RED-WINGED BLACKBIRD MIGRATION DISTANCE AND ITS RELATIONSHIP WITH REPRODUCTION

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Red-winged blackbirds (*Agelaius phoeniceus*) are one of the most commonly researched birds in North America. My study aims to build upon what is known about migration patterns and reproduction in this species. My first objective was to determine if individuals that breed together travel to similar overwinter locations and to investigate the similarities or differences in the timing of migratory movements. My second objective was to examine the relationship between spring migration distance and reproduction. In short, I found that female blackbirds travel to more southern overwinter locations than males. Males and females may be leaving the North Dakota region at similar times during the fall, but males return to the breeding grounds approximately one month before females. Within sexes, there was not a correlation between migration distance and reproduction in females; however, males that migrated a shorter distance returned to the breeding ground with higher levels of baseline testosterone.

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CHAPTER 1. INTRODUCTION

Animal migration is awe-inspiring even to those who pay little attention to the natural world around them. It is hard to believe that animals possess the ability to appropriately time and coordinate feats of migration as they move from one life history stage to the next. Previously, migration has been studied primarily by observation or band and recapture efforts. These studies provide valuable information, but do not enable a researcher to easily follow an individual throughout the year because once an individual leaves the breeding grounds, it is very difficult to track them (*1*). With recapture rates as low as 0.31% and 0.51% for red-winged blackbird (*Agelaius phoeniceus*) movement studies (*2*, *3*), we only know information about a small subset of the population in specific locations with researchers available to trap birds. Approximately 20 years ago, the use of stable isotopes of carbon, nitrogen, and hydrogen provided researchers with an opportunity to estimate overwinter habitat and migration distance when birds are caught during the spring. Furthermore, advances in GPS and geolocator technology allow year-round tracking of individual songbirds. The movement data provided by these techniques can then be combined with physiological data to understand the consequences and benefits of different overwinter locations.

Species

My study species, the red-winged blackbird, is a polygamous species that nests in forests, fields, and ditches but prefers nesting in wetlands (*4*). Individuals breed throughout the United States but the Prairie Pothole Region (PPR) in North Dakota has an abundance of cattails (*5*), which provides great habitat for roosting and breeding red-winged blackbirds. From the end of April to the end of May, red-winged blackbirds are engaged in mating and incubation (*6*). This involves finding a mate and building woven cup nests over water. From the end of May to early

July, both parents care for nestlings and fledglings through feeding or nest defense (*6*). The mean number of females per male has been calculated at 2.48 (*7*), but this number varies among different populations. The harem is composed of primary females (the first to nest in the territory) (*8*), secondary, tertiary, and beyond. For example, the quaternary female is the fourth female to nest on a territory. The number of females within a territory varies with the quality of the habitat. It is likely that the primary female was the first to arrive because the first females to arrive are typically the first to initiate nesting (*9*). The incubation period for this species is 12.6 days with a range of 11-13 days (*10*), and females typically lay 2-4 eggs. Although red-winged blackbirds are a polygamous species, males invest in paternal care by feeding young, maintaining vigilance against predators, and defending young from predators (*11*). While much is known about the breeding behavior in this species, less is known about these individuals outside of the breeding season.

Current knowledge on migration patterns

Over the winter, red-winged blackbirds form large roosts in the southern United States with European starlings (*Sturnus vulgaris*), yellow-headed blackbirds (*Xanthocephalus xanthocephalus*), common grackles (*Quiscalus quiscula*), and brown-headed cowbirds (*Molothrus ater*). These roosts are common in Arkansas, Mississippi, Louisiana, and along the Atlantic Coast (*12*). In 1975 a nationwide estimate found 137 roosts containing over 1 million birds in the southern United States (*13*). When caught, birds from the North Dakota region primarily occupied roosts in Texas, but could also be found from Arkansas to northern Colorado (*2*, *14*). However, these locations were determined based on recapture information that may be biased toward areas with active banding stations.

In early spring, winter roosts break up and individuals travel to their breeding grounds. Overall, birds travel from the end of February until the end of April (*14*). Males are the first to arrive at the breeding site, and they establish their breeding territories. Next, females arrive and choose a territory occupied by a male. Young male red-winged blackbirds return to the breeding grounds after their more mature counterparts (*15*). Differences in arrival date may be due to individuals leaving the overwinter location at different times or traveling from different distances. Since females typically spend the winter farther south than males, it follows that they would arrive later in the season.

Once the breeding season is over, individuals begin to prepare for molt and fall migration. The exact timing of fall migration varies for different latitudes and locations, but in North Dakota this likely occurs from mid-October until early December (*14*). It is hypothesized that males are the first to complete their fall molt and leave the breeding grounds; females and hatch year birds are the last to leave (*15*). This time period aligns with the maturation of sunflowers in North Dakota. Blackbirds cause significant damage to sunflower crops as they fuel for their flight south. Through aerial marking, it is known that some individuals from North Dakota will utilize a stopover at Cheyenne Bottoms, South Dakota in the fall (*16*), but more details about other locations used by birds from the time that they leave their breeding site and when they arrive at their overwinter location is still needed to understand their movement patterns.

Factors influencing migration distance and timing

With GPS tags and stable isotopes researchers are beginning to investigate the migratory connectivity of species (*17*), which is the linkage of species both temporally and spatially. A species with individuals that breed together and travel to their overwinter grounds together has high migratory connectivity. Understanding the similarity in migratory distance and timing is difficult because, until recently, our technology was not capable of tracking small songbirds. The advent of small GPS tags now enables researchers to ask questions related to migratory connectivity. Our study investigates the migratory connectivity of red-winged blackbirds that breed together in North Dakota, with a goal to better assess similarities and differences in their migration patterns. Additionally, this research aimed to address potential consequences of different migration decisions (i.e., spring migration distance) to determine if they influence reproduction.

There are many factors that influence the distance that an individual within a flock migrates, including both size and sex. Birds follow Bergmann's rule which tells us that larger body size is favored at higher latitudes (*18*). Large body sizes have less surface area for their mass and are typically more immune to the cold weather. In red-winged blackbirds there is strong sexual dimorphism with females weighing approximately one-third less than their male counterparts (*19*, *20*). This size difference may explain why females have been noted to migrate a farther distance than males (*19*). It is also thought that younger males will travel to more southern latitudes than older males due to intra-sex competition and the pressure for older, territory-holding males to arrive at the breeding site early in the spring (*21*).

Consequences of different arrival times or overwinter locations

Individuals may try to decrease their spring migration distance by spending the winter at a more northern location in order to arrive at the breeding ground earlier. Birds are unable to invest the energy to be fully prepared to breed while they are migrating. For males, it takes approximately one month for testicular regrowth from a regressed condition (*22*) and females also wait until they arrive to the breeding ground to enter the final stages of reproduction preparation (*23*). In this case, early arrival to the breeding ground may allow an individual to breed early in the season. There is pressure to arrive early because early-breeding females are often able to produce the most offspring (*24*, *25*) and males arriving early may be able to claim the best territory (*21*).

In the Ipswich sparrow (*Passerculus sandwichensis*), it was found that males that traveled a shorter distance were in better condition upon arrival to the breeding grounds and established territories earlier compared to males that traveled a longer distance (*26*). However, in this same study, females that had traveled a longer distance had earlier lay dates. This indicates that migration distance may influence males and females differently, and much about this relationship is still unknown. Potentially, different overwinter distances correlate with different overwinter habitat that affect breeding phenology. For instance, individuals traveling from higher-quality habitats have been shown to arrive earlier (*27*, *28*) and achieve overall increased reproductive success (*29*).

In our study species, the red-winged blackbird, there are likely species-specific benefits of breeding early. First, more aggressive, early-arriving females are able to obtain better territories than later-arriving, less aggressive females (*8*). Males tend to spend more time perched by the nest of primary females to engage in predator defense (*11*), and these males also tend to spend more time feeding young of the primary and secondary nests than later nests (*30*). By breeding early, a female may also be able to renest if the first nesting attempt fails due to predation or unfavorable weather conditions. Additionally, the young born early in the season also have more time to mature before fall migration.

Importance of red-winged blackbirds

We specifically are interested in understanding migratory connectivity in red-winged blackbirds and its relationship with reproduction; populations are stable in the Prairie Pothole Region of North Dakota (*31*) and causing significant damage to agriculture (*32*, *33*). Red-winged blackbirds cause 3.5 million dollars of damage to sunflower crops and 1.3 million dollars of damage to corn annually (*34*). Reproduction is important because young-of-the-year blackbirds are a significant portion of the local late season population (*35*). Many of these individuals will not survive to the next breeding season, but in the fall they consume sunflower seeds to prepare for their molt and migration south (*35*). Therefore, a better understanding of the factors that facilitate high reproductive success and production of the age class most destructive to sunflower crops is important to sunflower producers (*36*).

Due to their high destruction of sunflower crops, there is an interest in managing the populations that destroy producers' fields. However, red-winged blackbird populations are declining in much of the USA and Canada (*31*, *37*). It is important to more clearly understand if declining populations will be affected by management strategies. Following damage-causing individuals throughout the annual cycle should help inform future management proposals. For instance, there may be a large roosting colony that researchers have highlighted as a place for management, but tracking data will be able to indicate if birds from high damage areas are traveling there.

Research objectives

In chapter 2, I discuss migratory patterns and the migratory connectivity of individual red-winged blackbirds that breed in eastern North Dakota. Using stable isotopes and GPS data loggers, I analyze differences in spring migration distance both between and within sexes and the general timing of spring and fall migration. I also analyze the habitat used by red-winged blackbirds throughout the year. In chapter 3, I detail the relationship between migration distance and reproduction in this species. Using stable isotopes of hydrogen, I examine the relationship between migration distance and the reproductive hormones estradiol and testosterone to gauge how migration distance may influence reproductive development. I also follow those same individuals to investigate if migration distance influenced timing of reproduction and reproductive success, measured as harem size and fledglings produced for males and lay date and fledglings produced for females. Together my research provides insight into red-winged blackbird migration patterns for birds from North Dakota and how differences in these patterns, i.e. migration distance, may influence reproduction.

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CHAPTER 2. REVISITING RED-WINGED BLACKBIRD MIGRATION: ANNUAL MOVEMENT PATTERNS OF BIRDS BREEDING IN EASTERN NORTH DAKOTA

Abstract

Previous knowledge about migration patterns of red-winged blackbirds (*Agelaius phoeniceus*) comes from banding and recapture efforts in the middle of the 20th century. While useful, these methods are biased by the locations of banding stations with dedicated researchers capable of capturing the birds and only provide a single snapshot of where a bird was captured or recaptured. Little to no information on the individual between captures can be obtained. With our current technology, researchers have the opportunity to record multiple locations that target birds visit per year using small GPS tags. To gather a larger sample size, researchers can measure stable isotopes in inert tissues to estimate the latitude of where each bird was located a few weeks or months prior to capture. Our study used both stable isotopes of hydrogen and GPS data loggers to study the annual movements of red-winged blackbirds that breed together in eastern North Dakota. We found that females travel significantly farther than males to their overwintering grounds, concurring with previous banding studies. Further, we also observed that both between and within sexes, individuals breeding together spend the winter in different locations. Our study was conducted on a stable population within a declining species that causes significant damage to sunflower crops; thus, knowledge of where these individuals travel outside of the breeding season may have important implications for both conservation and population management practices.

Introduction

Recent studies indicate that bird populations across all biomes in North America have declined by 29% since the 1970s, while the New World Blackbird family (*Icteridae*) has decreased by as much as 44% or 439.8 million birds (*1*-*3*). However, in the northern Great Plains, red-winged blackbird (*Agelaius phoeniceus*) breeding adult populations are remaining steady (*4*) and are still causing significant economic damage in this and other regions (*5*). For instance, it is estimated that red-winged blackbird damage to sunflower crops in North Dakota accounts for 3.5 million US dollars annually (*6*) or 18.7 million US dollars annually when indirect costs such as the impact of crop loss on the regional economy are also accounted for (*7*). Additionally, blackbird damage is consistently one of the leading reasons why farmers stop planting sunflowers in North Dakota (*8*). Therefore, both ecologists and farmers have an interest in what leads to the success or decline of these individuals at both the local population and the species level. Annual movements and the habitats used by this migratory species may provide information about what is necessary for the success of this species.

