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Behavioural Mechanisms of Protandrous Spring Migration in a Nearctic-Neotropical Songbird

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Abstract

Most seasonally migrating songbirds have a protandrous migration pattern, meaning that males arrive to the breeding grounds before females. The proximate mechanisms that underlie this pattern are largely unknown for most species. In this thesis, I evaluate the behavioural mechanisms of protandry in the black-throated blue warbler *Setophaga caerulescens* using onset of migratory restlessness as a proxy for departure from the wintering grounds, migratory restlessness intensity as a proxy for motivation to fly, and stopover duration of radio-tagged birds. The onset of migratory restlessness and stopover duration was similar in both sexes. Males displayed higher intensity wing whirring than females, suggesting that protandry might be achieved through sex differences in nocturnal flight duration. Stopover duration was negatively correlated with migratory restlessness intensity on the last night the birds were in captivity, validating migratory restlessness as an accurate proxy for motivation to migrate at the individual level.

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Co-Authorship Statement

All work presented in this thesis was completed under the supervision of Dr. Yolanda E. Morbey and Dr. Christopher G. Guglielmo at the University of Western Ontario. All work was done in collaboration with Dr. Yolanda E. Morbey and Dr. Christopher G. Guglielmo, who helped develop the study objectives and methodology. Data collection and analysis was completed by Jessica E. Deakin. This thesis has been written by Jessica E. Deakin and will be published with Yolanda E. Morbey and Christopher G. Guglielmo.

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List of Abbreviations

AHY – After Hatch Year

GLM – General Linear Model

GPS – Geographic Positioning System

HY – Hatch Year

QMR – Quantitative Magnetic Resonance

TC/EA – High Temperature Conversion Elemental Analyzer

VHF – Very High Frequency

VSMOW – Vienna Standard Mean Ocean Water

δ^2H – Stable Hydrogen Isotopes

δ^2H_F – Stable Hydrogen Isotopes of Feathers

δ^2H_P – Stable Hydrogen Isotopes of Precipitation

INTRODUCTION

1.1 Defining migration and its ultimate causation

Diurnal and seasonal periodicity, caused by the Earth's rotation on its axis and the 23.5° tilt of the axis relative to the sun's position, are associated with constant changes in environmental conditions. As such, animals have adapted to overcome challenges that are inherent to inhabiting highly variable environments. Migration, a specialized form of movement, is a common adaptive response to seasonal periodicity. It is found throughout the animal kingdom, takes place in numerous types of media, and encompasses various types of locomotion such as walking, swimming, or flying. There are six defining characteristics of migration that separate it from other forms of movement (from Dingle 2014): (1) persistent locomotion, (2) straightened out pathways, (3) inhibited responses to resources, (4) specialized behaviour associated with departure and arrival, (5) triggering by surrogate cues such as photoperiod, and (6) shifted resource and energy allocation to support movement. These broadly defined characteristics are differentially expressed and are highly variable among taxonomic groups. For instance, the classically viewed seasonal to-and-fro migration pattern can occur on massive scales that cover large proportions of the planet, such as the humpback whale (*Megaptera novaeangliae*) swimming greater than 8,461 km semiannually between the Antarctic Peninsula and Central America (Rasmussen et al. 2007), or on small scales, such as the bighorn sheep (*Ovis canadensis*) that traverse between mountain slopes in the summer and valleys in the winter (Geist 1971). Migration can also occur as a one-way trip, where individuals have unidirectional migration but the population completes the journey, such as the lady butterfly (*Vanessa cardui*) taking 6 generations to fly between Europe and Africa (Stefanescu et al. 2012).

Despite the diversity of migratory behaviour across taxa, the ultimate reason an organism migrates is to increase fitness (Dingle 2014). This may mean moving to exploit certain food sources, to find more favourable habitats for refuge, and/or to find resources for breeding and rearing young. As such, migrants are faced with the challenging decisions of 'when to leave?' and 'where to go?'. In the field of behavioural ecology, a

decision refers to the act of an animal choosing one behaviour from a set of alternative behaviours to maximize fitness (Dill 1987). A well-adapted individual is thought to be able to compare the costs and benefits of the alternative behaviours, and trade them off, to make the optimal decision. Decisions can be intrinsic, where alternative behaviours have been evaluated by selection over evolutionary time, or extrinsic, where individuals make decisions in response to present conditions. Theory suggests that individual migrants should make decisions that optimize time, energy, and/ or safety (Alerstam and Lindström 1990). For most species, the proximate factors that regulate these decisions (what processes cause the immediate behaviour) are still poorly understood. However, we do know that migrants rely on intrinsic and extrinsic cues to provide them with information such that they can match their migration timing to the phenology of resources at their destination. These cues have the potential to become unreliable under the threat of a changing environment, potentially resulting in detrimental phenological mismatches (Walther et al. 2002; Mayor et al. 2017). Thus, the proximate factors underlying migration are highly relevant in advancing our understanding of migration and to properly manage and conserve at-risk migratory species. The migratory bird system provides an excellent model to study these factors because of the sheer amount, with up to 50 billion birds migrating per year (Berthold 1993), and diversity displayed both among and within species.

1.2   The phenomenon of bird migration

The migratory bird system has long been of interest to birders, ornithologists, and biologists alike. The astonishing 1600 known bird species that are migratory (Kirby et al. 2008) originate in various geographic regions around the world and include a wide variety of bird groups, such as raptors (Accipitriformes), shorebirds (Charadriiformes), waterfowl (Anseriformes), and songbirds (Passeriformes). Migratory behaviour and the role that migration plays in life histories varies both across and within bird species. These differences can occur with respect to the presence or absence of migratory behaviour, the ultimate reason to migrate, and the degree or type of activity that takes place. For instance, European robins (*Erithacus rubecula*) undergo partial migration meaning that some individuals migrate and others remain on the breeding grounds year-round. Some

species migrate to find refuge habitats, such as shelducks (*Tandorna* spp.) that undergo moult migration to locations with few predators and rich resources that are needed to regrow feathers after completing moult (the process of replacing feathers) (Dingle 1996). Other species migrate to exploit ephemeral conditions, or habitats that are intermittently rich in resources because of ecological succession or unpredictable variations in climate. For example, a high density of Australian pelicans (*Pelicanus conspicillatus*) establish in Cooper Creek, Australia after unpredictable rainfall events that flood the normally dry river (Kingsford et al. 1999). The most widespread type of bird migration, referred to as seasonal migration, is driven by seasonal changes in essential resources required for survival and reproduction. Seasonal migration involves moving between two or more distinct locations that are resource rich during alternate times of the year (Dingle 2014). Typically, this involves a to-and-fro migration pattern between a summer breeding location and a different location for the remainder of the year, as seen in most songbirds.

A songbird ('passerine') is a bird from the order Passeriformes, meaning "perching bird" which includes more than half of all bird species in the world. The overwintering and breeding sites for many migratory songbirds are separated by thousands of kilometers, as seen in many Nearctic-Neotropical migrators (species that migrate between the temperate zones of North America and tropical areas of Central America, South America and the Caribbean Islands). For instance, blackpoll warblers (*Setophaga striata*) undergo a non-stop transoceanic migration ranging from 2270 to 2770 km as part of its overhaul migratory journey between northeastern North America and northern South America (DeLuca et al. 2015). This highly energetically demanding migratory journey is primarily powered by internal fat stores that are initially accumulated prior to migratory departure (King 1961).

Interestingly, most songbirds remain diurnal during the breeding and overwintering stages, foraging during the day and sleeping at night. During fall and spring migration however, they remain active during the day but will also become active at night to migrate. Nocturnal migration is believed to be more advantageous than daytime migration (diurnal migration) because foraging time can be maximized and energy deposition can occur prior to and after nighttime flights (Alerstam 2009).

Successful migration is complicated by unpredictable extrinsic factors experienced *en route* such as poor weather, competition, and predation (Alerstam and Lindström 1990). For many species, movement between breeding and wintering sites requires stopovers, meaning that birds will stop at specific locations (termed stopover sites) along their migratory route to rest and replenish fuel reserves before carrying on their migratory journey. Most of the time and energy spent on migration takes place during stopovers (Wikelski et al. 2003). Decisions made at stopover such as where to stopover and how long to stopover for can greatly influence arrival timing to the breeding areas. Thus, the success of an individual throughout the rest of its annual cycle greatly hinges on decisions made during stopover (Sillett and Holmes 2002; Smith and Moore 2005).

Birds are under increasing pressure from human-made disturbances such as habitat loss, habitat fragmentation, building collisions, cats, and climate change. Many bird species are shifting their migration phenology earlier in the spring as a response to climate change (Jonzén et al. 2006). This is essential since proper arrival timing to the breeding grounds should be timed such that the bird is not at excessive risk of death by harsh climatic conditions, starvation, predation, or parasitism (Møller et al. 2010). The timing of seasonal migration has evolved to match peak food availability for the young; however, recent evidence suggests that species and trophic phenologies can shift at differing rates (Mayor et al. 2017). Since individual fitness depends on arrival timing to the breeding areas (Smith and Moore 2005), phenological mis-matches caused by climate change may have detrimental fitness consequences and could potentially impact northern breeding migratory populations. Thus, understanding the proximate mechanisms underlying bird migration timing and how flexible these mechanisms are will help us to predict how species will respond to environmental variability.

1.3   Historic and current methods to study bird migration

There are many challenges in studying bird migration. The most apparent challenge is trying to follow a small bird that is continuously on the move throughout much of its annual cycle – from breeding territories to migratory stopover sites to overwintering territories. One of the earliest methods used to track avian migration, and the factors influencing this migration, was capture-recapture. In this method, birds are caught,

marked (e.g. with a coloured or numbered leg band), released, and then hopefully recaptured again. However, the recovery rate for many passerine species is <1% (Berthold 2001). Bird observatories located at high density migratory stopover locations capture and band birds that are passing through. Data obtained from these banding stations can be used to track seasonal migration times and patterns, and habitat preferences during migration. Another challenge in studying migration is estimating true stopover durations. Empirical data of stopover duration come mostly from capture-recapture studies that assume that time of arrival to stopover is the same as when the bird was first captured (Cherry 1982; Morris et al. 1996). However, this minimum stopover duration method most likely underestimates true stopover duration. Through advances in technology, new methods have been developed to track birds throughout their annual cycle, including large scale movements between breeding and wintering sites and small scale movements at stopover sites.

1.3.1 Tracking bird migration

Birds can be tracked with satellite tags, data loggers with Geographic Positioning System (GPS) capabilities, and light-level geolocators (hereafter geolocators), each of which are harnessed onto a bird and either emit or receive location data. The common practice in the field is to use tags that weigh <5% body mass to reduce drag and to minimize potential added energy expenditure (Barron et al. 2010). Satellite tags are small miniature transmitters that are harnessed to a bird like a backpack and are detected by satellite, using GPS, to determine a birds' location (Higuchi et al. 1998). The smallest satellite tags with automated GPS Argos positioning capabilities (locations can be automatically downloaded online – no need for tag recovery) is 3.5 g. Based on the 5% rule, the smallest bird that could be equipped with an automated GPS tag is currently 70 g. Due to the heavy weight of these tags and the price for each tag (>$3000), studies are limited by which bird species that can be tracked and how many tags can be deployed. Data loggers with GPS positioning capabilities provide accurate location data (± 30 m), with the bonus of being light weight (~1 g). However, these tags must be recovered to retrieve the location data. Since the probability of recapturing a bird is low, this method is typically only plausible for species that display high levels of site fidelity. For instance, Hallworth

and Marra (2015) had a 31% GPS tag (~1 g) recovery rate for ovenbirds (*Seiurus aurocapilla*), a Nearctic-Neotropical migratory songbird that has high site fidelity. Geolocators record ambient light level to estimate sunrise and sunset times, which can be used to estimate day length and thus, in combination with time, latitude and longitude (Stutchbury et al. 2009). Currently, these tags are as little as 0.3 g, however, they must be recovered to retrieve location data and have a low spatial resolution. For instance, Hallworth et al. (2015) had a 24.7% recovery rate of geolocators harnessed on ovenbirds. Using stationary geolocators, Fudickar et al. (2012) found that latitude position estimates ranged from 23 km to 390 km off target in a forested habitat.

Very high frequency (VHF) radio-telemetry is frequently used to track local movements of animals with high location estimate accuracy and does not require recapture to retrieve location information (reviewed in Bridge et al. 2011). Radio tags are harnessed onto the animal and emit pulsed signals in the VHF electromagnetic spectrum. Manual radio-telemetry requires following tagged birds by foot, car, or plane with specialized antennas and receivers that are used to detect the tags. Traditionally, each tag emitted a radio signal on a different frequency. Researchers were limited in the number of birds that could be tracked since the antenna had to be tuned to different frequencies and typically only one bird could be tracked at a time. Radio-telemetry can be used to track the smallest songbirds (<20 g) since tags can be very small and light weight (<0.21 g). The tag's pulse frequency (rate of radio signal emittance) is typically between 2-10 times per minute (Taylor et al. 2017), which allows for the continuous monitoring of movement and provides an excellent method to study stopover behaviour.

The recent development of automated radio telemetry systems allows for the continuous recording of animal movement without the need of following the animal. In these systems, receiver stations are in fixed positions and are continuously listening for signals emitted by tags. Tags can now be coded to emit radio signals at the same frequency, which unlike conventional radio-telemetry, allows automated radio receivers to detect hundreds of tags at the same time. Coordinated arrays of automated receiver stations, such as Motus Wildlife Tracking System (hereafter Motus), are arranged such that local and regional scale movements can be tracked when an animal is detected by

multiple towers (Taylor et al. 2017). Typically, Motus using researchers use special tags (nanotags) that emit a unique coded sequence of radio transmissions (pulses), repeated at fixed intervals (burst rate) (Taylor et al. 2017). The combination of unique pulse and burst rate allows for individuals to be identified. Previous studies have used Motus to study fine-scale movement behaviour of songbirds at stopover. For instance, Dossman et al. (2016) used Motus to study the departure decisions of American redstarts (*Setophaga ruticilla*) and yellow-rumped warblers (*S. coronata coronata*) during spring migration and (Morbey submitted) used Motus to determine the onset and end of diel activity in black-throated blue warblers (*S. caerulescens*) and magnolia warblers during spring stopover.

1.3.2 Using feathers to establish bird origins

A major component of a bird's migration is where it breeds and overwinters. Stable isotopic markers in animal tissues have been used in numerous studies to determine migratory connectivity, or the geographic linking of individuals between one life cycle stage and another (Rubenstein et al. 2002; Hobson et al. 2009). Most commonly, stable hydrogen isotopes (δ^2H) of feathers have been used to assign birds to geographic origins (e.g. Hobson and Wassenaar 2008). Stable hydrogen isotopic markers in animal tissues can accurately predict the geographic origins of North American birds because of the well-known and predictable patterns of hydrogen isotopes in rainfall across the continent (Hobson and Wassenaar 2008). The hydrogen isotope signature of rainfall translates into plant biomass (Yapp and Epstein 1982), which then travels up the food chain to higher trophic level consumers (Cormie and Schwarcz 1994). The δ^2H composition of a bird's diet is translated into metabolically inert feathers during feather moult (Hobson and Clark 1992). For instance, for birds that grow their feathers at the breeding site, the δ^2H of their feathers is highly correlated with δ^2H values of growing season precipitation at this location (Chamberlain et al. 1996). Thus, δ^2H analysis of feathers collected from birds at non-breeding regions (i.e., stopover sites, overwintering sites) can be used as a tool to determine migratory connectivity.

1.3.3 Recording migratory restlessness

It has been known for centuries that when a migratory bird is kept in captivity it will display migratory restlessness (adapted from the German word 'Zugunruhe'). This behaviour is characterized by extensive wing whirring, grounding, hopping, and jumping at night and has been referred as "migration on perches" (Berthold and Querner 1988). Wing whirring refers to wing fluttering at high frequency but low amplitude while sitting on a perch (Berthold 1993). This behaviour is particularly evident in nocturnal migrants, who are completely inactive at night during the breeding and wintering stages, but will suddenly become active at night during the migratory stages. The first written accounts of migratory restlessness were those from (Naumann 1795) and (Brehm 1828). It has since been observed in more than 100 different species (Berthold 1996), which has led to the establishment of migratory restlessness as a model study system to explore the timing, physiology, and mechanisms that underlie migration (Berthold 1993; Berthold 1996; Dingle 1996; Gwinner 1996; Dingle 2014).

Numerous studies demonstrate that the occurrence and intensity (amount) of migratory restlessness displayed by captive species matches the migration patterns observed in free-living birds (Gwinner 1986). For instance, the onset of migratory restlessness of captive individuals has been shown to commence at the same time as migration in wild conspecifics. European willow warblers (*Phylloscopus trochilus*) initiate spring migratory restlessness during February and March, the normal time of their return southern migration (Gwinner 1986). Similar results have also been observed in long-distance migrating warblers (Berthold 1973) and flycatchers (Gwinner and Schwabl-Benzinger 1982). Additionally, the intensity of migratory restlessness in captive birds mirrors migration distance in wild conspecifics. In a comparative study of 13 species of *Sylvia* warblers, Berthold (1984) found that the intensity of migratory restlessness (measured by number of half hours of activity per night) was positively correlated to the migratory distance in free-living conspecifics. Moreover, the temporal pattern of intensity of migratory restlessness relates to the migratory route of wild conspecifics. As shown in a comparative study of eight *Sylvia* warbler species, Gwinner (1986) found that the long-distance migrants had right-skewed temporal intensity patterns

with peak intensity near the beginning of the season, which matches the timing wild conspecifics would normally cross the Sahara. In contrast, the short-distance migrants that normally have no large ecological barriers along their migration routes, had relatively flat temporal intensity patterns without any pronounced peaks.

Various methods have been used to quantify migratory restlessness. One of the earliest methods for recording nocturnal locomotion was by spring loaded cages coupled to a counter, for which the number of movements were read off in the morning (Wagner 1930). Since then, technological advancements have allowed for the further development and fine-tuning of methods used for quantifying migratory restlessness. Most commonly, migratory restlessness has been quantified by using registration cages equipped with mobile perches that are connected to micro-switches. Any time a bird hops, it pushes the perch down, which activates the switch to send an impulse to a registration apparatus (e.g. computer or event recorder). Air movements have been used to quantify the wing whirring behaviour of migratory restlessness (Czeschlik 1974). In this method, a brass rod is loosely suspended inside a vertical brass tube and air movement causes the rod to press against the rim of the tube. Each time the rod touches the rim, an electrical impulse is generated, which operates an event recorder. More recently, highly sensitive microphones have been used. In this method, the microphone is attached to a cage wall and vibrations produced by bird movement and sound are recorded as activity counts (Maggini and Bairlein 2010, 2012; Eikenaar and Bairlein 2014; Bulte et al. 2017). Migratory restlessness has also been quantified with the use of infrared sensors (Coverdill et al. 2011; Vandermeer 2013; Berchtold et al. 2017). Here, an infrared emitter and an infrared detector are attached on opposite ends of a perch. Every time a bird moves, it breaks the beam, which transmits this information to a data logger. Arguably one of the most comprehensive ways to quantify behaviour, however, is through video analysis. Video analysis allows researchers to separately quantify the various types of behaviours associated with migratory restlessness (e.g. Coverdill et al. 2008). A major drawback to video analysis becomes apparent when analyzing migratory restlessness over long periods of time (e.g. over the course of a typical migratory period) as video analysis is considerably time consuming.

1.3.4 Orientation studies

Studying how a migratory bird maintains specific direction, or orientation, typically involves one of three methods: observations of free living birds, experiments on free living birds (e.g. displacement studies), and perhaps most commonly, orientation cages. Kramer (1957) demonstrated that red-backed shrikes (*Lanius collurio*) and blackcaps (*Sylvia altricapilla*) orient their migratory restlessness toward the side of the cage that corresponded to their normal migration direction. Later, funnel cages were developed to study the orientation behaviour of migratory birds, including the most commonly used Emlen cage (Emlen and Emlen 1966). The Emlen cage originally consisted of a blotting paper funnel with an inkpad base and a screen top. A bird would then be placed inside of the funnel and the preferred direction of the bird could be determined from the density of black footprints left behind as the bird hopped onto the side of the funnel. Other studies have used telemetry in combination with displacement to study the orientation behaviour of migratory birds. For instance, Kishkinev et al. (2017) used Motus to track post-displacement migration in white-throated sparrows (*Zonotrichia albicollis*).

1.3.5 Fuel load and accumulation

Researchers use body composition as a measure of quality of birds, the habitats they occupy, and the current annual stage they are in. For instance, one of the major characteristics of a bird in the migratory state is their pronounced fat storage (Berthold 1993). Most birds will start to over-eat prior to migration, termed hyperphagia (King 1961; Bairlein 1985), which causes the bird to develop fat deposits that are needed to fuel their energetically expensive migratory flights (Blem 1980). As such, fuel load and fuel accumulation can be used a proxy for the pre-migratory and migratory states. Measuring total body mass and determining fat score are two of the simplest methods to monitor fuel load and fuel accumulation (i.e. an increase in total mass and/or fat score indicate increased fuel load and accumulation). Previous studies have used fat score as a proxy for fuel load (Goymann et al. 2010; Dossman et al. 2016). Fat scores can be determined through visual observation of subcutaneous fat (Pettersson and Hasselquist 1985; Kaiser 1993). In the Kaiser (1993) technique, 31 classes (nine main classes: 0-8 index) are used to characterize the fat deposition of a bird through visible observation of the furcular

depression, neck, and breast muscle. A fat score of 0 represents virtually no fat deposits and a fat score of 8 characterizes a bird with such large fat deposits that the flight muscles are not visible and a fat layer covers the underside/ventral side of the bird completely (Kaiser 1993). More recently, Quantitative Magnetic Resonance (QMR) has been used to obtain precise and accurate measurements of fuel load (Seewagen and Guglielmo 2010). QMR is a non-invasive method that can determine fat, lean mass, and total water of non-anaesthetized animals (Guglielmo et al. 2011). Additionally, plasma metabolite analysis can provide insight into individual refueling rate since triglyceride levels rise, while ß-OH-butyrate levels fall, in the blood plasma of refueling birds (Guglielmo et al. 2005).

