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# STABLE HYDROGEN ISOTOPE ANALYSIS OF AMERICAN REDSTART RECTRICES

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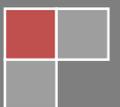
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## Abstract

Understanding avian migration patterns between breeding, stopover, and wintering sites is crucial for the creation of conservation efforts. Stable isotope analysis is rising in popularity due to its ability to track avian migration without the use of satellites and traditional banding methods. Hydrogen isotope signatures vary by latitude due to fractionation during evaporation of ocean water. As warblers molt on their breeding grounds, the hydrogen isotope signatures are incorporated into their growing feathers through trophic-level interactions. We used a stable hydrogen isotope analysis of American Redstart (*Setophaga ruticilla*) rectrices using feather samples collected from the Powdermill Avian Research Center in Pennsylvania. We found no significant difference in  $\delta D$  values and migration timing between the two sample years (2010 and 2011). A significant difference in migratory patterns between adult male and adult female warblers was present, where males appear to migrate before their female counterparts. This pattern may be a result of the need for females to recover after the breeding season and gain fat reserves before strenuous migration. Within female migration, juvenile females arrived at the stopover site significantly earlier than adult females. There was no significance between juvenile male and juvenile female migration as well as adult male and juvenile male migration. This pattern may be the result of the competition for desirable wintering grounds by all subgroups of redstarts. This information is important in the conservation of Neotropical migratory birds in light of the recent decline of many species. If scientists can connect breeding, stopover, and wintering sites, they can create conservation efforts to protect those specific areas important for helping passerines thrive.

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## Executive Summary

American Redstarts (*Setophaga ruticilla*) are one of the most numerous New World warbler species found in North America. They are easily distinguishable by their distinctive appearance and high-pitched vocalizations during their breeding season (Sherry and Holmes 1997). They breed over much of northern, northeast, northwest, eastern, and southern regions of North America as well as Canada, and winter in the Caribbean, especially Greater Antilles and western Mexico (Pashley and Martin 1988; Sherry and Holmes 1997). Their breeding habitats include deciduous woodlands and secondary forests (Sherry and Holmes 1997).

In eastern North America, fall adult migrants and juveniles follow a route along the coastal plain between the Appalachian Mountains and the West Atlantic Ocean (Sherry and Holmes 1997). Migration is an extremely taxing process for passerines and demands a high amount of energy. Many migratory birds must replenish and restore their energy at locations called stopover sites (Bayly et al. 2012). Stopover sites are areas along migration paths that allow migratory birds to rest and regain fat reserves lost while in transit (Johnson and Winker 2008).

In the last fifty years, American Redstart populations have declined slightly within their widely distributed breeding regions (-0.34% per year, Sauer et al. 2014; Sherry and Holmes 1997). Within some fragmented and urbanized landscapes, American Redstart populations have declined mainly due to the deterioration of breeding habitats (Peterjohn 1989; Sherry and Holmes 1997). Many strategies are available to track bird migration. The emergence of stable isotope analysis (SIA) has aided researchers to successfully study avian migration patterns like that of American Redstarts (Inger and Bearhop 2008; Wunder 2012). SIA enables researchers to understand how breeding populations of birds move between their breeding, stopover, and wintering habitats which

is critical in the creation of conservation efforts to protect vulnerable or declining species like American Redstarts (Bowen 2010).

The goal of our study was to examine the relationships between breeding latitudes and the timing of migration of American Redstarts. In this study, we sought to: (1) determine if more northern populations migrate first (Paxton, 2007), (2) determine if adult male American Redstarts arrive earlier than adult females and if juvenile males arrive earlier than juvenile females (Tottrup and Thorup 2008), and (3) determine if female juvenile American Redstarts migrate later than their adult female counterparts and if juvenile male American Redstarts migrate later than their adult male counterparts (McKinnon et al. 2013).

Our study measured stable isotope ratios of hydrogen in the tail feathers of American Redstarts in order to examine the relationship between breeding latitudes and the timing of migration of American Redstarts. Successful conservation efforts aim to preserve American Redstart's natural habitat at breeding and wintering grounds, as well as stopover sites. Without information on the location of these habitats and timing of American Redstart migration, this task is impossible (Masalaite 2012). SIA has the potential to fix this problem.

In this study, Dr. Andrew Vitz, Massachusetts State Ornithologist, and his collaborators collected feather samples at the Powdermill Avian Research Center (PARC) in Pennsylvania during fall migration in 2010 and 2011. Researchers collected one outer rectrix from each bird, placed a USGS bird band onto their ankle, and recorded pertinent data such as date, sex, and age. We chose to study American Redstarts due to the large sample size of rectrices PARC provided. We used tail feathers due to their large size in relation to body feathers. We prepared the feather samples in the Worcester Polytechnic Institute Project Laboratory using the washing and weighing

protocols provided by the Colorado Plateau Stable Isotope Laboratory (CPSIL). Once washed, we sent the feather samples to CPSIL in order to determine the hydrogen isotope ratios.

Once we obtained the  $\delta D$  values, we performed various statistical analyses. We started by transforming  $\delta D$  values to fit a precipitation isoscape created by Meehan et al. (2004) ( $\delta D_p$ ). We designed an American Redstart Breeding Group Distribution ISOMAP to identify the breeding areas of our species. We used a previously made isoscape of growing season precipitation with 10% contours (Meehan et al. 2004). We ran a Shapiro-Wilk test to check for data normality and a t-test to check for differences between the  $\delta D$  values from 2010 and 2011. We used box and whisker plots to observe the relationship between age and migration dates. In these box and whisker plots, we observed juvenile and adult birds of both sexes separately, as well as male and female birds of each age group separately. To examine if age was related to arrival date, we ran separate t-tests for adult and juvenile males and for adult and juvenile females, with age as the dependent variable and Julian date as the independent variable. Before we ran t-tests, we ran F-tests for two-sample variance. To examine if sex had a relationship to arrival date, we repeated these tests on adult and juvenile males and females.

We found no statistical difference between the hydrogen isotope values from feathers collected in the two different years ( $t_{99} = -1.21$ ,  $P = 0.230$ ) thus we pooled the data for both years ( $n = 101$ ). No statistical significance was found between the Julian dates and  $\delta D_p$  values ( $F_{1, 99} = 0.49$ ,  $P = 0.487$ ), although a slight positive relationship was visible. This could indicate that northern breeding locations (more negative  $\delta D_p$ ) migrated later than southern breeding locations (less negative  $\delta D_p$ ). We found no statistical difference between the timing of migration for juvenile males and adult males ( $t_{55} = 1.260$ ,  $P = 0.213$ ). We found a significant difference in the arrival time of adult female and juvenile female redstarts, with juveniles arriving earlier than their adult

counterparts do ( $t_{42} = 2.649$ ,  $P = 0.011$ ). This could be due to adult females needing more time to recover and gain fat reserves after breeding and before starting fall migration. In addition, juveniles may leave early to avoid dominant adults on breeding grounds (Woodrey and Chandler 1997). Morris and Glasgow (2001) observed this pattern in their study on passerine migration, specifically with American Redstart female juveniles migrating significantly earlier than their adult female counterparts do. We found a significant difference in arrival time at stopover sites of adult males and females ( $t_{37} = -3.046$ ,  $P = 0.004$ ), with adult males arriving at the stopover site before adult females. This could indicate that females need more time to recover and gain fat reserves after a late breeding season. We found no significant difference in arrival time between juvenile males and females ( $t_{57} = 0.638$ ,  $P = 0.526$ ). The majority of our samples came from the -99 to -50  $\delta D_p$  area, the southern portion of the areas studied. The northern populations, -100 to -118  $\delta D_p$ , may have had a smaller sample size in our study due to northern populations taking a coastal route of migration, and therefore bypassing PARC. Within our data, only 3% of the redstarts migrated from -119 to -110  $\delta D_p$ , and no redstarts originated in the -109 to -100  $\delta D_p$  range. Norris et al. (2006) found that many adult redstarts molted south of their breeding habitat, accounting for the lack of adults in the northern contours.

From the isotopic ratios collected, the breeding distribution of American Redstarts included Southern Canada, the Great Lakes states, Maine, and Alaska. Although the exact location of these birds is difficult to pinpoint, previous studies based the potential origin areas on past research of known American Redstart breeding locations and the north-south migration patterns of passerine birds (Sherry and Holmes 1997). We suspect that some species could have come from outside the mapped area due to previous data collected at PARC.

In conclusion, this study on American Redstarts can provide researchers with valuable knowledge on the connectivity of breeding, wintering, and stopover habitats as well as important migratory information. This will allow for the creation of conservation efforts such as the preservation of American Redstart natural habitats and locating critical migratory habitats for declining populations. Without information from this study and other similar studies, these tasks would be impossible. This study can help us take a step in the right direction to uncovering the missing links between migratory patterns and conservation efforts.

# Chapter 1

## Problem Statement

Neotropical migratory birds are a subcategory of birds that breed within the United States and Canada where they also migrate to Mexico, Central America, South America, and the Caribbean for the winter (Rohwer et al. 2009). Some of these birds include plovers, warblers, hawks, cranes, sparrows, and terns (Finch 1991). There are 341 Nearctic-Neotropical migratory birds breeding regularly in the United States and Canada (Cotton et al. 2009; Rohwer et al. 2009). Out of these 341 species, 127 have shown a significant decline in their population (Cotton et al. 2009). In addition, 60 of these species have seen a decline of 45% or more of their population in the past 40 years (Cotton et al. 2009).

Neotropical migratory birds face many threats including loss and fragmentation of their breeding, staging, stopover, and wintering habitat (Kirby et al. 2008). This loss and fragmentation is partially due to habitat degradation, development, deforestation, and land conversion (Kirby et al. 2008). Other threats include collisions with tall structures, such as buildings and antennae, poisoning by chemical toxins, climate change, and predation (Kirby et al. 2008). All of these threats either directly or indirectly affect birds during their migration. A bird may be directly affected by a large antennae or building, or indirectly affected by the destruction of their intended wintering or breeding ground (Kirby et al. 2008).

Although some Neotropical birds have an uncertain future, scientists have established conservation efforts to slow population declines (Finch 1991). The Neotropical Migratory Bird Conservation Act (NMBCA), established in 2000, has spread knowledge through their funding of research, monitoring of projects, and support of conservation initiatives internationally (Cotton et al. 2009). These projects span from the United States and Canada, to Latin America and the

Caribbean (Cotton et al. 2009). In 2002, the U.S Fish and Wildlife Service joined the conservation movement with their financial support (Cotton et al. 2009). The NMBCA also collaborates with Partners in Flight, the North American Bird Conservation Initiative, the U.S. Bird Conservation Joint Ventures, and the U.S. Shorebird Conservation Plan (Cotton et al. 2009). The NMBCA has invested money for projects in Puerto Rico and other Caribbean countries to protect American Redstart's and other migratory bird's habitats (Cotton et al. 2009).

### **American Redstart**

Distributed throughout the world, warblers are small, perching songbirds containing two families, one in the New World (*Parulidae*) and one in the Old World (*Sylviidae*) (Freedman 2014). Old World warblers live in Europe, Asia, and Africa, while New World warblers, a family of 126 species, live within the Western Hemisphere, or Americas (Freedman 2014). New World warblers are small birds with nine primaries, or outer flight feathers, and often have colorful plumage (Mertz 2004). Found in North America and primarily during the breeding season, American Redstarts (*Setophaga ruticilla*) are one of the most numerous New World warbler species (Sherry and Holmes 1997). They are easily distinguishable due to their distinctive appearance and high-pitched vocal presence during their breeding season (Sherry and Holmes 1997).

American Redstarts are identified as Neotropical migrants due to their nesting in the United States and Canada ("Nearctic" region) and migration south to the tropical regions of Mexico, Central America, South America, and the Caribbean ("Neotropics") for the non-breeding season (U.S. Fish and Wildlife Service Forensics Lab 2015). American Redstart populations are locally abundant within their breeding range, which contains ideal habitat qualities (Sherry and Holmes 1997). American Redstarts' breeding grounds cover much of the northern, northeast, northwest,

eastern, and southern regions of North America (Figure 1) (Sherry and Holmes 1997). However, with very small numbers breeding occasionally along the northwestern United States, the breeding distribution remains inconsistent. The most abundant breeding regions include habitats located in southern Canada, northern parts of the Great Lakes states, and the northeastern United States through New York, Vermont, New Hampshire, and Maine (Price et al. 1995). These breeding habitats include secondary forests and deciduous woodlands (Sherry and Holmes 1997).