The genus of red-winged blackbirds, *Agelaius*, is derived from the Ancient Greek word agelaios which means "belonging to a flock" and many people have noted migratory movements and overwinter roosting sites of large groups (i.e. flocks) of blackbirds. Banding and recapture studies provided information on movement patterns of red-winged blackbirds throughout North America; however, there are limitations to what we can learn from these records. Often, birds banded in a large geographic region are grouped together in migration analysis and have recapture rates as low as 0.31% and 0.51%. Therefore, these efforts provide the location of a small subset of those banded birds (*9*, *10*). The recaptures are also biased to locations and time periods, both daily and seasonally, that are convenient for researchers capturing birds. A study using banding records from 1924 to 1974 found that birds captured in the summer in the broadly defined northern Midwest (North Dakota, South Dakota, Nebraska, eastern Montana, eastern Wyoming, and north eastern Colorado) spent the winter in Texas, Louisiana, Oklahoma, Arkansas, Kansas, Colorado, and South Dakota (*11*). This provides an account of red-winged blackbird migration that happens over a long period of time and lumps many states together. The data for this study included 700,000 banded birds and yielded only 11,000 recoveries; the vast majority of banded birds are never recaptured and their movements remain unknown.

Additionally, many, but not all (*12*, *13*), of these large banding and recovery studies used data collected prior to the 1980's. With increasing overwinter temperatures and habitat alterations, the current migratory pathways and overwinter locations for red-winged blackbirds in North Dakota may be quite different than they were 50 years ago. This change in migratory behavior can be seen in other bird species that are changing their overwinter locations or have ceased to migrate at all (*14*, *15*). However, even the more recent studies of red-winged blackbirds by Homan et al. in 2004 and 2005 (*12*, *13*) were limited to mark and recapture limitations of aerial marking which means that recapture rates are low and birds are only found where researchers are present.

This individual variation may also have crucial implication for management. Most damage to sunflower crops in North Dakota occurred in August and September (*16*); implicating local birds near natal territories as the source of damage. Therefore, a local focus is needed to understand the movement behavior of the birds responsible for the most damage within one season. Therefore, we need to have a more local focus on individuals to better understand where birds travel outside the breeding season as this may influence various aspects of their life history that could be the key to understanding why populations of red-winged blackbirds in North Dakota are stable. It may also provide information on how, where, and when to best manage this population that causes a lot of sunflower damage.

Another limitation of band and recapture data was that it does not address differences in overwinter location for individuals that breed together in the same habitat and populations. When data is aggregated from banding efforts across an entire state or group of states, we cannot tell if individuals that breed together show variation in their migration distance or if the variation in overwinter location is caused by variation in their breeding locations. This individual variation in overwinter location may also have crucial implication for management. To combat blackbird damage to crops, birds are sometimes managed at stopover sites, overwinter locations, or sunflower fields (*17*). Attempting to remove red-winged blackbirds through culling methods at winter roost sites did not make a large impact on the regional red-winged blackbird populations (*18*). This minimal effect of culling of blackbirds at winter roost sites may be because the majority of the birds in these roosts were not resident birds of the Prairie Pothole Region likely responsible for the depredation. To begin to examine this hypothesis, we need to examine whether blackbirds that breed together are overwintering together. If they do not seem to converge on a single location, it is unlikely that they can effectively be managed at an overwinter location. Therefore, individual tracking is necessary.

To closely investigate individual-level movements across the annual cycle for individuals breeding in the same location, we deployed GPS-data logging tags on birds breeding in the same wetland. These devices provide more precise data of the movements and locations utilized by individuals from the same breeding habitat throughout the year and remove the bias of active trapping times and locations (see above). While GPS tags provide accurate locations, their size and weight constrain their ability to be deployed on smaller songbirds. These weight constraints necessitate data logging as opposed to transmission of ‘real time data’ on smaller songbirds, limiting the number of points a GPS tag can log on a single deployment and requiring re-capture of the same individual to retrieve data. Additionally, the high cost per unit limits large-scale deployment and data collection.

To obtain a larger sample size that does not require recapture of individuals, we analyzed stable isotopes of hydrogen (δD) obtained from claw samples upon return to the breeding grounds. This data can be used as an estimate of overwinter latitude and migration distance (*19-21*). As birds drink water at their overwinter locations they ingest stable isotopes of hydrogen from the water which are retained in metabolically inert tissues such as feathers and claws. These δD values vary in the United States from the southeast to the northwest portion of the country due to the continual evaporation and precipitation of water moving north from the equator and altering the ratio of deuterium to protium (*22*). Since these δD values vary for different regions, they provide an approximation of the latitude where the claw was grown (*23*).

Here, using GPS data loggers and stable isotopes we aim to provide a detailed account of overwinter locations and movements of individuals breeding together in the same wetland. Our study system, the red-winged blackbird is a species in decline in much of their range, but without declines observed in many breeding populations of this species. Specifically, we deployed GPS loggers on breeding birds to track locations throughout the annual cycle. Further, we estimated differences overwinter location and spring migration distance for male and female red-winged blackbirds breeding in the same wetland using δD values from claws.

Methods

Field site

The data from GPS data logging tags and stable isotopes of hydrogen were collected from red-winged blackbirds breeding at the Alice Waterfowl Production Area (WPA) (46° 46'36"N, 97° 30'31"W) near Alice, North Dakota, USA during the spring and summer of 2018 and 2019.

This field site in the Prairie Pothole Region of Cass County, North Dakota was selected because it has ample stands of cattail for red-winged blackbird breeding territories, and red-winged blackbirds from this region are thought to cause significant damage to sunflowers in the fall (*17*). In 2018 at our Alice WPA field site, the first male was observed on 11 April and the first male was captured on 28 April. The first observation of a female occurred on 21 April and the first female was captured on 3 May. In 2019, the first male was observed on 29 March and the first male was captured on 5 April. The first female was observed on 24 April and the first female was captured on 25 April.

Capture

Males and females were trapped in a walk-in trap or mist net located in or adjacent to their territories. Females were also trapped in nest traps (*24*) during incubation. Each captured bird was banded following the appropriate banding guidelines (*25*) and received a unique combination of a single USGS aluminum band and three color bands for later identification during the nesting phase. We estimated the age of males as second year or after second year for each bird using molt patterns and epaulette coloration. Older individuals typically have bright, red epaulettes and dark black body feathers in breeding plumage (*26*).

GPS tags

In the summer of 2018, PinPoint10 tags (<1.4 g; Lotek Wireless, Inc. Newmarket, Ontario, Canada), a store-on-board GPS logger, were placed on 21 females and 8 males using a leg-loop harness (*27*). Birds were captured as previously described. Only territory-holding males and females with a nest at our field site were tagged to better ensure likelihood of return. Additionally, only targeting breeding individuals helped control for age in our sample. All males were determined to be after second year based on plumage (see above) and territory holding status; males typically do not breed until they are after second year individuals (*28*). Females were determined to be at least second year birds based on plumage and the fact that they were of breeding age. The following year, 2019, birds were recaptured to retrieve the data. The tags can record up to 80 GPS points with an accuracy of 10 meters. These tags were configured to primarily capture information about overwinter location and spring migration, but also recorded 9 points per female and 14 points per male during the fall (see Appendix A). We recovered tags from 6 males and 6 females for a recovery rate of 41%. Due to damage to the tags, only 3 tags from females and 2 tags from males yielded data (see Results section below). Both males had been tagged as after second year. Two of the females were tagged as after second year and one female was tagged as a second year. All points with a low Horizontal Dilution of Precision (HDOP) value, ≥ 20, were removed from analysis ($n = 9$ location data points). This left 258 points for analysis from the five tags with a range of 49 to 54 points per individuals with a mean $\pm$ SE of 51.6 ± 0.93.

Stable isotopes

After birds were banded, we took a 2-3 mm sample from the distal portion of the middle claw from 71 females (2018= 26; 2019 = 45) and 89 males (2018= 35; 2019 = 54) until nesting began in each year, approximately the third week of May. Of these individuals, we restricted our analysis to birds that spent the summer at our study site. We determined that birds spent the summer at our field site if they were found breeding there or if they were captured in 2018 and recaptured in 2019. Additionally, if a bird was captured on multiple days within a year, they were considered to be a local bird. The range of days between the first and last capture for an individual within a year varied from 3 days to over a month. All birds that we considered local but weren't associated with a nest or territory were caught multiple times in May. As this is the month that breeding begins at our field site, we feel confident that they were not migrating through our study site. The ending sample size included 55 unique females and 31 unique males. Four females and 8 males were captured before the breeding season in both 2018 and 2019.

Claw samples were processed at the University of Regina. For δD analysis, dried washed claw material was weighed (c. 0.35 mg) into silver capsules and reduced to H_2 by reaction with Cr under helium flow at 1030 °C with a Eurovector 3000 (Milan, Italy – www.eurovector.it) elemental analyzer. The resultant H_2 gas was measured for δD in an Isoprime (Crewe, UK) continuous flow stable isotope mass spectrometer and corrected for H exchange using the comparative equilibration technique of Wassenaar and Hobson using two keratin calibrated standards: Caribou Hoof Standard (EC1, δD = -197 per mil) and Kudu Horn Standard (EC2, δD = -54.1 per mil) (*29*). All measurements are reported in δ-notation as the non-exchangeable feather H component in parts per thousand relative to the Vienna Standard Mean Ocean Water (VSMOW)–Standard Light Antarctic Precipitation (SLAP) scale. Based on replicate within-run measurements of standards, measurement error was estimated to be ± 2 parts per thousand difference.

A study on song sparrows (*Melospiza melodia*) estimated that the distal portion of the claw represents tissue grown three months prior to sampling (*30*) and another found that distal claw samples from warblers may provide information on the pre-migratory habitat for at least three to four weeks after arrival (*31*). Since claws continue to grow during migration and incorporate new isotopic signatures (*32*), we stopped claw collection in late May, approximately 8 weeks after the first male arrived. This should prevent bias in the δD values.

Differences in female and male δD values were examined using a Student's t-test to determine if males and females travel different distances in the spring. To confirm that migration distance is related to the δD values, we ran a linear model to look at the relationship between the δD values and spring migration distance using data from 5 GPS tags (see below). The model includes distance to the overwinter site using the GPS point that is the farthest distance from the breeding site as the independent variable and δD as the dependent variable. To identify if δD values and correlated with body size, we ran a linear model with δD values as the dependent variable and tarsus length as the independent variable. Tarsus length was chosen as it is a more reliable indicator of avian body size than mass.

Habitat use

ArcGIS Pro 2019 Version 2.4.0 was used to analyze habitat types. We used the USA NLCD Land Cover Map, which organizes the environment in the United States into twenty habitat types, to identify the habitat surrounding our red-winged blackbird locations. Each habitat block was broken into 3 m by 3 m squares. Only data points with a horizontal dilution of precision (HDOP) value of 5 or less (n = 235) were deemed to have adequate accuracy for habitat analysis (*33*) (see Appendix B). Habitat types within a buffer of 50 meters around each fix were recorded to conservatively account for inaccuracy in the GPS point fix. Additionally, a red-winged blackbird could easily travel 50 m in one day and our GPS birds could have interacted with any of the habitat types in their 50 m buffer zone. To test if red-winged blackbirds are choosing the habitats found near our points or if they are simply occupying the habitat available to them, we generated random points located within 74 km of the GPS point. This distance was chosen because although blackbirds typically forage within 2-3 km of roosts (*13*), they are capable of traveling 74 km or more in one day while foraging (*34*). We used linear models with each habitat type as the dependent variable and whether or not the point was from a

GPS tag or random point as the independent variable to see if the habitats recorded by the GPS tags differed from the habitats available.