1.4   What influences migration?

1.4.1 Endogenous biological clocks

The physiology, behaviour, and biochemical processes of most living organisms show repeated peak-to-peak intervals, or periods of activity that are synchronized with the environment. An oscillator is a system of interacting components (molecules, cells, and tissues) that produce a rhythm with a specific period length (Bell-Pedersen et al. 2005). For example, circadian oscillators have an endogenous period close to 24 hours and form the circadian biological clock. Environmental cues, known as Zeitgeber, entrain circadian oscillators to local time through the process of entrainment (Roenneberg et al. 2003). Entrainment refers to "causing another to fall into synchronization with", such that the endogenous timing of peaks and troughs synchronize with an environmental reference point. The light:dark cycle (L:D) of day and night is the main Zeitgeber for most organisms. As an example, a bird will have peak singing approximately at dawn in the spring. Circadian clocks control circadian rhythms of gene expression, which results in corresponding biological activity (Bell-Pedersen et al. 2005). Likewise, the circannual rhythm corresponds to annual cycles that are believed to be entrained to photoperiod (Gwinner 1989). These rhythms are repeated approximately every 365 days when an organism is experimentally placed under constant photoperiod, such as 12 h of light and 12 h of dark (12L:12D) (Gwinner 2003). However, both the circadian and circannual rhythms will drift or run free after a while in the absence of the environmental cues that entrain them (Gwinner 1996).

In birds, the circadian clock functions to control daily patterns of sleep and wakefulness, and the circannual clock controls orientation, seasonal patterns of song, reproduction, and migration (Gwinner 1986; Berthold 1996: 199). Along with exogenous factors, the endogenously controlled circannual rhythm controls the onset of outward and return migration in seasonal migratory birds as well as the onset and amplitude of migratory fattening. Evidence for this comes from captive studies of birds placed under constant photoperiodic conditions (12L:12D). For instance, *Sylvia* warblers and flycatchers (family Muscicapidae) display migratory restlessness at the appropriate times of year when placed under 12L:12D (Gwinner 1986). Further, birds have been observed showing spontaneous seasonal patterns of mass gain like wild conspecifics when placed under constant 12L:12D (Piersma et al. 2008; Maggini and Bairlein 2010).

1.4.2 Photoperiodism

Since seasonal migrators restrict their breeding to a specific time of year, the timing of migration and schedule of reproductive physiology are fundamentally linked. Photoperiod is an important extrinsic cue used by songbirds to broadly tune the timing of seasonal changes in reproductive physiology and behaviour (Wingfield et al. 1983; Hau et al. 1998; Gwinner and Scheuerlein 1999). The annual cycle of reproductive physiology involves three distinct phases: photostimulated, photorefractory, and photosensitive (MacDougall-Shackleton and Hahn 2007). As photoperiod increases as spring progresses, birds become photostimulated, which results in recrudescence of the reproductive axis (increased gonad size and sex steroid hormone levels, as well as the initiation of reproductive behaviour). When birds are exposed to these long photoperiods over time (i.e., as summer progresses), the birds are said to be photorefractory, meaning that their reproductive systems become insensitive to the normally stimulating long photoperiods and their gonads regress and become inactive. Only after exposure to short photoperiods (i.e. as late fall progresses to winter) will the birds become photosensitive again.

1.4.3 Extrinsic and intrinsic factors

Together with endogenous rhythms, various extrinsic and intrinsic factors have been shown to play an important role in influencing the course of bird migration. For instance,

weather conditions (e.g. wind, air pressure, cloud cover, precipitation, temperature) play an important role in migratory departure decisions and departure direction (Jenni and Schaub 2003). European robins are more likely to depart during favourable tail winds, when it is not raining, and with rising air pressure (Dänhardt and Lindström 2001). Dossman et al. (2016) show that American redstarts and yellow-rumped warblers are more likely to depart from migratory stopover on nights with high tailwind assistance. Finally, the probability of exhibiting autumn migratory restlessness and its intensity was higher in captive birds kept in cooler temperatures than warmer temperatures (Berchtold et al. 2017). Food availability and fuel stores influence migration timing. Recent studies have shown that migratory restlessness is positively correlated with fuel load at capture and negatively correlated with fuel accumulation in northern wheatears (*Oenanthe oenanthe*) during spring stopover (Eikenaar and Schläfke 2013). Comparable results have been obtained by Sjöberg et al. (2015) and Dossman et al. (2016), both of which found that high fuel load at capture reduced stopover duration. Additionally, Eikenaar and Bairlein (2014) found that birds at stopover were more motivated to migrate, quantified by migratory restlessness, under low food availability. Circulating hormone levels have been also shown to influence departure decisions. As an example, Lõhmus et al. (2003) found that baseline corticosterone levels were positively related to amount of activity displayed in orientation cages in red-eyed vireos (*Vireo olivaceus*). Testosterone levels are positively related to amount of spring migratory restlessness in white-throated sparrows (Vandermeer 2013), suggesting that testosterone levels influence migratory timing.

The age of a migratory bird influences the timing, direction, and duration of migration in many species. For example, older male American redstarts arrive to the breeding grounds in the spring before younger males (Lozano et al. 1996). Using light level geolocators, McKinnon et al. (2014) observed earlier departure from overwintering sites of adult wood thrushes (*Hylocichla mustellina*) compared to juveniles. Using an automated telemetry array, Crysler et al. (2016) found that juvenile Ipswich sparrows (*Passerculus sandwichensis princeps*) depart earlier, have different migratory routes, and more frequent and longer stopovers than adults. For juvenile birds on their first migratory journey, migratory patterns are initially controlled by the endogenous circannual rhythm

and later modified by experience (Berthold 2001). As such, many age-specific differences in migratory patterns have been attributed to learning (Sergio et al. 2014).

1.5 The influence of sex on migration

There is a widespread occurrence of sex-biased timing strategies throughout the animal kingdom, where males and females arrive to the breeding grounds according to different schedules. Protogyny, the earlier arrival of females than males, is the less common strategy. Protogyny has been reported in silver-haired bats (*Lasionycteris noctivagans*) where females arrive to the summer grounds before males (Jonasson and Guglielmo 2016), and in sex-role reversed birds, such as female spotted sandpipers (*Actitis macularis*) that arrive to breeding areas in the spring before male conspecifics (Oring and Lank 1982). Protandry, meaning males before females, is the most common sex-biased arrival timing strategy and is seen throughout the animal kingdom. For instance, male ground squirrels emerge from hibernation in the spring earlier on average than females (Michener 1984), male butterflies tend to emerge earlier on average than females (Wiklund and Fagerström 1977), male jumping spiders (*Servaea incana*) mature before females (Mendez et al. 2017), and male Pacific salmon (*Oncorhynchus* spp.) arrive earlier than females to the breeding grounds (Morbey 2000). Perhaps one of the most fascinating taxon to exhibit protandrous timing is birds (Francis and Cooke 1986; Spina et al. 1994; Rubolini et al. 2004; Morbey 2013).

1.5.1 Evolutionary functions of protandry

Morbey and Ydenberg (2001) classified and reviewed seven different, but not mutually exclusive, hypotheses for the adaptive significance of protandry. These hypotheses were divided into two groups: 1) indirect selection hypotheses, which describe how selection could act on arrival timing within the sexes, and 2) direct selection hypotheses, which describe how natural selection could act on arrival timing between sexes as a result fitness consequences for males or females. Protandry in birds was originally thought to evolve through intra-specific competition in the most territorial sex (usually males) for nest sites, termed the "rank advantage" hypothesis (Kokko 1999; Morbey and Ydenberg 2001). Empirical support for this hypothesis comes from the fact that the territorial sex is

typically the first to arrive. Earlier arrival would ensure a rank advantage over later arrivals; whether it be males competing for vacant territories, or females competing for occupied territories, both resources become scarce over time. The current favoured hypothesis for avian protandry however, is the "mate-opportunity" hypothesis. This hypothesis predicts that male-male competition for access to females, drives the earlier arrival of males compared to females (Morbey and Ydenberg 2001; Kokko et al. 2006; Møller et al. 2009). For instance, earlier arriving males may have greater opportunity for extra-pair mating or re-nesting after previously failed attempts (Kokko et al. 2006). Indeed, Rubolini et al. (2004) found that protandry in 22 sub-Saharan migratory species was driven by sexual selection, with degree of protandry strongly positively related to degree of sexual dichromatism. Among the abovementioned hypotheses, the ultimate function of protandry is to maximize individual fitness (Kokko 1999; Morbey and Ydenberg 2001; Kokko et al. 2006). For instance, Lozano et al. (1996) found that earlier arriving male American redstarts were more likely to attract a mate than later arriving males. Similar results have been demonstrated in great reed warblers (*Acrocephalus arundinaceus*), where male arrival order was positively related to pairing success, fledging success, and territory attractiveness rank (Hasselquist 1998).

1.5.2 Proximate causes of protandry

With the evolutionary explanations for protandry firmly grounded, attention is now focused on the behavioural mechanisms underlying protandry (Morbey and Ydenberg 2001; Morbey et al. 2012). In other words, how does individual behaviour scale up to cause protandry at the population level? Protandry is the norm for migratory songbirds and can be expressed at stopover sites and in arrival to the breeding sites (Møller 1994; Rubolini et al. 2004; Tøttrup and Thorup 2008; Coppack and Pulido 2009; Saino et al. 2010). Little is known about species-specific behavioural mechanisms that underlie protandry. There are at least three non-mutually exclusive hypotheses to explain the proximate mechanisms of protandry (Coppack and Pulido 2009). First, latitudinal sexual segregation, meaning that males could overwinter closer to the breeding grounds than females. If males and females began migrating at the same time, males would have shorter distances to travel and would likely arrive to stopover sites and breeding sites

before female conspecifics. Latitudinal sexual segregation has been reported in a variety of species such as the dark-eyed junco (*Junco hyemalis*) (Holberton 1993), the western sandpiper (*Calidris mauri*) (Nebel et al. 2002), savannah sparrows (*Passerculus sandwichensis*) (Woodworth et al. 2016), and Chiffchaffs (Catry et al. 2005). Using specimen records from 35 natural-history museums, Komar et al. (2005) found that males of 9 different Nearctic-Neotropical migratory species predominated in the northern part of the species' overwintering ranges. Second, males could have a faster rate of migration compared to females, realized through faster flight speeds (attributed to sex-specific wing shape and length) and/or shorter stopover durations. For instance, sexual dimorphism in wing length and shape is associated with differences in protandry between subspecies of northern wheatears (Schmaljohann et al. 2016). Male willow warblers have more pointed wings than females (Hedenström and Pettersson 1986), suggesting faster flight speeds by males than females. Lastly, and perhaps most commonly, males could initiate migration earlier and depart from their wintering sites before females. For instance, male European robins depart for migration in the spring before females (Catry et al. 2004). Recent studies using light level geolocators found that male northern wheatears left the wintering grounds before females (Schmaljohann et al. 2016). Male common redstarts (*Phoenicurus phoenicurus*) have an earlier onset of spring migratory restlessness in captivity compared to females, suggesting an earlier departure for spring migration by males than females (Coppack and Pulido 2009). Maggini and Bairlein (2012) observed an earlier onset of migratory restlessness under constant 12L:12D in male northern wheatears compared to females, providing evidence that earlier departure of males is endogenously controlled for this species.

1.5.3 Protandry in Parulidae warblers

Parulidae warblers (wood warblers) are mostly small, sexually dimorphic, insectivorous, medium to long distance Nearctic-Neotropical migrants. Parulidae are a group of birds that consistently show protandry; protandrous arrival to migratory stopover sites averages 4.2 days (range = 0.7 – 7.8 days; N = 37 estimates from 22 species; (from Francis and Cooke 1986; Kissner et al. 2003). This means that mean arrival date of males is 4.2 days earlier than that of females. However, only a few studies in Parulidae warblers have

observed sex differences in migratory behaviour that are associated with the three main hypotheses on the behavioural mechanisms of protandry (earlier departure, faster migration, geographic segregation). For instance, Marra et al. (1998) found that male American redstarts departed wintering grounds of Jamaica earlier than females in only one of two years and in one of two habitat types. Using plasma metabolite analysis, Seewagen et al. (2013) found that male yellow-rumped warblers and common yellowthroats (*Geothlypis trichas*) had faster refueling rates than female conspecifics during stopover in New York City, suggesting a faster migration rate in males. However, most studies have failed to find sex differences in stopover durations (Morris et al. 2003; Macdade et al. 2011; Dossman et al. 2016).

The black-throated blue warbler (*S. caerulescens*) is a small (8-13 g) long-distance Nearctic-Neotropical migrant, mostly insectivorous, territorial, and exhibits strong site fidelity in both its overwintering and breeding grounds (Holmes et al. 2005) (see Fig. 1 for a range map). Black-throated blue warblers have an average estimated protandrous arrival timing of 5 days at a migratory stopover site (Francis and Cooke 1986). They are one of the most sexually dichromatic wood warbler species and commonly exhibit extra-pair mating, despite being socially monogamous (Chuang et al. 1999; Murphy 2015). As sexual dimorphism and extra-pair mating are predicted to favor the evolution of protandry (reviewed in Morbey et al. 2012), black-throated blue warblers make an ideal model species to study the proximate causes of protandry. Currently, no study has evaluated the proximate causes underlying protandry in this species.

1.6   Study objective and overview

The objective of my thesis was to evaluate sex differences in the behavioural mechanisms that might underlie protandry in the black-throated blue warbler. I tested two established non-mutually exclusive hypotheses. To test the hypothesis that males depart from the wintering sites before females, I used the onset of migratory restlessness in the spring as a proxy for departure timing from the wintering grounds. To test the hypothesis that males have a faster overall migration speed than females, I measured the intensity of migratory restlessness and stopover duration as proxies for speed of migration, with the assumption
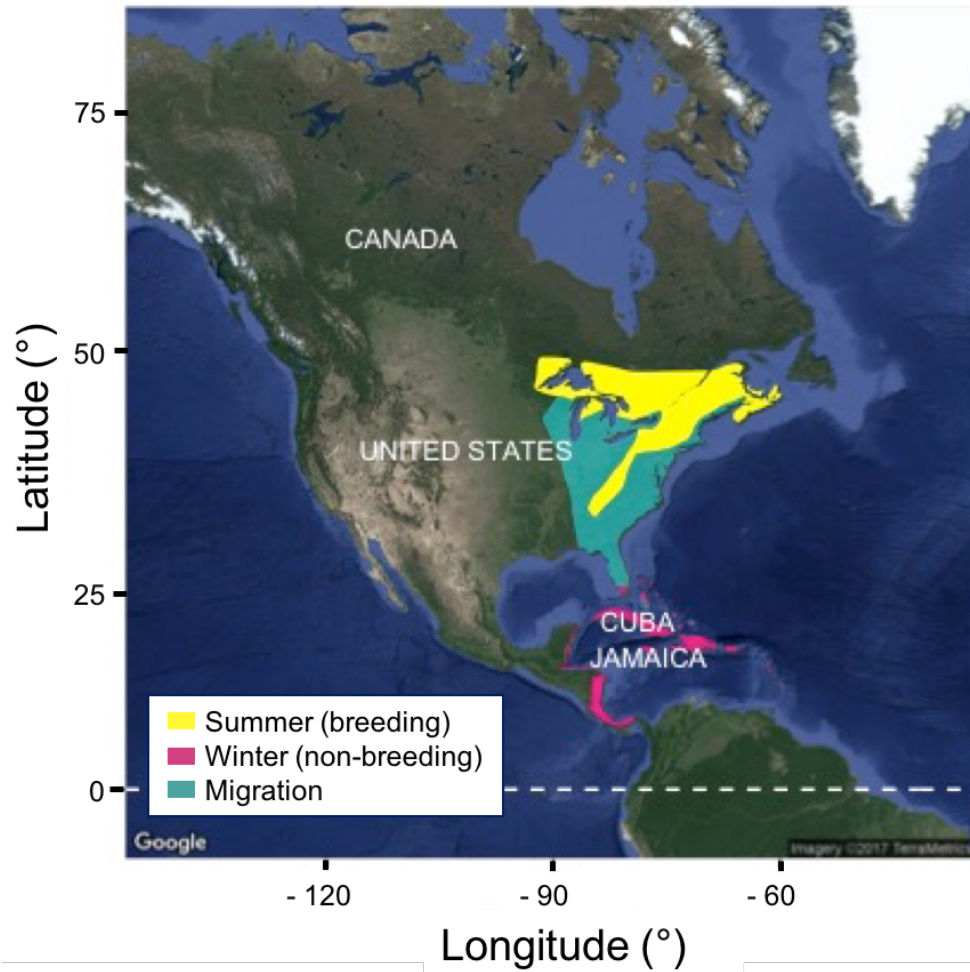
Figure 1. Range map of black-throated blue warblers (*Setophaga caerulescens*).
Shown are summer breeding grounds (yellow), overwintering non-breeding grounds
(pink), and areas in which they are located during spring and fall migration (teal).

that stopover duration at one site would be indicative of intrinsic differences between sexes across all sites.

To test whether migration behaviour differs between the sexes in this species, I captured black-throated blue warblers during their southern fall migration at Long Point Bird Observatory, ON, Canada. The birds were held in captivity over winter and I manipulated their photoperiod so that they became photostimulated in the spring at the appropriate time that they would normally begin preparing for migration in the wild. During this time, I video recorded their nocturnal behaviour using infrared lights to illuminate them and used Noldus Behavioural Analysis software to quantify the onset and intensity of their migratory restlessness. The birds were then fitted with digital nanotags and released at the stopover site they were captured the previous fall. Both manual radio-telemetry and Motus were used to precisely determine stopover duration for each bird. I predicted that 1) males would begin migratory restlessness in the spring before females, 2) males would have more intense migratory restlessness than females, and 3) males would have shorter stopover durations than females.

METHODS

2.1 Study birds

Black-throated blue warblers were captured during 2015 fall migration at the Old Cut station located at Long Point Bird Observatory, Long Point, Ontario (42°34'58.5"N 80°23'54.5"W; Fig. 2). All birds were captured with mist nets following the banding protocol of Long Point Bird Observatory with nets opening half-hour before sunrise and closing 6 h after sunrise each day. Age and sex were determined by plumage and molt characteristics following Pyle (1997). Birds were classified as after hatching year (AHY), meaning that they hatched before 2015 but otherwise hatch year is unknown, or hatching year (HY), meaning that they hatched during the 2015 breeding season. Wing chord length (mm), body weight (± 0.01 g), and fat score (0-8 scale index) (following Kaiser 1993) were recorded. The birds received an aluminum United States Fish and Wildlife Service/Canadian Wildlife Service leg band with a unique identification number. Following banding, the birds were transported in animal carriers (60.96 cm x 65.96 cm x 152.4 cm) equipped with small branches for perching, a water dish, and live mealworms to the Advanced Facility for Avian Research located at Western University, London, Ontario (43°00'37.1"N 81°16'48.2"W; Fig. 2). Sex-differences in wing chord length and body weight were analyzed with a two sample t-test and sex-differences in fat scores were analyzed with a general linear model. All analyses in this study were conducted using R statistical software version 3.2.2 (R Core Team, 2016) and alpha levels of 0.05 were set for all statistics. Means are presented as mean ± standard error.

