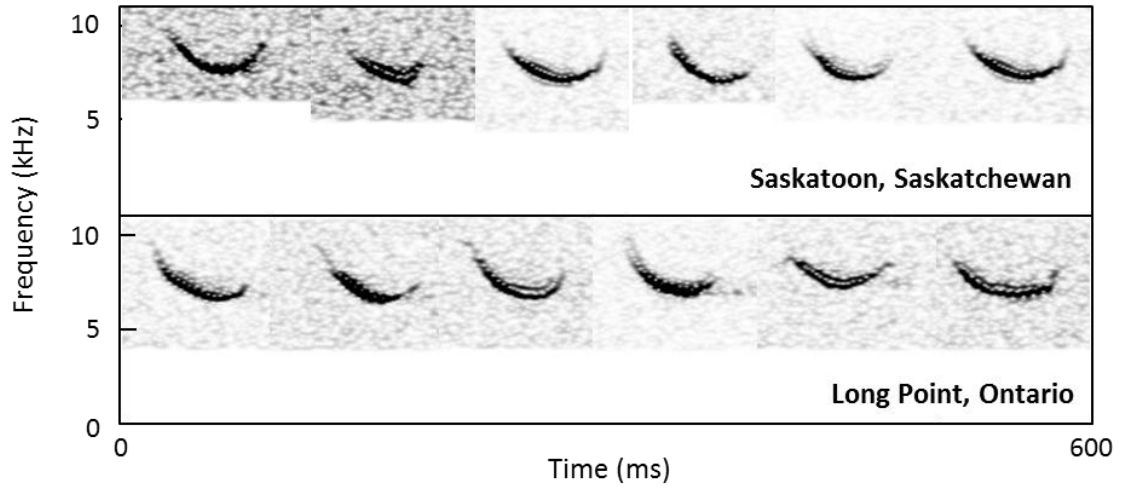
**Figure 3.4** Map of the 13 recorders deployed across North America to sample for geographic variation in the nocturnal flight calls of songbirds. Two recorders detected low traffic levels of target species and were excluded from analysis.



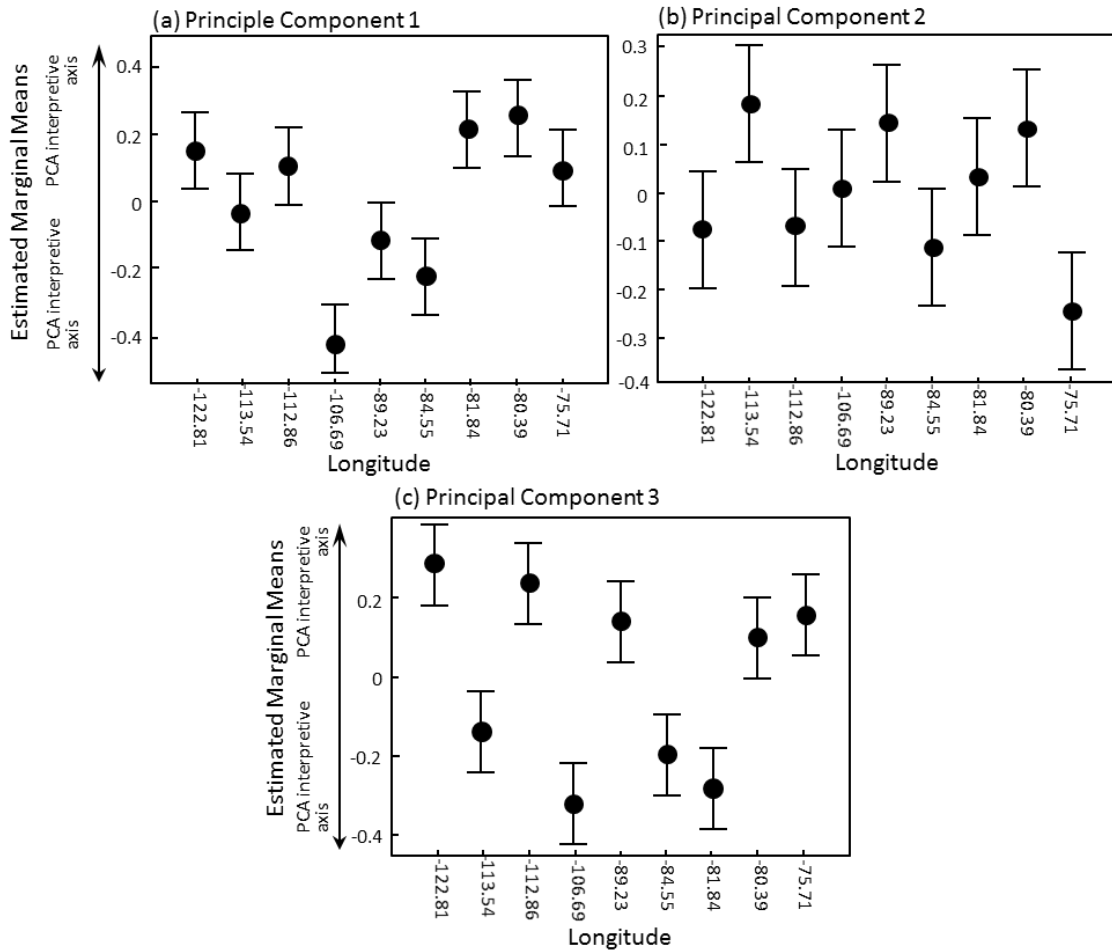
**Figure 3.5** Examples of American Redstart flight calls recorded at two locations (Saskatoon, Saskatchewan and Bon Portage Island, Nova Scotia). Flight calls were produced by different individuals and are representative of the magnitude of variation in fine structure within these vocalizations at these locations.