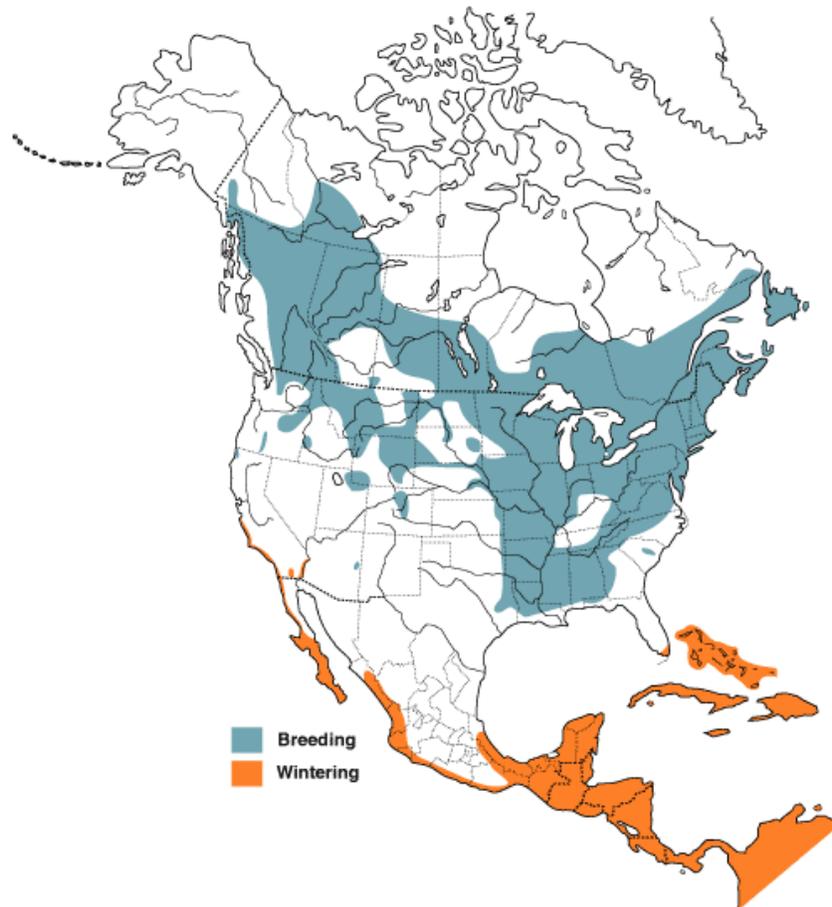


Figure 1: Distribution of American Redstart (Sherry and Holmes 1997)

In the non-breeding season, American Redstarts spend their time in low to mid elevation forest habitats. Wintering habitats span southern Baja California, southern and western Mexico, southern Florida, the Bermuda and Bahama Islands, southern Middle America (including offshore

Caribbean islands), and northern South America including Venezuela, Colombia, Ecuador, and Brazil (Sherry and Holmes 1997). As seen in Figure 1, the most abundant non-breeding regions include habitats located in the Caribbean, especially Greater Antilles and western Mexico (Pashley and Martin 1988; Sherry and Holmes 1997). These non-breeding habitats include woods, primary forests, secondary forests, plantations, and isolated trees in urban areas (Sherry and Holmes 1997).

In the fall, these warblers begin departing breeding areas in early July and arrive on the coast of the Gulf of Mexico in late July (U.S. Fish and Wildlife Service Forensics Lab 2015). However, most redstarts pass through the United States by October and will arrive south of the United States by November (Sherry and Holmes 1997). In eastern North America, fall adult migrants follow a route along the coastal plain between the Appalachian Mountains and the west Atlantic Ocean (Sherry and Holmes 1997). Possibly disoriented, some juvenile redstarts follow a route along the eastern Atlantic Coast in late August through early October (Sherry and Holmes 1997).

Migration is extremely taxing and energetically demanding for migratory birds. Because of this, many migratory birds will replenish and store energy at stopover sites along their migration routes (Bayly et al. 2012). Stopover sites are areas along migration paths that allow migratory birds to rest and regain fat reserves lost while in transit (Johnson and Winker 2008). Some stopover sites for American Redstarts include islands off both the Atlantic and Pacific coasts, as well as other coastal areas (Johnson and Winker 2008; Morris and Glasgow 2001). Adult American Redstarts may also utilize sites further inland than juveniles (Morris and Glasgow 2001). The utilization of stopover sites varies during fall vs spring migration. Birds may use some sites more often during fall migration because they are farther from the breeding grounds and the birds have traveled longer distances at this point before stopping (Morris and Glasgow 2001). In addition, they may

need to stop more often to gain fat reserves before traveling south (Morris and Glasgow 2001). American Redstarts do not always take advantage of stopover sites during spring migration. This may be because these stopover sites are close to breeding grounds and birds' migrations have just begun (Morris and Glasgow 2001).

In the last fifty years, American Redstart populations have slightly declined within their widely distributed breeding regions (Sherry and Holmes 1997). Within some fragmented and urbanized landscapes, American Redstart populations have declined mainly due to the deterioration of preferred breeding habitats (Peterjohn 1989; Sherry and Holmes 1997). The North American Breeding Bird Survey also found evidence of a small, though non-significant trend of decline within North America (-0.34% per year, Sauer et al. 2014). Figure 2 provides a trend map displaying the changes in American Redstart breeding abundance within North America. The abundance trend map of American Redstarts contains many blue areas indicating greater than 1.5% change per year, but most importantly, contains a number of red areas indicating areas of less than -1.5% change per year between 1966 and 2014 (Sauer et al. 2014).

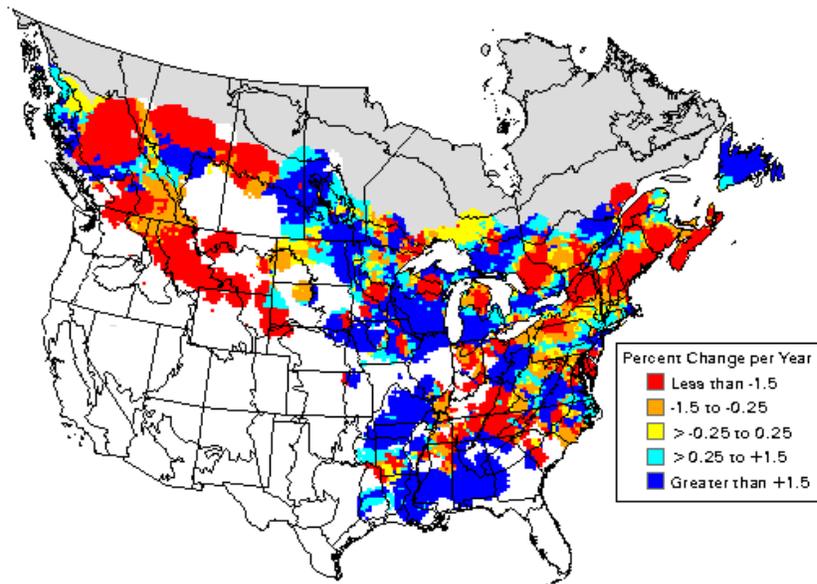


Figure 2: American Redstart Breeding Bird Survey Trend Map, 1966-2014 (Sauer et al. 2014)

In comparison to all other warbler species, adult female and male American Redstart plumages identify as one of the easiest to distinguish from one another (Sherry and Holmes 1997). The females generally have light gray on the head, gray to olive green on the back, and a whitish color below, accompanied by pale yellow patches on the tail, wings, and sides (Figure 3) (Sherry and Holmes 1997). Juvenile male American Redstarts show delayed plumage maturation and are similar in appearance to females (Debruyne et al. 2006). As seen in Figure 4, after two years, adult male redstarts develop a definitive glossy black plumage with bright salmon-orange patches on the base of their outer tail feathers, base of their wings, and sides (Ryan 2011; U.S. Fish and Wildlife Service Forensics Lab 2015). The tail feathers, or rectrices, and wing feathers, or remiges, are stiff, asymmetrically shaped feathers that are important for flight (Ryan 2011; U.S. Fish and Wildlife Service Forensics Lab 2015).



Figure 3: Adult Female American Redstart (*Setophaga ruticilla*) (Kevin T. Karlson n.d.)



Figure 4: Adult Male American Redstart (*Setophaga ruticilla*) (Vitz 2008)

The period between a warblers hatching and the end of that year is the bird's hatch year (Woodrey and Moore 1997). At this time, the bird is a juvenile (Woodrey and Moore 1997). Once this first year concludes, the warbler is then an after hatch year individual and is considered an adult (Woodrey and Moore 1997).

In this study, it was important to differentiate migration by age because propensity to migrate varies between individuals in a population (Gow and Wiebe 2014). These differences are

based on sex, age, or other factors and are important to understand when trying to identify and protect habitats used by American Redstarts. Unlike adult birds, juvenile birds are typically inexperienced foragers and need more time to store energy before the start of fall migration. Therefore, juveniles arrive later than their adult counterparts do (Morris 1996). Juveniles are migrating for the first time without directional assistance, and therefore juveniles might leave the breeding region at a more delayed time than adult birds (A.C. Vitz personal communication).

Studying differential migration by sex is important, because like differential migration by age it is vital to understand the patterns of American Redstart migration to aid in conservation efforts. During fall migration, females and males migrate at the same time (Morris and Glasgow 2001). This is because both sexes must compete for resources and high quality territory on the wintering grounds (Morris and Glasgow 2001). However, migration patterns are highly variable among passerine species. For example, Swanson et al. (1999) found that in Ruby-crowned Kinglets (*Regulus culendula*) females migrate before males during fall migration. This is because females are smaller and less hardy than males, and therefore would need to depart before resources decline in wintering grounds, as well as females winter farther south and need to travel farther than males (Swanson et al. 1999). American Redstarts are strongly segregated by sex in the wintering grounds, resulting from behavioral dominance of males (Marra and Holmes 2001). This segregation by sex can lead to differential patterns of migration in the species, with one sex arriving earlier than the other at the wintering grounds.

### **Strategies for Tracking Bird Migratory Patterns**

Before the popularization of the new migratory pattern tracking method, stable isotope analysis, other methods are also successful in answering various types of questions in regards to

bird migratory patterns. Although there are many methods, three of the most popular are capture-mark-recapture, band recovery, and radio telemetry (McClintock and White 2012).

During capture-mark-recapture (CMR) and mark-resighting studies, animals are captured, marked for later identification, and released back into the wild (McClintock and White 2012). Band recovery is one of the most common methods used in wild birds CMR (Silvy et al. 2005). This technique involves the application of a metal or colored plastic band to a bird's leg at a designated study location (Sutherland et al. 2004). Within the United States, the Bird Banding Laboratory (BBL), the U.S. Geological Survey (USGS), and the Patuxent Wildlife Research Center work together to direct the North American Bird Banding Program (USGS 2015). The BBL also works closely with the Canadian Bird Banding Office (BBO) to share responsibilities and data retrieved from all of North America (USGS 2015). In order for researchers to begin banding practices they must obtain a permit from the BBL (USGS 2015). Upon request, the BBL evaluates the cost versus benefits for each banding activity (USGS 2015). From 2010 to 2015, the Bird Banding Laboratory tracked the banding of 27,884 American Redstarts within the United States (USGS 2015). This large collection of data has the ability to provide valuable information on migration patterns, as well as information on species' survival rates (USGS 2015). The Powdermill Avian Research Center (PARC) is one location that works towards researching and banding migratory birds. This center is more than 50 years old, and as of 2001, the program has banded more than 500,000 birds. The bird-banding program at PARC may be the longest running, year round, professional bird-banding program in the United States (Carnegie 2016).

In order to counteract the low percentage of birds recaptured, another method referred to as radio telemetry was developed (USGS 2015). In radio telemetry, a researcher attaches a radio transmitter to an animal and track the signal in order to observe the animal's movements (USGS

2015). A traditional radio transmitter is a simple very high frequency (VHF) radio transmitter weighing between 0.4 and 12 grams (Warnock and Takekawa 2003). Technological advances have resulted in the development of the platform terminal transmitter (PTT) and the global positioning system (GPS) transmitter which operate using the basic principles of VHF, but have much greater capabilities (White and Garrott 2012). However, despite newer technologies that surpass the abilities of VHF radio telemetry, the first generation equipment continues to be a research staple (Migratory Connectivity Project 2012). VHF radio telemetry is so valuable due to its low cost and ability for use on animals of varying sizes (Migratory Connectivity Project 2012).

PTTs often weigh 12-18 grams and GPS transmitters weigh 30-60 grams (Whitworth et al. 2007). The scientific community understands that a radio transmitter should not exceed 3-4% of the bird's body mass due to the negative bearing a large addition of weight may have on an animal (Whitworth et al. 2007). It is for this reason that PTT and GPS transmitters are not yet possible for migratory studies of small bird species. The lightweight characteristic of the VHF radio telemetry transmitter makes it ideal for the study of large species within migration studies. However, this method is not appropriate for small species such as American Redstarts due to limited range and the weight of the transmitter (Migratory Connectivity Project 2012).