2.2 Bird husbandry and housing

For their first few days in captivity, each bird was fed copious amounts of live mealworms mixed into a synthetic mash diet to encourage eating and reduce weight loss due to stress. The birds were fed a mash diet (comprised of 16.29% glucose, 3.62% caesin, 1.63% agar, 1.59% Brigg's Salt, 0.54% vitamin mix, 72.39% water, 3.08% oil, 0.87% cellulose) (Guglielmo et al. 2017) *ad libitum* for the duration of their time in captivity. In addition, every morning all birds were given 3-5 live mealworms for enrichment as well as fresh water for drinking and bathing. Since black-throated blue
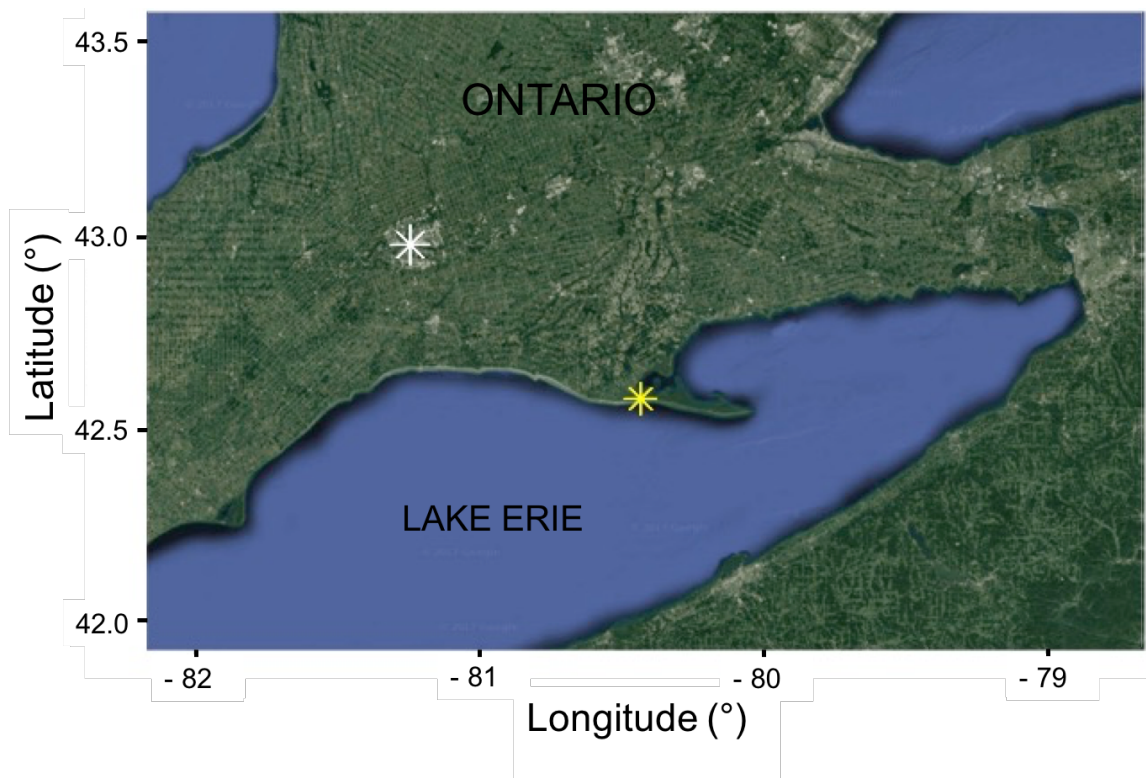
Figure 2. Map of southern Ontario showing where the birds used in this study were captured and where they were held in captivity overwinter. The black-throated blue warblers were captured at Old Cut station at The Long Point Bird Observatory, located in Long Point, Ontario (yellow star). The birds were transported and held in captivity overwinter at The University of Western Ontario, London, Ontario (white star).

warblers' diet is predominantly comprised of arthropods in the spring and summer months (Robinson and Holmes 1982), the birds were weaned from their mash diet to a 100% mealworm diet beginning May 9, 2016 to ensure their guts were properly prepared for release to the wild on May 19, 2016 (Afik and Karasov 1995). The birds experienced a temperature of 21° C (± 4° C) during the duration of the experiment.

The birds were initially held in Euro Cages (Corners Ltd., Lansing MI, USA; 39 cm x 34 cm x 42 cm) in groups of two (one male and one female) at the Advanced Facility for Avian Research (see Section 2.4 for details). Each cage was equipped with two small plant saucers (one for food and one for bathing water), two hanging water dishes for drinking, and two circular wooden perches (Fig. 3). On October 15, 2015, the birds were transported in modified animal carriers (60.96 cm x 65.96 cm x 152.4 cm) to the animal facility located in Collip Medical Building at Western University where they were housed in Euro Cages (39 cm x 34 cm x 42 cm) in one of two rooms. The birds were housed in the same groups of two and the cage set up remained the same.

On November 19, 2015, the birds were transferred to one of two free flight indoor aviaries (274 cm x 305 cm x 244 cm). To ensure a sex ratio of 1:1, the sexes were assigned to an aviary with an online list random generator. The first six males and six females of the list were transferred to aviary 1 and the rest were transferred to aviary 2. The aviaries were set up to provide the birds with comfort and stimulation while remaining a semi-natural environment. Tree branches were set up in a jungle-gym fashion in each of the aviaries to create perches of various heights to mitigate any dominance hierarchy issues (i.e. both dominant and submissive birds would have a place to perch). Since birds spend most of their time perching, the branches were of various diameters to prevent foot problems. Large buckets filled with sand were used to prop thick branches upright to create a base for smaller branches to be fastened to. Zip ties and duct tape were used to fasten the smaller branches to create either a stationary perch or a swinging perch (i.e. the perch would move based on amount of mass applied). Additional tree branches were scattered around the floor of the aviaries. Each aviary was equipped with two large plant saucers for the mash diet and mealworms, an additional large plant s

Figure 3. Picture of the inside of a birds' cage. Contents of each cage: two plant saucers (one food, one bathing water), two hanging water dishes (far left and right hand sides of cages; second is hidden by observed cage information card), and two wooden perches.

saucer for bathing water, and an automatic watering fount for unlimited drinking water. Refer to Fig. 4A – 4D for pictures of the free flight aviary setup.

On March 20, 2016, the birds were randomly transferred into modified Euro Cages (34 cm x 18.25 cm x 17 cm) in a single room (427 cm x 488 cm) to begin the migratory restlessness experiment (Fig. 5). Each cage was divided into two equally sized compartments with a piece of white opaque corrugated plastic sheet, secured with white duct tape, so that each side of the cage housed one bird. To reduce contrast noise during video analysis, all the dishes used were a light colour to ensure the bird remained the darkest entity in the cage. Each compartment was equipped with two beige coloured small plant saucers (one for food and one for bathing water) and one white hanging water dish for drinking. The standard wooden perches remained in each cage (one for each compartment) and a white painted circular wooden dowel (0.64 cm^2) was added to each compartment to provide additional perching opportunities. To add the extra perch, a hole the size of the dowel was cut in the centre of the opaque barrier. One side of the dowel was then tightly fit into the hole and the other side of the dowel rested on top of the original perch. Each cage was uniquely numbered with large, black, bold face font on a white background to allow for easy distinguishability between the birds during the video analysis.

2.3   Assigning study birds to place of origin

2.3.1   Stable isotope analysis

Analysis of δ^2H from feathers (hereafter δ^2H_F) was conducted to determine the breeding locations of the birds used in this study. One outer-tail rectrix was plucked from each bird on February 11, 2016 for analysis. Since the feather shaft has been shown to represent lower δ^2H values in comparison to the vane (Wassenaar and Hobson 2006), a subsample from each feather was taken by cutting a 2-cm section from the distal tip of the vane. All feathers were cleaned following methods from Hobson and Wassenaar (2008). A 2:1 chloroform:methanol solvent was used to clean all feather surface oils by soaking each feather independently in a vial filled with 2 mL of solvent for 1 hour. The solvent was then decanted and the feather was left to dry inside a fume hood overnight.
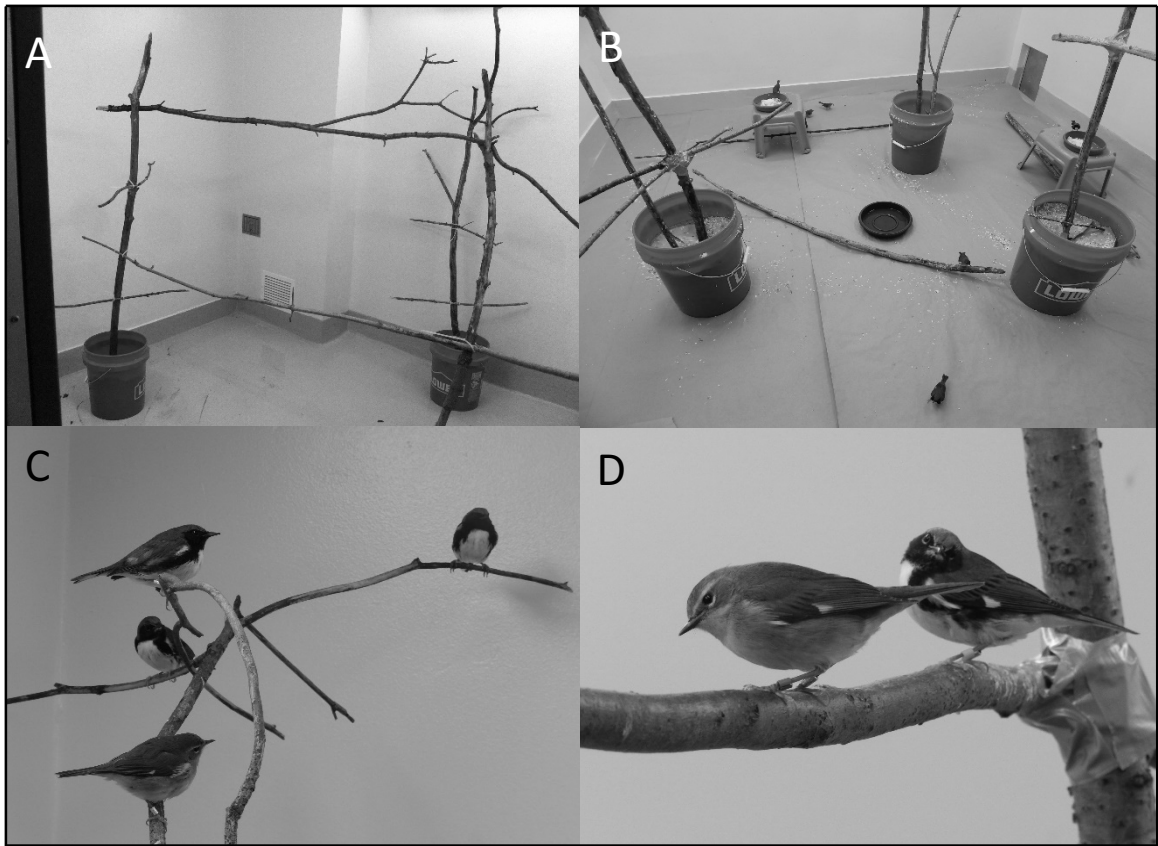
Figure 4. Pictures of the free flight aviary setup for the black-throated blue warblers (*Setophaga caerulescens*). A) Picture showing the placement of the tree branches. B) Picture showing the food and water dish setup and additional ground branches for perching. C) Picture example of a "swinging perch" whereby the perch would move based on the amount of mass applied. D) Picture example of a stationary perch. Note the duct tape securing the branches together.

Figure 5. Picture of the black-throated blue warblers (*Setophaga caerulescens*) cage setup during the migratory restlessness experimentation period (March 20, 2016 – May 17, 2016). Each Euro Cage (34 cm x 18.25 cm x 17 cm) was divided into two equally sized compartments with a piece of white opaque corrugated plastic sheet secured to the cage with white duct tape. Each cage was labelled with a unique number to allow for proper identification of the birds during the video analysis.

For each sample, 0.35 ± 0.03 mg was weighed out and sent to the LSIS-AFAR stable isotope laboratory at Western University, Ontario, Canada for analysis.

Each feather was analyzed using a High Temperature Conversion Elemental Analyzer (hereafter TC/EA; Thermo Scientific) coupled to a Delta V Advantage isotope ratio mass spectrometer (hereafter mass spectrometer; Thermo Scientific) operated in continuous flow-mode using a ConFlo IV with helium as the carrier gas. More specifically, each feather was converted to a H^2 gas sample in a pure helium atmosphere in the TC/EA via high temperature conversion, or pyrolysis. From here, the helium carrier gas transported the H^2 sample from the TC/EA through tiny capillary tubes to the mass spectrometer, where measurements of the ratio of heavy to light hydrogen isotopes takes place. The raw hydrogen isotope values were calibrated to VSMOW (Vienna Standard Mean Ocean Water international standard scale) using two United States Geological Survey reference materials, Caribou Hoof Standard and Kudu Horn Standard. Final δ^2H_F results were expressed in the typical delta notation, units per mil (‰).

2.3.2 Assignment to geographic origins

The assignment of the study birds' origins was computed via likelihood methods (Royle and Rubenstein 2004; Hobson et al. 2009; Van Wilgenburg and Hobson 2011). Algorithms relating variation in δ^2H_F to variation in δ^2H precipitation (hereafter δ^2H_P) were used to assess the potential origin of the birds following Hobson et al. (2012). First, a GIS-based model of expected growing-season δ^2H_P from Bowen et al. (2005) was converted into a δ^2H_F model (hereafter isoscape). Since black-throated blue warblers primarily forage in the lower to mid-strata of the canopy during breeding season (Holmes 1986), the isoscape was calibrated for "non-ground foraging Neotropical migrants" with $\delta^2H_F = -27.09 + 0.95\ \delta^2H_P$ (Hobson et al. 2012). To constrain the assignment to biologically plausible origins, the isoscape was cropped to the birds' breeding range (BirdLife International) using functions in the raster package (Hijmans 2016). For each bird, the likelihood that each cell in the isoscape represented a potential origin for that bird was assessed. Following a 2:1 odds ratio that the bird had truly originated from within that range, cells of the isoscape were identified that defined the upper 67% of estimated probabilities of origin and subsequently coded as 1, while all other cells were

coded as 0. The sum of all individual assignments were mapped on the δ^2H_F isoscape using map and plot functions. All methods used to determine origin were conducted using a R script (from Environment and Climate Change Canada, Saskatoon) which followed methods outlined in Hobson et al. (2014).

Since protandry is observed in arrival timing of black-throated blue warblers at stopover in Long Point during spring migration (Morbey et al. submitted), I expected to see similar spring behavioural patterns in birds captured at the same location during fall migration. However, an assumption is that spring and fall black-throated blue warblers at Old Cut originate from the same population. To test this assumption, the δ^2H_F values of birds in my study were compared to δ^2H_F values of black-throated blue warblers captured in Long Point in the spring of 2014 and 2015 that followed similar bird capturing methods (Morbey unpublished data). δ^2H_F values of spring and fall were compared with a two sample t-test.

2.4 Triggering the migratory state

To trigger the birds to enter a migratory state, the birds experienced 3 photoperiodic conditions throughout the duration of their time in captivity. All photoperiods the birds were exposed to were chosen to mimic the natural conditions experienced by wild conspecifics on their wintering grounds. Previous studies on black-throated blue warblers suggest that individuals that breed in the northern portion of their breeding range predominantly overwinter in the western Caribbean islands, whereas the birds that breed more southerly overwinter in the easterly islands (Rubenstein et al. 2002). Consequently, the various photoperiods the birds were exposed to in this study were calculated to mimic civil dawn and civil dusk times in Havana, Cuba (23°05'24.5"N 82°20'24.1"W). All photoperiods were calculated with an Excel VBA (Greg Pelletier, NOAA Sunrise/Sunset and Solar Position Calculators, http://www.ecy.wa.gov/programs/eap/models.html).

From their first day of capture until December 22, 2015, the birds were exposed to a photoperiod of 12 h light and 12 h dark. This timing simulated conditions wild conspecifics would experience upon arrival to their overwintering grounds in late October. From December 23, 2015 to March 19, 2016, the birds were exposed to a

photoperiod of 11.5 h light and 12.5 h dark. This extended exposure to a typical short day-length ensured the birds became photosensitive. To photo-stimulate the birds to become migratory, the light phase of their photoperiod gradually increased daily by 1-3 min from March 20, 2016 to May 19, 2016 (Fig. 6). This steady increase reflects a normal progression of day length that the birds' wild conspecifics would be exposed to. Additionally, the photoperiod the birds experienced on May 19 was timed so that lights on and light off corresponded to the start of civil dawn and civil dusk times of Long Point on May 19, 2016. This ensured the birds timing would be properly entrained to the natural photoperiod post release from captivity.

2.5 Determining seasonal changes in mass

To study the seasonal patterns of mass change associated with migratory preparation in black-throated blue warblers, mass (± 0.01 g) was recorded for each of the study birds from March 20, 2016 to May 19, 2016 with a digital scale (CS200; OHAUS, Parsippany, New Jersey). Measurements were recorded every 5-10 days. To reduce stress, the birds were hand captured in the dark with the use of a small handheld flashlight prior to taking their mass measurements. To minimize a time of day effect on body weight, all weighing occurred within 3 h after morning feeding. To test whether sex and date influenced pre-migratory mass gain of the birds, a linear mixed effects model for repeated measures was used. Sex and date were used as the predictor variables (fixed effects) and date and bird ID were specified as the random effects to allow for individual differences in mass gain over time.

2.6 Spring migratory restlessness

Overview: to determine the migratory restlessness patterns of black-throated blue warblers, the study birds were video recorded at night and the videos were analyzed with computer software to quantify various types of behaviour. These data were analyzed to determine if black-throated blue warblers had sex differences in migratory restlessness behaviour.
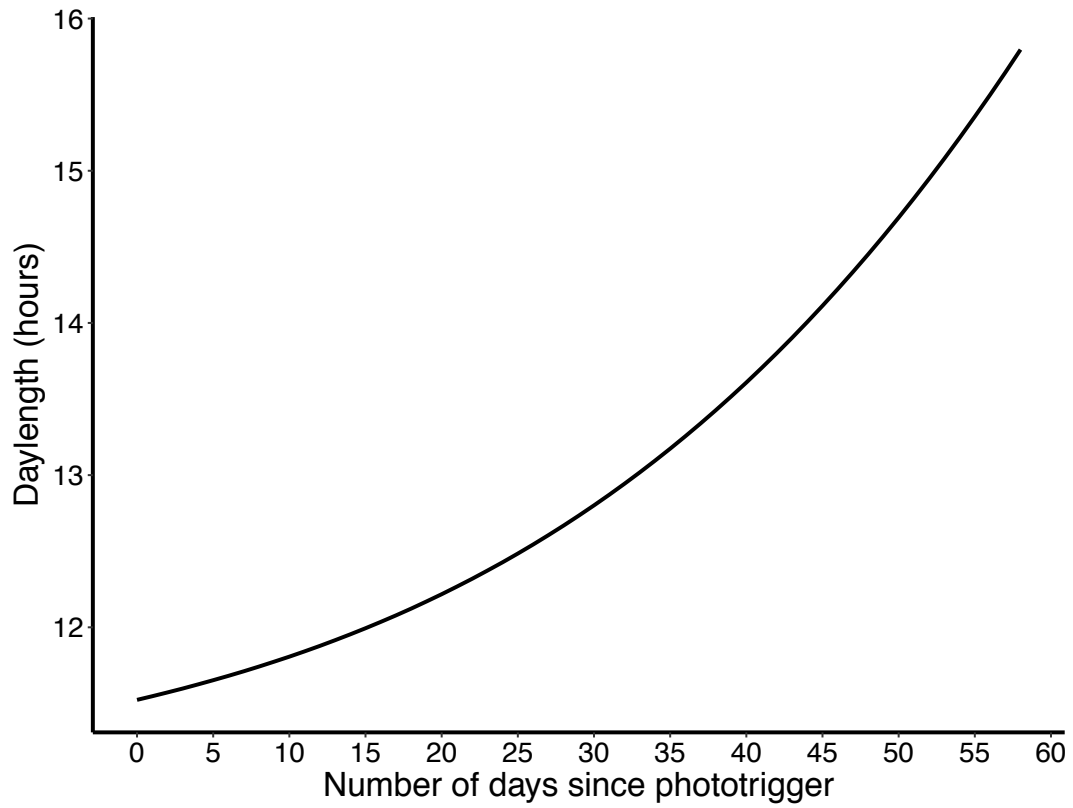
Figure 6. Photoperiod the black-throated blue warblers (*Setophaga caerulescens*) experienced during spring 2016. The number of hours of light the birds experienced from the first day of migratory photo-stimulation (March 20, 2016; represented as Day 0) until the day the birds were released back to the wild (May 19, 2016).

2.6.1 Video-recording nocturnal behaviour

The black-throated blue warblers were video recorded for the duration of the night from March 20, 2016 until May 17, 2016. Each bird was recorded from lights off to lights on (range 12.25-8.25 h per bird over the duration of the observational period). Infrared lights (Smart B-Series) with a wavelength of 850 nm and illumination angle of 130º were used to illuminate the birds at night, allowing the video camera (Black and White High Res Ex View Micro Video, Super Circuits, Austin, Texas, USA) to record their nocturnal behaviour (Fig. 7A). Both the infrared lights and video cameras were secured to a metal wire shelving unit that was set up parallel to the cages (approx. 125 cm apart; Fig. 7B). The bird cages were grouped together in such a way as to create five groups of four cages and one group of two cages. For each group of cages, one video camera and one infrared light was used to record the nocturnal behaviour (e.g. Fig. 7C). Thus, in total there were six video cameras coupled with six infrared lights that were used in this study. However, since the behavioural analysis software used (described below) only had the capacity to simultaneously analyze videos for up to 4 different cameras at one time, a color quad processor (American Dynamics, USA) was used to combine the videos of three of the groups into one new group comprising 10 cages. The video cameras and quad processor were hooked up to a computer, which allowed the videos to be saved in real time.