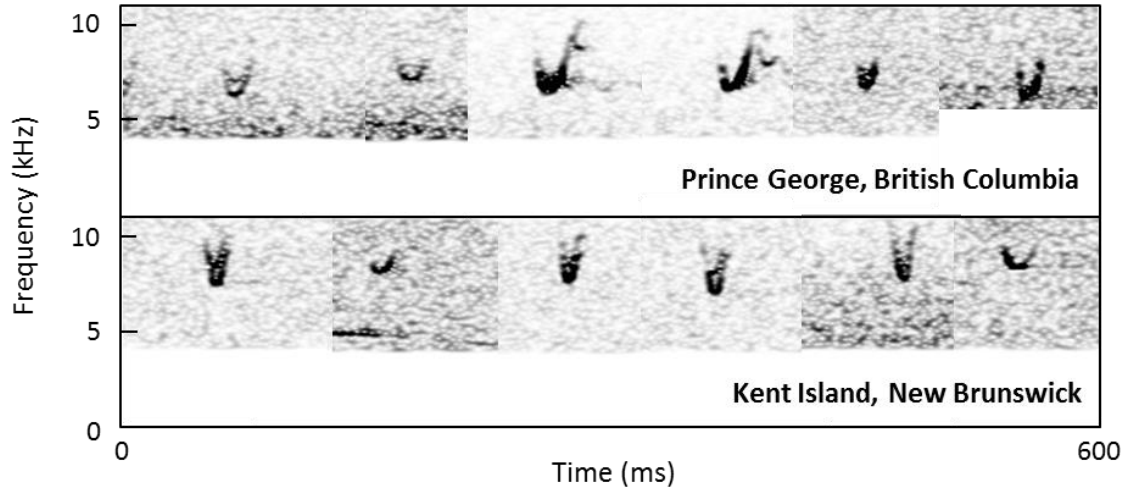
**Figure 3.6** A multivariate analysis of variance of American Redstart mean principal components revealed significant variation but no consistent geographic variation in call properties (Wilk's Lambda=0.83;  $F=2.56$ ;  $p < 0.001$ ; partial eta squared=0.05; observation power=1). The high Wilk's Lambda score indicated a large proportion of the variation in call structure is not explained by changes in longitude.