These older methods have been able to shed some light on the migratory mystery of birds. However, the aforementioned methods have failed to provide researchers an understanding of the relationship between wintering and breeding habitats (Robertson 2004). Stable hydrogen isotope analysis has the ability to fill in the gaps of previous and ongoing studies, having an influential impact on migratory research.

## **New Strategy for Tracking Migration**

### **Stable Isotope Analyses**

The emergence of stable isotope analysis (SIA) has aided researcher's ability to study broad avian migration patterns (Inger and Bearhop 2008; Wunder 2012). Previously mentioned conventional approaches track animal movements through extrinsic markers. SIA alternatively uses intrinsic markers such as fatty acid profiles, molecular DNA analyses, and the measurement of naturally occurring stable isotopes in animal tissues (Hobson 1999). This approach is extremely valuable for small or non-game animals, such as American Redstarts, who may be unable to carry large extrinsic markers (Hobson 1999). Although researchers cannot use SIA to examine the detailed history of movement, it can reveal broad migration patterns, which can help determine the general breeding areas of a species captured on non-breeding grounds (Hobson 1999; Whitworth et al. 2007). Landmark studies displayed the immense potential of SIA in tracking migration through "systematic geographic patterning in stable isotopes located in tissues of animal populations" (Best and Schell 1996; Chamberlain et al. 1997; Bowen et al. 2005; Wunder 2012).

An isotope is a form of an element containing the same number of protons as the standard, but a different amount of neutrons, creating a variation in mass (Robertson 2004). The common elements that possess isotopes are carbon, hydrogen, oxygen, and nitrogen (Robertson 2004). In addition, some elements can have more than one isotope, leading to stable or unstable forms (SFU Museum of Archaeology and Ethnology 2010). Unstable isotopes decrease or decay over a period, whereas stable isotopes do not change (SFU Museum of Archaeology and Ethnology 2010).

During the evaporation of ocean water, kinetic fractionation of stable isotopes occurs, due to one isotope having a larger mass than the other (Bowen et al. 2005). The heavier isotope is slightly harder to evaporate due to its larger mass. This correlates with latitudes. The higher the

latitude, the lower the amount of this isotope form present, producing more negative isotope ratios (Bowen et al. 2005). This singularity is present in annual growing seasonal precipitation. Local vegetation uses water, which insects eat (Bowen et al. 2005). Birds then consume these insects, making their feathers a carrier of the stable isotope ratio for the region. Birds also drink water, contributing to about 26-32% of their feathers isotopes (Bowen et al. 2005).

### **Stable Hydrogen Isotope Analysis**

Hydrogen isotope analysis is based off the fact that “the ratio of naturally occurring light stable isotopes (D/H,  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ ,  $^{34}\text{S}/^{32}\text{S}$ ) in dietary items may vary predictably among biomes, which organisms inhabit” (Bowen et al. 2005). Hydrogen isotopes are measured using delta notation ( $\delta\text{D}$ ), where  $\delta\text{D}$  is the hydrogen isotope ratio<sub>sample</sub>/ hydrogen isotope ratio<sub>standard</sub> (Kelly et al. 2002). An organism’s tissues show the isotopic signatures from the intake of food and water (Bowen et al. 2005). These signatures can also vary depending on geographic location (Bowen et al. 2005). To make a connection between organisms and their origin in North America, researchers compare hydrogen isotope analyses ( $\delta\text{D}$ ) to “large-scale isotopic contours of growing-season average  $\delta\text{D}$  values in precipitation” (Hobson 1999).

Researchers measure stable isotope ratios in parts per thousand (Bowen et al. 2005). There are a few isotope forms of hydrogen, including protium ( $\text{H}^1$ ), deuterium ( $\text{H}^2$  or D), and tritium ( $\text{H}^3$ ) (Kendall and Caldwell 1998). Deuterium has one neutron and one proton and is twice the mass of protium (Kendall and Caldwell 1998). When an isotope ratio is positive, there is more deuterium present than the standard (Bowen et al. 2005). If the isotope ratio is negative, there is less deuterium than the standard (Bowen et al. 2005). In Figure 5, a contour world map shows the average deuterium isotope ratios from precipitation (Kelly 2015).

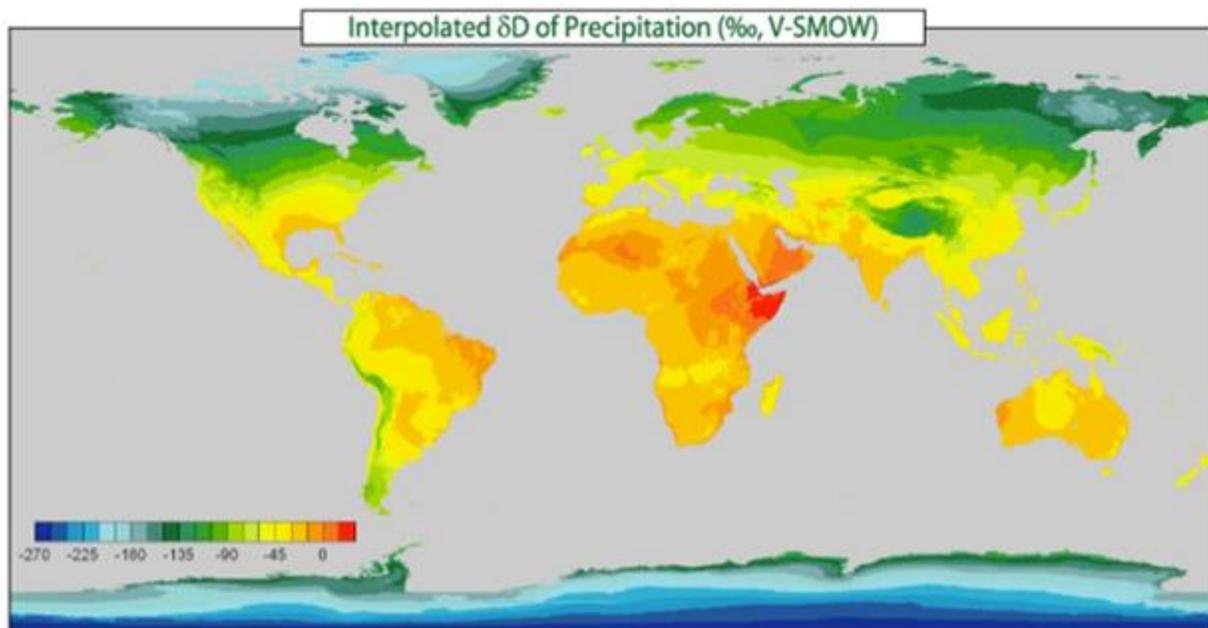


Figure 5: Contour world map of average  $\delta D$  isotope ratios of precipitation (Kelly 2015)

An example of an isoscape (i.e. a spatiotemporal distribution of isotopes) with the locations of the collection sites of feather samples is present below in Figure 6 (Hobson 2012). The isoscape emulates Bowen's (2010) model and shows the weighted average of stable hydrogen isotopes ( $\delta^2H$ ) in precipitation across North America. The authors concluded that there was a strong relationship between tissue  $\delta^2H$  and "global hydrologic  $\delta^2H$  patterns" (Hobson 2012). This seminal paper demonstrates that stable hydrogen isotope analysis can accurately predict where a feather grows (Hobson 2012).

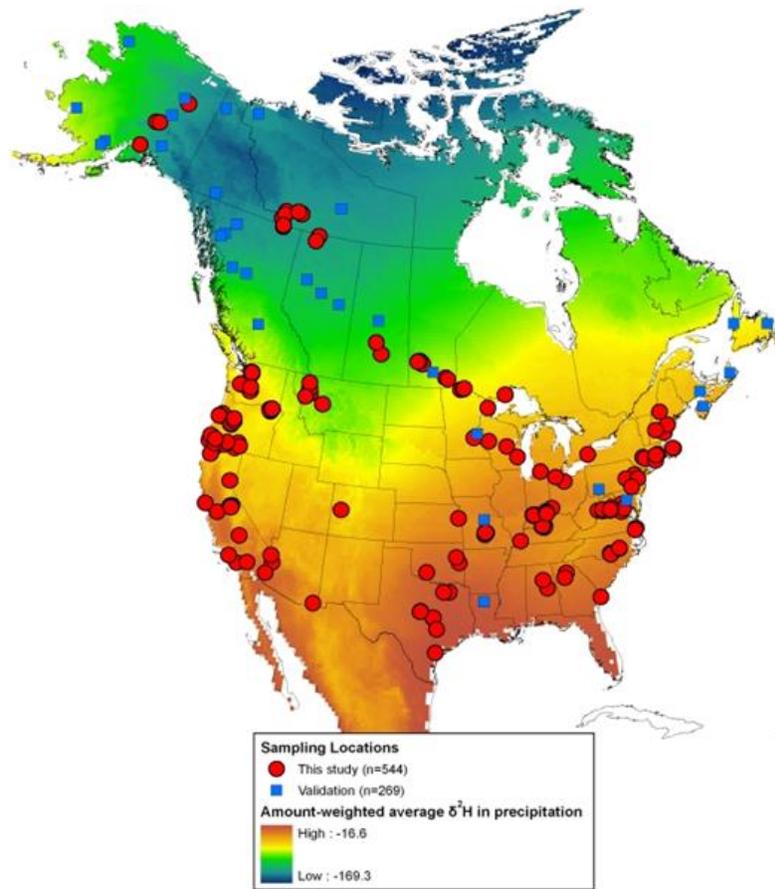


Figure 6: Isoscape with location of feather sample collection sites (Hobson 2012)

In addition, a study by Hobson and Wassenaar (1996), observed that there was a positive relationship between the hydrogen isotope signature of feathers and the hydrogen isotope signature of the breeding locations. Once this relationship was established, the authors were then able to link breeding and wintering grounds of the Black-Throated Blue Warbler (*Setophaga caerulea*; Hobson and Wassenaar 1996). Their study also revealed that the hydrogen isotope signature of beetle chitin, commonly eaten by warblers, positively related to the signature of the rainwater at that latitude (Hobson and Wassenaar 1996). In additional studies, authors found that some of the hydrogen is non-exchangeable and remains bound to the tissue. It then becomes fixed when the tissue is synthesized (Fan and Dettman 2015; Meehan et al. 2004). To analyze the  $\delta^2\text{H}$  values in a

feather tissue, researchers analyze exchangeable hydrogen where water vapor exchanges in the surrounding area (Fan and Dettman 2015). The  $\delta^2\text{H}$  values of bird feathers mirror those of the “growing-season precipitation” in the area where the feathers grew (Kelly et al. 2002; Meehan et al. 2004). This allows researchers to determine the migratory movements of birds by comparing the ratios to maps of growing-season  $\delta^2\text{H}$  (Meehan et al. 2004).

## **Molting in Birds**

Molting in birds is a cyclical activity that replaces most of their feathers once their current ones wear out (Howell 2010). All birds need to molt at least once a year to replace worn feathers and acquire new feathers seasonally (Howell 2010). A majority of bird species molt just after the breeding season (Pyle 2008). This single, predominant molt is the prebasic molt (Pyle 2008). For most passerines, the adult prebasic molt is a complete molt where they replace all body and flight feathers, while the first prebasic molt is less than complete (Pyle 2008). Some bird species, including American Redstarts, molt more than once a year, and this second molt is the prealternate molt (Pyle 2008). The prealternate molt occurs in both juvenile and adult birds prior to the next prebasic molt, and is a molt of some or all body plumage and occasionally flight feathers (Pyle 2008). In most cases, the alternate plumage of males differ from their basic plumage, while in females, both plumages are usually similar in appearance (Pyle 2008).

There are two types of feathers: downy (Figure 7a) and nondowny (Figure 7b) (Howell 2010; Yue et al. 2005). Downy feathers are weak and have a poorly developed shaft and no interlocking barbs (Howell 2010). Nondowny feathers, also known as contour feathers, produce the contour (outline) of the bird, have stronger, well-developed shafts, and interlocking barbs that form a firm vane (Howell 2010). Downy feathers are usually found on chicks, and close to the body of adult birds, while contour feathers are found exposed on the outside of the bird, and include

the flight and tail feathers (Howell 2010). These nondowny flight and tail feathers are the feathers that are usually associated with molting (Howell 2010).