2.6.2 Using Noldus EthoVision software to quantify behaviour

EthoVision XT Noldus behavioural analysis software (Noldus Information Technology, Wageningen, NL, www.noldus.com) was used to quantify the nocturnal behaviour of the birds. The static subtraction setting was used as the detection method and the sample rate of the videos was set to 1.49 samples per second. This sample rate was selected to ensure that only migratory restlessness was quantified, by reducing noise created from small non-migratory restlessness behaviour (e.g. a bird ruffling feathers at night). Each night was broken into 15-minute intervals, or trial, starting when lights went off and ending when the lights came back on. When a night was unable to have an even number of 15-minute intervals, the last interval would end when the lights came on regardless of the time.
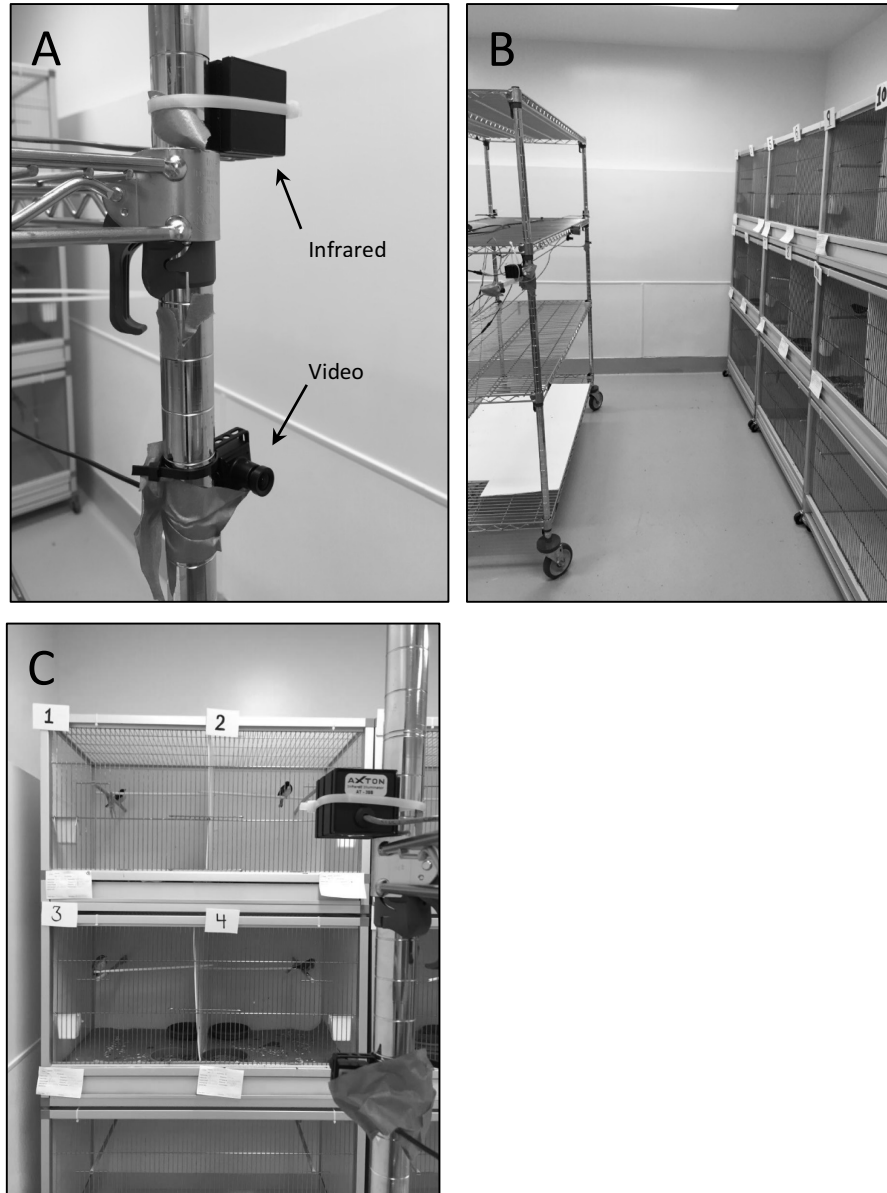
Figure 7. Migratory restlessness experimental setup. A) Video cameras and infrared lights were attached via zip ties and duct tape to a metal wire shelving unit. The video cameras were connected to a computer that saved the video in real time. B) The metal shelving unit was situated 125 cm apart from the bird cages. C) The camera and infrared lights were situated centrally between 4 bird cages.

Noldus software computed two movement variables for each 15-minute interval: 1) centre-point movement and 2) continuous mobility count (Fig. 8). Centre-point movement was calculated based on the amount of time (s) for which the centre-point of the bird changed location (hereafter referred to as "moving"). For each sample of the 15-minute period, the centre-point of an individual was classified as moving when it had a velocity greater than a user defined threshold. Continuous mobility count (s) was calculated based on both body area fluctuations and centre-point movement. For this variable, an individual was classified as mobile when both the total body area changed by a user defined threshold and the centre-point movement was greater than a user defined threshold.

I defined the threshold for classifying a centre-point movement as >2 cm/s and the threshold for classifying a total body area fluctuation (i.e. a mobile bird) as >20%. The centre-point movement primarily represented hopping and/or jumping behaviour. The continuous mobility count (hereafter called "total movement") represented various types of migratory restlessness movement including hopping, jumping, and wing whirring. A third type of movement termed body contour movement (s) was calculated for each 15-minute interval as: continuous mobility count (s) – centre-point movement (s). This type of movement was calculated to independently assess wing whirring behaviour.

2.6.3 Analysis of migratory restlessness

The first interval of each night was excluded from the analyses to allow the birds to adjust to the abrupt switch from lights on to lights off (e.g. settling movement from feeding dish to perch should not be classified as migratory restlessness) and to allow the Noldus software to adjust to the new light settings. The relationship between movement and date for both males and females were analyzed with mixed effects models for repeated measures. Date was used as the predictor variable and was inputted as the fixed effect in the model. Bird ID and date were specified as the random effects in the model to allow for individual differences in movement over time. Intraclass correlation coefficients were calculated to assess the variation caused by the random effects (Tabachnick and Fidell 1996). To analyze the seasonal onset, nighttime onset, and time of
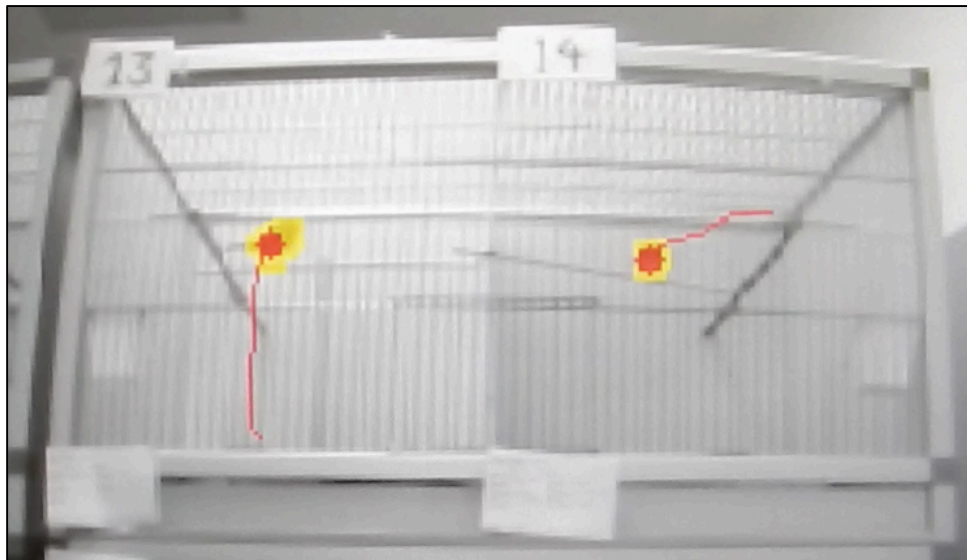
Figure 8. Depiction of Noldus EthoVision analysis software detecting centre-point movement and continuous mobility count. Note, this is a screenshot taken from a video clip at night. The birds are illuminated with infrared light. The red dot is on the centre of the bird and the trailing red line shows where the bird was located within the last two seconds. The yellow is showing the detection of the body area of the bird.

peak migratory restlessness, total movement of each bird was used. See Table 1 for a summary of the variables used in the statistical analysis.

Migratory restlessness: To be classified as displaying migratory restlessness on a given night, a bird had to have ≥10% movement. This threshold of 10% has previously been used in other migratory restlessness studies (Maggini and Bairlein 2010, 2012).

Seasonal onset of migratory restlessness: The date of seasonal onset of migratory restlessness was defined as the first day out of 5 consecutive days a bird displayed migratory restlessness. If the bird did not display migratory restlessness on one of these 5 days, the process began again at the next date the bird displayed migratory restlessness. Sex differences in seasonal onset of migratory restlessness were analyzed using survival analysis. Cox semiparametric regression in a proportional hazard modelling framework was conducted with both sex and age as factors using the functions coxph and Surv. The importance of model covariates was assessed with 95% confidence intervals.

Nighttime onset of migratory restlessness: The nighttime onset of migratory restlessness was defined as the first trial in which a bird displayed at least the 10th percentile of its movement on nights in which it was defined as displaying migratory restlessness. Individual percentiles of movement over each night were averaged using the functions group_by and mutate from the plyr package, abs package, and ddply package. Sex differences in nighttime onset were analyzed with a Mann-Whitney U test for non-parametric data.

Time of nighttime peak of migratory restlessness: Time of peak migratory restlessness was defined as the trial with the highest amount of migratory restlessness. Time of peak migratory restlessness was computed for each bird using the packages ddply and plyr, and was averaged over the duration of the season. Male and female mean time of peak migratory restlessness was compared with a two-sample t-test.

Intensity of migratory restlessness: Intensity of migratory restlessness was defined as the total cumulative time the bird moved on each night that the bird was exhibiting

Table 1. Description of variables used for statistical analysis of migratory restlessness

Dependent Variable	Temporal Scale	Type of variable	Description	Independent Variable(s)	Handling pseudo-replication
Moving	1 sample (0.67 s)	categorical	$Velocity > 2\ cm/s$	-	-
Mobile	1 sample (0.67 s)	categorical	$Total\ body\ area\ fluctuation > 20\%$	-	-
Centre-point movement	trial (15 min)	categorical	$\Sigma\ time\ spent\ moving$	-	-
Total movement	trial (15 min)	categorical	$\Sigma\ time\ spent\ moving + time\ spent\ mobile$	-	-
Body contour movement	trial (15 min)	categorical	$Total\ movement - centre\text{-}point\ movement$	-	-
Migratory restlessness	night	categorical	$\dfrac{\Sigma\ movement}{hours} \geq 10\%$	-	-
Seasonal onset	experimental period	continuous	First day with 5 consecutive days of migratory restlessness	sex + age + $\delta^2 H_F$	-
Nighttime onset	night	continuous	Trial with 10th percentile of cumulative movement*	sex	median
Time of nighttime peak	night	continuous	Trial with maximum movement*	sex	mean
Intensity	night	continuous	Cumulative movement*	sex + date	bird ID as random effect

*Calculated on nights when the bird displayed migratory restlessness

migratory restlessness. Intensity was computed for all three movement types: centre-point, continuous mobility count, and body contour. The relationship between intensity of migratory restlessness (measured in h) and sex was analyzed with linear mixed effects models for repeated measures. Sex and date were used as the fixed effects (predictor variables) and bird ID was used as the random effects to allow for individual differences of intensity over time. Intraclass correlation coefficients were calculated to assess the variation caused by the random effects (Tabachnick and Fidell 1996).

2.7 Release back to the wild: stopover behaviour

2.7.1 Automated radio-telemetry: stopover duration

On May 18, 2016, each bird was equipped with a 0.29 g digitally encoded radio transmitter (model NTQB-1, Lotek Wireless, www.lotek.com) with a 4.9 s pulse rate (Fig. 9). The radio transmitters were attached with an over-the-leg elastic loophole harness (Rappole and Tipton 1991). Depending on mass at time of tagging, the birds received an elastic harness of 30, 32, or 34 mm in length. The elastic string was secured to the radio transmitted with super glue (LePage UltraGel Contact, www.LePage.com). Each radio transmitter emitted a unique identifiable code that allowed all the birds to be synchronously tracked. On May 20, 2016, the birds were transported in animal carriers (60.96 x 65.96 x 152.4 cm) equipped with branches for perching, a water dish, and live mealworms to Long Point Bird Observatory, where they were captured the previous fall. The birds were synchronously released at 12:00 pm behind the Old Cut station.

To determine the birds' stopover duration, an array of two automated radio-receiving stations was used (Motus Wildlife Tracking System, www.motus.org; Taylor et al., 2017) (Fig. 10). Both towers had nine-element directional Yagi antennas oriented in various directions and a detection radius of approximately 20 km under optimal conditions. Each receiving station continuously detected the birds' location every 4.9 seconds. Each detection included the tag identification number, site (coordinates of tower detection), antenna number, and signal strength. The birds were considered to have departed from stopover when they left the detection range of the towers at Old Cut and
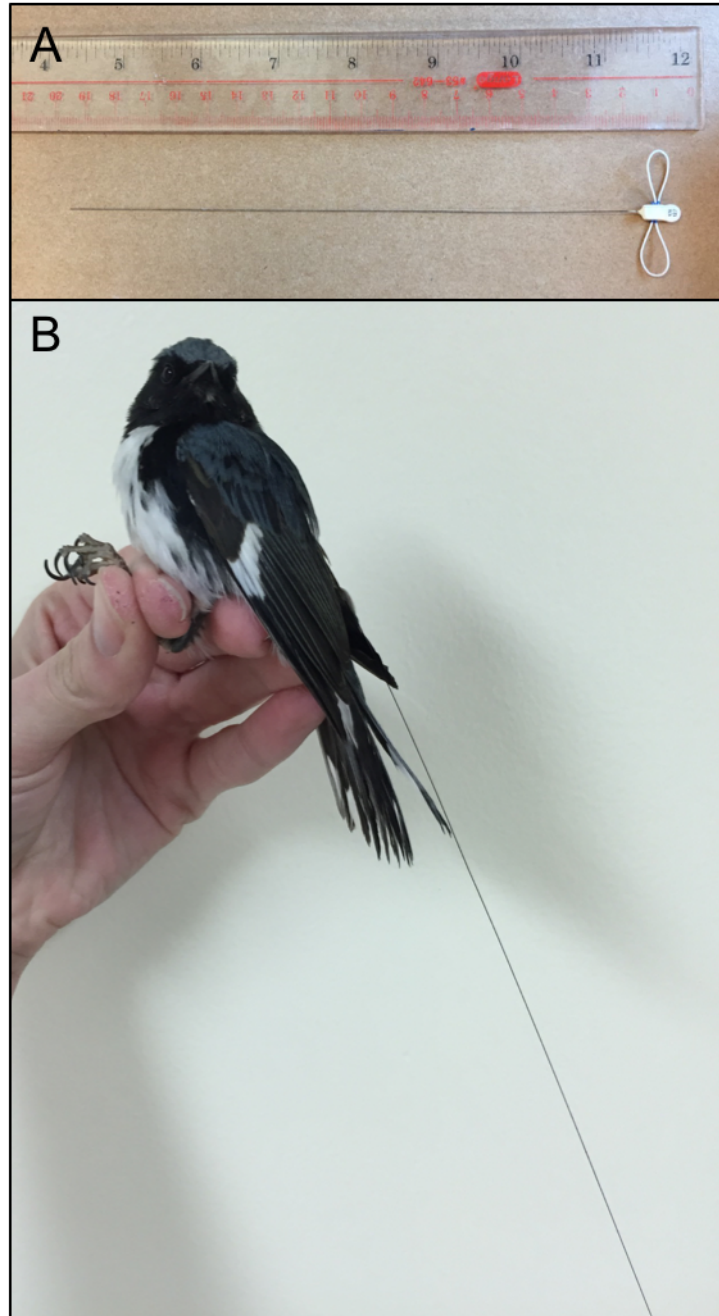
Figure 9. Photo of a nano radio transmitter and accompanying harness. A) A nano radio transmitter (0.29 g) that the birds in this study were harnessed with. B) A male black-throated blue warbler harnessed with a nano radio transmitter.
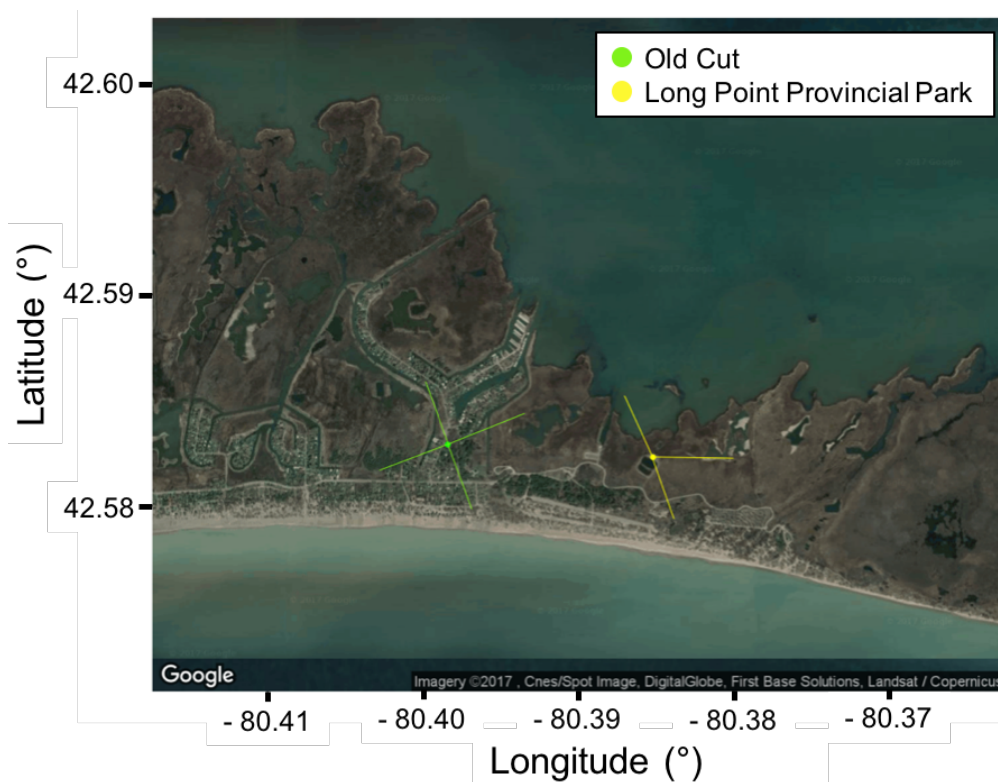
Figure 10. Map displaying the locations of the Motus Wildlife Tracking System receiving stations used in this study. The receiving stations were used to determine the stopover duration of black-throated blue warblers (*Setophaga caerulescens*) in spring 2016. Each receiving station is represented by a colour, and the estimated bearings of the Yagi antennas on the receiving stations are represented by the lines.

Long Point Provincial Park. Stopover departure was further validated when a bird went undetected with manual telemetry (see Section 2.7.2) the following day. Stopover was modelled using a survival analysis framework. A Cox Proportional Hazards model was used to analyze the risk a bird would depart the study site and included sex, age, the intensity of total movement on May 17, 2016 (min), and mass (g) on the morning of release as covariates. The importance of model covariates was assessed with 95% confidence intervals.

2.7.2 Manual radio-telemetry: habitat use

Each bird was tracked daily to visually observe their behaviour (e.g. movement, foraging, singing, habitat use). From May 20, 2016 to June 1, 2016, the birds were searched for daily with a 5-element Yagi antenna and a Lotek SRX 600 receiver. Upon visual location of the bird, geographic UTM coordinates, GPS error ($\pm$ 5 m), and time of day were recorded. When visual location of the bird was not possible (e.g. bird was in thick forest or flying around too quickly), coordinates were recorded if the receiver was displaying a high signal strength and low gain which indicates the bird was close in proximity. High signal strength and low gain were defined as S$\geq$175 and G$\leq$50 on the receiver, respectively. The telemetry search area covered an approximate 1.6 km^2 area (perimeter 15 km) of the Long Point peninsula centred around Old Cut station where the birds were released (Fig. 11).

Habitat types of the search area were characterized by land-cover metrics. Through visual observation of satellite imagery (Google Earth, version 7.1; TerraMetrics), land-cover metrics were broadly categorized into one of the following: 1) dense forest, 2) sparse forest, 3) marsh, 4) scrub, 5) residential, 6) dune savannah, 7) lawn, and 8) meadow. Characterization criteria for these categories were as follows. Dense forest was characterized as areas with densely packed trees (very little to no ground visible beneath the trees; typically comprised of evergreen trees) and sparse forest was characterized as the areas with visible ground beneath the trees (predominantly deciduous trees). Marsh was characterized as marsh areas. Dune savannah was areas predominantly covered in sand with few tall grass patches and few trees. Areas predominately covered with manicured grass without a building in close proximity and
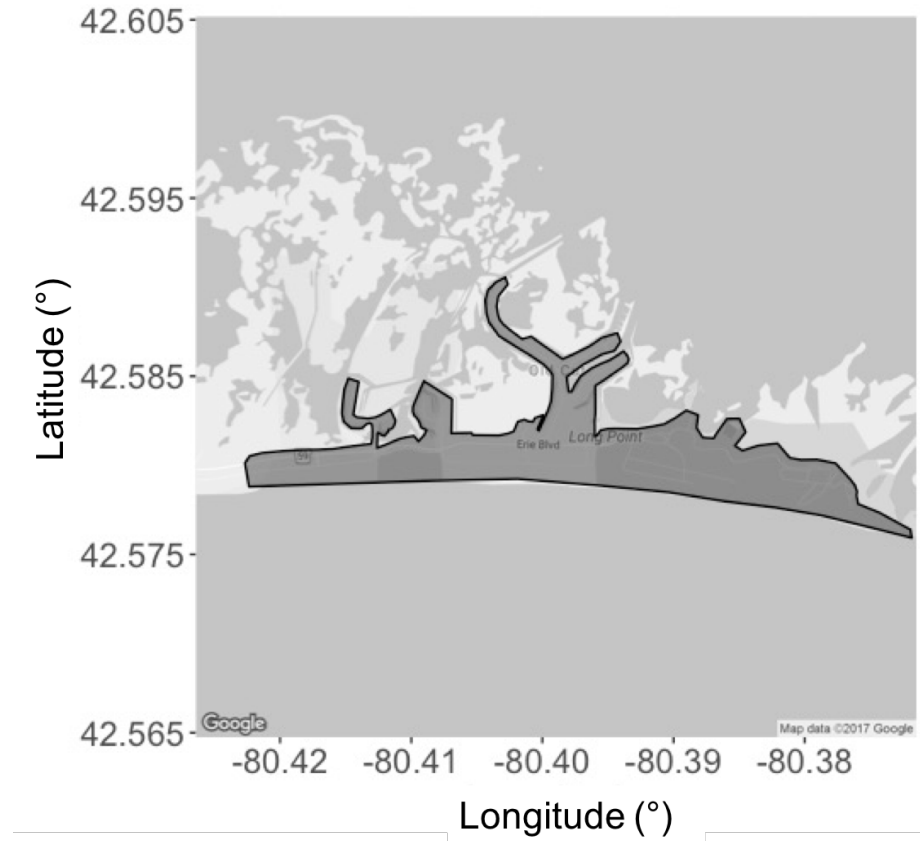
Figure 11. Approximate telemetry search area. Search area (dark grey) in Long Point, Ontario where the black-throated blue warblers were tracked daily between May 20, 2016 and June 1, 2016.

short wild grass was classified as lawn habitat. Scrub habitat was classified as areas with a combination of thick grasses, shrubs, and few trees. Any area with a man-made structure (e.g. house, shed, etc.) and the accompanying yard around the structure (e.g. manicured lawn, sand around cottages) was classified as residential habitat. Lastly, meadow was classified as any area that had predominantly tall wild grass. Habitat type specific polygons (.kml file format) were created by outlining specific areas in Google Earth following these criteria. Each polygon was converted to a shapefile (.shp) using ArcMap (version 10.5; Environmental Systems Research Institute (ESRI), 2016). A map of the various habitat type locations was then created by plotting the polygons onto a satellite image of Long Point Peninsula with the packages ggmap and ggplot2.