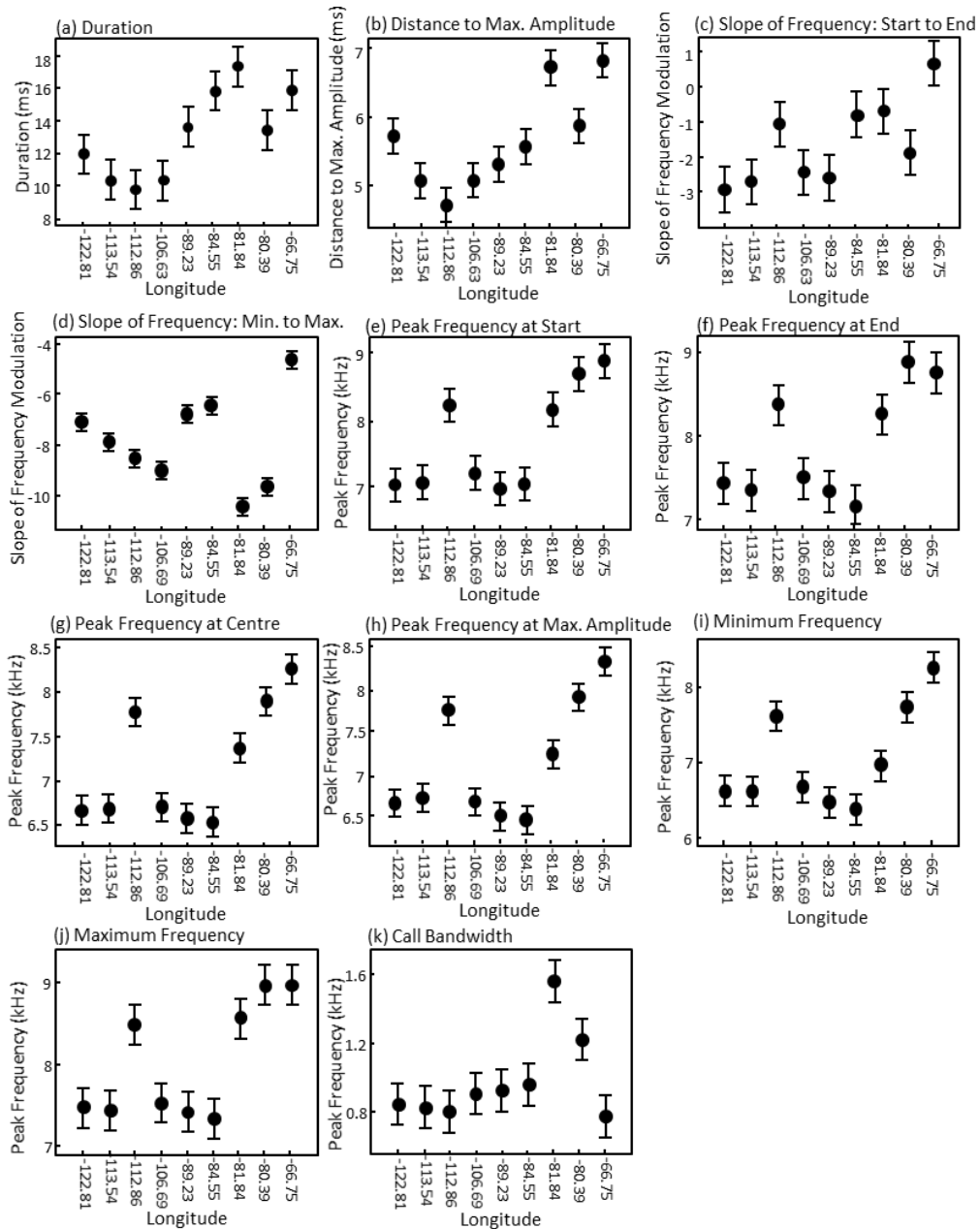
**Figure 3.7** Examples of American Tree Sparrow flight calls recorded at two locations (Saskatoon, Saskatchewan and Long Point, Ontario). Flight calls were produced by different individuals and are representative of the magnitude of variation in fine structure within these vocalizations at these locations.



**Figure 3.8** A multivariate analysis of variance of American Tree Sparrow mean principal components revealed significant variation but no consistent geographic variation in call properties (Wilk's Lambda=0.89;  $F=2.21$ ;  $p \leq 0.001$ ; partial eta squared=0.04; observation power=0.99). The high Wilk's Lambda score indicated a large proportion of the variation in call structure is not explained by changes in longitude.



**Figure 3.9** Examples of Dark-eyed Junco flight calls recorded at two locations (Prince George, British Columbia and Kent Island, New Brunswick). Flight calls were produced by different individuals and are representative of the magnitude of variation in fine structure within these vocalizations at these locations.