Results

GPS data

Males and females did not have significant differences in their variance in overwinter distance from the breeding site ($F = 0.95$, $df = 1, 2$, $n = 5$, $p = 0.87$) A Student's t-test revealed that males and females tend to travel different distances from the breeding site ($t = -2.62$, $df = 3$, $p = 0.08$). In January, a time when both males and females have reached their overwinter locations (Figure 1), males were on average 1,150 km from the breeding site ($n = 2$) while females were 1,769 km away ($n = 3$). The GPS tag recorded the first male at the breeding site on 29 March; however, he could have arrived as early as 26 March since the GPS unit took a point every 4 days. The first GPS female was captured on 05 May, but could have arrived as early as 02 May since the GPS unit took a point once every 3 days.

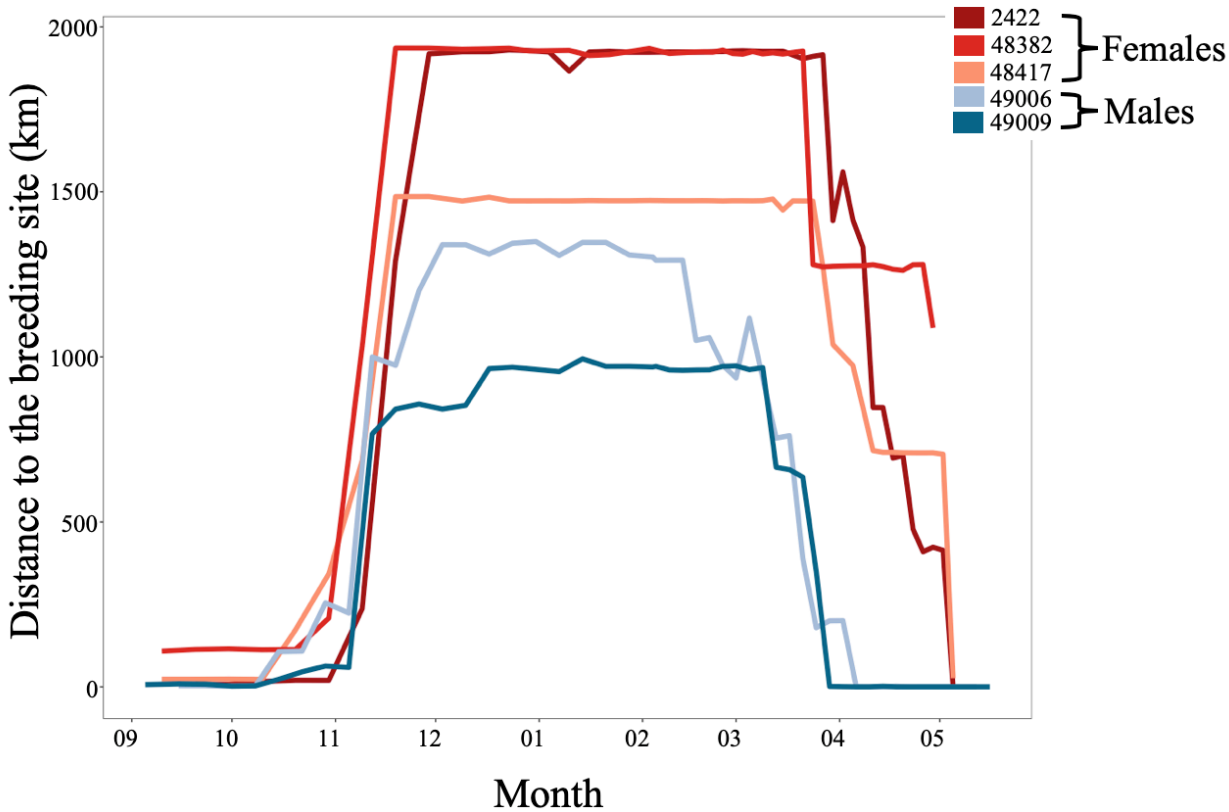

Figure 1. Data from GPS data loggers deployed in the breeding season of 2018 and collected from birds in the breeding season of 2019 show that female red-winged blackbirds (n = 3) tend to travel farther from the breeding site in the winter and arrive back to the breeding grounds later than male red-winged blackbirds (n = 2) ($t = -2.62$, $df = 3$, $p = 0.08$). Legend numbers correspond to unique individual tag numbers.

Females were located in North Dakota from May through September, although one female moved to the northeast edge of South Dakota in September. In October females were located in Minnesota, South Dakota, and North Dakota. In November females were found in South Dakota, Iowa, Missouri, Oklahoma, and Arkansas. In December, females were found in Oklahoma, Arkansas, and Texas. In January, females were located in Texas and Oklahoma, and in February they were located in Texas, Louisiana and Oklahoma (Figure 2). In March females were located in Texas, Oklahoma, and Kansas. In April, females were located Texas, Oklahoma, Kansas, Iowa, and Minnesota. In May females were found in Iowa, Minnesota, and North

Dakota as they returned to the breeding site. They remained at the breeding site in North Dakota until the tags were recovered.

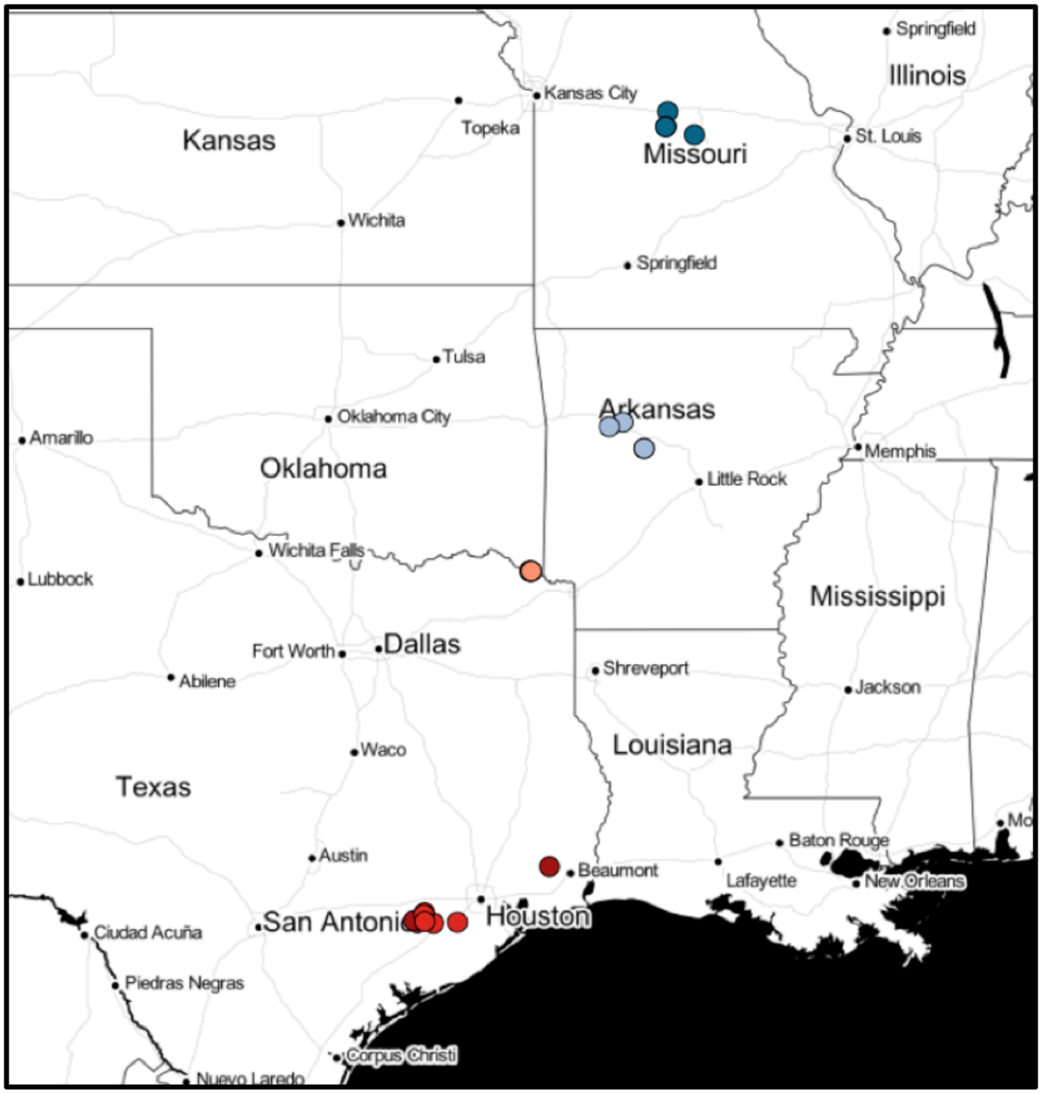

Figure 2. Locations of GPS-tagged individuals in January. Shades of red represent the three females and shades of blue represent the two males.

From May through September, all GPS tagged males were in North Dakota. In October males were located in Minnesota, South Dakota, and North Dakota. In November males were found in North Dakota, Minnesota, Missouri, Arkansas. In December, males were located in Missouri and Arkansas and remained there through February. In March, males were found in Oklahoma, Missouri, Iowa, Minnesota, South Dakota, and North Dakota as they moved north to the breeding grounds. By April males were found in Minnesota and North Dakota and they stayed in North Dakota until the tags were recovered. See Appendix C for a table displaying this information.

The tags indicated that all individuals were located in the Prairie Pothole Region during the early fall with an average distance from their breeding site of 114 km south for males (n = 2) and 61 km south for females (n = 3) for the month of October (Figure 1). We are unable to assign a fall migration time due to the time between recorded points, but the two males were located 224 and 59 km from Alice, North Dakota on 05 November. This was the first point taken in November. On 26 November, the last point taken in November, these same males were near their wintering location: 1,200 and 857 km away from their breeding site. On the 30 October, females were 19.74, 342.66, and 208.75 km from the breeding site. By 29 November, these females were at their overwinter sites: 1,918, 1,485, and 1,935 km from the breeding grounds. This change in the distance from the breeding sites indicates that individuals made the majority of their movement south for the winter during November.

Stable isotopes

To confirm that migration distance is related to the δD values, we ran a linear model that included distance to the overwinter site (using the GPS point that is the farthest distance from the breeding site) as the independent variable and δD as the dependent variable (see Appendix D). The model showed that migration distance from GPS tags significantly predicted δD values, with a short migration distance being associated with more negative δD values ($p < 0.05$, $F = 10.59$, $df = 1, 3$, n = 5, adjusted $R^2 = 0.70$).

For 2018 and 2019 combined, the mean δD value for males was -52.8 with a range from -37.13 to -72 (n = 31) and for females the mean was 33.8 with a range from -6 to -51 (n = 28). A

Student's t-test showed that the mean of the δD values for males and females were significantly different ($p < 0.001$, df = 57, t = 7.0635). The mean of male δD values were significantly more negative than the mean for female δD values (Figure 3). When comparing tarsus length, a reliable indicator of body size in birds, to overwinter locations as estimated by δD values, there is a significant relationship when males and females are both included ($p < .001$, $F = 37.87$, $df = 1, 51$, n = 53, adjusted $R^2 = .41$). However, there is no significant relationship between male tarsus length and δD value ($p = .99$, $F = 0.09$, $df = 1, 25$, n = 27, adjusted $R^2 = -0.04$) or between female tarsus length and δD value ($p = .67$, $F = .18$, $df = 1, 24$, n = 26, adjusted $R^2 = -0.03$).