RESULTS

3.1 Study birds

A total of 12 females and 12 males were captured during fall 2015 migration. Of females, 9 were HY birds and 3 were AHY birds, and of males, 8 were HY birds and 4 were AHY birds. Upon capture, males and females had a mean mass of 9.8 ± 0.14 g and 9.4 ± 0.19 g, respectively. There were no sex differences in mass at time of capture (t_{22}=1.85, P=0.08). Males had a mean wing chord length of 63.7 ± 0.86 cm and females had a mean wing chord length of 61.3 ± 0.4 cm. Males had significantly longer wing chord lengths than females (t_{22}=2.24, P=0.02). Fat score did not differ significantly between males and females at time of capture (GLM: $F_{1,22}$ = 0.45, P=0.51). The median date of arrival of all the birds was September 22, 2016 (Fig. 12).

3.2 Feather δ^2H isotope analysis

A total of 22 birds (N=12 females; N=10 males) were used for δ^2H_F analysis. Two of the males died prior to the feather collection and so they were excluded from the analysis. The birds showed a relatively narrow range in δ^2H_F (–117 to –85 ‰). Thus, most of the birds were largely isotopically consistent with origins associated with the northern portion of their breeding range (Fig. 13). Indeed, likelihood-based assignment of the individuals to the δ^2H_F isoscape resulted in most of the birds being associated with origins in mid-Southern Ontario, Quebec, and New Brunswick, Canada and Maine, USA (Fig. 14). There were no differences in δ^2H_F values between the study birds used in this study and the black-throated blue warblers previously captured during spring migration (t_{69} = 1.5, P = 0.13).

3.3 Pre-migratory mass gain

Analysis of pre-migratory mass gain was restricted to dates after March 25, 2016 and prior to April 15, 2016 (Fig. 15; Table 2). The first 5 days after migratory trigger were excluded to reduce possible variation caused by switching the birds to individual cages from free-flight aviaries. Dates after April 15 were excluded to ensure only
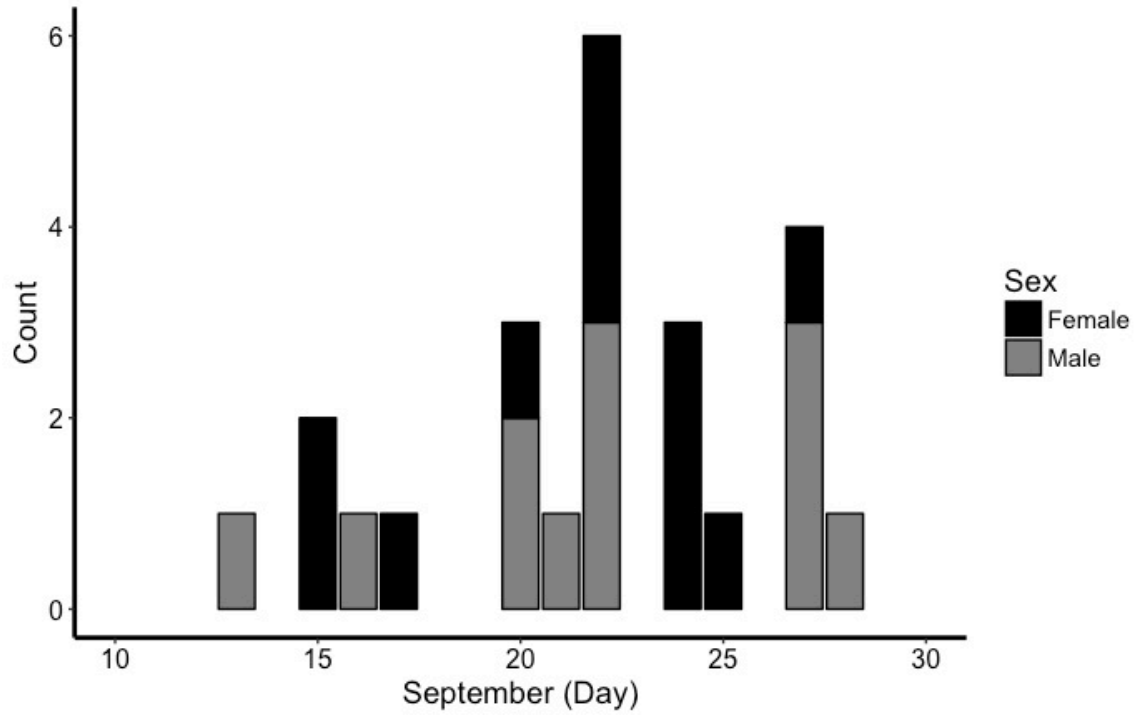
Figure 12. Distribution of the birds' arrival dates. Arrival date was estimated as the first day of capture at Long Point Bird Observatory, Long Point, Ontario (N= 12 males; N=12 females).
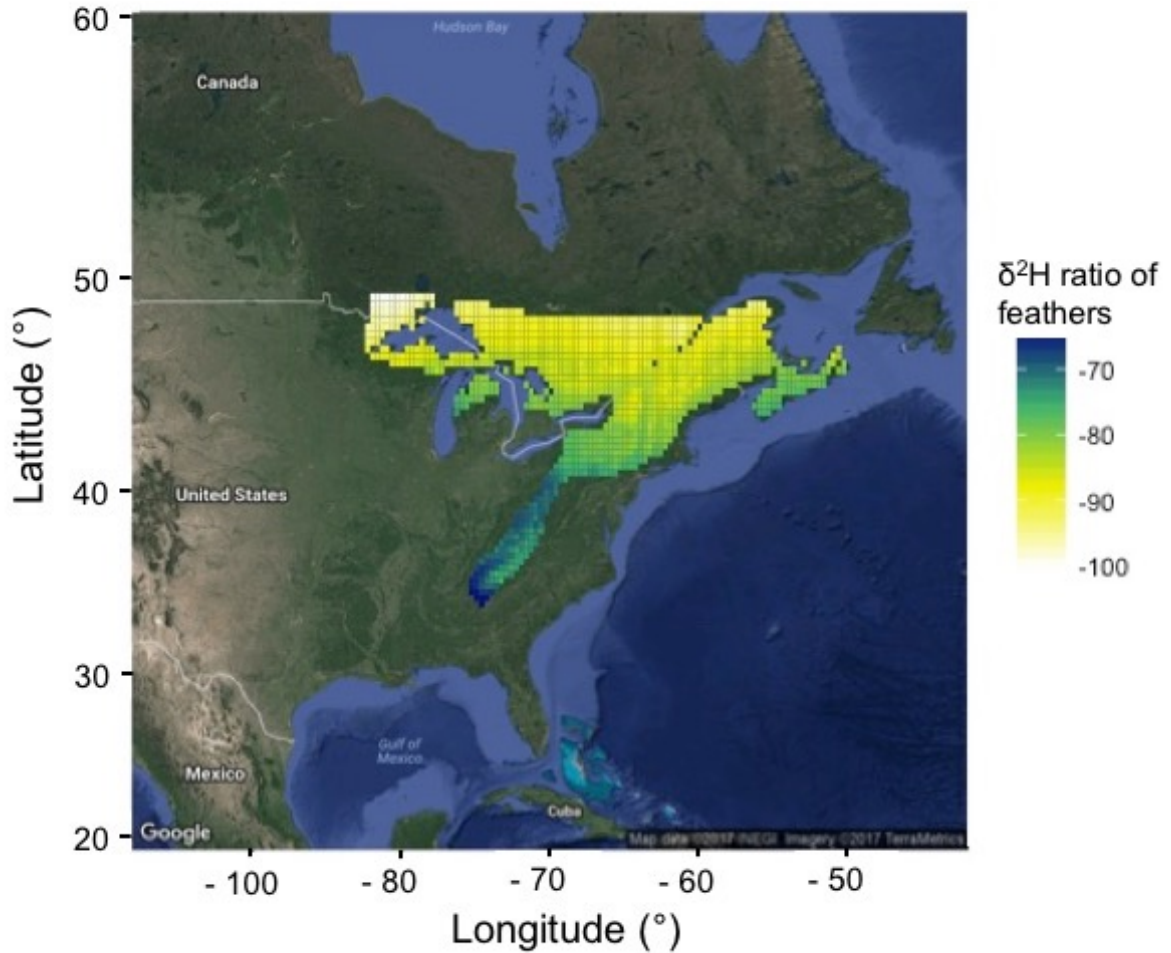
Figure 13. Predicted geographic distribution of the stable hydrogen (δ2H) isotope ratios of black-throated blue warbler feathers. The predicted origins are constrained to within the published species breeding range. Predicted values were calculated by calibrating the geospatial predictions of amount-weighted growing season δ^{2}H precipitation values (Bowen et al. 2005) into δ^{2}H from feathers following Hobson et al. (2012) (see Method section 2.3.2).
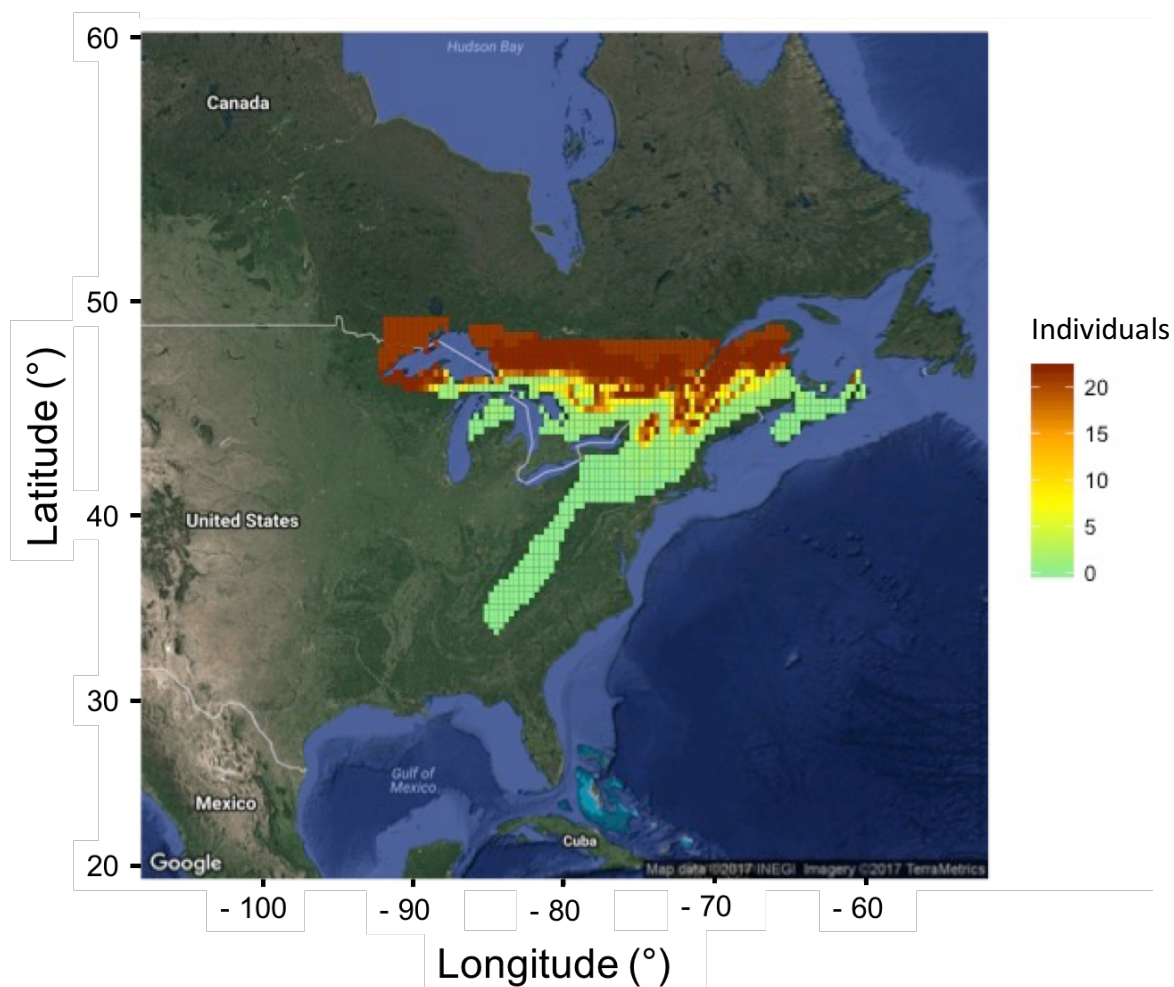
Figure 14. Geographic distribution of assigned sites of origin for black-throated blue warblers (*Setophaga caerulescens*) (N=22) captured at Long Point, Ontario during fall migration. Assignments were based upon stable isotope analysis of feathers using a likelihood based comparison. Values on the legend represent the number of birds in the sample that were isotopically consistent with similarly coloured portions of the map at our selected (2:1) odds ratio (see Methods section 2.3.2 for details).
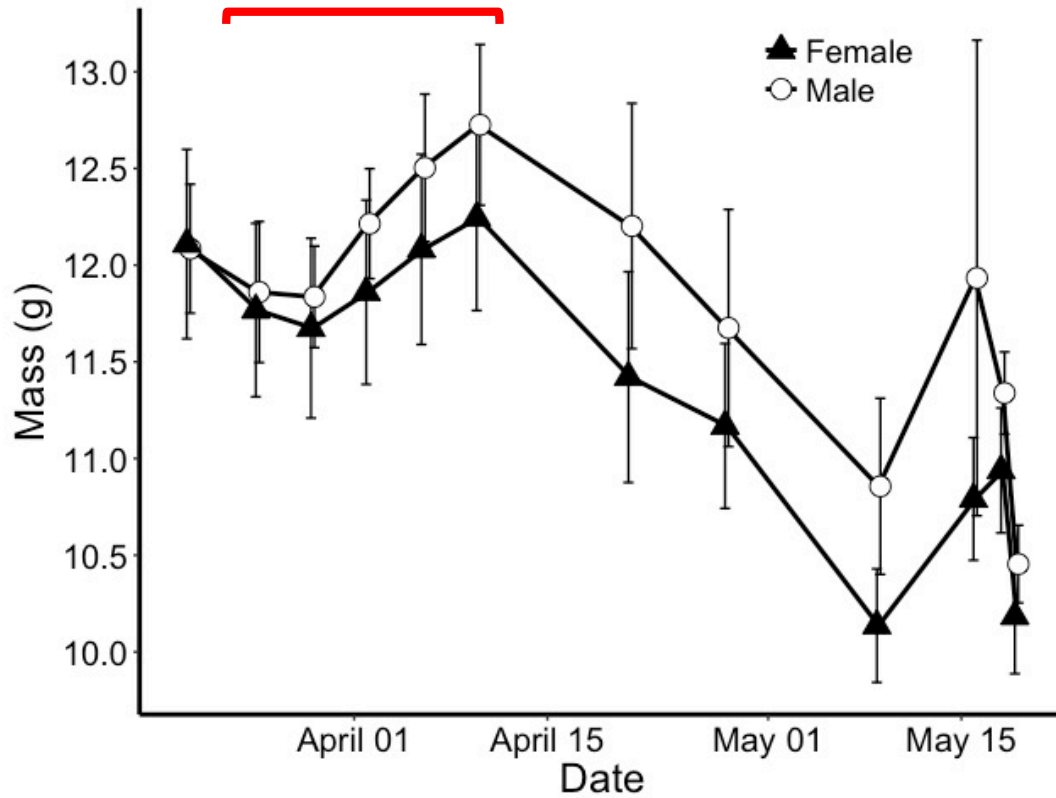
Figure 15. Seasonal variation of black-throated blue warbler *(Setophaga caerulescens*) mass. The red line indicates the dates that were used in the analysis of pre-migratory mass changes. Data points represent mean mass ± SE (g).

Table 2. Descriptive summary of results indicating the measured variables with their associated proxies and the present or absence of protandry in black-throated blue warblers.

Measured variable	Proxy	Protandry
Mass accumulation	Preparation for migratory departure timing from the wintering grounds	No
Migratory restlessness seasonal onset	Departure timing from the wintering grounds	No
Migratory restlessness intensity	Nocturnal migratory flight distance	Yes
Migratory restlessness nighttime onset	Nocturnal migratory flight departure timing	No
Migratory restlessness peak nighttime activity	Nocturnal migratory flight departure timing	Yes
Stopover duration	NA	No

"pre-migratory" masses were used in analyses. There was an effect of date on mass ($F_{3,57}=2.83$, P=0.047), but no effects of sex on mass ($F_{1,20}=0.39$, P=0.54) and no interaction between date and sex ($F_{3,57}=0.85$, P=0.47). During this two week period, females had a mean mass of 11.9 ± 0.2 g while males had a mean mass of 12.3 ± 0.2 g. The mean mass of all birds on March 25 was 11.8 ± 0.3 g, which increased to 12.45 ± 0.3 g on April 10.

3.4 Migratory restlessness in black-throated blue warblers

3.4.1 Movement overview

Linear mixed effects models for repeated measures were run separately for males and females for each of the three movement types to evaluate the temporal pattern of movement throughout the course of spring. Mean total movement per trial gradually increased throughout the experimental period in females ($F_{1,668}=135.5$, P<0.0001; Fig. 16) from 83 ± 29 s to 309 ± 32.9 s, and in males ($F_{1,475}=54.1$, P<0.0001) from 166 ± 38 s to 347 ± 56 s. Mean centre-point movement per trial gradually increased throughout the experimental period in females ($F_{1,668}=119.7$, P<0.0001; Fig. 17) from 35 ± 13 s to 186 ± 22 s, but did not change in males (mean = 62 ± 3 s; $F_{1,475}=3.2$, P=0.07). Mean body contour movement per trial significantly increased throughout the experimental period in males ($F_{1,475}=84.9$, P<0.0001; Fig. 18) from 102 ± 30 s to 223 ± 56 s, and in females ($F_{1,668}=57.7$, P<0.0001) from 48 s ± 19 s to 123 ± 26 s. In the female models, individuals accounted for 40% of the variation of the total movement, 30% of the variation of the centre-point movement, and 0.00% of the variation of body contour movement. In the male models, individuals accounted for 20% of the variation of the total movement, 25% of the variation of the centre-point movement, and 0.00% of the variation of body contour movement.