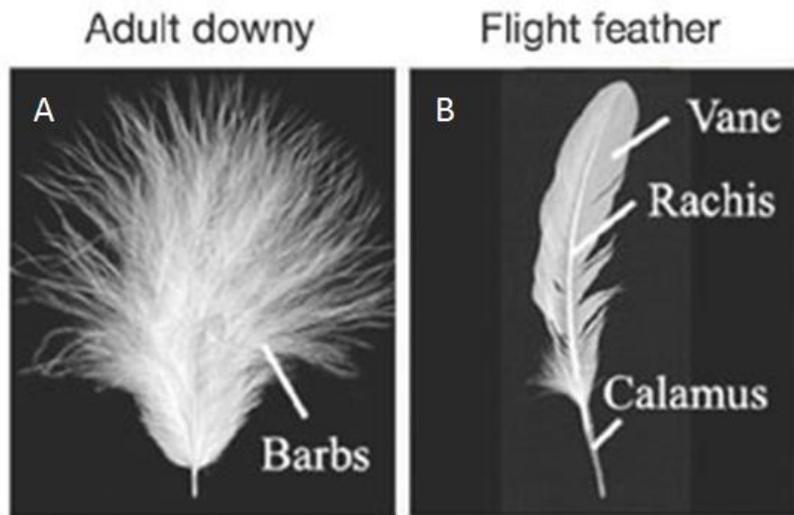


Figure 7: An adult downy feather; B adult flight feather (Yue et al. 2005)

Feathers grow in patches called tracts that spread out and cover naked areas of the body (Howell 2010). Muscles more easily control these tracts (Howell 2010). Feathers develop within a protective wax and keratin sheath that protects and supplies nutrients to the feather as it grows (Howell 2010). As the feather grows, it emerges from the sheath and expands to the full width, and once the feather is fully mature, the sheath flakes off from the feather base (Howell 2010). At this point, the feather is cut off from the bird's circulatory system and is dead tissue where the isotope is now inert (Howell 2010). The feather is no longer able to grow, and is replaced with another feather through molting (Debruyne et al. 2006).

Downy feathers are most notable for being the first feathers birds grow. When chicks first hatch, downy feathers cover them (Howell 2010). During the first year, the chick's follicles produce a coat of vaned feathers known as the juvenile plumage (Howell 2010). This juvenile

plumage is relatively weak and another molt replaces it (Howell 2010). This molt following the juvenile plumage is all contour feathers (Howell 2010).

### **The Benefits of using Stable Isotope Analysis**

In recent years, SIA has proven to have the ability to tie together the lives of migratory birds in which other tracking methods have previously failed (Robertson 2004). It has been successful because “well-known and predictable continental patterns of hydrogen isotopes in rainfall ( $\delta^2\text{H}_p$ ) are often closely reflected in tissue  $\delta^2\text{H}$  values” (Bowen 2010). The ability to understand how breeding populations of birds move between their summer and winter habitats is critical in protecting any vulnerable or declining species (Bowen 2010). Identifying population declines in distinct breeding populations allows researchers to link factors such as habitat loss and degradation to these patterns (Boulet et al. 2006). By studying migration patterns of animals, researchers are successfully able to track populations at any given time. This allows them to identify and protect the habitats that these birds use more efficiently as well as prioritize habitat protection and restoration (Hovick et al. 2016; Davis 2003; Hart et al. 2014).

American Redstart populations have declined within their widely distributed breeding regions due to habitat loss (-0.34% per year, Sauer et al. 2014; Sherry and Holmes 1997; Masalaite 2012). In the study presented here, we measured stable isotope ratios of hydrogen in redstart feathers to examine relationships among breeding latitudes and the timing of migration of American Redstarts. Specifically we sought to: (1) determine if more northern populations migrate first, (2) determine if adult male American Redstarts arrive earlier than adult females and if juvenile males arrive earlier than juvenile females, and (3) determine if juvenile female songbirds migrate later than their adult female counterparts and if juvenile male songbirds migrate later than their adult male counterparts.

Performing a stable hydrogen isotope analysis on American Redstart feathers can provide researchers with important migratory information, allowing for the creation of conservation efforts (Masalaite 2012). Effective methods of conservation include preserving American Redstart's natural habitats at breeding grounds, wintering grounds, and stopover sites, and tracking changes in populations. Another method is locating critical migratory stopover habitats for declining populations based on information from the research we could conduct and existing American Redstart population trend maps. However, without information on the location of these habitats and timing of American Redstart migration, this task is impossible (Masalaite 2012). In conclusion, SIA has the ability to uncover the missing links between migratory patterns and conservation efforts.

### **Statistical Analyses**

In order to analyze results to support or deny hypotheses, scientists must perform a series of statistical analyses. Researchers start by testing the normality of their data. To do this, many scientists use the Shapiro-Wilk test. This test utilizes the null hypothesis to check if a sample came from a normally distributed population (Ghasemi and Zahediasl 2012). Researchers use t-tests to identify if there are any significant differences between the means of two independent groups of interest. Specifically, a t-test seeks to reject the null hypothesis that researchers identify before a study. If a t-test has significant results then researchers need to reject the null hypothesis. There are two types of t-tests, assuming either equal or unequal variance. An F-test for two-sample variance must precede a t-test to predict this variance.

Scientists use linear regressions to predict one variable based on the value of another variable (Lane 2016). The variable being predicted is called the criterion variable, while the variable the prediction is based on is the predictor variable (Lane 2016). An R-squared value is

present along with the equation of the line to measure how close the data fit to the created line (Lane 2016). An R-squared value for a linear regression is “good” when it is close to one (Lane 2016).

A box and whisker plot is important in showing the distribution of a range of data, its central value, and its variability (Stapel 2014). Furthermore, box and whisker plots are a visual representation of the median and quartiles of a set of data (Stapel 2014). Quartiles 1 through 5 represent the minimum (bottom line), the center data point between the minimum and median (bottom border of the box), the median (the center line), the center data point between the median and maximum (top border of the box), and the maximum (top line), respectively (Stapel 2014).

## Chapter 2

### Introduction

The effectiveness of conservation efforts relies heavily on data concerning breeding, stopover, and wintering sites. Little data currently exist in regards to connecting these concerns, although passerines are most vulnerable, pushing themselves to the edge of their physiological limits during this time (Blem 1980). Tracking differences in the routes of diverse populations can determine how habitat degradation along these routes will affect breeding populations and therefore stand as a footprint for conservation efforts (Paxton 2007).

Many strategies are available to track migration patterns, including traditional banding techniques and GPS/radio transmitters. However, both of these methods have produced only a small amount of information on migratory routes. Recent studies have examined the technique of stable isotope analysis in animal tissue to differentiate between breeding populations passing through the same stopover sites (Chamberlain et al. 1997). Stable isotope analysis using hydrogen ( $\delta D$ ) is a popular method because  $\delta D$  in precipitation highly correlates with  $\delta D$  in the body tissue, and therefore feathers of passerines (Chamberlain et al. 1997). This is due to the trophic interactions between passerines, their prey, and the local precipitation where more northern latitudes have more negative  $\delta D$  values than southern latitudes (Chamberlain et al. 1997). Getting insight into passerine migration is especially critical with respect to the recent decline in some species throughout Canada and the United States in the recent decades (National Audubon Society 2016).

We examined differential migration of American Redstarts (*Setophaga ruticilla*) during fall migration, based on breeding location derived from stable hydrogen isotope analysis from samples from the Powdermill Avian Research Center. American Redstarts molt and regrow their

tail feathers on breeding grounds, so the isotope signature of a tail feather is representative of their most recent breeding ground. American Redstarts breed in Northern United States and Southern Canada, and winter in Central America, the Lesser Antilles and northern South America (Sherry and Holmes 1997). Over the years, there has been no detected change in annual migration patterns of this species (Stanley 2012). This is helpful with respect to increasing overall sample size by combining samples from multiple years.

The goal of our study was to examine the relationships among breeding latitudes and the timing of migration of American Redstarts. Specifically we sought to: (1) determine if more northern populations migrate first, (2) determine if adult male American Redstarts arrive earlier than adult females and if juvenile males arrive earlier than juvenile females, and (3) determine if juvenile female American Redstarts migrate later than their adult female counterparts and if juvenile male American Redstarts migrate later than their adult male counterparts.

## **Methodology**

### **Feather Collection**

Dr. Andrew Vitz collected feather samples at the Powdermill Avian Research Center in Pennsylvania (+40.16363313,-79.26748042) during September and October 2010 and 2011. He used 12-meter long; 30 mm mesh mist-nets. Researchers set up about 68 of these mist-nets at sunrise for 6 hours each day (A.C. Vitz personal communication). Researchers from the laboratory distributed the nets throughout a 30-acre area that was composed of young forest habitat surrounding three man-made ponds. These nets were present 6 days a week, unless there were poor weather conditions. Then every 40 minutes, birds were transported back to the laboratory and fitted with a USGS bird band with a nine digit unique banding number and recorded information such as sex, age, fat, and morphological measurements (A.C. Vitz personal communication). Banders collected one outer rectrix from each bird and placed it into a small coin envelope with the individual's information.

### **Identifying and Tallying Feather Samples**

We chose American Redstarts as our sample set due to the high number of tail feather samples provided by the Powdermill Avian Research Center for this species. We obtained the band number, date collected, sex of the individual, and age (adult, or juvenile). We assigned new ID numbers to each sample for our own records and then used these numbers again when sending our samples to the Colorado Stable Isotope Laboratory (CPSIL). We used American Redstart tail feathers because these feathers are larger than body feathers, and easier to pull, wash, weigh, and sample.

### **Preparing Feather Samples for Hydrogen Isotope Analysis**

To prepare the samples for hydrogen isotope testing, we followed the CPSIL protocols for washing and weighing bird feathers (Appendices A and B). In the Life Science Project Lab located on the Worcester Polytechnic Institute campus, we created a “soap” and a Chloroform/methanol solution to wash the feather samples. The “soap” solution was a mixture of Extran MA02 Liquid Detergent and DI water. We cleaned each sample according to the wash protocol provided above. We placed the feather samples in capped plastic test tubes labeled with their ID numbers to transport them to WPI’s Gateway Graduate Chemistry Laboratory for weighing.

We used the Mettler Toledo MT5 microbalance located in the laboratory. We weighed the feathers according to the weighing procedure mentioned above. We used 3.5 x 5.0 mm silver capsules, the smallest capsules that would fit and hold the feather samples. We added or removed feather material until we reached the target weight of  $350 \mu\text{g} \pm 20 \mu\text{g}$ . Once the sample matched the target weight, we crimped the top of the capsule shut and folded the top tightly. We compacted the silver capsule into a small, tight cube and made sure that there were no stray edges or loose sides. Unfortunately, the error of the scale was  $500 \mu\text{g}$ , which was larger than the desired weight. We measured twelve (12) samples; however, due to instrument failure, we could not weigh the remaining samples and we sent them to CPSIL to be weighed on an appropriate scale.

### **Stable Hydrogen Isotope Analysis**

Researchers measure stable isotopes by gas isotope-ratio mass spectroscopy, but the samples must undergo a few procedures beforehand. They are first converted to gas by high temperature pyrolysis then the gas is ionized (stripped of electrons) to make the molecules positive. The charged particles enter a bent flight tube that has a magnet over it. This allows the molecules to separate by mass. Those with the heavier isotope ratios bend less than the ones with the lighter

isotope ratios. At the end of the tube, a Faraday collector measures the intensity of each ion beam (CPSIL 2007). The abundance of a stable isotope is “expressed as the ratio of the two most abundant isotopes in the sample compared to the same ratio in an international standard.” CPSIL collected several masses at once so that the ratios could be accurately calculated. If the ratio was greater than the international standard, the delta values were positive, but if they were less, the delta values were negative (CPSIL 2007).

At CPSIL, they analyzed hydrogen isotopes in solid inorganic/organic sample via automated continuous-flow through a combination of equipment. CPSIL used a Thermo Scientific TC/EA preparation system to convert the samples into gas molecules ( $H_2$  in this case) via high temperature pyrolysis (CPSIL 2007; TFS 2010). With the help of the Thermo Scientific ConFlo IV universal interface, the gases were separated and transferred into an isotope-ratio mass spectrometry (IRMS) (TFS 2010). The laboratory used the DELTA V plus spectrometry to measure the hydrogen isotope ration ( $\delta D$ ) from each feather sample (CPSIL 2007; TFS 2009).

### **Statistical Analysis**

First, we checked the data for normality using the Shapiro-Wilk test. We converted  $\delta D$  to altitude-corrected growing-season precipitation values ( $\delta D_p$ ) using the equation  $\delta D_p = \delta D - 25\%$  (Kirchman et al. 2011; Mazerolle et al. 2005) and compared these to the growing season  $\delta D_p$  map for North America constructed by Meehan et al. (2004). We transformed arrival dates into Julian dates (i.e., January 1 = day 1, years not considered) using a Julian date conversion table. Then we performed a t-test to check for differences between the  $\delta D_p$  (Hydrogen ratios) from 2010 and 2011. To test if  $\delta D_p$  could predict arrival date at the stopover site, we ran a regression with  $\delta D_p$  as the independent variable, and arrival date as the dependent variable.