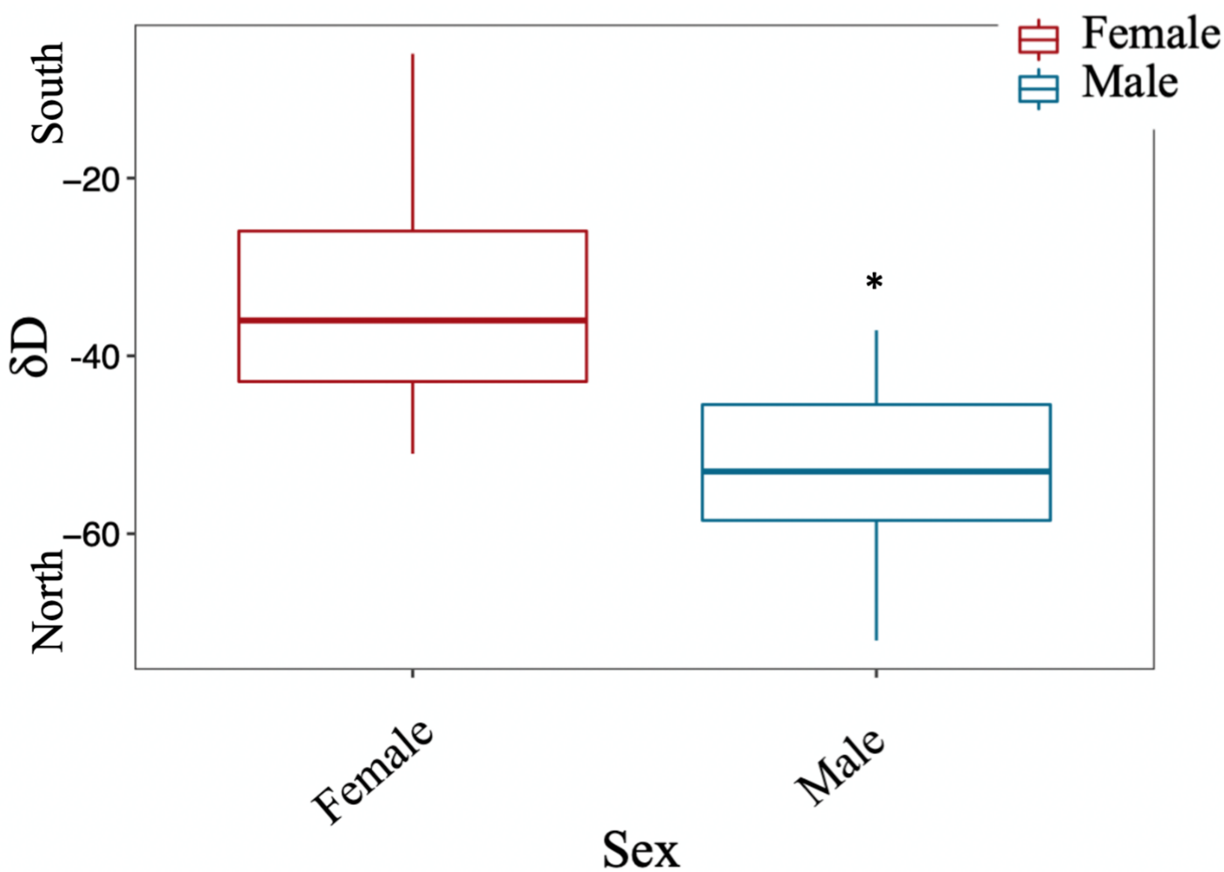

Figure 3. In the years 2018 and 2019, female claw samples (red) had more positive δD values than male claw samples (blue) indicating that females traveled to a more southern overwinter location ($p < 0.001$).

Habitat types

Of all the habitat types available, birds were recorded spending the majority of their time in cultivated crops (41%) and pasture and hay areas (27%) (Figure 4). The next most common habitat types were emergent herbaceous wetlands (12%) and open water (6%). The percentage of pasture and hay areas and cultivated crops found in the buffer zones of GPS tag points did not significantly differ from random points ($p = .38$ and $p = .72$, respectively). However, the percentage of emergent herbaceous wetlands and open water did indicate that red-winged blackbirds were selecting those habitats ($p < .001$ and $p = .05$, respectively). Additionally, individuals seemed to be avoiding deciduous ($p < .001$), evergreen ($p < .001$), and mixed forests ($p < .01$) which represent the only forest types included on the USA NLCD Land Cover Map.

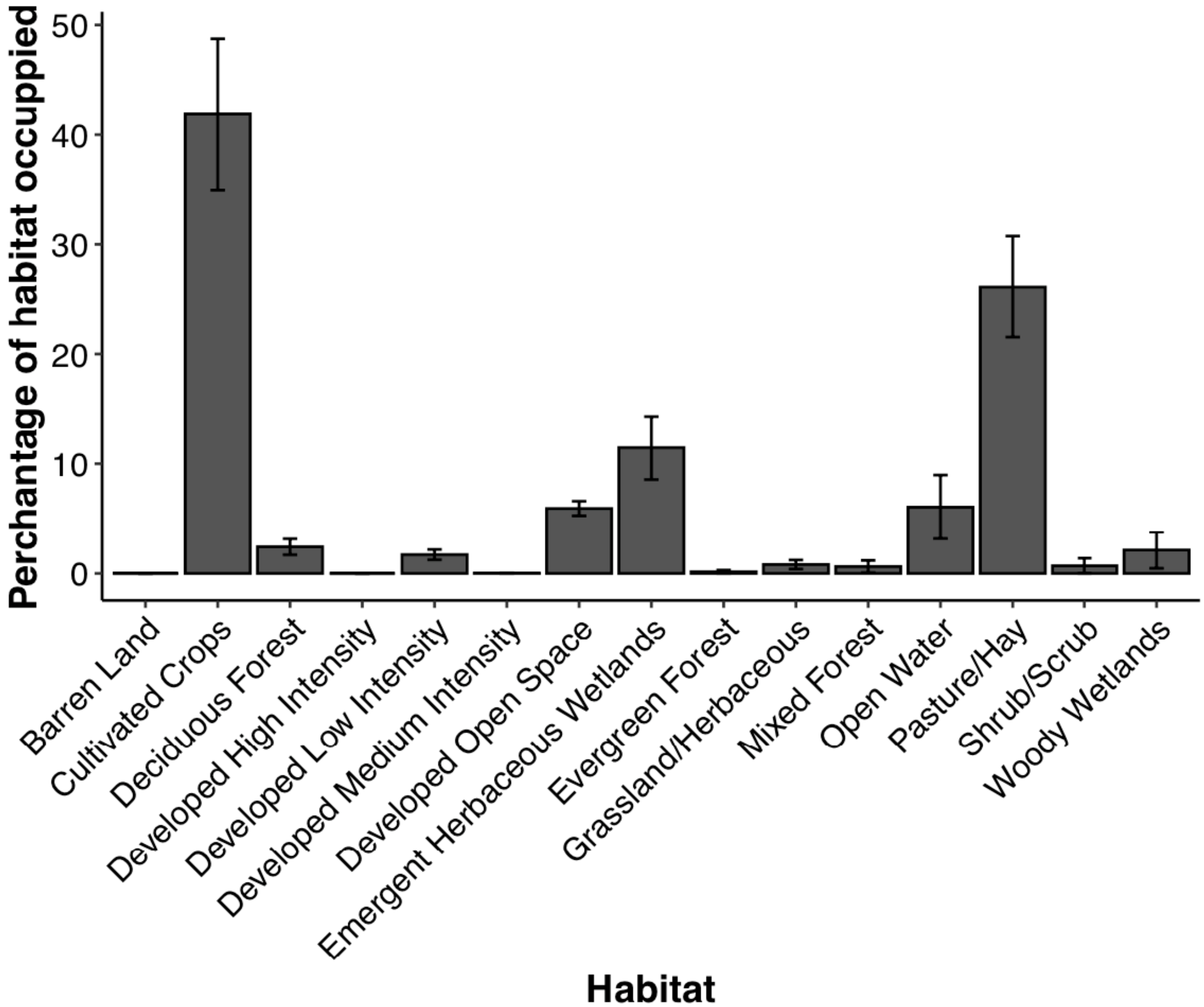

Figure 4. Percentage of habitat types within 50 meters of GPS points for three female and two male red-winged blackbirds throughout the year. Individuals were most often found in cultivated crops (41%) and pasture/hay habitats (27%).

Discussion

Fall location

Dolbeer found individual birds migrating in the fall around November (*35*). This also agrees with recapture evidence from Homan et al. that birds breeding in North Dakota could be recaptured in North and South Dakota in late October (*13*). However, in 1983, a study examining molt patterns in birds roosting in the fall at our study site at the Alice WPA suggested that resident birds had left the region by the end of September and the birds located in northeastern

North Dakota in October were transients (*28*). Our data, however, indicates that resident birds are still present in the region during this time. It is possible that the number of migratory birds in the region during October diluted the ability to observe residents that were still present in the region. Alternatively, increasing overwinter temperatures could have delayed the fall departure in this species. Although the effects of climate change on autumn phenology have received far less attention than spring phenology (*36*), this delay in fall migration has been observed in other short-distance migrants (*37*, *38*). With the use of GPS tags, we are able to show that individuals breeding in eastern North Dakota are located close to their breeding areas during times of high sunflower depredation (Aug-Oct).

Overwinter areas

Although the sample size is small, our data from 5 GPS tagged individuals indicates that δD values are correlated with overwinter latitude. δD values showed that females traveled significantly farther to their overwinter location than males, confirming a general pattern previously described for this and many other songbird species (*39*-*42*). This difference in migration distance could allow males to return to the breeding site early and claim the best territory (*40*). Dolbeer proposed that the difference in migration distance for males and females was due to the sexual dimorphic size differences between males and females in this species (*39*). Males on average weigh 50% more than females (*43*). Larger body size confers benefits for both heat conservation (*44*) and social competition with conspecifics (*45*). In support of this, larger male red-winged blackbirds had higher survival between years (*46*). Thus, it is thought that smaller individuals of a species, such as female red-winged blackbirds, tend to migrate to a more southern overwinter location with warmer winter conditions and potentially greater food availability (*40*). There is a significant relationship between tarsus length and δD value when males and females are both included, but there is no significance within sex in our study (see Appendix E). Males and females had relatively similar variation in their δD values. Males had a range of 34.87 (-37.13 to -72.00) and females have a range of 46.00 (-6.00 to -51.00). Our three GPS tagged females showed that two females went to a similar overwinter location just west of Houston, Texas, but the third female traveled to northeastern Texas approximately 400 km north of the location of the other two females. Thus, using our GPS and stable-isotope data, our data indicate that individuals, even of the same sex, that breed together are traveling to a range of overwinter locations.

Previously, banding records showed that birds banded across the United States and Canada (including Alberta, Minnesota, Illinois, Indiana, Michigan, Wisconsin, New York, South Dakota, Kansas, and North Dakota), were recaptured in the southern rice growing states of Arkansas, Mississippi, Louisiana, and Texas during the winter (*47*). Our data, suggesting a wide-range of overwintering locations for birds from our breeding study site, in conjunction with the data provided by Meanley indicates that overwinter roosts likely are comprised of birds from a variety of locations that may not necessarily include conspecifics from their breeding grounds (*47*). Thus, as individuals from the same breeding grounds overwinter in different locales, winter-roost management aimed at reducing individuals predating sunflower crop is likely to be unproductive. This may be why Dolbeer found that overwinter management did not make an impact on regional populations (*18*). If birds are forming heterogenous roosts with populations from broad geographical ranges, including those breeding and preparing for migration in regions outside of the sunflower producing regions, declining, non-target populations may be affected by culling in winter roosts.

However, it is worth noting that the overwinter location for two GPS females were close to the overwinter location of a female in a previous banding study (*9*). This female was banded in Kenton, North Dakota sometime between May and October and was found spending the winter in Colorado County, Texas. In our study two of the three females with GPS tags resided overwinter in that general area. One female spent time in Colorado County and the other was located in the adjacent Fort Bend and Wharton Counties.

Habitat use

Habitat data from GPS tags revealed that individuals were spending the majority of their time in cultivated crops (41%) and pasture/hay fields (27%). Red-winged blackbirds are known to feed in crop fields so it is expected that they would be found in those habitats. Pastures are also known to provide an alternative food source for these blackbirds during the spring/summer months; weed seeds and bristle grass were found in 68% of gizzards (*48*). These seeds were found in more gizzards than any other food item. Thus, our data suggest that pastures and hay fields may provide additional areas where birds forage on their wintering grounds as well.