3.4.2 Seasonal onset of migratory restlessness

The onset of seasonal migratory restlessness could be determined for 19 out of 22 birds (N=10 females, N=9 males; N=5 AHY, N=14 HY). Three of the birds (all HY) failed to reach the criteria for migratory restlessness onset. While all birds remained healthy, two males died during the experimental period due to unknown causes (veterinary histology
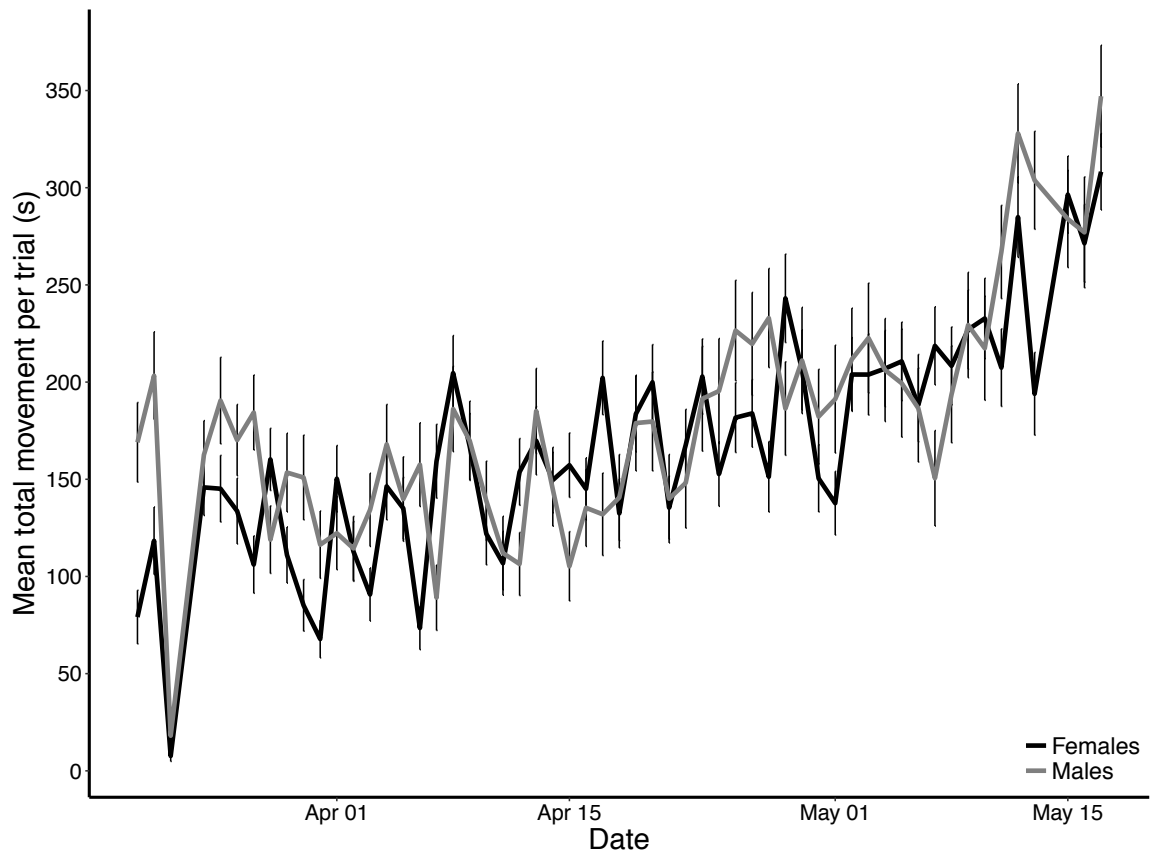
Figure 16. Black-throated blue warbler mean total movement per nighttime trial during spring 2016. Total movement was calculated as the time (s) a bird had a centre-point velocity >2cm/s and body area fluctuations >20%. Females are represented by the black line and males by the grey line. Data points are the mean of individual means with 95% confidence intervals.
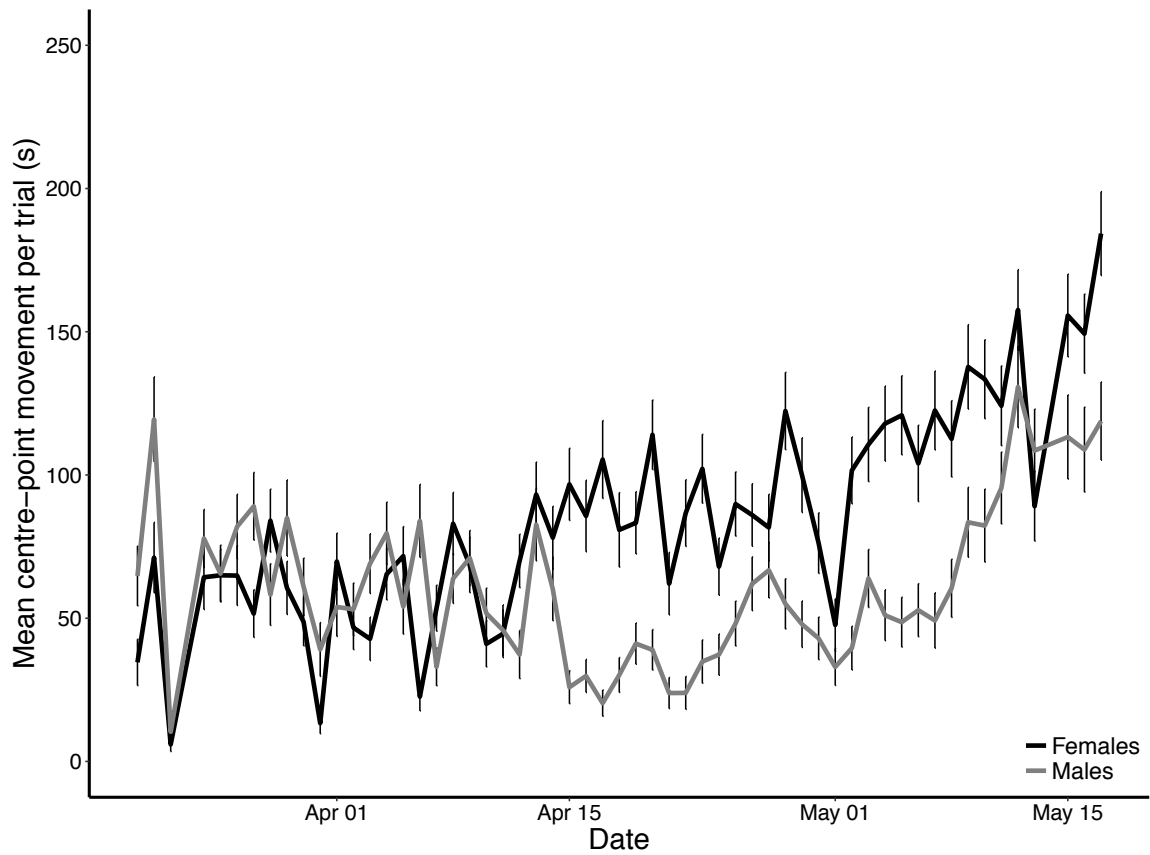
Figure 17. Black-throated blue warbler mean centre-point movement per nighttime trial during spring 2016. Centre-point movement was calculated as the time (s) a bird had a centre-point velocity >2cm/s. Females are represented by the black line and males by the grey line. Data points are the mean of individual means with 95% confidence intervals.
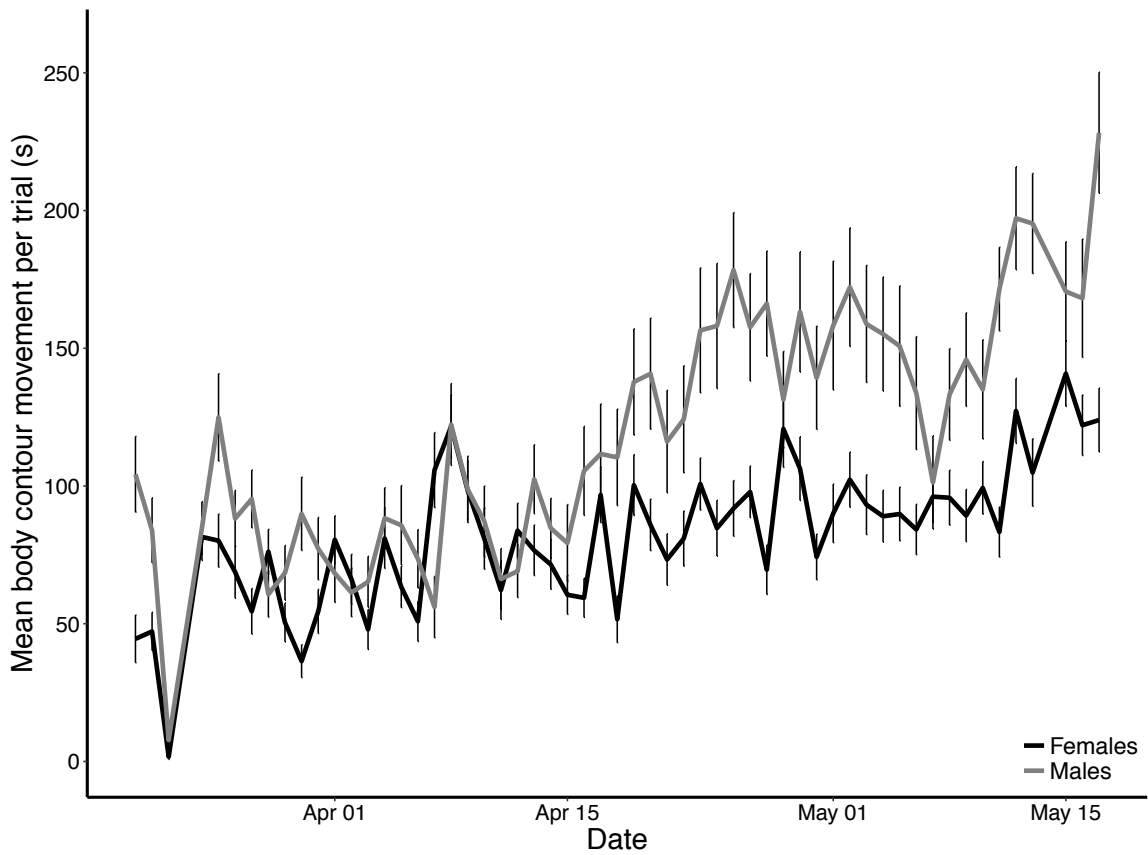
Figure 18. Black-throated blue warbler mean body contour movement per trial during spring 2016. Body contour movement was calculated for each bird as the time (s) displaying body area fluctuations >20%. Females are represented by the black line and males by the grey line. Data points are the mean of individual means with 95% confidence intervals.

returned inconclusive) after the photoperiod was extended. Both male birds died at night while exhibiting migratory restlessness, and so it is possible that death was due to the increased levels of stress associated with the migratory state. Of the 19 birds that reached the criteria for migratory restlessness, males had a median onset of migratory restlessness 5 days post migratory trigger and females had a median onset of 19.5 days post migratory trigger (Fig. 19; Table 2). Of the 19 birds that reached the criteria for migratory restlessness, hatch year birds had a median onset of migratory restlessness 23 days post migratory trigger and after hatch year birds had a median onset of migratory restlessness 5 days post migratory trigger (Fig. 20; Table 2). In the hazard model of seasonal onset of migratory restlessness (including the 3 birds that failed to reach onset as right censored), the hazard for beginning migratory restlessness depended on age but not on sex or $\delta^2 H_F$ (Table 3). The hazard of migratory restlessness onset for HY birds was 14% of the hazard for AHY birds, indicating later onset of migratory restlessness by HY birds than AHY, regardless of sex.

3.4.3 Nighttime onset

Nighttime onset of migratory restlessness was determined for the 19 birds that reached the criteria for seasonal onset of migratory restlessness (N=9 males; N=10 females). Males had a median nighttime onset of migratory restlessness of 5 ± 0.4 trials (equivalent to 1.3 ± 0.1 h), while females had a median nighttime onset of migratory restlessness of 6.3 ± 0.5 trials (equivalent to 1.6 ± 0.1 h). There were no sex-differences in the nighttime onset of migratory restlessness (W=64.5, P>0.5; Fig. 21; Table 2).

3.4.4 Nighttime peak

Nighttime peak was calculated for each bird that reached the criteria for the seasonal onset of migratory restlessness and only on nights displaying migratory restlessness. Males and females reached their peak migratory restlessness at significantly different times of the night (t_{17}=3.2, P<0.005; Fig. 22; Table 2). Males displayed the most migratory restlessness at 17 ± 1.5 trials after lights off (equivalent to 4.3 ± 0.3 h) and females peaked at 23 ± 1.5 trials after lights off (equivalent to 5.8 ± 0.4 h).
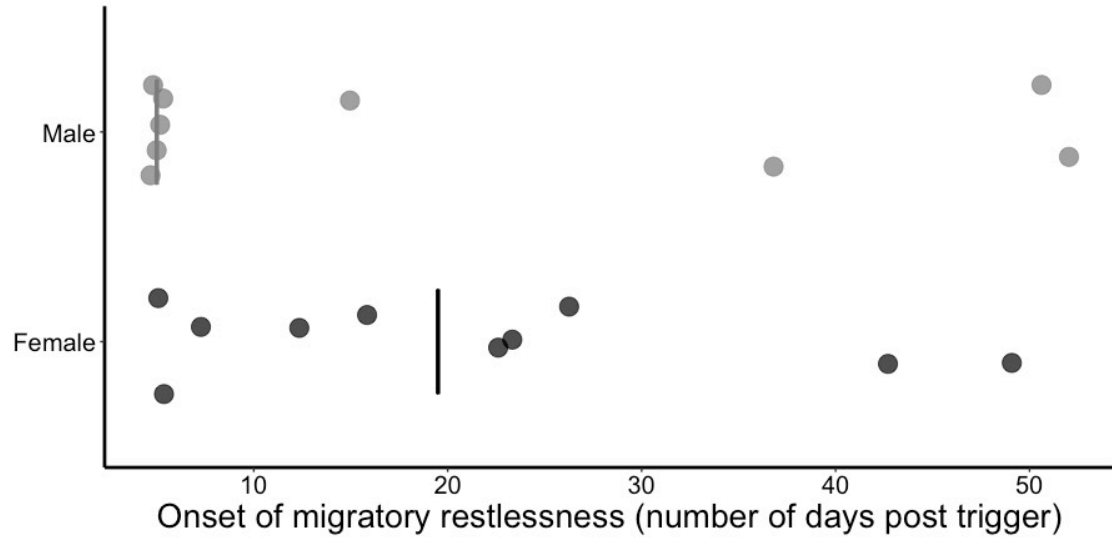
Figure 19. Onset of spring migratory restlessness in male and female black-throated blue warblers (*Setophaga caerulescens*). Data points represent the first day the birds displayed migratory restlessness. Crossbars represent the median values.
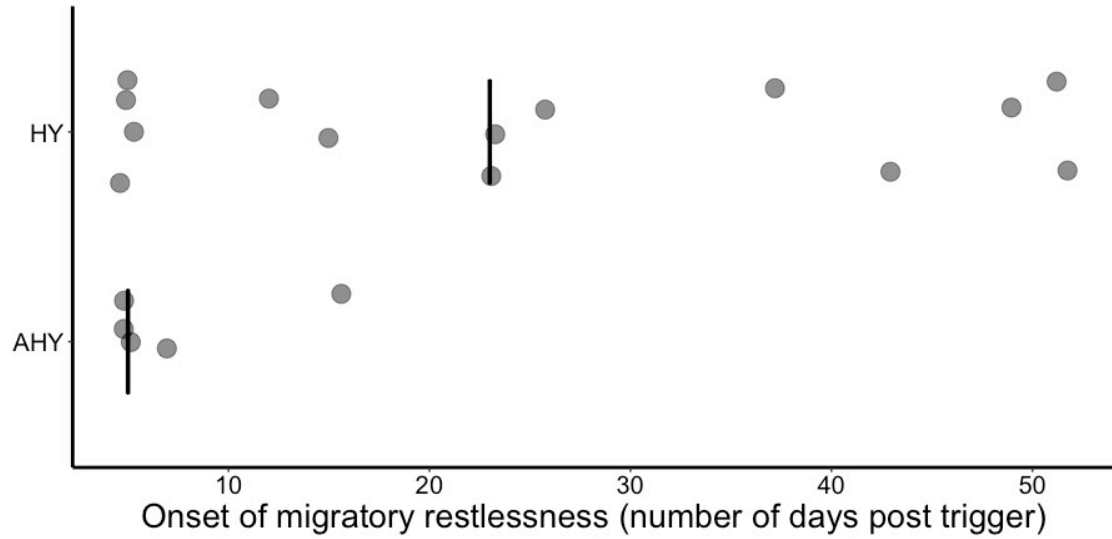
Figure 20. Onset of spring migratory restlessness in hatch year (HY) and after hatch year (AHY) black-throated blue warblers (*Setophaga caerulescens*). Data points represent the first day displaying migratory restlessness after the birds were photo-triggered. Crossbars represent median onset.

Table 3. Parameter coefficients β ± SE of the Cox regression model of seasonal onset of migratory restlessness in black-throated blue warblers (*Setophaga caerulescens*). Also shown are the 95% confidence intervals for β, p-values, and risk ratio.

Variable	β ± SE	95% C.I.	p-value	Risk Ratio
Sex (M)	0.31 ± 0.56	-0.78, 1.40	>0.5	1.36
Age (HY)	-1.95 ± 0.79	-3.51, -0.39	0.01	0.14
$\delta^2 H_F$	0.02 ± 0.04	-0.07, 0.10	>0.5	1.02
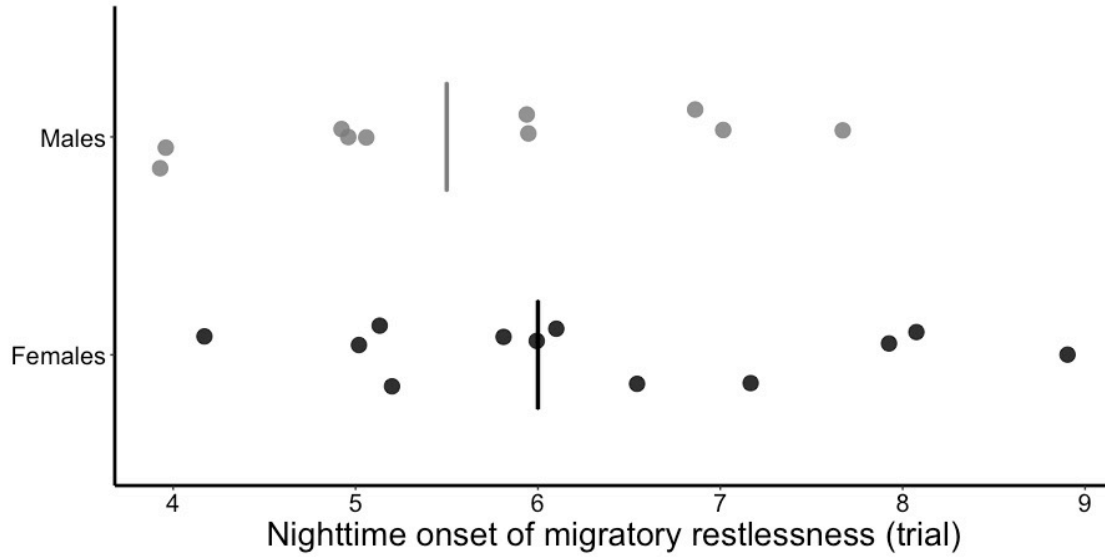
Figure 21. Nighttime onset of migratory restlessness in black-throated blue warblers (*Setophaga caerulescens*). Trials are 15-minutes long. Data points represent the nighttime onset of migratory restlessness and the crossbars represent the median values.
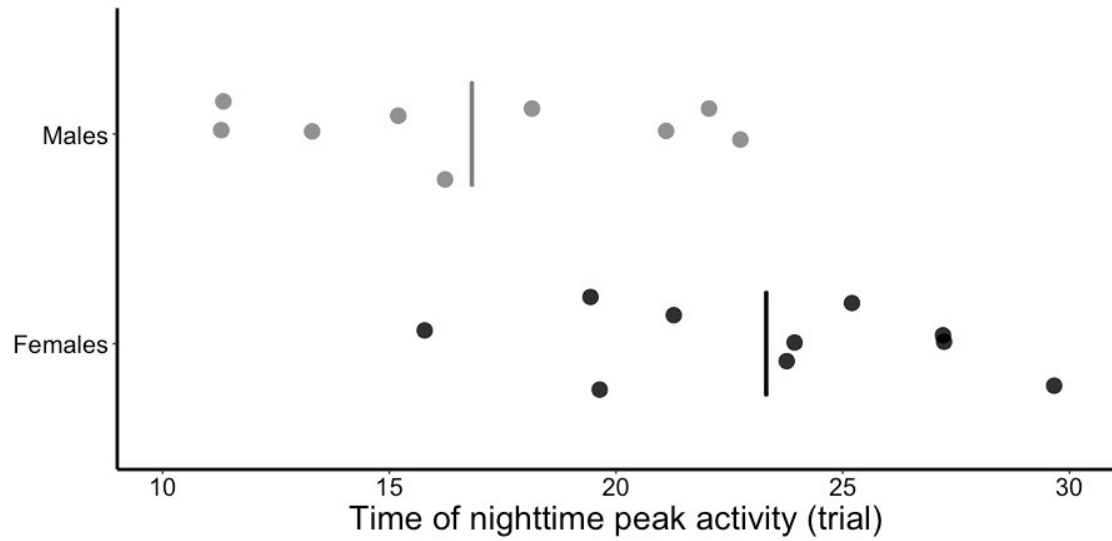
Figure 22. Time of night black-throated blue warblers (*Setophaga caerulescens*) reached peak migratory restlessness. Data points represent the mean trial (15-min trials) with the highest amount of total movement for each bird between March 20, 2016 and May 17, 2016. The crossbars represent mean values.

3.4.5 Intensity of migratory restlessness

Intensity was quantified for each sex on the days of migratory restlessness (Fig. 23; Table 2). Sex significantly influenced the intensity of body contour movement ($F_{1,17}$=5.68, P=0.03), with males having more body contour movement than females by 0.56 ± 0.22 h. Date did not significantly affect the intensity of body contour movement ($F_{1,449}$=2.85, P=0.09), however, there was a significant interaction between sex and date ($F_{1,449}$=2.85, P=0.005). Individuals accounted for 32% of the variation of body contour movement. Sex did not significantly influence the intensity of centre-point movement ($F_{1,17}$=0.93, P=0.35), however, there was a significant effect of date on centre-point movement ($F_{1,409}$=6.63, P=0.01) and there was a significant interaction between sex and date ($F_{1,409}$=3.98, P=0.047). Individuals accounted for 19% of the variation in centre-point movement. Sex and date did not significantly influence the intensity of total movement (sex: $F_{1,17}$=0.72, P=0.41, date: $F_{1,669}$=0.03, P>0.8), nor was there an interaction between date and sex ($F_{1,669}$=0.81, P=0.37). Individuals accounted for 35% of the variation in total movement.

3.5   Stopover behaviour

3.5.1 Stopover duration

Radio transmitters were deployed on 20 black-throated blue warblers (N=11 female; N=9 male). Stopover duration for 8 birds (N=5 female; N=3 male) was quantified. Four of the birds (ID=14,22,23,28) remained in the study region for more than 3 weeks (approximate transmitter battery life), however, they continued to move around the study region during this time. Three of the birds lost their tags post release. Two of the three dropped tags (ID=19, 32) were found intact to the harness with no bird (or feathers) in sight. The third dropped tag (ID=29) was never located, however, the male was located without a tag in the same small habitat patch as he was prior to dropping his tag. One of the birds (ID=16) was never located with manual telemetry and the signal strength from the Motus towers remained consistent (flat-line) from shortly after time of release on May 20 until June 13, 2016, indicating that the transmitter was stationary. Data were missing from 3
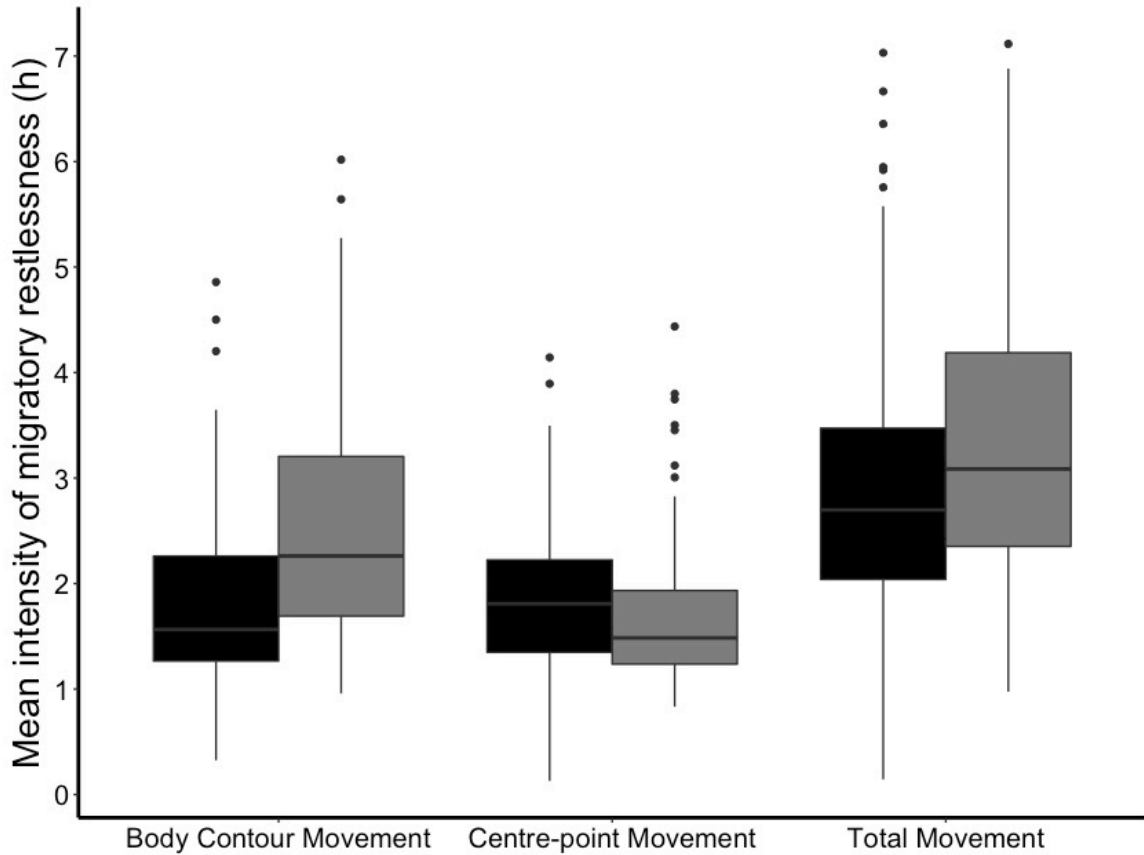
Figure 23. Intensity of migratory restlessness in black-throated blue warblers. Intensity of migratory restlessness was calculated as the cumulative time a bird displayed body contour movement, centre-point movement, or total movement per night between March 20, 2016 and May 17, 2016. Males are in grey and females in black.

birds tagged due to technical difficulties with the radio-transmitters (possibly un-activated or faulty tags).

Stopover duration of the eight birds with known departure dates ranged from 4 – 20 days (median 7.5 days). Including the 4 birds that did not depart as right censored, the probability of departure from stopover was influenced by the intensity of migratory restlessness prior to release (total movement on May 17, 2016), but was not significantly influenced by mass on May 17, 2016, sex, or age (Table 4; Fig. 24; Table 2). Each minute of intensity of total movement increased the probability of departure by 4 percent.

3.5.2 Habitat use

Between 2 and 20 positions were collected for 15 black-throated blue warblers at Long Point stopover post release from captivity, for a total of 139 manual position estimates (Fig. 25). The most utilized habitat type was dense forest, with 73 position estimates, followed by residential (29 positions), sparse forest (21 positions), scrub (12 positions), marsh (3 positions), and meadow (1 position) (Fig. 26). Most individuals remained relatively localized before departing the stopover site. The number of habitat types a bird occupied before departing ranged from 1 to 4 habitat types (median = 2 habitat types).

3.5.3 Migratory trajectories

Five of the black-throated blue warblers (N=2 HY, N=3 AHY; N=1 male, N=4 females) were detected by Motus towers located north of Long Point, Ontario. See Fig. 27 for mapped estimated migratory pathways based on the shortest line distance between towers with detections.

Table 4. Parameter coefficients (β ± SE) of the Cox regression model of departure from stopover in black-throated blue warblers (*Setophaga caerulescens*) at Long Point, ON, during spring migration. Also shown are 95% confidence intervals for β, p-values, and risk ratio. Intensity is of migratory restlessness measured as total movement on May 17, 2016. Mass of birds was measured on May 17, 2016.

Variable	β ± SE	95% C.I.	p-value	Risk Ratio
Sex (Males)	-0.05 ± 1.11	-2.21, 2.13	>0.5	0.96
Intensity	0.04 ± 0.02	0.007, 0.072	0.018	1.04
Mass	0.20 ± 0.17	-0.12, 0.53	0.22	1.23
Age	-0.09 ± 0.94	-1.94, 1.75	>0.5	0.91
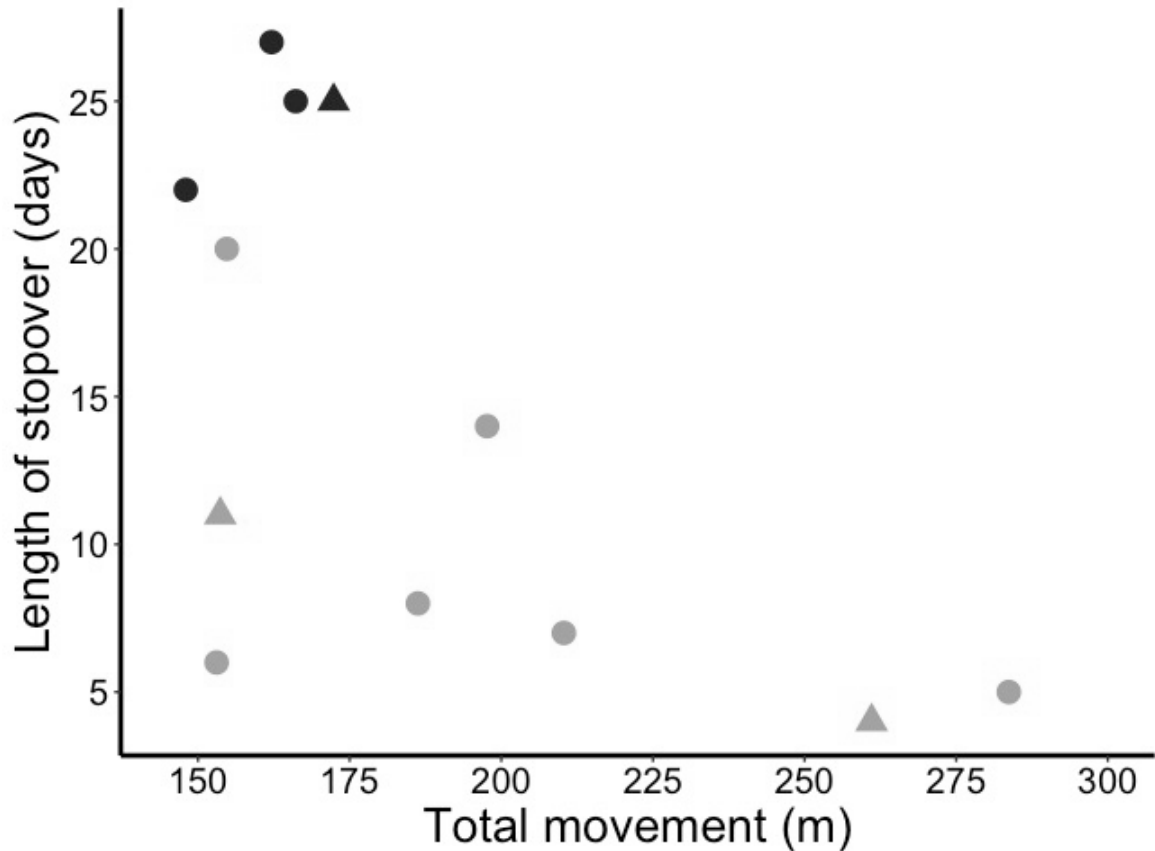
Figure 24. Relationship between black-throated blue warbler (*Setophaga caerulescens*) total migratory restlessness while in captivity on May 17, 2016 and subsequent stopover duration after release at Long Point, ON. Triangles represent males and circles represent females. Grey represents birds with known departure dates, while black represents birds that did not depart as of June 16, 2016 (approximately 3 weeks after release).
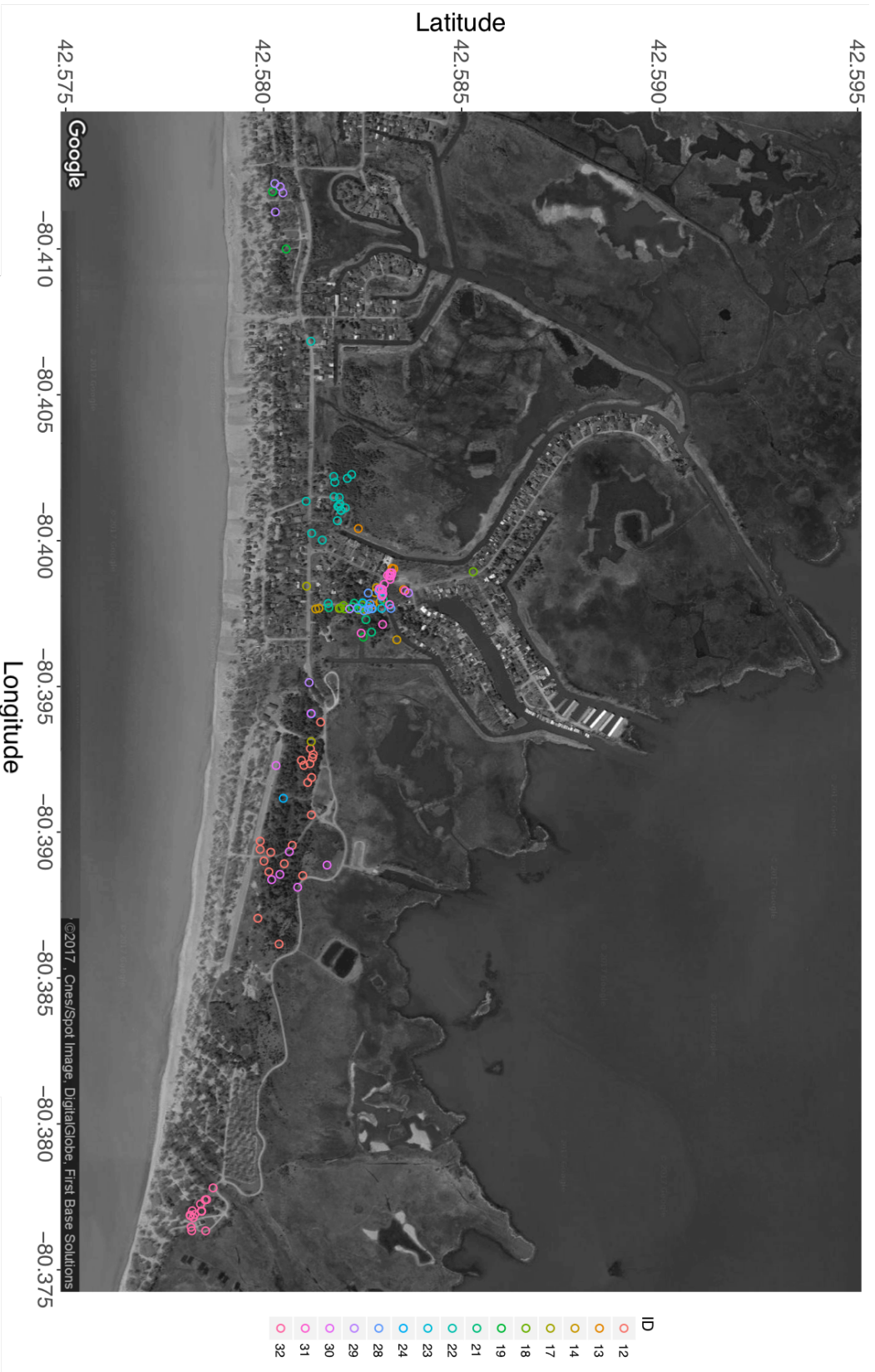
Figure 25. Black-throated blue warbler (*Setophaga caerulescens*) positions at Long Point, Ontario. Birds were tracked daily between May 20, 2016 – June 1, 2016 with manual radio-telemetry. Colours indicate different birds.
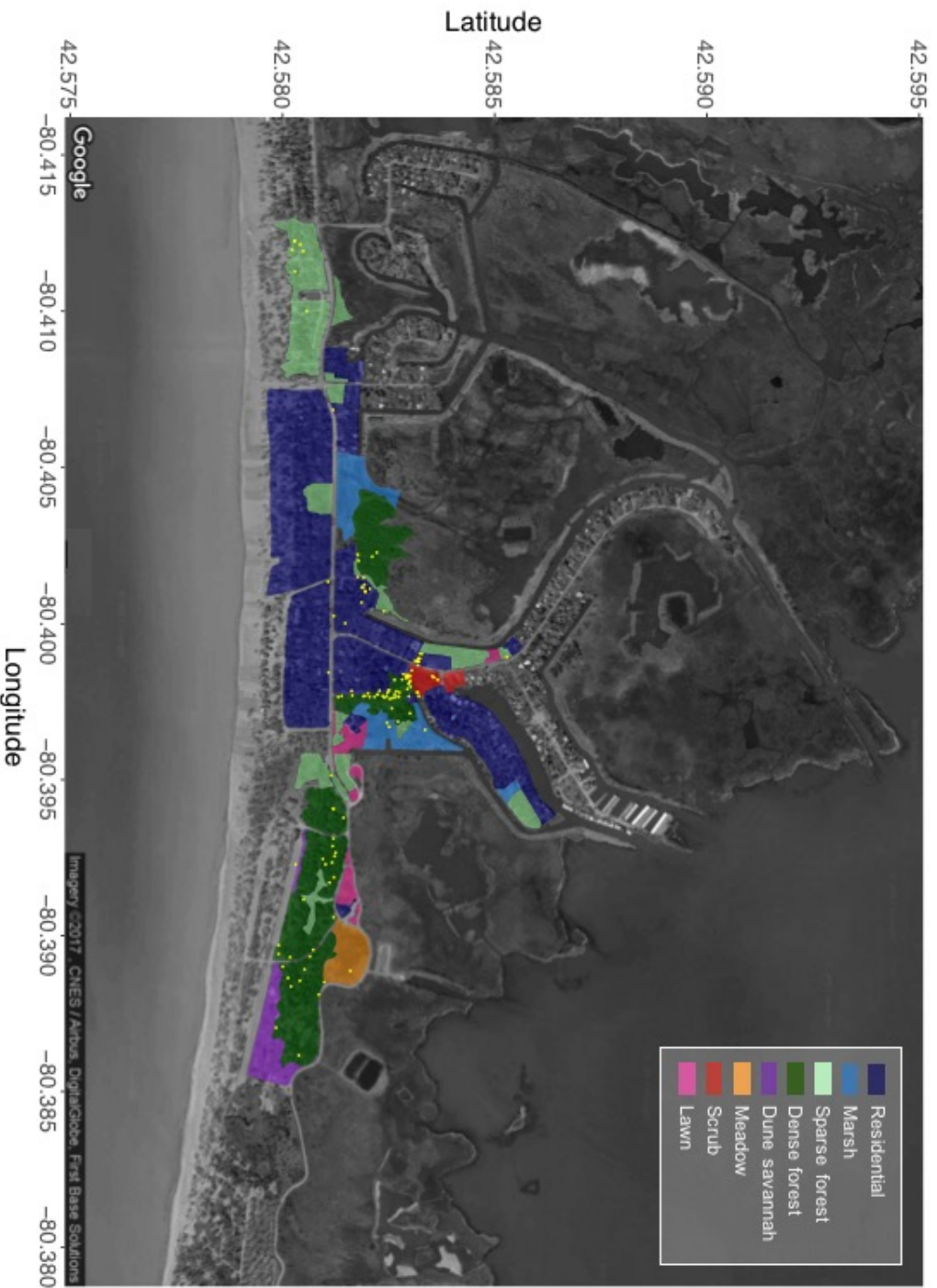
Figure 26. Habitat types of Long Point, Ontario. Yellow dots represent black-throated blue warbler (*Setophaga caerulescens*) positions during spring 2016 migration
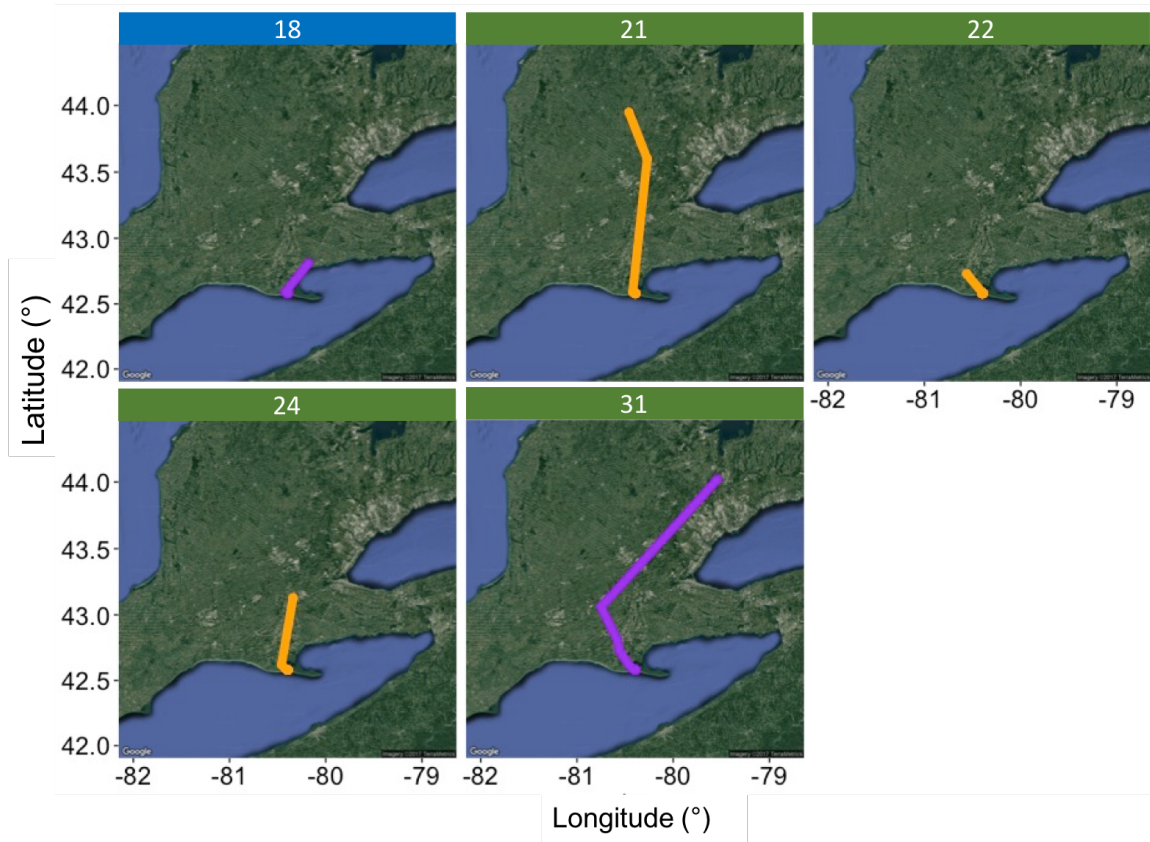
Figure 27. Migratory trajectories of black-throated blue warblers (*Setophaga caerulescens*) during spring 2016 migration. The coloured box above each map panel indicates either male (blue) or female (orange) individuals, as well as tag ID. Trajectories were estimated by the shortest line distance between Motus Wildlife Tracking receiving stations that had detections. Trajectory colour represents either birds that were hatched prior to (orange) or during (purple) the 2015 breeding season.