Next, we created box and whisker plots to observe the relationship between age and migration dates. Quartiles 1 through 5 represented the minimum (bottom line), the center data point between the minimum and median (bottom border of the box), the median (the center line), the center data point between the median and maximum (top border of the box), and the maximum (top line), respectively (Stapel 2014). We looked at males and females of both age classes (adults and juveniles) separately. We ran separate t-tests, one for adult and juvenile males and one for adult and juvenile females, where age was the dependent variable and Julian date was the independent variable. F-tests for two-sample variance preceded the t-tests in order to check for equal or unequal variances.

We created box and whisker plots to observe the relationship between sexes and date of arrival. In these box and whisker plots, we observed juvenile and adult birds of both sexes separately. We ran separate t-tests, one for adult males and females and one for juvenile males and females where sex was the dependent variable and Julian date was the independent variable. We ran F-tests for two-sample variances before our t-tests to determine the variances. We accepted significance for these statistical tests at  $P < 0.05$ .

## Results

The data received from CPSIL did not have a perfectly normal distribution (see raw data in Appendix C); however, the statistical analyses used were robust to these slight deviations. There was no statistical difference between the hydrogen isotope values from feathers collected in the two different years ( $t_{99} = -1.21$ ,  $P = 0.230$ ) thus the data for both years were pooled together to increase overall sample size ( $n = 101$ ).

### American Redstart Breeding Ground Distribution (ISOMAP)

Values of  $\delta D_p$  for American Redstarts ( $n = 101$ ) ranged from  $-118.2\text{‰}$  to  $-57.15\text{‰}$  (mean =  $-78.97\text{‰}$ ). The estimated breeding areas of our species included regions north of PARC, and extending well to the east and west (Figure 8). The  $\delta D_p$  range of  $-119\text{‰}$  to approximately  $-79\text{‰}$  encompasses the area of southern Canada and northern parts of the Great Lakes states while the  $\delta D_p$  range from  $-69\text{‰}$  to  $-50\text{‰}$  includes the northern United States (Figure 8).

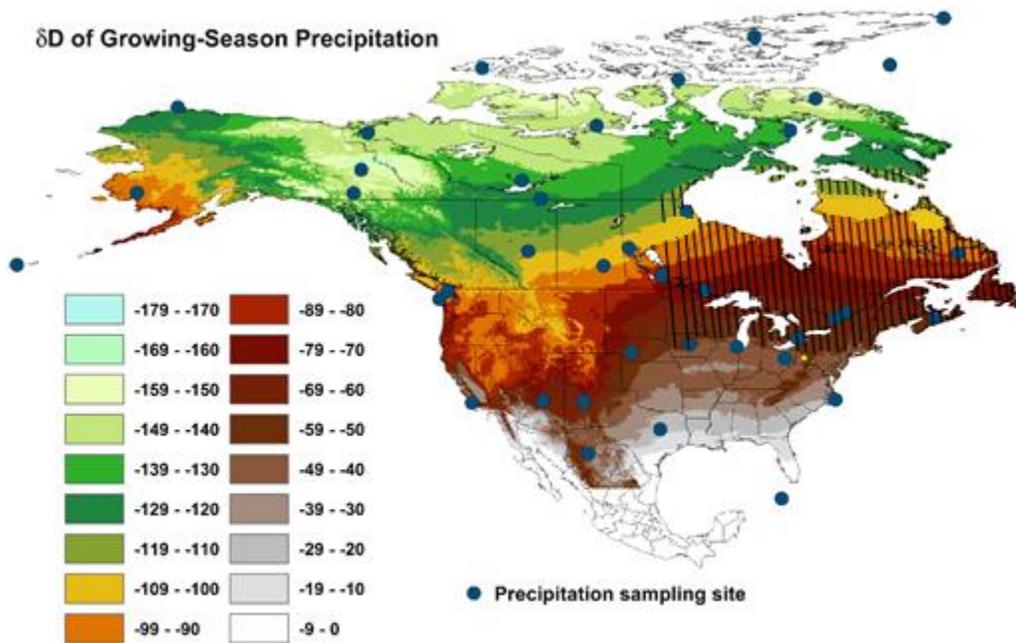


Figure 8: Breeding range for American Redstart captured at the Powdermill Avian Research Center. Black hash marks indicate the gradient of stable hydrogen isotope values in growing season precipitation ( $\delta D_p$ ) with 10‰ contours. The yellow square indicates the location of PARC. Original isomap from Meehan et al. (2004).

As seen in Figure 9, we divided the percentages of age and sex by breeding contours. Red, blue, green, and yellow represent adult males, adult females, juvenile males, and juvenile females respectively. Each pie chart is representative of only the specific 10‰ contour that its arrow delegates. The colors within the pie chart represent the portion per latitude while the gray samples represent the remaining regions. Only 3% of the migrants came from the -119‰ to -110‰ region, all of which were juveniles, and 0% from the -109‰ to -100‰ (Figure 9). The largest group of migrants came from the -79‰ to -70‰ and -89‰ to -80‰ regions (Figure 9). Similar amounts of males, females, juveniles, and adult migrants originated within each section of the graph (Figure 9).

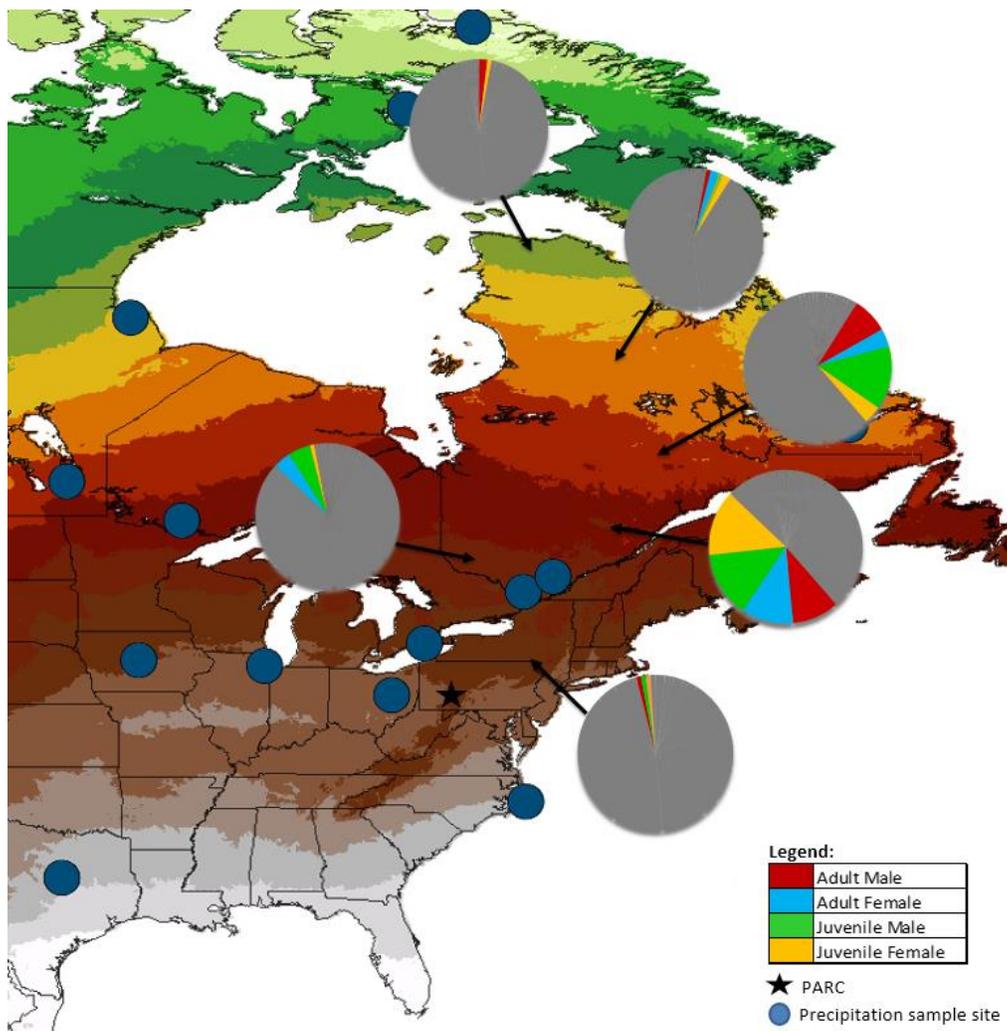


Figure 9: Distribution of migrants by age and sex. See percentages in Appendix D. Red = adult male, Purple = adult female, Green = juvenile male, Yellow = juvenile female. Original isomap from Meehan et al. (2004).

### Differential Migration by Breeding Latitude

There was no statistical significance between date of migration and  $\delta D_p$  values ( $F_{1, 99} = 0.49, P = 0.487$ ). However, there was a slight visible positive relationship with y-axis ranging from 0 to -140 (Figure 10).

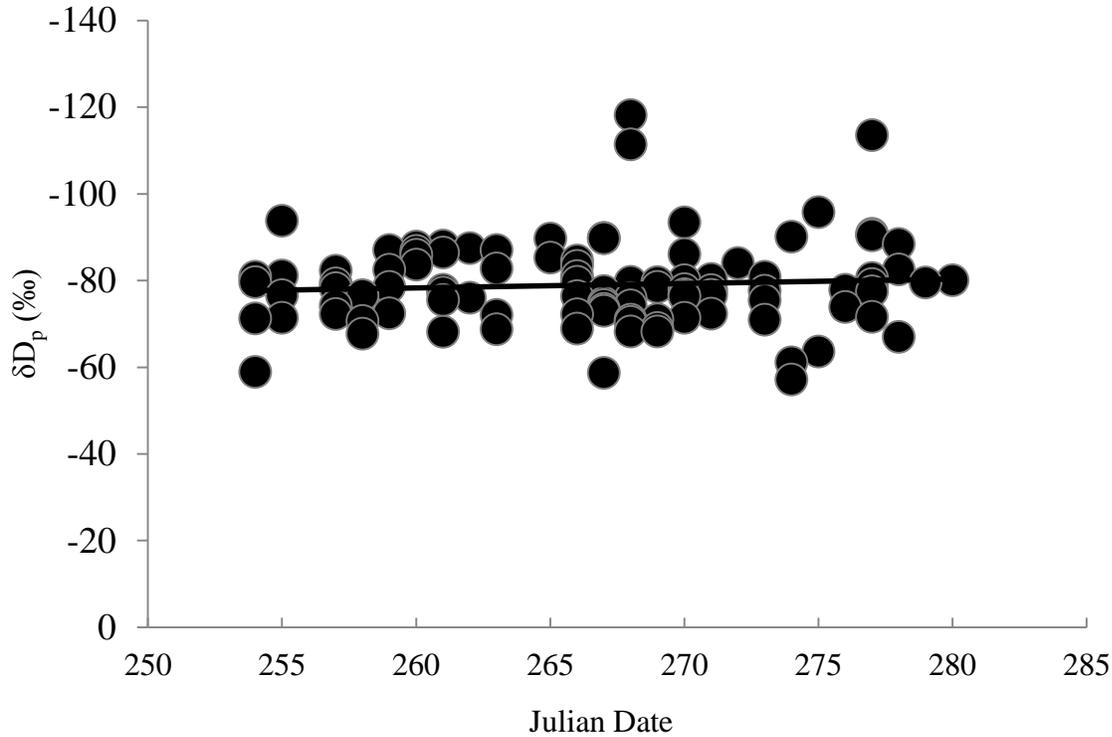


Figure 10: Regression of  $\delta D_p$  of migrating American Redstarts by Julian date, showing a slightly positive relationship between timing of migration and  $\delta D_p$  of migrating warblers.

### Differential Migration by Age and Sex

Juvenile and adult male warblers had an equal variance ( $F = 1.114$ ,  $P = 0.412$ ), and there was no significant difference in arrival time at stopover sites between the two ( $t_{55} = 1.260$ ,  $P = 0.213$ ) (Figure 11). Adult males showed peak migration between September 14th and 25th (day 257.5 and 268.5), while juvenile males showed peak migration between September 17th and 30th (day 260 and 273).