The next most common habitat types were emergent herbaceous wetlands (12%) and open water (6%). Red-winged blackbirds are known to breed in wetlands, but they also preferentially roost there throughout the entire year (*34*). Thus, this close proximity to water is expected. These results also reaffirm the importance of conserving these habitats for species whose populations are in decline.

Conclusions

Although, as a species, red-winged blackbirds are in decline, they are still causing significant damage to crops (*49*). Populations in North Dakota provide a great research perspective because red-winged blackbirds in this region are stable and causing a significant economic impact to crop production (*7*). Declines in the red-winged blackbird populations can be linked to habitat loss (*50*) and it is possible that the loss of natural foraging habitat in overwintering locations and throughout the year could increase the need for birds to forage on cultivated crops such as corn, rice and sunflowers. This study begins to lay out a baseline for the individual, temporal and spatial movements of red-winged blackbirds from one key sunflower producing region, North Dakota. Although it is important to understand the movements of both sexes, future work should focus on females and reproduction. Females control when breeding occurs and they also breed in their second year. Males rarely breed until after their second year. Therefore understanding how differences in individual female migration patterns and overwinter habitat influence reproductive fitness may provide important knowledge about how movement patterns influence population numbers of red-winged blackbirds.

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CHAPTER 3. MIGRATION AND REPRODUCTION: IS THERE A REPRODUCTIVE ADVANTAGE TO A SHORTER MIGRATION?

Abstract

To avoid harsh conditions at their breeding grounds, many birds migrate south during the winter. Individuals migrating to a southern location may experience more favorable overwinter conditions but at the cost of a long, energetically-demanding migration that may delay their spring reproductive development. The relationship between migration distance and reproduction has primarily been investigated in monogamous species with a focus on early season testosterone production in both males and females. Our study presents a new perspective by assessing the importance of migration distance in a polygynous species with increased pressure for both males and females to arrive and breed early. Males that arrive early are able to compete for high-quality territories while the nests of females that lay earlier receive increased paternal care. To estimate migration distance and assess reproductive status upon arrival, we collected blood and claw samples from male and female red-winged blackbirds (*Agelaius phoeniceus*) before breeding in a managed wetland in eastern North Dakota. We measured baseline levels of estradiol and testosterone in females and baseline and GnRH-induced testosterone levels in males to estimate preparedness to breed. Further, a subset of individuals were fitted with GPS data logging tags that were collected the following year. A linear model revealed that migration distances provided by the GPS tags were strongly related with stable isotope values. We found males with shorter migration distances have higher baseline testosterone levels upon arrival. However, migration distance was not correlated with GnRH-induced testosterone, larger harems, or the number of fledglings produced. Female migration distance was not correlated with baseline estradiol or

testosterone levels, number of eggs laid, timing of reproduction, or the number fledglings produced.

Introduction

Migration is an adaptive response that allows species to travel to new environments throughout the year, and it can be observed in a variety of taxa including mammals, birds, fish, and butterflies. For instance, tens of thousands of monarchs can be seen each year in Long Point, Ontario as they migrate to their overwintering grounds in Mexico (*1*). Similarly, large flocks of thousands of blackbirds can be seen migrating to their breeding grounds in the spring. However, we know little about the origins or destinations of the individuals within these flocks. Now, technology provides the opportunity to track an individual for an entire year and view the locations of the bird during different seasons. This is needed to understand the potential benefits and costs of these annual movements and how individual variation in distance between breeding grounds and winter habitat may influence individual reproductive success.

There are many benefits to traveling south during the winter. Birds migrate to a latitude that provides food resources (*2*) and removes the threat of freezing temperatures that are a known cause of mortality in birds (*3*). Migratory individuals residing close to their breeding grounds and non-migratory individuals are exposed to colder winter temperatures and the associated thermoregulatory costs of maintaining their body temperature (*4*).

However, there are also benefits to a shorter migration distance. First, species staying near the breeding site avoid the energetic cost of traveling to and from a distant overwinter location. Birds must acquire large fat stores before they migrate that they quickly burn through on their way to their destination. Second, it has been found that the arrival of short distance migrants is more likely to correlate with regional breeding temperatures than long distance migrants (*5*). This indicates that short distance migrants may be more capable of shifting their lay dates in response to climate change (*6*).

While different species migrate different distances, individuals within a species often show variation in migration distance as well. Just as short distance species are better able to time reproduction than long-distance species, individuals of a species that live closer to their breeding site may be better able to assess the conditions found at the breeding grounds to properly time migration and reproduction than their longer-distance conspecifics. Previous work using banding and recapture data in red-winged blackbirds (*Agelaius phoeniceus*) provides snap shots of individuals that breed in multiple states. These records indicate that individuals from different age and sex classes migrate different distances and that females and younger individuals tend to travel longer distances than their male counterparts (*7*, *8*). However, individuals within the same sex and age class and from the same breeding site have also been shown to migrate different distances (Stonefish, unpublished). Identifying whether or not individuals from the same breeding area overwinter together is important to note because management strategies at a given overwinter roosting site may only affect a small portion of the targeted breeding population.

In addition to potentially allowing individuals access to external physical properties such as high-quality territories and mates, a shorter migration distance may provide physiological advantages. Lengthening photoperiods in the spring stimulate an increase of reproductive hormones and gonadal maturation (*9*) through the activation of the hypothalamic–pituitary–gonadal (HPG) axis. However, the process leading to full reproductive maturity in songbirds does not happen quickly. It takes approximately a month for a male to regrow his testes from a regressed state (*10*). It is also well documented that reproductive success in birds tends to decline throughout the breeding season with earliest breeding females typically producing the most offspring (*11*, *12*). Therefore, birds must begin breeding preparation early.

A shorter migration has been proposed as a mechanism for earlier laying (*13*) which may mean that hormones important for reproduction, such as testosterone and estradiol, should also be higher on arrival to the breeding grounds in individuals that travel a shorter distance. In support of this, studies have found that male and female song sparrows (*Melospiza melodia*) with a shorter migration distance may produce more testosterone early in the spring (*14*) and testosterone correlates with testes mass and spermatogenesis during migration in garden warblers (*Sylvia borin*) (*15*). Estradiol, a compound synthesized from testosterone and one of the most common estrogens (*16*), is necessary for both reproductive behaviors and physiological breeding preparation for female songbirds. Due to sampling constraints (e.g. blood volume from smaller birds), many studies on the trade-off between migration distance and reproduction have assessed testosterone and not estradiol. Even though testosterone has been researched more than estradiol, the role of testosterone and reproductive fitness in females warrants further research (*17*). Although testosterone is a precursor to estradiol, there may be a negative relationship between testosterone levels and reproductive success in females (*18*). Our study will measure baseline estradiol and testosterone in females. This will provide information on the relationship between those two hormones in our study system and if there is a relationship between migration distance and estradiol in females.

The carry-over effects of overwinter locations on reproduction has been conducted primarily in species that are predominantly monogamous (*14*, *19*, *20*). While polygyny has been noted in song sparrows (*Melospiza melodia*), prairie warblers (*Setophaga discolor*), and American redstarts (*Setophaga ruticilla*), monogamy is most commonly observed (*21*-*23*). Red-winged blackbirds are a highly polygynous species, with observations of up to 90% of males and 99% of females breeding in polygynous associations (*24*). In polygynous species, such as the red-winged blackbird, males with larger harems produce more offspring (*25*). Therefore, there is strong competition over territories capable of supporting multiple females. This creates an additional pressure to arrive to the breeding grounds in an endocrine state conducive to fighting and defending. In support of this, male red-winged blackbirds holding high-quality territories were sampled at multiple times throughout the breeding season and had, on average, higher testosterone levels than males with low-quality territories (*26*).

This study investigated the influence of migration distance, estimated using stable isotopes of hydrogen, on reproductive hormones by measuring the level of pre-breeding baseline and post-Gonadotrophin releasing hormone (GnRH) testosterone levels in males and baseline estradiol and testosterone in females. Baseline blood samples indicate circulating levels of testosterone or estradiol in individuals at the time of sampling. The levels of these sex steroid hormones fluctuate on daily endogenous rhythms (*27*, *28*), but they can also change due to social interactions (*29*, *30*). To remove daily variation and identify the maximum amount of testosterone that a male can produce at the time of sampling, we gave males an injection of GnRH. This stimulated the HPG axis and caused downstream production of testosterone (*31*). We tracked individuals throughout the breeding season to measure harem size and the number of fledglings produced for males and the number of eggs and fledglings produced for females.

Our study aims to further address the relationship between migration distance and reproduction. We hypothesize that the relationship between migration distance and reproduction will be magnified in a polygynous breeding bird and show that 1) within sexes, those migrating a shorter distance will have elevated hormone levels upon arrival, 2) females that travel a shorter distance will have earlier lay dates and fledge more offspring, and 3) males that travel a shorter distance will have larger harems and fledge more offspring.

Methods

Capture

We studied a population of red-winged blackbirds at the Alice Waterfowl Production Area (46° 46'36"N, 97° 30'31"W) near Alice, North Dakota during the spring and summer of 2018 and 2019. This site has ample, consistent, wetland habitat with large areas of cattail for red-winged blackbird breeding. This species is protandrous with males arriving to the breeding site approximately a month before females (*32*). In 2018, the first observed male arrived on 11 April and the first male was captured on 28 April. The first observed female was sighted on 21 April and the first female was captured on 03 May. In 2019, the first observed male was on 29 March and the first male was captured on 05 April. The first female arrived on 24 April and the first female was captured on 25 April. Elevated walk-in traps, ground walk-in traps, and mist nets were used to capture individuals early in the breeding season. We followed the reproductive success of birds in 11 male-territories in 2018 and 18 territories in 2019. Each captured bird received a unique combination of a USGS aluminum band and color bands for later identification during the nesting phase. We estimated age as second year or after second year for males using molt patterns and epaulette coloration, as older males typically have brighter epaulettes (*33*). All of our resident males were after second year. Female age was not included in our analysis; older females tend to have brighter epaulets, but this can also be influenced by diet (*34*). Measurements of tarsus length, wing chord, mass and the presence or absence of a brood patch were recorded for females. Measurements of tarsus length, wing chord, cloacal protuberance width, and mass were recorded for males. Only known breeders or individuals captured multiple times at our study site were used in the analyses.

Blood sampling and GnRH injections

A blood sample was taken from the brachial (wing) vein of each individual and collected in capillary tubes before the first lay date of the season. For females in both years, a blood sample was taken shortly after capture to measure testosterone and estradiol. For males in 2018, a baseline sample for testosterone was taken. In 2019, we took both a baseline and post-GnRH sample to measure testosterone in males 30 minutes after the GnRH injection was administered (see below). To prepare the GnRH, we diluted 5 mg of chicken GnRH (Bachem catalog number H-3106) with 200 ml of phosphate-buffered saline solution (PBS) to a final dilution of 25 ng/μL. This GnRH was injected into the pectoral muscle at a dose of 2 mg/kg. All samples were taken between 06:30 am and 11:00 am CDT.

Testosterone enzyme immunoassay

Testosterone was measured using a testosterone ELISA kit (Enzo Life Sciences ADI-900-065) following manufacturer's instructions. Briefly, 30 μL of plasma samples were extracted two times with ether. The extracted sample was then dried with N_2 gas at 25°C and reconstituted with 300 μL of assay buffer. The assay kit instructions were followed and all samples were run in duplicate (100 μL per well). Plates had intra-plate variation of 0.00%, 8.66%, 9.49%, 9.56%, 9.68%, 10.55%, 23.36%, and inter-plate variation of 14.57%. Samples with undetectable values were assigned the lowest value detectable by the kit which was 5.67 pg/mL (n = 2 for females and n = 0 for males). Only hormone values with a %CV of less than 10 were included for analysis (n = 3 males excluded).

17β-estradiol enzyme immunoassay

Estradiol levels were measured using a 17β-estradiol high sensitivity ELISA (enzyme-linked immunosorbent assay) kit (Enzo Life Sciences ADI-900-174) following manufacturer's instructions. Briefly, 100 µL of plasma samples were extracted three times with ether. The extracted sample was then dried with N_2 gas at 25°C and reconstituted with 250 µL of assay buffer. All samples were run in duplicate. The estradiol plates had an intra-plate variation of 4.35% and 10.64%, and an inter-plate variation of 7.86%. One sample with an undetectable value was assigned the lowest value detectable by the kit which was 14 pg/mL. Only hormone values with a coefficient of variation less than 10 were included for analysis ($n = 3$ females excluded).

Estimation of winter location

After blood sampling, we took a 2-3 mm claw sample. Claw samples were processed at the University of Regina. For δD analysis, dried washed claw material was weighed (c. 0.35 mg) into silver capsules and reduced to H_2 by reaction with Cr under helium flow at 1030 °C with a Eurovector 3000 (Milan, Italy – www.eurovector.it) elemental analyzer. The resultant H_2 gas was measured for δD in an Isoprime (Crewe, UK) continuous flow stable isotope mass spectrometer and corrected for H exchange using the comparative equilibration technique of Wassenaar and Hobson using two keratin calibrated standards: Caribou Hoof Standard (EC1, $\delta D = -197$ per mil) and Kudu Horn Standard (EC2, $\delta D = -54.1$ per mil) (*35*). All measurements are reported in δ-notation as the non-exchangeable feather H component in parts per thousand relative to the Vienna Standard Mean Ocean Water (VSMOW)–Standard Light Antarctic Precipitation (SLAP) scale. Based on replicate within-run measurements of standards, measurement error was estimated to be ± 2 per mil.

Claw samples provide a stable hydrogen isotope ratio (δD). These ratios vary in the United States from the southeast to the northwest portion of the country (*36*) and provide an estimation of the overwinter location where the claw was grown (*37*). A study in warblers estimated that claw samples provide information on the pre-migratory habitat for at least three to four weeks after arrival (*38*) and a study in song sparrows (*Melospiza melodia*) found that their claw tips represented tissue grown three months prior to sampling (*39*).

In the summer of 2018, PinPoint10 tags (<1.4g), a store-on-board GPS logger, was placed on 21 females and 8 males using a leg-loop harness (*40*). We recovered 12 of the 29 tags for a recovery rate of 41%. Data was recovered from 5 GPS tags (2 males and 3 females) the following summer. The data from these tags were used to investigate the relationship between δD values and the distance to the winter site (using the farthest distance from the GPS points) and δD and the most southern latitude from GPS points.

Reproductive observations

We searched cattail wetlands for nests and observed nests throughout the breeding season to investigate the relationship between reproductive fitness and migration distance. Once a nest was located, it was checked every three days to determine clutch initiation and completion. Parents were identified by capturing the females in a nest trap (*41*) and by noting which male and female defended the nest as an observer approached. During incubation, each nest was visited 3, 6, 9, and 12 days after the first egg was laid until hatchlings were observed. If hatchlings were not present on day 12, we returned 3 days later. Upon hatching, individuals were weighed to confirm hatch date (*42*). Nests were visited on nestling days 3, 6, 9, and 12. Mass and tarsus length were measured on day 6. The nestling period ranges from 9-12 days with an average of 12 days (*43*). The number of fledglings were calculated based on the number of nestlings in the nest on the day 9 check. However, the nest was recorded as producing zero nestlings if the nest showed evidence of depredation at or before the day 12 check. A nest found with only cowbird eggs in it was not included in the harem number calculated for males (n = 2). Additionally, nestlings that were suspected to be brown-headed cowbird nestlings (if more nestlings hatched than there were red-winged blackbird eggs in the nest) were not included in the number of fledglings produced by a parent (n = 5).

Statistical analysis

All statistical analyses were performed using R version 3.5.2 (*44*). All hormone values were log-transformed to meet assumptions of a normal distribution. Fisher's F test was used to test for equal variance among groups. Differences in female and male δD values were examined using a Student's t-test to determine if males and females are traveling different distances in the spring. To confirm that migration distance is related to the δD values, we ran a linear model to look at the relationship between the δD values and spring migration distance using data from 5 GPS tags. The model includes distance to the overwinter site (using the GPS point that is the farthest distance from the breeding site) as the independent variable and δD as the dependent variable.

Linear models for females examined the relationship among the dependent variables of pre-breeding hormones levels, baseline estradiol and testosterone, with the independent variables of δD values, capture date, and year as fixed effects. Linear models for males examined the relationship among the dependent variables of baseline testosterone with the independent variables of δD values, capture date, and year as fixed effects. Linear models for males examined the relationship among the dependent variables of GnRH-induced testosterone with the independent variables of δD values and capture date. Year was not included in the model for

GnRH-induced testosterone because this variable was only measured in 2019. Linear mixed models were also used to investigate the relationship between the dependent variables of capture date, harem size, and number of young produced with the fixed effect δD values. To examine the relationship between baseline testosterone and estradiol for a female, we ran a linear model with estradiol as the dependent variable and testosterone as the independent variable. Bird ID was not included in linear models as a random effect because there were only 4 males and 2 females with δD values in both 2018 and 2019.

Results

Migration distance and pre-breeding hormones

There was equal variance in male and female stable isotope values (F = .50). A Student's t-test revealed that males on average traveled a significantly shorter distance than females as estimated by the δD ($p < 0.001$, t = 7.0635, $df = 57$) as shown and described in Ch. 2 (Figure 3, page 32). Males with δD values that indicate they traveled a shorter distance have higher baseline testosterone ($p < 0.05$, $F = 2.7$, $df = 3, 25$, n = 29, adjusted $R^2 = 0.16$). There was no significant relationship between male post-GnRH testosterone and δD values ($p = 0.78$, $F = 2.71$, $df = 2, 12$, n = 15, adjusted $R^2 = 0.20$) but the day of capture was significant in that model ($p < 0.039$). Later captures had higher GnRH response than earlier captured males. There was no relationship between δD values and baseline estradiol ($p = 0.36$, $F = 7.82$, $df = 3, 11$, n = 15, adjusted $R^2 = 0.59$) or baseline testosterone ($p = 0.84$, $F = 3.82$, $df = 3, 21$, n = 25, adjusted $R^2 = 0.26$) in local females, but year was significant in both models with values $p < 0.001$ and $p < 0.005$, respectively.

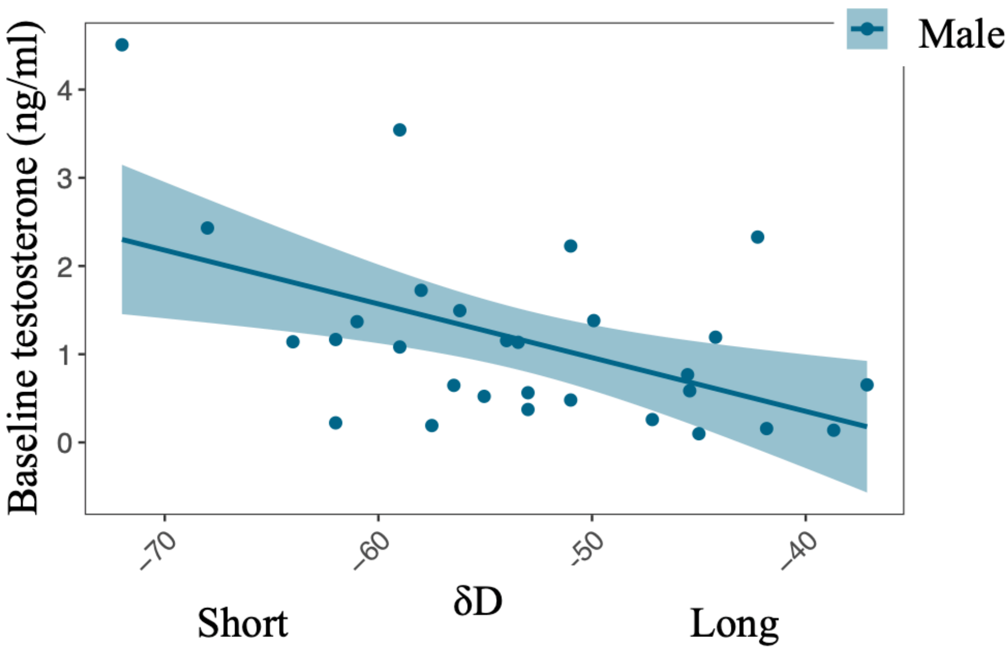

Figure 5. Relationship between baseline testosterone levels upon arrival to the breeding site and δD values from claw tips in local males in 2018 and 2019. Males that travel a shorter distance during spring migration have higher early season baseline testosterone than males that travel a longer distance ($p < 0.05$, $F = 2.7$, $df = 3, 25$, n = 29, adjusted $R^2 = 0.16$).

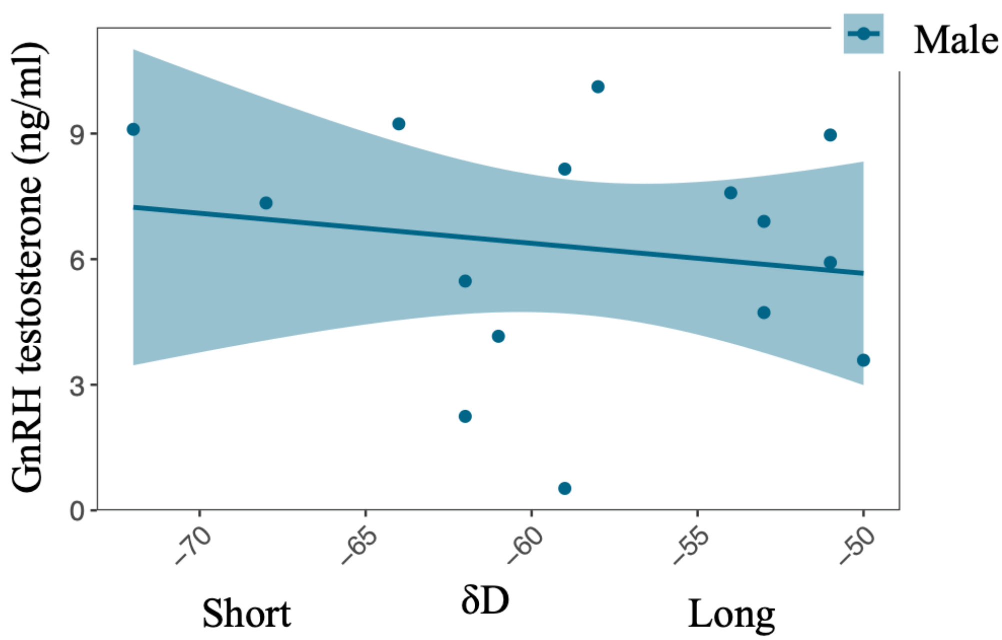

Figure 6. Relationship between GnRH-induced testosterone levels upon arrival to the breeding site and δD values from claw tips in local males from 2019. GnRH-induced testosterone values of resident males do not correlate with migration distance ($p = 0.78$, $F = 2.71$, $df = 2, 12$, n = 15, adjusted $R^2 = 0.20$).

Baseline levels of testosterone and estradiol for an individual female were compared and lower baseline levels of testosterone were correlated with higher levels of estradiol ($p < 0.05$, F = 4.856, df = 1, 12, n = 14, adjusted $R^2 = 0.23$) (Fig. 4).

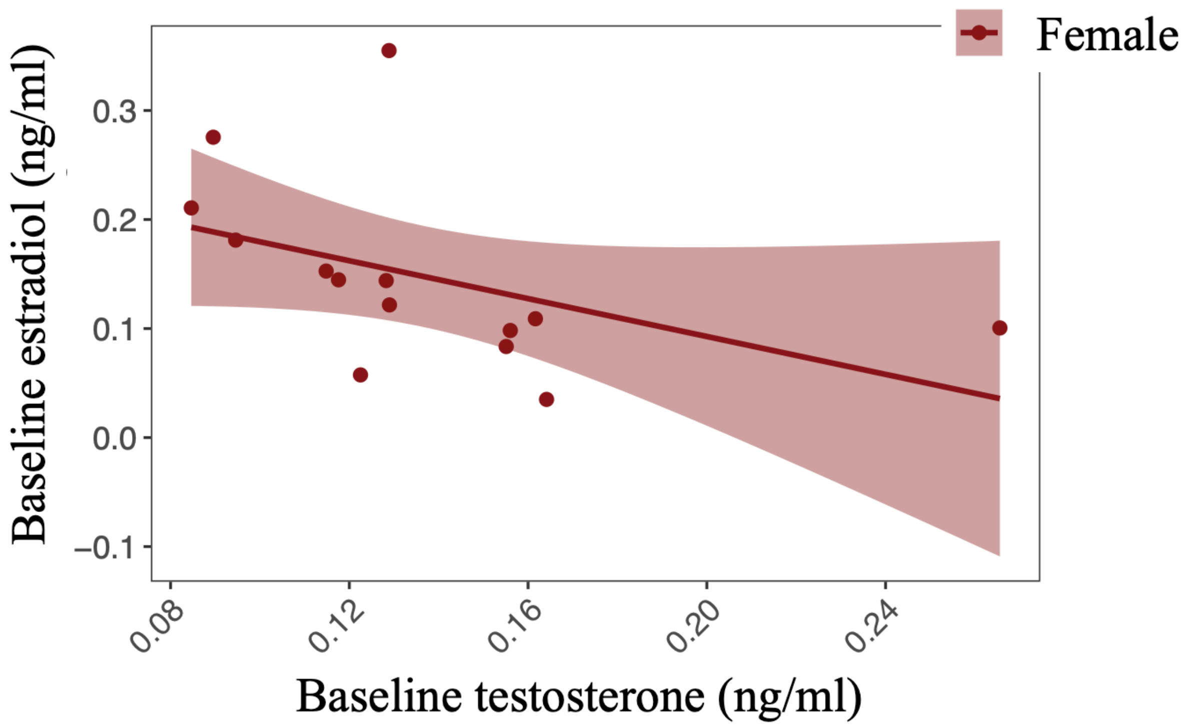

Figure 7. Relationship between baseline estradiol and baseline testosterone levels upon arrival to the breeding site in local females in 2019. Resident females with lower testosterone values have increased estradiol levels ($p < 0.05$, $F = 4.856$, df = 1, 12, n = 14, adjusted $R^2 = 0.23$).

Migration distance and reproductive success

Of all of the nests that we followed in 2018, there was a nest failure rate of 42%. In 2019 there was a nest failure rate of 54%. These numbers are for all nests that we followed in our nesting population and include nests where we were not able to confirm parent ID. We used linear models to investigate the relationship between δD and reproductive fitness. Female δD values as an independent variable were not correlated with the dependent variables lay date ($p < 0.13$, $F = 2.527$, df = 1, 15, n = 17, adjusted $R^2 = 0.09$,), number of eggs ($p < 0.61$, $F = 0.27$, df =

1, 16, n = 18, $p < 0.61$, adjusted $R^2 = -0.04$), or number of fledglings ($p < 0.88$, $F = 0.02$, $df = 1, 16$, n =18, adjusted $R^2 = -0.06$) in their respective models.

Similarly, linear models were used to investigate the relationship between δD and reproductive fitness in males. δD values and year were included as fixed independent effects with harem size and number of fledglings as dependent variables in their respective models. δD values were not related with harem size ($p = 0.54$, $F = 2.75$, $df = 2, 16$, n = 19, adjusted $R^2 = 0.16$), but year was significant in that model ($p < 0.04$) with larger harems occurring in 2018. δD values were also not significant in the model for number of fledglings produced ($p < 0.21$, $F = 3.90$, $df = 2, 16$, n = 19, adjusted $R^2 = .24$), but year was significant in that model as well with more fledglings produced in 2018 ($p < 0.025$).

Stable isotope values compared with GPS points

We ran a linear model to look at the relationship between the δD values and spring migration distance using data from the 5 GPS tags. One model includes distance to the overwinter site (using the GPS point that is the farthest distance from the breeding site) as the independent variable and δD as the dependent variable. Individuals traveling from a shorter distance had more negative δD values than individuals that traveled a longer distance ($p < 0.05$, $F = 10.59$, $df = 1, 3$, n = 5, adjusted $R^2 = 0.70$) This model confirmed that δD values in this small subset of samples were significantly related to migration distance.

Discussion

As predicted, we did find that males and females had significantly different δD values. Female values indicated more southern overwinter locations. This follows the trend that was found in previous studies. This difference in migration distance for males and females could be due to sexual dimorphic size differences between males and female in this species (*7*) because males weigh approximately 50% more than females (*45*) and larger body size may provide benefits for heat conservation (*46*). Males may also stay closer to the breeding ground overwinter in order to arrive early and claim the best territories (*8*).

We hypothesized that for both males and females, individuals migrating a shorter distance would arrive with higher levels of reproductive hormones (estradiol and testosterone), suggesting earlier onset of reproductive readiness than conspecifics within their sex that migrated a longer distance. While this pattern was true for baseline testosterone in males, there was no correlation between δD and GnRH-induced testosterone in males or δD values and baseline reproductive hormones in females. The relationship between higher baseline testosterone levels in males that migrated a shorter distance follows what has been found in a previous study in song sparrows (*14*), and these differences in testosterone levels could occur because, as seen in black-and-white warblers (*Mniotilta varia*), males are preparing to breed during migration (*47*). This was predicted due to the fact that male red-winged blackbirds holding a territory tend to have higher levels of testosterone than floater males (*26*).

Although there was a relationship between baseline testosterone and migration distance, there was no relationship between GnRH-induced testosterone and migration distance. This result was unexpected given that GnRH levels are repeatable and useful for assessing relationships between testosterone and traits of interest (*27*, *48*). Our results indicate that individuals traveling from different distances are capable of producing similar amounts of testosterone, but that individuals traveling from a shorter distance are producing more testosterone at their baseline. Potentially this is occurring because individuals that travel a shorter distance are higher quality males which arrive early and acquire high-quality territories. These males may need to defend this coveted resource more than males with low-quality territories and these aggressive interactions may have resulted in these males having higher levels of testosterone when they were sampled. Unfortunately, our study did not collect data on territory disputes to confirm or refute that hypothesis.

Looking past hormonal levels to the reproductive success of males, a shorter migration distance did not influence the harem size or number of fledglings produced per territory. Previous studies have shown that males with higher levels of baseline testosterone have larger harems and show a trend toward fledging more offspring (*49*). However, we did not find a relationship between baseline or GnRH-induced testosterone and harem size or fledglings produced.

Migration distance in females was not correlated with baseline testosterone, estradiol, lay date, number of eggs, or number of fledglings. Additionally, early spring baseline reproductive hormones were not correlated with any of our measures of reproductive fitness in females, although previous studies indicated that females migrating a shorter distance will have increased testosterone levels (*14*). In white-crowned sparrows (*Zonotrichia leucophrys*), it was found that ovaries and testes had begun development when individuals arrived to the breeding ground, but they did not fully develop until after arrival during territory establishment and courtship (*50*). Similarly, levels of testosterone and estradiol have been found to be low in newly arrived females (*51*). It is possible that the levels of testosterone and estradiol found in females when they first arrived are similar because their HPG axis is not active during migration (*52*). Future work is needed to address whether later in the season there would be a difference once their HPG axis is elevated.

In females, testosterone levels were inversely correlated with estradiol levels. Females with lower levels of testosterone had higher levels of estradiol. The relationship between estradiol and testosterone is interesting given that less is known about estradiol in wild songbirds during the breeding season. In pooled, post-GnRH plasma samples, it has been found that individuals with higher estradiol levels also had higher testosterone levels (*53*) but another study in the same species did not show a relationship between estradiol and testosterone in post-GnRH blood samples for individuals (*54*). The negative relationship between testosterone and estradiol in our study indicates that testosterone may not be a reliable indicator of HPG activity early in the spring. However, this relationship is complicated and will require further research of both baseline and post-GnRH testosterone and estradiol levels. This information will help future researchers decide whether testosterone, estradiol, or the levels of both are needed to answer questions about early breeding season reproductive preparedness and migration distance.

While we predicted that the trade-off between reproduction and migration distance would be stronger in our polygynous species than in previous work done in monogamous species, this did not occur. Our study demonstrates that migration distance may influence reproductive hormones upon arrival to the breeding grounds in males in a polygynous species, but females showed no relationship between migration distance and reproductive hormones or reproductive success. Among other factors, the northern location of our field site may have added some additional barriers to seeing this relationship. It is possible that individuals were staging at early spring stopover locations and arrived at the breeding site in a similar breeding state. Future work is needed to determine the role of breeding site latitude in the trade-off between spring migration distance and reproduction.

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CHAPTER 4. CONCLUSIONS AND FUTURE DIRECTIONS

Conclusions

The story of the passenger pigeon (*Ectopistes migratorius*) is well known in the United States. Estimates indicate that individuals of this species composed a quarter of the continent's avifauna at one point (*1*) but from 1870 to 1899, the species plummeted to extinction. The cause of this extinction could have potentially been linked to loss of habitat and interference with nesting colonies (*2*), but this is still under debate among researchers. Similarly, today, red-winged blackbirds are known as one of the most common birds in North America, breeding across the United States and Canada. However, their population numbers, along with other bird species, have been decreasing during the last half century (*3*). The cause of red-winged blackbird decline is hypothesized to be linked to habitat loss and population control (*4*).

Our research provides valuable baseline information on migration patterns of individual red-winged blackbirds breeding in the Prairie Pothole Region of eastern North Dakota. Further, this research addresses relationships between migration distance and reproduction. We found that red-winged blackbirds breeding together in the summer disperse to broad overwintering areas, even within the same sex. Our five GPS tagged individuals breeding in the same cattail wetlands in Alice, North Dakota, including two male and three female red-winged blackbirds, could be found in Texas, Oklahoma, Missouri, and Arkansas in January. Stable isotope values, serving as a proxy for latitude of overwinter locations, also showed variation that indicates both males and females travel from a range of overwinter locations. This relates to previous work conducted with geolocators that showed males breeding in the same county as our field study, Cass County, overwintered in Missouri (4), Oklahoma (2), Texas (2), Louisiana (2), Mississippi (1), Arkansas (1), and Illinois (1) (Stonefish, unpublished; see Appendix F). Males cause more sunflower damage than females (*5*), so differences in migration patterns between males and females, especially in the fall, may be important to sunflower producers. These differences in migration distance are also likely important for conservation efforts and future research studies.

In a previous study by Gammell et al., a single female banded in Kenton, North Dakota sometime between May and October was found spending the winter in Colorado County, Texas (*6*). Our study also showed that two of the three females with GPS tags resided overwinter in that general area. One female spent time in Colorado County and the other was located in the adjacent Fort Bend and Wharton Counties. Stonefish (unpublished) also showed that a male was overwintering in southern Texas. This region may be important for understanding local red-winged blackbird population in North Dakota.

Young of year blackbirds contribute a significant portion of the local late season population that consume sunflower seeds in the fall (*5*). Due to this, we asked if migration distance/overwinter locations may relate to production of young of year birds. We found that migration distance was positively correlated with baseline testosterone in males, but not in post-GnRH testosterone levels. There was not a relationship between migration distance and the reproductive hormones estradiol and testosterone for females. We also did not find a relationship between an individual's migration distance and their reproductive success. Migration distance did not significantly relate to the harem size and fledglings produced for males or lay date and fledglings produced for females.

Future directions

Future research using GPS, geolocator data, or other tracking equipment should continue building on our knowledge of red-winged blackbird migration patterns, especially in females, to better understand the carryover effects of overwinter habitats and migration differences on reproduction. There may be specific locations that are crucial for the red-winged blackbird populations in general that provide important information for red-winged blackbird population dynamics and inform future management strategies. Additionally, our study on reproduction and migration in red-winged blackbirds could be replicated at a more southern breeding ground. In our study, few measurements of reproductive success were correlated with migration distance. This could be because individuals had long stopovers before they arrived at the northern breeding grounds. It is possible that red-winged blackbirds breeding at a more southern breeding latitude could provide some insight into if individuals of northern breeding populations arrive at the breeding grounds in more similar condition than southern breeding populations.