**Figure 3.10** A multivariate analysis of variance of Dark-eyed Junco flight call properties revealed significant differences between locations (Wilk's Lambda=0.3;  $F_{24}=21.6$ ;  $p \leq 0.001$ ; Partial Eta squared=0.33; observation power=1; Pillai's Trace=0.842;  $F_{24}=17.22$ ;  $p \leq 0.001$ ; Partial Eta squared=0.28; observation power=1).



## **Chapter Four:**

### **General Summary**

Nocturnal flight calls have numerous potential applications for ecologists and conservation biologists studying migratory birds. To date, nocturnal flight call detections have been used to monitor migratory movements across broad geographic ranges (e.g. Evans and Rosenberg 2000; Sanders and Mennill 2014) and to study the anthropogenic effects on migratory songbirds (Watson et al. 2016). Multiple studies have validated the use of nocturnal flight calls for monitoring bird populations through comparisons of flight call detections with other monitoring approaches, including radar and bird banding (Farnsworth et al. 2004; Horton et al. 2015; Sanders and Mennill 2014). Yet there remains considerable gaps in our knowledge regarding flight calls, particularly in relation to variation in calls of closely-related taxa, and variation in relation to sex, age, and geographic variation. In this thesis, I strove to fill some of these gaps.

In my first data chapter (Chapter 2), I recorded birds held for banding, at bird observatories in southern Ontario during April-May and August-October 2015-2017, to collect nocturnal flight call recordings from individuals of known species and used three bioacoustic comparison approaches to investigate the differences in flight call characteristics across the nine “zeep” warbler species. Analysis of flight call properties found two species, Louisiana Waterthrush (*Parkesia motacilla*) and Bay-breasted Warbler (*Setophaga castanea*), to be significantly different in one or more acoustic properties from the eight other “zeep” species. Further, canonical discriminant analyses revealed levels of classification higher (73%) than expected by chance (36%). My results indicated that maximum frequency and inter-peak duration are important properties for discerning these calls. My results indicate some flight calls produced by the “zeep” species-group can be identified to the species level based on fine structure. This research will allow biologists to collect detailed information, through passive

acoustic monitoring, on two migratory birds, including a species-at-risk (i.e. Louisiana Waterthrush).

In my second data chapter (Chapter 3), I investigated intraspecific variation in the nocturnal flight calls of migratory songbirds. I recorded birds held for banding during spring (April-May 2016-2017) and fall (August-October 2015-2016) at two bird observatories in southern Ontario to investigate sex- and age-based variation in call structure. My results revealed no significant differences in flight calls produced by males or females of two species: American Redstart and Magnolia Warbler (*Setophaga magnolia*). I found no significant differences in calls produced by adult versus juvenile birds of three species: American Redstart, Magnolia Warbler, and Yellow Warbler (*Setophaga petechia*). During fall migration (15 August-15 November) 2015-2016, I used an array of recorders deployed across North America (Nanaimo, British Columbia to Paradise, Newfoundland) to sample for geographic-based variation in flight calls. I found significant variation in flight calls between some locations, but I found no consistent pattern of geographic variation in the flight calls of two migratory birds: American Redstart and American Tree Sparrow (*Spizelloides arborea*); however, my analysis revealed a complex pattern of inconsistent differences in calls produced by Dark-eyed Junco (*Junco hyemalis*) along an east-west axis.

In order to make the most of nocturnal flight calls as a population-monitoring tool, it is critical to continue researching aspects of these calls, and expanding the line of inquiry that I have applied in my thesis. The responsiveness of birds held for banding to calls broadcast by loudspeaker provides some support for the function of these calls (i.e. maintaining contact with flock mates), although more research into the biological function of these calls is necessary. Recent evidence suggests flight calls may have the potential for individual or kin recognition (Griffiths et al. 2016); however, it is necessary to thoroughly quantify the degrees of within-

individual variation within these vocalizations before examining potential individuality in the fine structure of nocturnal flight calls. Although we found no evidence for geographic variation in the flight calls of two species, we discovered evidence of a complex pattern of geographic variation in the calls of one widespread species. It is important to recognize, however, that these results cannot be extrapolated necessarily to other birds. Therefore, it may be beneficial to investigate potential geographic variation in the calls of other widespread migrants. Geographic variation may be especially likely in species where there are different subspecies found in eastern versus western North America.

Nocturnal flight calls may not be able to provide information beyond the species-composition of nocturnally migrating birds. Nevertheless, there are a number of species whose flight calls, if any, have not yet been described. Nocturnal flight call research has been primarily restricted to the Northeastern United States, and we do not have information concerning the flight calls of many western bird species in North America, as a result. Further, research on nocturnal flight calls to date has focused primarily on songbirds. Many birds in other taxonomic groups also produce these vocalizations, including rails (*Rallidae*) and sandpipers (*Scolopacidae*); however, there is a dearth of information concerning these vocalizations, such as the description and verification of many species' nocturnal flight calls. There remains substantial untapped potential for nocturnal flight call studies to begin monitoring other taxonomic orders, if only recordists would collect and analyze more species across a broader geographic area.

In addition to quantifying variation within these calls, it is necessary to improve the identification approaches used by nocturnal flight call studies. Currently, nocturnal flight call studies use teams of trained analysts to process and identify recordings (e.g. Sanders and Mennill 2014). Manual approaches for processing acoustic recordings, while accurate, are significantly more time consuming, which may limit the amount of data available to be included

in analyses (Swiston and Mennill 2009). While there are continuing efforts to develop automated recognition programs to process flight call recordings (e.g. Damoulas et al. 2010), automated processing of acoustic recordings has been shown to produce a large amount of false positives and to identify significantly less vocalizations (Swiston and Mennill 2009; Venier et al. 2017). Since current automated approaches are less comprehensive, manual processing of nocturnal flight call recordings is currently necessary to collect accurate data on the population trends of species. Computation advances, especially use of random forests and machine learning, suggest that a technical break-through may improve reliability and permit the use of automated processing for nocturnal flight call research, which would reduce the time and human resources necessary to process recordings.

In order for the broad implementation of automated processing for nocturnal flight call research, two issues need to be addressed. First, there is a critical need for further development of automated processing techniques addressing the current limitations of false positives and missed detections when detecting and identifying animal sounds (Brandes 2008; Swiston and Mennill 2009; Venier et al. 2017). Second, it is necessary to expand the existing library of nocturnal flight calls by collecting more recordings from birds of known species, age, and sex to allow more investigations into the pressures driving variation in these calls across and within species. The development of a comprehensive library of flight calls and efficient acoustic processing approaches is necessary before long-term flight call monitoring projects can be developed and implemented.

Currently, migration monitoring stations across North America typically use either diurnal monitoring approaches (e.g. diurnal censuses and bird banding) or nocturnal monitoring approaches (e.g. acoustic recording or radar). However, each monitoring approach possesses different strengths and weaknesses, and reliance on sampling during a specific time of day

cannot provide a complete picture of the migratory movements through a region. For example, many raptors and cranes migrate during the day to take advantage of thermal soaring, but many small birds, including most passerines, will complete at least parts of their migration at night when conditions are more favourable to long distance flight (Alerstam 2009). While the development of efficient approaches for processing acoustic recordings and radar data is ongoing, the recording of nocturnal flight calls and collection of radar data could begin to be incorporated into the protocol of existing and future migration monitoring stations. The ideal protocol for a network of migration monitoring stations of the near future will require multiple approaches to sample the local bird abundance and movements during both the day and night: bird banding and censuses (diurnal), and acoustic monitoring and radar (nocturnal). This would provide a more comprehensive view into the populations and movements of many migratory birds, including species that are not often detected by traditional monitoring stations (e.g. Louisiana Waterthrush and Worm-eating Warbler).

The nocturnal flight calls of migratory songbirds offer a powerful ornithological tool for the study of bird populations and migration behaviour. My results revealed that discrete differences do exist between some flight calls produced by the “zeep” species-group allowing discernment of some of these calls based on fine structure. Further, I presented evidence that the nocturnal flight calls of songbirds do not contain information concerning the sex of an individual, contrary to earlier research. I presented further evidence that flight calls do not contain information about the age of a bird. Finally, I presented the first study, to my knowledge, of geographic variation in the nocturnal flight calls of migratory songbirds across North America. This research serves to enhance the capabilities of acoustic recording for monitoring migratory birds while improving our understanding of the forces driving acoustic variation in the calls of songbirds.

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