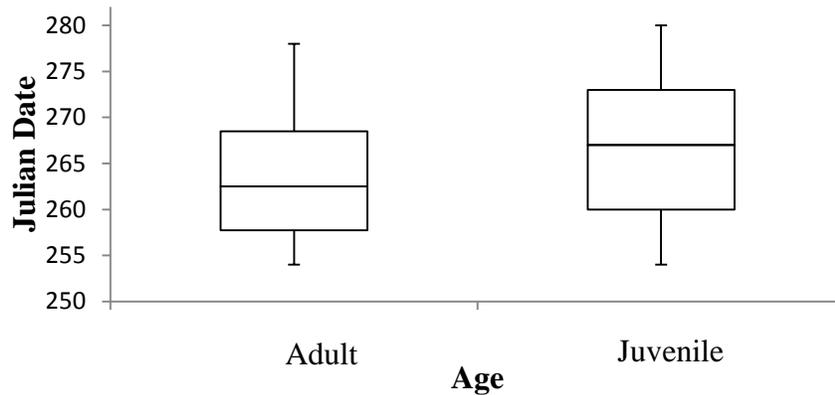


Figure 11: The median arrival date of adult ( $n = 20$ ) and juvenile ( $n = 37$ ) males when collected at the Pennsylvania Avian Research Center during fall migration in 2010 and 2011. Day 1 = January 1, 2010/ 11. The lower and upper boundaries of these boxes show the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively.

Juvenile and adult female warblers had an unequal variance ( $F = 0.910$ ,  $P = 0.416$ ), and juvenile females arrived at stopover sites significantly earlier than adult females ( $t_{42} = 2.649$ ,  $P = 0.011$ ) (Figure 12). Adult females showed peak migration between September 24<sup>th</sup> and October 2<sup>nd</sup> (day 267.25 and 275), while juvenile females showed peak migration between September 20<sup>th</sup> and 26<sup>th</sup> (day 263 and 269.75).

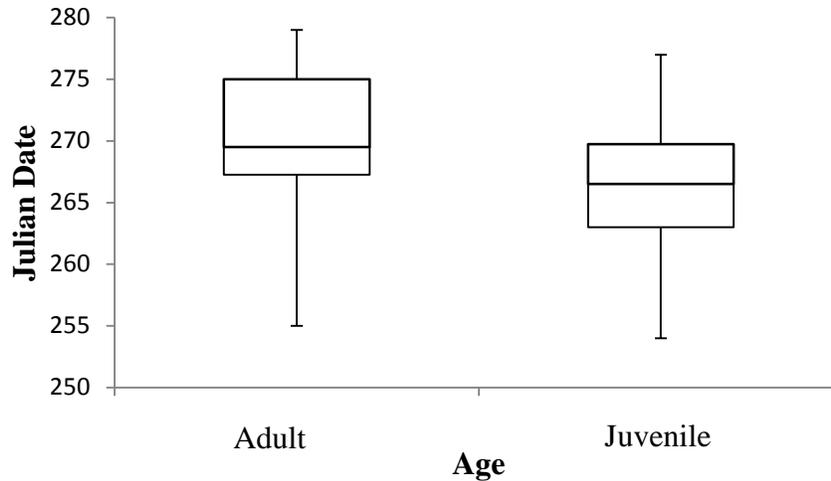


Figure 12: The median arrival date of adult ( $n = 22$ ) and juvenile ( $n = 22$ ) females when collected at the Pennsylvania Avian Research Center during fall migration in 2010 and 2011. Day 1 = January 1, 2010/11. Lower and upper boundaries of the boxes show the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively.

Adult male and female warblers had an unequal variance ( $F = 0.678$ ,  $P = 0.193$ ), and adult males arrived at stopover sites significantly earlier than adult females ( $t_{37} = -3.046$ ,  $P = 0.004$ ) (Figure 13). Adult males showed peak migration between September 14th and 25th (day 257.75 and 268.5), while adult females showed peak migration between September 24th and October 2nd (day 267.25 and 275).

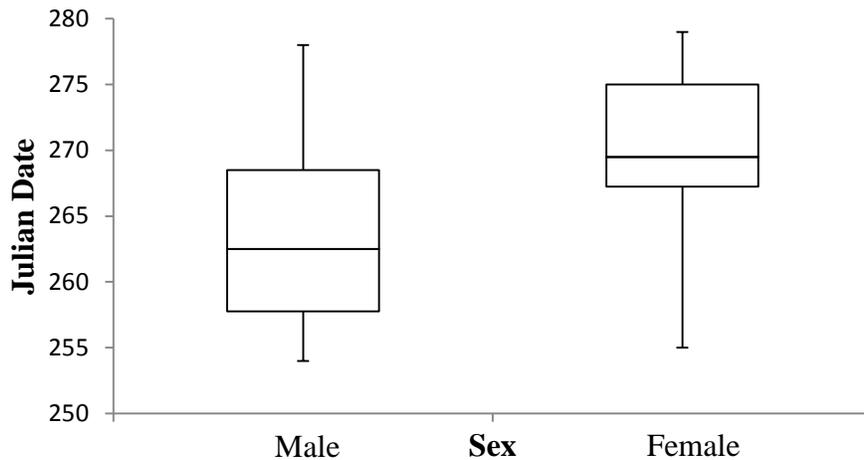


Figure 13: The median arrival date of male ( $n = 20$ ) and female ( $n = 22$ ) adult warblers when collected at the Pennsylvania Avian Research Center during fall migration in 2010 and 2011. Day 1 = January 1, 2010/11. Lower and upper boundaries of the boxes show the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively.

Juvenile male and female warblers had an unequal variance ( $F = 1.496$ ,  $P = 0.165$ ), and there was no significant difference in arrival time at stopover sites between the two ( $t_{57} = 0.638$ ,  $P = 0.526$ ) (Figure 14). Juvenile males showed peak migration between September 17<sup>th</sup> and 30<sup>th</sup> (day 260 and 273), while juvenile females showed peak migration between September 20<sup>th</sup> and 26<sup>th</sup> (day 263 and 269.75).

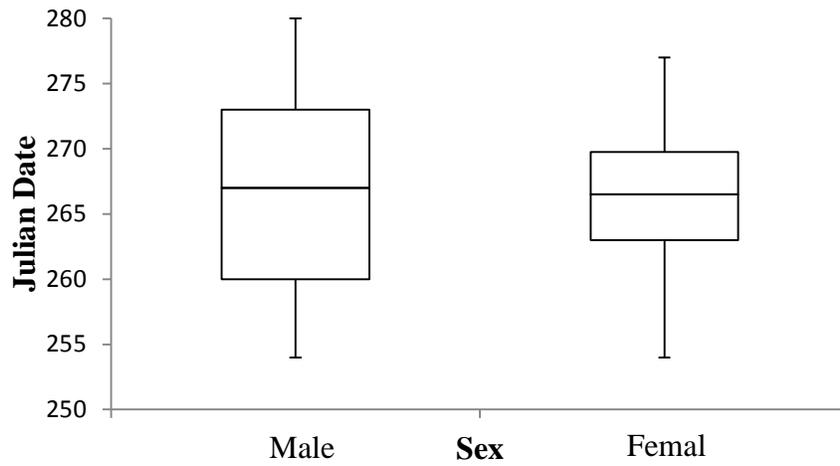


Figure 14: The median arrival date of male ( $n = 37$ ) and female ( $n = 22$ ) juvenile warblers when collected at the Pennsylvania Avian Research Center during fall migration in 2010 and 2011. Day 1 = January 1, 2010/11. Lower and upper boundaries of the boxes show the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively.

## Discussion

### Breeding Distribution

Based on the isotopic ratios collected in this study, the estimated breeding distribution of American Redstarts includes regions north of PARC, and extending to areas of southern Canada (Figure 8). Within the United States, the breeding area, or area of origin, extends out west encompassing portions of the Great Lakes states and extending out east as far as Maine (Figure 1). Some subspecies captured at PARC are known to breed as far west as Alaska and as East as coastal Canada (A.C. Vitz personal communication). Figure 1 displays the probability of origin represented by the isotopic ratio values not necessarily the actual origin of the sample. The deuterium isoscape for North America contains a gradient that is stronger in latitude than longitude (Gonzales 2011). Therefore, the isotope approach does not allow distinguishing among all potential origin areas within a given  $\delta D_p$  contour and all interpretations of the depiction require some caution (Gonzales 2011; Kirchman 2011; Hobson 2009). As a result, it is not possible to identify precisely how far east or west the birds that stopped over at PARC may have originated (Kirchman, 2011). Most passerine species, American Redstarts included, generally migrate in a north-south direction (Kirchman 2011). The most abundant American Redstart breeding regions are located in southern Canada, northern parts of the Great Lakes states, and northeastern United States through New York, New Hampshire, and Maine (Sherry and Holmes 1997; Price et al. 1995). We suspect the origin areas we mapped do not overestimate the western or eastern extent of true breeding areas. However, we suspect that populations originating near the very edge of our breeding areas are not passing through PARC. We acknowledge that some individuals may have come from outside our suspected mapped area (Figure 8) due to previous subspecies data sampled at PARC (A.C. Vitz personal communication). Isotopic approaches that enable greater clarification

of longitudinal origins would be valuable in the depiction of migratory routes, stopover sites, and breeding origins in a species whose breeding range spreads over a wide range of longitudes (Gonzales 2011; Boulet et al. 2006; Chamberlain et al. 1997).

### **Differential Migration by Breeding Location**

Previous research suggests that there is a relationship between breeding location and timing of fall migration (Paxton 2007). No significant relationship was present between the timing of migration and  $\delta D_p$  of the feather samples collected at the Powdermill Avian Research Center. However, there was a visible positive relationship seen by the slope of the regression line in the results. This could indicate that northern breeding locations (more negative  $\delta D_p$ ) migrated later than southern breeding locations (less negative  $\delta D_p$ ). Our data did not align with previous research done by Kelly et al. (2002) where southern breeding populations migrated first in a “leap frog” fashion. Within our data, only 3% of the redstarts migrated from -119 to -110  $\delta D_p$ , and no redstarts originated in the -109 to -100  $\delta D_p$  range. This may be due to the location of the sampling station at PARC. In a study by Jahn et al. (2012) on general passerine migration, northern breeding populations often took more coastal routes of migration, which may account for the majority of our samples having less negative  $\delta D_p$  values. In a previous study by Norris et al. (2006), the authors suggested that some adult American Redstarts molted slightly south of their breeding grounds while juveniles molted directly on their breeding grounds. This could account for no adults being north of -99  $\delta D_p$ . With a larger sample size ( $n > 101$ ), there may have been more significance in our results. In addition, samples collected at more coastal banding stations may provide more information on the more northern breeding populations.

## Differential Migration by Age

With respect to migration, juvenile female warblers arrived at the stopover site significantly earlier than the adult females. Juvenile females arrived at a median Julian date of 266.5 (September 23/24) whereas adult females arrived at a median Julian date of 269.5 (September 26/27), three days apart from each other. Typically, juvenile birds are inexperienced foragers and need more time to store energy before the start of fall migration, therefore arriving later than their adult counterparts do (Morris 1996). Adults often migrate at a higher speed than juveniles as well (Ellegren 1993). However, we did not observe this in our results. We found that adult female warblers migrated later than juvenile females. Morris and Glasgow (2001) also observed this pattern in their study on passerine migration, specifically with American Redstart female juveniles migrating significantly earlier than their adult female counterparts do. This could indicate that juveniles leave early to avoid dominant adults on breeding grounds (Woodrey and Chandler 1997). In addition, adult females that breed later into the summer, or have multiple broods, often need more time to recover and gain fat reserves before fall migration (Morris and Glasgow 2001). A study by Benson (2006) supported our results showing juvenile Wilson's Warblers (*Cardellina pusilla*) migrating before their adult counterparts. Similar studies by P. P. Marra (2001) showed that juveniles arrived at stopover sites more rapidly than adult birds. We also found no significance in migration timing between adult males and juvenile males, which is consistent with the results of previous studies (Morris and Glasgow 2001). Proximity of stopover sites to breeding grounds may play a role in the data collected, with closer sites to breeding location disrupting patterns (Benson 2006). We based our results on collection from only one stopover site over two years, and we acknowledge that the use of long-term data from additional sites would improve our results. Previous studies also suggest that differential migration between age and sex of American Redstart

migration alters between years (Woodrey and Chandler 1997). In addition, the date of capture may not accurately represent the first day of arrival to the stopover site, as juveniles often remain at stopover sites for longer periods of time (Morris and Glasgow 2001).