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APPENDIX A. GPS SCHEDULES

Females - One point was taken on the day of deployment. A point was taken every 10 days from August 1st until December 11th. From December 17th until February 16th, a point was taken every 6 days. From February 19th until capture, a point was taken every 3 days.

Males - One point was taken on the day of deployment and once every 21 days over the summer until August 1st. A point was taken every 11 days from August 3rd until September 15th. From September 15th until October 1st, a point was taken every 4 days. From October 1st until December February 4th, a point was taken every 7 days. From February 5th until capture, a point was taken every 4 days.

APPENDIX B. HORIZONTAL DILUTION OF PRECISION (HDOP) VALUES FOR PINPOINT10 GPS TAGS AND THEIR RELIABILITY.

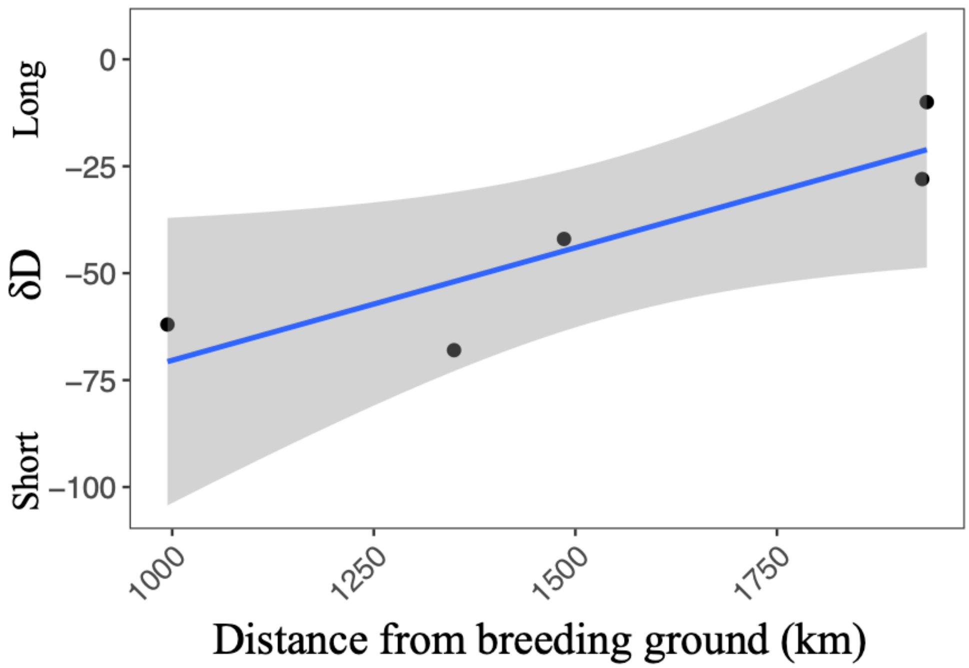
Description of DOP Values

DOP Value	Rating	Description
< 1	Ideal	Highest possible confidence level to be used for studies demanding the highest possible precision at all times.
1 to 2	Excellent	At this confidence level, positional measurements are considered accurate enough to meet all but the most sensitive study requirements.
2 to 5	Good	Represents a level recommended as minimum for good study data.
5 to 10	Moderate	Positional measurements could be used for calculations, but the fix quality could still be improved. A more open view of the sky is recommended.
10 to 20	Fair	Represents a low confidence level. Positional measurements should be discarded or used only to indicate a very rough estimate of the current location.
>20	Poor	At this level, measurements are inaccurate by as much as 300 meters with a 6-meter accurate device (50 DOP × 6 meters) and should not be used.

APPENDIX C. STATE LOCATIONS OF RED-WINGED BLACKBIRDS THROUGHOUT THE YEAR BASED ON GPS DATA

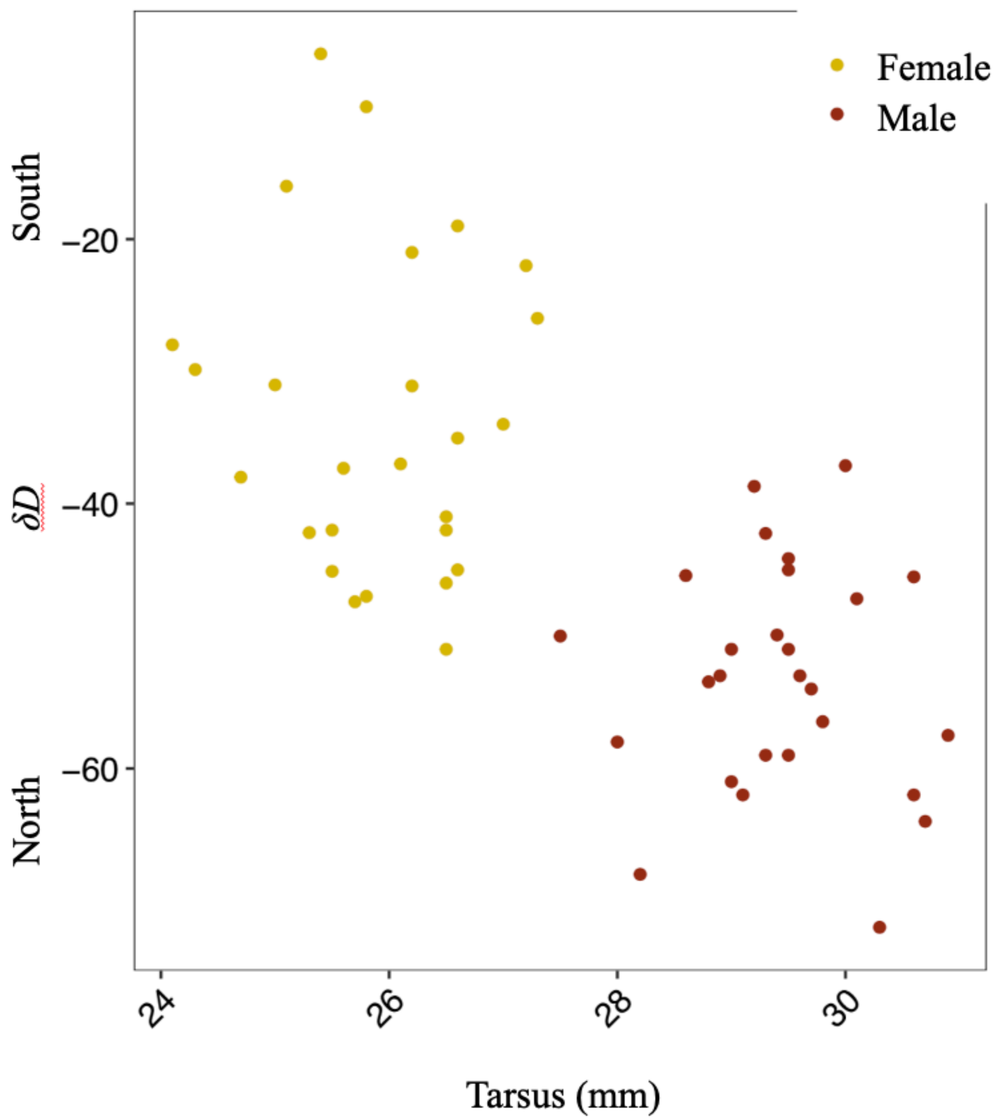
Month	Females	Males
January 2019	Texas, Oklahoma	Missouri, Arkansas
February 2019	Texas, Louisiana, Oklahoma	Missouri, Arkansas
March 2019	Texas, Oklahoma, Kansas	Oklahoma, Missouri, Iowa, Minnesota, South Dakota, and North Dakota
April 2019	Texas, Oklahoma, Kansas, Iowa, Minnesota	Minnesota, North Dakota
May 2019	Iowa, Minnesota, North Dakota	North Dakota
June 2018	North Dakota	North Dakota
July 2018	North Dakota	North Dakota
August 2018	North Dakota	North Dakota
September 2018	South Dakota, North Dakota	North Dakota
October 2018	Minnesota, South Dakota, North Dakota	Minnesota, South Dakota, North Dakota
November 2018	South Dakota, Iowa, Missouri, Oklahoma, Arkansas, Texas	North Dakota, Minnesota, Missouri, Arkansas
December 2018	Oklahoma, Arkansas, Texas	Missouri, Arkansas

APPENDIX D. OVERWINTER DISTANCE FROM THE BREEDING LOCATION PROVIDED BY GPS DATA LOGGERS AND CORRELATED ΔD VALUES PROVIDED BY CLAW SAMPLES



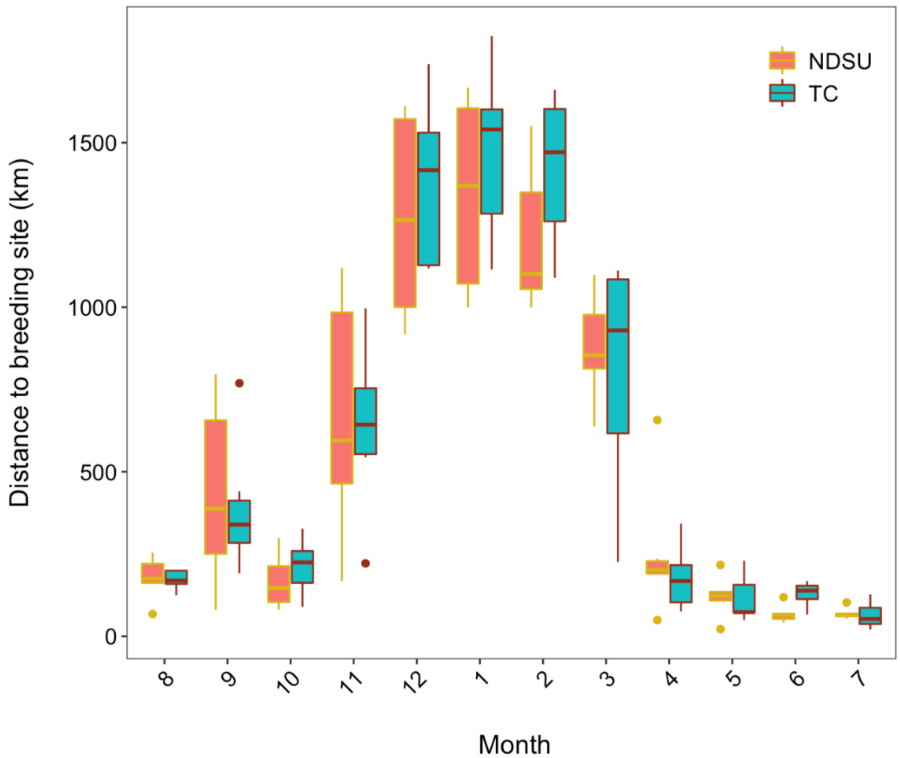
This indicates that δD values from claw tips collected soon after arrival are correlated with spring migration distance in red-winged blackbirds ($p < 0.05$, $F = 10.59$, $df = 1, 3$, n = 5, adjusted $R^2 = 0.70$).

APPENDIX E. RELATIONSHIP BETWEEN TARSUS LENGTH (MM) AND OVERWINTER LATITUDE AS ESTIMATED BY STABLE ISOTOPES OF HYDROGEN



There is not a relationship within sexes between overwinter latitude, estimated by stable isotopes of hydrogen, and tarsus length. However, males (n = 27), which have larger tarsus measurements than females, spend the winter in a more northern location than females (n = 26) ($p < .001$, $F = 37.87$, $df = 1, 51$, n = 53, adjusted $R^2 = .41$).

APPENDIX F. THE AVERAGE DISTANCE THAT A MALE IS FROM THE BREEDING GROUND IN A GIVEN MONTH BASED ON GEOLOCATOR DATA (N = 13) FROM 2010-2011



Individuals have been grouped by their breeding sites. One of these breeding sites was located near NDSU (orange) and the other breeding site was located near Tower City, North Dakota (noted as TC in the legend and shown in blue).