DISCUSSION

Seasonal migrators are under pressure to match their arrival timing to the breeding grounds such that death due to starvation or harsh climatic conditions is minimized and arrival and length of stay maximizes reproductive possibilities. Protandrous arrival timing theory has provided insight into the benefits associated with early arrival and how intraspecific and interspecific selection factors may drive the earlier arrival of males compared to females (Morbey and Ydenberg 2001; Section 1.5.1). Researchers are now particularly interested in understanding the behavioural mechanisms that result in protandry at the population level (Coppack and Pulido 2009; Schmaljohann et al. 2016). Three non-mutually exclusive hypotheses have emerged concerning the behavioural mechanisms underlying protandry in birds (Coppack and Pulido 2009). However, there is a shortage of data that test these proximate hypotheses empirically, especially for Nearctic-Neotropical migrating wood-warbler species. In my thesis, I address this lack of data by evaluating the behavioural mechanisms of protandry in a Nearctic-Neotropical migrating species, the black-throated blue warbler (family Parulidae). More specifically, I test the hypothesis that 1) males have earlier onset of spring migration than females and that 2) males have faster overall migration speed than females.

4.1   Key findings

4.1.1 Onset of migratory restlessness

My results suggest that sex differences in the onset of spring migration may not play an important role in causing protandry in black-throated blue warblers. Males and females had similar pre-migratory total mass gain and similar onset of migratory restlessness, suggesting no intrinsic differences in migratory preparation nor in departure for migration between the sexes from the wintering grounds. It has been suggested that onset of spring migration is one of the most important mechanisms of protandry (Coppack and Pulido 2009). Maggini and Bairlein (2012) observed sex-differences in the onset of migratory restlessness and the onset of pre-migratory fuel accumulation in the spring under constant light conditions (12L:12D) in a long distance Palearctic-African migrator, the Northern wheatear, suggesting that sex differences in departure timing are endogenously

controlled. Further, there is evidence for day length being the main zeitgeber for migration timing in other species. For instance, in controlled laboratory settings with simulated photoperiod, dark-eyed juncos (Ketterson and Nolan 1985), common redstart (Coppack and Pulido 2009), and *Sylvia* warblers (Terrill and Berthold 1990) display sex differences in the onset of their migratory restlessness, suggesting that sex differences in the timing of departure from the wintering grounds are caused by different photoperiodic sensitivities. My results indicate that male and female black-throated blue warblers have similar photosensitivity, but perhaps other cues or factors fine-tune the onset of spring migration in this Nearctic-Neotropical wood-warbler species.

It has been suggested that habitat quality can influence departure timing from the wintering grounds and arrival to the breeding grounds in Nearctic-Neotropical wood-warblers (Marra and Holberton 1998). Marra et al. (1998) observed that American redstarts occupying a higher quality habitat on Jamaican wintering grounds were in better condition, departed wintering grounds earlier, and arrived earlier to the breeding grounds, compared to birds occupying a poorer quality habitat. Since lower quality habitants departed earlier than higher quality habitants regardless of sex, Marra et al. (1998) concluded that a better physical condition simply allowed birds to depart sooner. American redstarts commonly display sexual habitat segregation on the wintering grounds, with old males forcing younger males and most females into poorer quality habitats (Sliwa 1991). Occupants of poor quality habitats are at risk for losing up to >10% body mass (Marra et al. 1998) and have increased levels of baseline corticosterone associated with increased foraging effort to meet increased energy demand (Marra and Holberton 1998), and as a result, would presumably take longer to prepare and fuel for migratory departure. Perhaps sexual habitat segregation on the wintering grounds is a proximate mechanism of protandry in black-throated blue warblers. Indeed, sexual habitat segregation has been observed in black-throated blue warblers wintering in Jamaica (Wunderle 1995), and sex ratio (number of males relative to females) increases with canopy cover (Spidal and Johnson 2016). Given that the sexes in my study experienced similar food quality and abundance, it is possible that differences in the onset of migration were masked if males and females had similar body conditions before migratory trigger.

I found that older birds had significantly earlier onset of migratory restlessness than younger birds, regardless of sex. This suggests that age influences departure timing from the wintering grounds in black-throated blue warblers, with older birds departing earlier than younger birds. Previous studies have found that arrival timing is influenced by age, as a process of learning (Sergio et al. 2014; Schmaljohann et al. 2016) and better tailwind selectivity for departure in older birds (Mitchell et al. 2015), but my results suggest that intrinsic differences in photo-responsiveness may also be important.

4.1.1 Intensity of migratory restlessness

I found that on the nights when birds displayed migratory restlessness, males had significantly higher intensity of body contour movement than females (~30 minutes more), while no sex differences were found in intensity of centre-point movement. Since the intensity of migratory restlessness mirrors distance flown in wild conspecifics (Berthold 1984), my results suggest that males might have longer nocturnal migratory bouts. Further, I found that on nights of migratory restlessness, males reached nighttime peak (calculated as total movement) significantly earlier than females (~1.5 hours earlier). This might suggest that males depart earlier in the night than females, which may cause protandry if earlier departure allows for longer nocturnal migratory flight bouts by virtue of having more time at night to migrate. Thus, my results suggest that speed of migration, realized through distance flown during nocturnal migratory bouts, may play a role in causing protandry in black-throated blue warblers.

4.1.2 Sex distinctive migratory restlessness behaviours

Methods used in previous migratory restlessness studies are typically unable to distinguish between the various behaviours of migratory restlessness (e.g. hopping, jumping, and wing whirring). In my study, quantifying seasonal migratory restlessness of captive black-throated blue warblers with Noldus EthoVision software revealed sex distinctions in nocturnal migratory behaviour. Both males and females displayed nocturnal centre-point movement and body contour movement throughout the experimental period, representing "hopping and jumping" and "wing whirring", respectively. Agatsuma and Ramenofsky (2006) characterized specific nocturnal

migratory restlessness behaviours of white-crowned sparrows (*Zonotrichia leucophrys*; family Emberizidae) and suggested that beak-up and beak-up flight, as opposed to flight and jump, were the most suggestive of restlessness behaviours in this species because they were confined to night. Similarly, the birds in my study displayed centre-point movement during the day and at night, while body contour movement was displayed only at night (Deakin unpublished data), suggesting that body contour movement is more indicative of restlessness behaviour in black-throated blue warblers.

In my study, male birds displayed higher levels of mean body contour movement per nighttime trial than females throughout most of the experimental period. This might suggest that males are overall more eager to migrate than females throughout the course of spring, and this makes sense from an evolutionary standpoint. The current favoured hypothesis for the adaptive significance of protandry suggests that intraspecific competition in males for access to females drives the earlier arrival of males compared to females (Kokko et al. 2006; Møller et al. 2009). For instance, early arriving males would have more opportunities for extra-pair mating, higher probability of breeding with high-quality mates, and be more likely to re-nest after failed attempts, by virtue of being in breeding areas before later arriving males. Thus, males have more pressure to arrive early at the breeding grounds compared to females.

The distinctive migratory restlessness behaviours observed between the sexes may exemplify different stop-and-go movement patterns made *en route* to the breeding grounds. For example, Swainson's thrush (*Catharus ustulatus*) and hermit thrush (*C. guttatus*) undertake landscape scale relocations at night from stopover sites that are up to 30 km from the site of initial capture (Mills et al. 2011). These 'stopover flights' represent movement between stopover sites over the landscape and do not result in a continuation of migration. Indeed, in a study at Long Point during fall migration, approximately half of the black-throated blue warblers captured exhibited 'stopover flights' with a mean distance of 6.1 km (Taylor et al. 2011). These relocations have been attributed to exploratory movements (Paxton et al. 2008) and changes in migration direction upon encountering harsh environmental conditions (Richardson 1978). Perhaps, centre-point migratory restlessness behaviour is more indicative of 'stopover flights'

throughout the landscape, while body contour movement is more indicative of continuous migratory flights. Based on my results, this may suggest that females partake in more 'stopover flight' movements throughout the landscape, while males partake in more continuous flights, which would afford males faster migration speeds.

4.1.3 Stopover duration

I quantified stopover duration in black-throated blue warblers using Motus automated telemetry and manual radio-telemetry in combination with the controlled arrival date at stopover. With the caveat that sample sizes were small and some birds lost radio tags, I found no differences in stopover duration between males and females, which suggests that intrinsic sex differences in stopover duration is an unlikely cause of protandry in black-throated blue warblers. Remarkably, even with a limited sample size, stopover departure probability was significantly correlated to migratory restlessness intensity before being released back to the wild. Comparable results have been found in northern wheatears by Eikenaar et al. (2014), finding that individuals that displayed more migratory restlessness on the night of capture at stopover were more likely to depart the following night. Thus, my results provide further validation that migratory restlessness can be used as an accurate proxy for the motivation to migrate at the individual level.

4.1.4 Effect of captivity on migratory behaviour

There appeared to be no negative effects of being held in captivity over winter for eight months with no exposure to natural cues on the migratory behaviour of the black-throated blue warblers. After being released back to the wild in the spring, both males and females were tracked daily and located in suitable habitat around Long Point, Ontario. Through visual observations, the birds were seen preening, singing, and flying between trees to forage and evade human birders. Since the stable hydrogen isotope analysis of feathers estimated that the birds in my study originated from the northern section of their breeding range, it would make sense for the birds to pass through Long Point in the spring in a northern direction to reach their breeding sites. Indeed, based on bird detections received by Motus towers north of Long Point, the black-throated blue warblers appeared to be heading in the appropriate direction. To my knowledge, this is the first study to hold

migratory birds in captivity over winter and then release and track their spring migration patterns with an automated radio-telemetry array.

4.2 Implications for conservation

For most seasonal migrators breeding in temperate habitats, breeding is restricted by the timing and overall schedule of migration (Both and Visser 2001). Over the past few decades, plant and invertebrate phenology has advanced in temperate areas (Root et al. 2003), likely due to climatic change and increasing spring temperatures. The proper timing of arrival to the breeding grounds is essential for migratory birds because it influences individual fitness. For instance, a study on American redstarts captured birds upon arrival and found that earlier arriving individuals had bred earlier and produced more nestlings than later arriving individuals (Møller et al. 2009). With a rapidly changing environment, bird phenology and their resource phenology have the potential to become mis-matched, and indeed, recent evidence suggests that the phenology of 9 out of 48 migratory birds studied are not advancing at the same rate as spring green up (Mayor et al. 2017). This begs the question of whether migrating birds will be able to adapt to rapidly changing environments.

To predict whether the evolutionary change of migration timing is possible, detailed knowledge on timing and the plasticity of the mechanisms that influence timing is required. Currently, little empirical evidence exists on the selection of migration timing and its genetic variation. Potti (1998) found no evidence for genetic variation in arrival timing in pied flycatchers, while Møller (2001) found that the arrival timing in barn swallows was moderately heritable. My thesis results provide essential information required to assess how black-throated blue warblers and other Nearctic-Neotropical wood-warblers will respond to rapidly changing environmental conditions. For instance, I have shown that photoperiodic sensitivity between male and female black-throated blue warblers is similar. If departure for migration is extrinsically cued by photoperiod and the sole mechanism of protandry in this species, then we would expect to see high occurrences of mis-matched timing to the breeding grounds since photoperiod remains consistent on a yearly basis. Further, I have shown that migratory birds can be captured, held in captivity overwinter, released, and still display appropriate migratory behaviour.

This result will allow researchers to conduct future laboratory experiments on wild caught birds and release them to assess migratory timing strategies, which can ultimately be used to predict how at-risk migratory species will respond to variable environmental conditions.

4.3   Study assumptions and limitations

There were various assumptions in my study that were out of my control, and if they disappeared my study would become otherwise irrelevant. For instance, my study operated on the assumption that the sample of birds I used were representative of the black-throated blue warbler population. This assumption is probably true for the population of birds with breeding sites in the northern most part of their breeding range (i.e. excluding black-throated blue warblers with breeding sites south of Long Point, Ontario) since the birds were captured at varying times of the morning (approximate range 6 am – 12 pm) and throughout the month of September. This is further confirmed by my stable hydrogen isotope analysis of the bird's feathers, which showed that their breeding sites were widespread throughout southern Ontario, Quebec, and Nova Scotia. Based on citizen science data (www.eBird.com), I assumed that black-throated blue warblers depart the wintering grounds for spring migration late March and thus photo-triggering the birds to become migratory occurred at an appropriate time of year. I also assumed that the birds were released back to the wild at an appropriate time of the year they would have passed through had they not been in captivity. Banding data from Long Point Bird Observatory of black-throated blue warblers suggests that peak spring migration occurs between mid-May (Long Point Bird Observatory 2008), and therefore this assumption is probably true.

There were several limitations to my study that were relatively out of my control. Being a long-distance Nearctic-Neotropical migratory bird, black-throated blue warblers fly through a particularly large geographic range during migration. Because of this, I was limited to specific locations I could capture birds for my study since it is impractical to use anything but a high-density stopover site with reliable capture rates. I was unsure how black-throated blue warblers would respond to long distance transportation, and so utilizing Long Point to capture my birds afforded me the shortest distance possible

between capture site and the facility in which I had access to for my study. This study is also limited by the sample size. To my knowledge there have been no attempts at holding black-throated blue warblers in captivity overwinter, and as a result, I was unsure how this species would respond to photoperiodic cues. Even though they have an overwintering range close to the equator (providing very little photoperiodic cues), I assumed that my birds were accurately photo-triggered to become migratory since evidence suggests that a difference in photoperiod of mere minutes can act as a reliable cue (Hau et al. 1998). The incomplete array of Motus towers throughout the breeding range of black-throated blue warblers has also limited this study since I was unable to fully track the birds to their breeding sites and that some the birds went undetected after departing from Long Point. Further, the analyses of stopover duration at only one site and close to the breeding grounds may not be an accurate representation of what occurs at all stopover sites throughout their migratory range. It is noteworthy that the results of this study represent intrinsic sex- and age-differences in the migratory behaviour of black-throated blue warblers, and are most likely conservative given the small sample size and the lack of many extrinsic cues responsible for controlling migratory timing.

4.4 Future directions

Future research should focus on repeating this study with a larger sample size to validate the results of the current study. Such a study should begin recording migratory restlessness in advance of the photoperiodic migratory trigger to assess what the baseline levels of nighttime activity are during the non-migratory state. The current study should be repeated with other species to provide a more in-depth evaluation of the proximate mechanisms of protandry. It has been suggested that degree of protandry is highly and positively correlated to degree of sexual dichromatism. Currently, most empirical studies on protandrous migration timing are biased towards sexually dichromatic species, which is largely due to the challenges associated with sex determination of monomorphic species in the field. To eliminate this sampling bias, molecular techniques can be used to accurately determine sex from feathers or blood. Alternatively, a study could use data from migration bird banding stations to correlate arrival timing to degree of dichromatism in numerous study species with a broad range of plumage characterizations.

Additional research is needed to further assess whether males and females have different departure timing, and what other potential extrinsic and intrinsic cues are responsible for fine-tuning this timing. Since black-throated blue warblers show site fidelity to their non-breeding grounds, a study could be conducted on the northern edge of the wintering grounds in Cuba to monitor body condition throughout the winter and habitat quality. A Motus tower could then be installed on the northern shore, which could be used to accurately monitor departure for migration. Male black-throated blue warblers would be predicted to occupy higher quality territories and be in better condition prior to migration than female conspecifics. An alternative study could involve using Noldus EthoVision software to determine the onset and intensity of migratory restlessness in birds subjected to various manipulations such as food quality/abundance, temperature, sex ratio, or predator threat. For example, manipulating the availability and/or quality of food given to captive birds could serve as a proxy for overwintering condition.

My results suggest that protandry in black-throated blue warblers is most likely caused by intrinsic sex differences in duration of nocturnal migratory flight bouts, which alters the overall speed of migration. Additional research is needed to confirm this suggestion. A study could involve tracking the birds from their wintering grounds to their breeding grounds to see whether males have longer flight bouts than females, and subsequently, if females stopover more often than males. Wild black-throated blue warblers range in mass between 8-12 g, and thus, this study would require a tag capable of long distance tracking with high precision location estimation that is no heavier than 0.4 g (based on 5% rule). Unfortunately, technology is currently the limiting factor for this study since the smallest GPS tags are ~1 g. An alternative study could use the hypobaric wind tunnel in the Advanced Facility for Avian Research to further assess intrinsic sex differences in nocturnal migratory fight distance. Comparing the duration of time in which males and females are capable of sustained flight in the wind tunnel could act as a proxy for migratory distance flown, since flight duration corresponds to fight distance. Further, this captive study could be repeated and compared among species with varying migration distances.

4.5   Conclusions

I evaluated two non-mutually exclusive hypotheses for the behavioural mechanisms of protandry in a Nearctic-Neotropical migrating species, the black-throated blue warbler. My results do not support the hypothesis that sex differences in the departure timing from the wintering grounds causes protandry in black-throated blue warblers. Male and female birds exhibited similar onset of seasonal migratory restlessness. I found evidence to support the hypothesis that sex differences in the speed of migration may cause protandry in black-throated blue warblers. Males exhibited higher intensity of body contour movement than females, which suggests that males have longer nocturnal migratory fights than female conspecifics. Since longer migratory flights would afford males faster overall migration speed than females, distance flown could potentially lead to protandrous arrival timing. There were, however, no intrinsic sex differences in stopover duration between the sexes. Future research is required to further evaluate the behavioural mechanisms of protandry in Nearctic-Neotropical migrating species.

My study offers the first set of data that suggests that migratory restlessness behaviour is sexually distinct. Throughout the course of spring, males typically displayed higher amounts of mean body contour movement ("wing whirring") per nighttime period, while females typically displayed higher amounts of mean centre-point movement ("hopping and jumping"). My study also provides evidence that migratory birds can be held in captivity overwinter, released, and still display appropriate migratory behaviour.

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Appendices

Appendix A. Animal Use Protocol

AUP Number: 2010-020

PI Name: Guglielmo, Christopher

AUP Title: Physiological Ecology Of Migratory Birds And Bats

Approval Date: 03/28/2014

Official Notice of Animal Use Subcommittee (AUS) Approval: Your new Animal Use Protocol (AUP) entitled "Physiological Ecology Of Migratory Birds And Bats" has been APPROVED by the Animal Use Subcommittee of the University Council on Animal Care. This approval, although valid for four years, and is subject to annual Protocol Renewal. 2010-020::5

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Laura Copeman on behalf of the Animal Use Subcommittee University Council on Animal Care

Curriculum Vitae

Jessica E. Deakin

Education

M.Sc. Biology, Sept. 2015 – Present
University of Western Ontario, London, ON

B.Sc. Honours Biological Science, June 2013
University of Guelph, Guelph, ON

Work Experience

Teaching Assistant, *University of Western Ontario*, London, ON, 2015 – 2017

Laboratory Research Assistant, *University of Western Ontario*, London, ON, 2015 – 2016

Laboratory Technician, *Activation Laboratories Ltd.*, Ancaster, ON, 2015

Field Research Assistant, *Abacus Consulting Services*, Milton, ON, 2013

Awards

Biology Travel Award, University of Western Ontario, 2017

Best Graduate Student Poster Presentation, Biology Graduate Research Forum, 2016
University of Western Ontario, London, ON

Peer-reviewed publications

Morbey, Y.E., Guglielmo, C.G., Taylor, P.D., Maggini, I., **Deakin, J.**, Mackenzie, S.A., Brown, J.M., and Zhao, L. (in press). Evaluation of sex differences in the stopover behavior and post-departure movements of wood-warblers. Behavioral Ecology.

Conference Presentations

Deakin, J., Morbey, Y.E., and Guglielmo, C.G. (2017) The propensity of a migratory bird to migrate. Animal Behavior Society. Scarborough, ON, Canada. [Oral]

Deakin, J., Morbey, Y.E., and Guglielmo, C.G. (2017) Migratory restlessness of black-throated blue warblers during spring migration. Canadian Society for Ecology and Evolution. Victoria, BC, Canada. [Oral]

Deakin, J., Morbey, Y.E., and Guglielmo, C.G. (2017) Mechanisms of protandry in the Black-throated Blue Warbler (*Setophaga caerulescens*). Comparative Physiology and Biochemistry Workshop. Rice Lake, ON, Canada. [Poster]

Deakin, J., Morbey, Y.E., and Guglielmo, C.G. (2017) Behavioural mechanisms of protandry in the Black-throated Blue Warbler (*Setophaga caerulescens*). Fallona Family Interdisciplinary Showcase. London, ON, Canada. [Poster]

Deakin, J., Morbey, Y.E., and Guglielmo, C.G. (2016) Behavioural mechanisms of avian protandry in the Black-throated Blue Warbler (*Setophaga caerulescens*). Biology Graduate Research Forum. London, ON, Canada. [Poster]

Outreach and Service

Biology Graduate Research Forum, Financial committee, 2017

Ontario Ecology, Ethology and Evolution Colloquium, Social committee, 2017

Society of Graduate Students SOGS representative, Sept. 2016 – Present

London Humane Society - fundraising events and animal care, 2013 – 2016