### **Differential Migration by Sex**

With respect to migration, adult male American Redstarts arrived at the stopover site significantly earlier than adult females. Adult males arrived at a median Julian date of 262.5 (September 19/20) and adult females arrived at a median Julian date of 269.5 (September 26/27), seven days apart from each other. This could indicate that adult females need to recover from a late breeding season to gain fat before fall migration (Morris and Glasgow 2001). American Redstarts in better physical condition were able to migrate earlier (Marra et al. 1998). Typically, male American Redstarts are larger in size, have larger wingspans, and are in better physical condition than females after the breeding season (Morris and Glasgow 2001). Because of this, they do not store fat on the breeding ground before fall migration, allowing them to migrate before females. We found that adult males did in fact migrate earlier than adult females; however, there was no significant difference in migration timing between juvenile males and juvenile females. As it is their first migration, juvenile males and females did not have the same experience as their adult counterparts, possibly accounting for the lack of significance in the data (McKinnon et al. 2014). In addition, Morris and Glasgow (2001) stated that there was no sex or age related difference in fall migration as both male and female redstarts are competing for the same wintering territories. At PARC from 1990-1991 the median capture date was September 10, much earlier than our data suggested (Sherry and Holmes 1997). This may be due to the noted differences in migration between years of redstarts (Morris and Glasgow 2001). We acknowledge that the collection of data from additional stopover sites and years would be beneficial in improving the

significance of our results. The proximity of stopover sites to breeding grounds may have also played a role in the data collected, with closer sites to breeding location disrupting patterns (Benson 2006).

In conclusion, this study on American Redstarts can provide researchers with valuable knowledge on the degree of connectivity of breeding, wintering, and stopover habitats as well as important migratory information. This will allow for the creation of conservation efforts such as the preservation of American Redstart natural habitats and locating critical migratory habitats for declining populations. Without information from this study and other similar studies, these tasks would be impossible. This study can help us take a step in the right direction to uncovering the missing links between migratory patterns and conservation efforts.

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## APPENDICES:

### Appendix A: CPSIL Protocol for Washing Bird Feathers

#### Materials Needed:

- 2 – 250 ml polyethylene wash bottles
- 1 – 500 ml polyethylene wash bottle filled with E-pure water (18 Mohm water)
- 2 – 500 ml pyrex beakers
- 1 – 250 ml pyrex beaker
- 1 – 100 ml pyrex graduated cylinder
- Extran MA02 Liquid Detergent (EMD Chemicals)<sup>1</sup>
- DI water
- E-pure water (18 Mohm water)
- Methanol (VWR – cat#VW4300-3; CAS#67-56-1)
- Chloroform (VWR – cat# VW1430-3; CAS#67-66-3)
- Forceps
- Watch glass plates
- Aluminum foil
- Sharpie
- Scotch tape and paper for labels
- Fume hood
- Drying Oven

1. Label each individual watch glass with sample labels. Using Scotch tape, adhere label to watch glass face up, on the UNDERSIDE of the glass plate.
2. Obtain 1- 250ml wash bottle. Add approximately 245 ml of DI water. Add approximately 2 ml of Extran MA 02 soap. Label the bottle “soap”.
3. Obtain 1- 500ml wash bottle. Fill with E-pure water. Label it “E-pure”.
4. Label the 2 - 500 ml beakers “waste soap + water” and “waste Ch:MeOH”
5. In a fume hood, measure 60 ml of methanol and add to empty 250 ml beaker. Using a graduated cylinder, measure 120 ml of chloroform and add to beaker containing methanol.
6. Fill a 250 ml wash bottle with Chloroform and methanol solution (Ch: MeOH). Label it “Ch: MeOH”.
7. Remove feather/nail from its sample envelope. Rinse the feather with E pure water from the wash bottle and collect rinse in beaker labeled “waste soap + water.”

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<sup>1</sup> Extran MA 02 Liquid Detergent is a neutral concentrate specifically designed for cleaning alkali-sensitive materials. Halogen-free universal cleanser delivers a highly effective but mild cleaning action. Dose: 20– 50mL per liter. pH of 2% solution: 7.2.

8. Place feather on its appropriately labeled watch glass plate.
9. Using the “soap” 250 ml wash bottle, soak the feather with the soapy solution. With forceps, gently move the feather so that you are removing all dirt. Let it stand for one minute.
10. Gently rinse the feather using the “E Pure” squirt bottle over the “waste soap + water” to collect the rinse water. Repeat this step as needed. **MAKE SURE THAT ALL SOAP IS RINSED FROM THE FEATHER.** Gently tap excess water from the feather and put it back on glass plate.
11. Dry the feather at 60° C in drying oven for 20 minutes.
12. **PERFORM THIS STEP IN THE FUME HOOD!** Using the Ch:MeOH wash bottle, soak feather with the solvent solution. With the forceps, gently stir feather in solvent solution on glass plate. Let it stand for 5 minutes.
13. Using the forceps, hold feather over the beaker labeled “waste Ch:MeOH”. With the “Ch:MeOH” wash bottle, gently soak the feather with Ch:MeOH one more time to assure complete lipid removal. Gently tap excess solvent from the feather. Empty all solvent mixture left on glass plate in the “waste Ch:MeOH” beaker. Rinse the feather using the “E Pure” wash bottle using the beaker labeled “waste water” to collect rinse water. **DO NOT POUR SOLUTION FROM “waste Ch:MeOH”**  
  
**BEAKER DOWN THE SINK. COLLECT THIS WASTE IN HAZARDOUS WASTE CONTAINER AND DISPOSE IN AN APPROPRIATE MANNER.** Rinse collected in “waste soap + water” can be disposed of in a sink drain.
14. Rinse glass plate with the E Pure water. Place feather back on watch glass plate.
15. Rinse feather with E pure water and gently stir.
16. Remove the feather from the glass plate and gently tap the feather to remove excess water. Place the feather back on the labeled watch glass and place in a 60-degree oven for one hour to overnight.
17. Remove the feather from the oven and wrap it in aluminum foil. Place the feather back to its appropriate labeled sample envelope.

## Appendix B: CPSIL Protocol for Weighing Bird Feathers

### Instructions for weighing feathers on a micro-analytical balance using silver capsules for stable hydrogen isotope (D/H) analysis.

**Materials Required** Microbalance, cleaned feather samples, clean culture tray(s), weighing utensils, methanol, Kimwipes, sample submission sheet, masking tape, marker, elastics, silver capsules.

1. Obtain a clean 96-position plastic culture tray (Elisa Plate), and our sample submission sheet.
2. **OPTIONAL** - Ensure feathers have been previously solvent cleaned (2:1 v/v chloroform/methanol 2x rinse) to remove surface oils. Rinse 2x using DI water. Air dry feathers in fume hood (>48 hours). If feathers are dirty, initially wash in mild detergent followed by DI rinse.
3. Cut off a small amount of feather vein material for analysis – always cut samples from the same location on different feather samples if possible (e.g. sample near tip)
4. Clean weighing utensils using methanol and Kimwipes, allow to dry. Do not use acetone!
5. Make sure the microbalance is clean and calibrated. Ensure the doors are closed when taring and weighing.
6. Tare a silver capsule (i.e., zero the balance so that the reported weight only incorporates the weight of the feather), handling only with tweezers, remove, and set on a clean metal surface. Use the smallest available silver capsule that will safely contain the sample (e.g. 3.5 x 5.0 mm).
7. Using a spatula or tweezers, transfer a small amount of feather material into the silver capsule.
8. Re-weigh, and continue adding or removing feather material until the target sample weight of  $350 \text{ ug} \pm 20 \text{ ug}$  is obtained (micrograms NOT milligrams). This will take practice to get a feel for the appropriate amount. With practice samples should take less than 5 minutes to weigh out.
9. To seal the silver capsule, crimp the top of the capsule shut with a pair of straight edge tweezers and then fold tightly (as if folding down from the top of a paper lunch bag). Then use the edge of the tweezers (use of 2 tweezers helps here) to gently compact the silver capsule into a small, tight cube. There should be no stray edges, loose sides or feather material poking out. Flattened capsules (rather than cube/ball-shaped) or capsules with stray or loose edges can jam the autosampler, crosscontaminate samples, and ruin analyses.

10. Record the final sample (feather) weight and sample name on sample submission sheet. Place the silver capsule into the corresponding position in the 96-position tray (e.g. Tray 1, position A5). Clean all utensils lightly with Kimwipes and methanol after completing each sample, air dry briefly.

When finished, secure the lid of the sample tray with rubber bands and masking tape (please no Scotch or duct tape) and label the tray appropriately so that it can be matched with its corresponding sample submission sheet.

11. Use 3.5 x 5.0 mm silver capsules designed for elemental isotope analysis. Our suppliers are

Costech (1-800-524-7219), Elemental Microanalysis (1-800-659-9885), or Isomass Scientific (1-800363-7823). Silver capsules are generally about \$0.50 each depending on the capsule size and how many you order.

## Appendix C: American Redstart Tracking Sheet

Key: A = American Redstart, ID = # of feather for personal reference, HY = Hatch Year (juvenile), AHY = After Hatch Year (adult), M = male, F = female,  $\delta D$  (‰) = Hydrogen ratio,  $\delta D_p$  (‰) = Hydrogen ratio corrected for precipitation

Species	ID	Band Number	Month	Day	Year	Julian	Age	Sex	$\delta D$ (‰)	%H	$\delta D_p$ (‰)
A	3	233055611	9	14	2010	257	HY	F	-104.3	5.8	-78.23
A	4	233055517	9	12	2010	255	HY	F	-125.1	5.3	-93.83
A	5	230055869	9	11	2010	254	HY	F	-107.8	5.6	-80.85
A	6	230055871	9	11	2010	254	HY	F	-106.2	5.6	-79.65
A	7	238038895	9	23	2010	266	HY	F	-108	5.8	-81
A	8	255096472	9	25	2010	268	HY	F	-94.6	5.6	-70.95
A	9	255097135	10	7	2010	280	HY	M	-106.8	5.6	-80.1
A	10	255096957	10	5	2010	278	AHY	F	-117.9	5.5	-88.43
A	11	255096864	10	3	2010	276	AHY	F	-103.8	5.6	-77.85
A	12	255096641	10	1	2010	274	AHY	F	-120.2	5.1	-90.15
A	13	255096653	10	1	2010	274	HY	M	-81.5	6.1	-61.13
A	14	238055672	9	16	2010	259	HY	M	-96.6	6.1	-72.45
A	15	233055671	9	16	2010	259	HY	M	-116.1	6	-87.08
A	16	233055627	9	15	2010	258	AHY	M	-102.2	5.9	-76.65
A	17	233055628	9	15	2010	258	HY	M	-94.2	5.8	-70.65
A	18	233055619	9	15	2010	258	HY	M	-90.3	5.8	-67.73
A	19	233055545	9	14	2010	257	HY	M	-109.7	5.9	-82.28
A	20	233055516	9	14	2010	257	AHY	M	-98.9	5.7	-74.18
A	21	233055608	9	14	2010	257	AHY	M	-105.8	7	-79.35
A	22	233055556	9	14	2010	257	AHY	M	-96.4	5.8	-72.3
A	23	233055523	9	12	2010	255	HY	M	-95.2	5.8	-71.4
A	24	233055524	9	12	2010	255	AHY	M	-102.2	5.6	-76.65
A	25	233055529	9	12	2010	255	AHY	F	-108.2	5.8	-81.15
A	26	230055868	9	11	2010	254	HY	M	-95	5.8	-71.25
A	27	230055877	9	11	2010	254	AHY	M	-78.6	5.8	-58.95
A	28	255096586	9	29	2010	272	HY	M	-112.1	5.7	-84.08
A	29	255096557	9	28	2010	271	AHY	F	-107.2	5.9	-80.4
A	30	255096540	9	28	2010	271	HY	F	-96.5	5.7	-72.38
A	31	255096490	9	26	2010	269	HY	F	-106.2	5.9	-79.65
A	32	255096477	9	25	2010	268	AHY	F	-91	5.7	-68.25
A	33	255096485	9	25	2010	268	AHY	F	-106.3	5.6	-79.73
A	34	255096488	9	25	2010	268	AHY	F	-101.3	5.3	-75.98

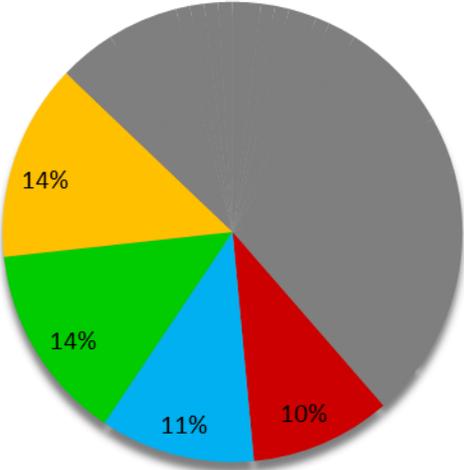
Species	ID	Band Number	Month	Day	Year	Julian	Age	Sex	$\delta D$ (‰)	%H	$\delta D_p$ (‰)
A	35	255096451	9	25	2010	268	AHY	F	-99	5.5	-74.25
A	36	255096469	9	25	2010	268	HY	M	-148.6	5.7	-111.5
A	37	255096456	9	25	2010	268	HY	M	-157.6	5.7	-118.2
A	38	255096434	9	24	2010	267	HY	M	-119.7	5.7	-89.78
A	39	255096406	9	24	2010	267	AHY	F	-99.8	5.5	-74.85
A	40	238038830	9	22	2010	265	HY	M	-113.7	5.7	-85.28
A	41	238038847	9	22	2010	265	AHY	F	-119.6	5.6	-89.7
A	42	238038776	9	19	2010	262	HY	M	-116.7	5.6	-87.53
A	43	238038781	9	19	2010	262	AHY	M	-101.4	5.6	-76.05
A	44	238038754	9	18	2010	261	HY	M	-115.3	5.8	-86.48
A	45	238038678	9	18	2010	261	AHY	M	-117.4	5.5	-88.05
A	46	238038663	9	17	2010	260	HY	M	-115.8	5.7	-86.85
A	47	238038715	9	17	2010	260	HY	M	-114.6	5.8	-85.95
A	48	238038635	9	17	2010	260	HY	M	-111.6	5.7	-83.7
A	49	238038643	9	17	2010	260	AHY	M	-117.1	5.6	-87.83
A	50	238038608	9	16	2010	259	HY	M	-104.7	5.8	-78.53
A	51	233055677	9	16	2010	259	HY	M	-110	5.8	-82.5
A	52	255096653	10	1	2010	274	HY	M	-76.2	6.7	-57.15
A	103	263036382	9	25	2011	268	AHY	M	-93.9	5.8	-70.43
A	104	263036426	9	27	2011	270	AHY	M	-114.8	5.7	-86.1
A	105	263036433	9	27	2011	270	AHY	M	-102.5	5.6	-76.88
A	106	263036498	9	30	2011	273	AHY	M	-94.6	5.7	-70.95
A	107	263036637	10	4	2011	277	AHY	F	-95.5	5.7	-71.63
A	108	263036769	10	6	2011	279	AHY	F	-106.1	5.3	-79.58
A	109	263036594	10	3	2011	276	AHY	F	-98.5	5.5	-73.88
A	110	263036585	10	2	2011	275	AHY	F	-127.7	5.9	-95.78
A	111	263036582	10	2	2011	275	AHY	F	-84.8	5.8	-63.6
A	112	263036439	9	27	2011	270	AHY	F	-101.9	5.5	-76.43
A	113	263036427	9	27	2011	270	AHY	F	-95.2	5.6	-71.4
A	114	263036412	9	26	2011	269	AHY	F	-91	5.8	-68.25
A	115	263036366	9	24	2011	267	AHY	F	-78.3	5.8	-58.73
A	116	263036326	9	23	2011	266	AHY	F	-91.9	5.3	-68.93
A	117	263036256	9	18	2011	261	AHY	F	-100.7	5.5	-75.53
A	118	263036327	9	23	2011	266	HY	F	-101.9	5.7	-76.43
A	119	263036332	9	23	2011	266	HY	F	-106.3	5.7	-79.73
A	120	263036354	9	24	2011	267	HY	F	-103.4	5.6	-77.55
A	121	263036365	9	24	2011	267	HY	F	-99.1	5.5	-74.33
A	122	263036406	9	26	2011	269	HY	F	-104.7	5.7	-78.53

Species	ID	Band Number	Month	Day	Year	Julian	Age	Sex	$\delta D$ (‰)	%H	$\delta D_p$ (‰)
A	123	263036622	10	4	2011	277	HY	F	-151.4	5.8	-113.6
A	124	263036425	9	27	2011	270	HY	F	-106.8	5.8	-80.1
A	125	263036465	9	27	2011	270	HY	F	-124.6	5.7	-93.45
A	126	263036486	9	28	2011	271	HY	F	-102.3	5.6	-76.73
A	127	263036490	9	28	2011	271	HY	F	-103.7	5.6	-77.78
A	128	263036257	9	18	2011	261	AHY	M	-103.8	5.7	-77.85
A	129	263036325	9	23	2011	266	AHY	M	-111.2	5.7	-83.4
A	130	263036339	9	23	2011	266	AHY	M	-112.8	5.5	-84.6
A	131	263036362	9	24	2011	267	AHY	M	-119.8	5.7	-89.85
A	132	263036729	10	5	2011	278	AHY	M	-110.1	5.6	-82.58
A	133	263036619	10	4	2011	277	AHY	M	-120.6	5.5	-90.45
A	134	263036287	9	20	2011	263	AHY	M	-116.1	5.8	-87.08
A	135	263036248	9	18	2011	261	HY	M	-90.9	5.9	-68.18
A	136	263036335	9	23	2011	266	HY	M	-96.3	5.8	-72.23
A	137	263036347	9	24	2011	267	HY	M	-97.4	5.7	-73.05
A	138	263036726	10	5	2011	278	HY	M	-89.3	5.7	-66.98
A	139	263036627	10	4	2011	277	HY	M	-105.6	5.6	-79.2
A	140	263036638	10	4	2011	277	HY	M	-103.3	5.7	-77.48
A	141	263036616	10	4	2011	277	HY	M	-107.5	5.8	-80.63
A	142	263036613	10	4	2011	277	HY	M	-121.1	5.7	-90.83
A	143	263036363	9	24	2011	267	HY	M	-98	5.5	-73.5
A	144	263036415	9	26	2011	269	HY	M	-94.7	5.6	-71.03
A	145	263036416	9	26	2011	269	HY	M	-92.1	5.3	-69.08
A	146	263036446	9	27	2011	270	HY	M	-104	5.5	-78
A	147	263036499	9	30	2011	273	HY	M	-100.6	5.4	-75.45
A	148	263036511	9	30	2011	273	HY	M	-103.5	5.4	-77.63
A	149	263036515	9	30	2011	273	HY	M	-107.8	5.3	-80.85
A	150	263036249	9	18	2011	261	HY	F	-103	5.6	-77.25
A	151	263036273	9	20	2011	263	HY	F	-91.7	5.4	-68.78
A	152	263036275	9	20	2011	263	HY	F	-96.1	5.4	-72.08
A	153	263036306	9	20	2011	263	HY	F	-110.4	5.6	-82.8

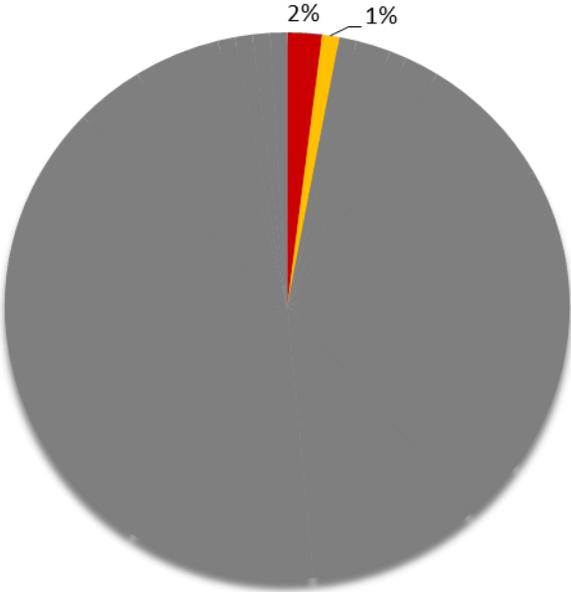
## Appendix D: Percentages of Age/Sex Divided by Breeding Contours

10% Contours	A. Male	A. Female	J. Male	J. Female	Total
neg 119 to neg 110	0	0	2	1	3
neg 109 to neg 100	0	0	0	0	0
neg 99 to neg 90	1	2	1	2	6
neg 89 to neg 80	8	4	14	4	30
neg 79 to neg 70	10	11	14	14	49
neg 69 to neg 60	0	4	5	1	10
neg 59 to neg 50	1	1	1	0	3
<b>Total</b>	<b>20</b>	<b>22</b>	<b>37</b>	<b>22</b>	<b>101</b>

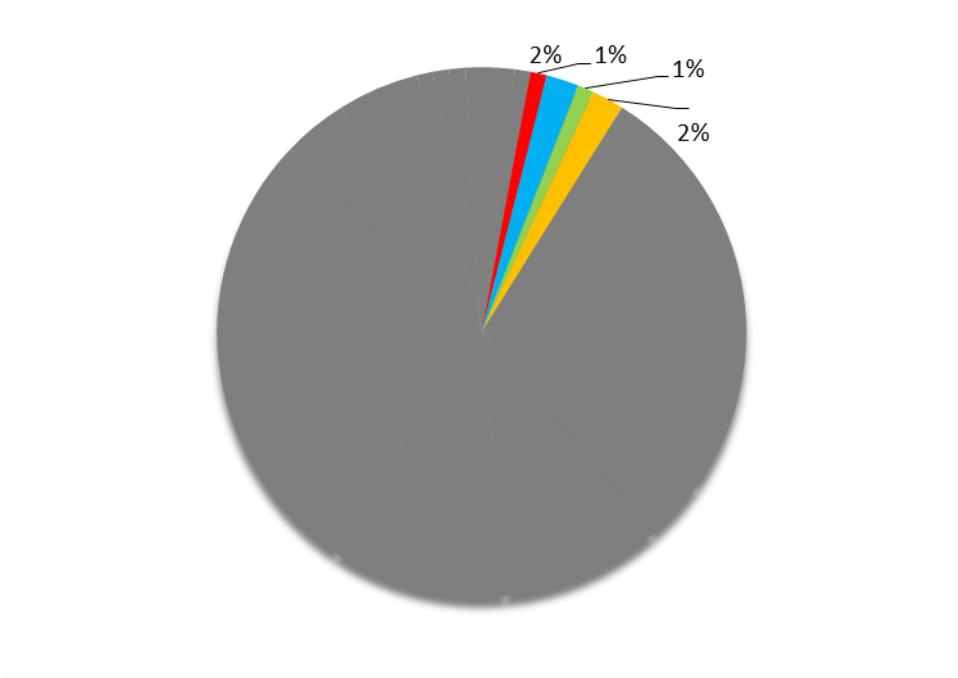
Distribution Data for -79 to -70 range:



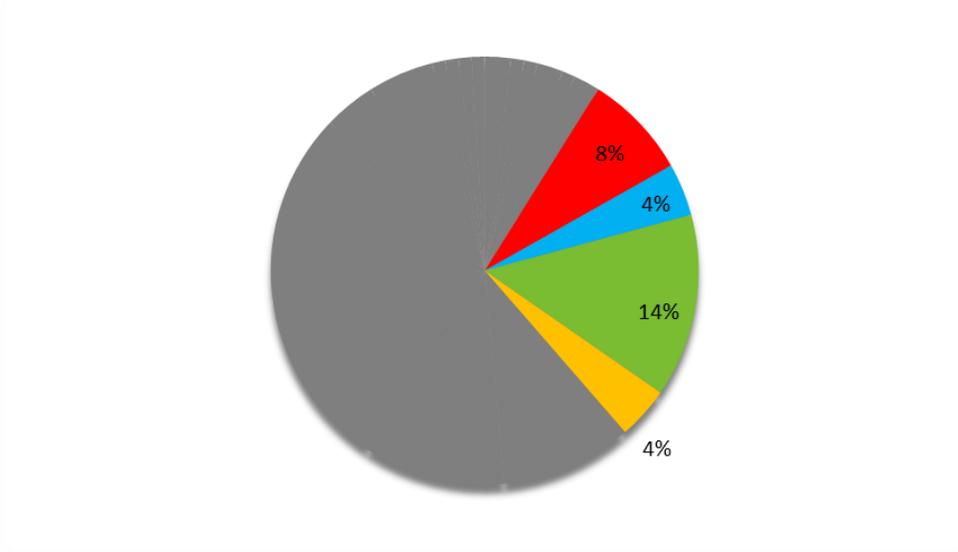
Distribution Data for -119 to -109 range:



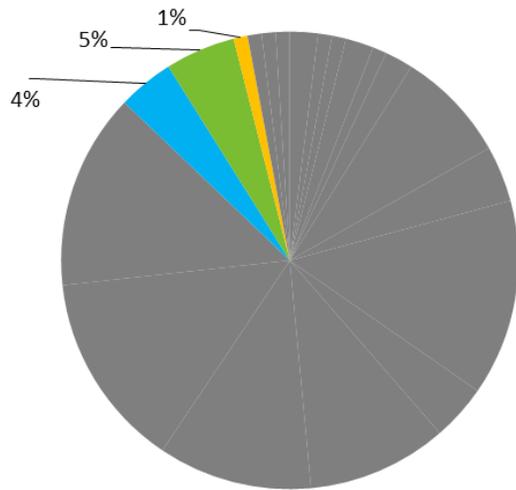
Distribution Data for -99 to -90 range:



Distribution Data for -89 to -80 range:



Distribution Data for -69 to -60 range:



Distribution Data for -59 to -50 range:

