ASSESSING POPULATION STRUCTURE AND MIGRATORY PATTERNS OF WHITE-THROATED SPARROW (*ZONOTRICHIA ALBICOLLIS*) BREEDING POPULATIONS IN WESTERN CANADA

by

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ABSTRACT

The objective of the present study was to investigate the genetic and migratory differences among White-throated Sparrow populations in western Canada in order to locate the presence of a migratory divide. Deuterium isotopes and molecular markers were used to assess the migratory differences between sparrows west (Central Interior BC) and east (Peace Region BC) of the Continental Divide. Head feather isotopes showed that both populations are overwinter wintering in either the Pacific Coast, New Mexico/Arizona or Colorado/Kansas areas. Microsatellites and mitochondrial markers did not show genetic structure among populations, however tail feather isotopes were significantly different. Analysis of migratory samples is congruent with Peace region birds migrating east of the divide. The Central Interior birds were not detected in any migratory monitoring locations. Data of the present study is congruent with a migratory divide and an east/west migration pattern between Central Interior and the Peace Region populations.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 MIGRATORY ROUTES AND CONNECTIVITY OF BIRD POPULATIONS

The study of migratory behaviour is important for determining the connectivity between breeding and wintering populations. Bird migration has been studied for many years, yet little is known about population specific migratory corridors and routes used by neotropical songbirds in North America. This lack of knowledge is of concern when human development is proposed along suspected migratory routes. In western Canada, increased interest in the development of wind energy is expanding in the northern Rocky Mountains (BC Hydro 2009). As this area also corresponds to the confluence of two migratory corridors, it is important to understand how human-made structures might impact migratory populations. In order to assess this impact, full understanding of the migratory routes and wintering areas used by breeding populations of migrant species is necessary. Once migratory connectivity is more fully understood, it will then be possible to assess the impact that a disruption on a migratory route could have on a specific breeding population.

A number of migratory routes have been recognized in North-America (Lincoln 1998). Even though it represents an oversimplification of a more complex situation, migratory routes have been grouped into four general flyways: Pacific, Central, Mississippi and Atlantic. The delimitation of these four migratory flyways has been used as a general tool to understand migratory behaviour. It should be noted that several migratory routes crossover each other and the exact routes change according to species (Lincoln 1998). The Pacific flyway extends

through the west from Alaska, following the Pacific coastline of British Columbia, Washington, Oregon, and California. The eastern extension of the Pacific flyway follows the eastern foothills of the northern Rocky Mountains where it eventually turns west heading to Oregon or California through several passes, including the Columbia or the Snake River valleys (Wythe, 1938). The Central flyway extends from breeding locations in the Northwest Territories around the Mackenzie River watershed and then follows the eastern foothills of the Rocky Mountains, where it overlaps with the eastern Pacific flyway. Instead of turning west towards the Pacific coast however, birds continue on the eastern aspect of the Rockies straight through the Great Plains to overwintering locations in Texas and Mexico (Lincoln 1998). The Mississippi flyway also extends from the Northwest Territories, originating at the Mackenzie River delta and continuing unimpeded by mountains for more than 3000 miles to the Mississippi River delta (Lincoln 1998). The Atlantic flyway follows the Atlantic coast to Florida and South America. The Atlantic coast wintering area receives birds from three or four interior migration paths: coastal region south of Delaware Bay; Central Canada coming through the south-easterly path of the Great lakes; Ontario region, following the Ohio river valley or flying south-east crossing the mountains to the Atlantic coast (Lincoln 1998).

Determining the migratory behaviour of an avian species can be very complex because birds can fly along the confluence of two or more migratory routes before heading to their final destination. In species' which utilize multiple migratory routes, these migratory overlaps complicate efforts to determine population-specific routes based solely on observational (i.e., banding station) information. However, it is essential to elucidate this complex network in

order to better infer which population(s) might be affected when human development is planned along known migratory pathways.

A very important aspect of understanding migratory routes and behaviour of avian species is the study and delimitation of migratory divides. A migratory divide is a geographic boundary between two or more breeding populations that follow different migratory routes from each other (Irwin and Irwin 2005). In North America, the Rocky Mountains have long been considered to be an important migratory divide for some species. In northern British Columbia, breeding populations of several songbird species in the Peace River Region and in Central Interior British Columbia lie in a migratory divide (Dunn *et al.* 2006). Located in this area, however, there are two important passages through the Rocky Mountains (Pine Pass and Peace River) that are used by some migratory birds during fall and spring migrations. These passes may allow populations to breach the migratory divide or lead to areas of migratory overlap, *i.e.,* between the Pacific and Central flyways. In both cases, movement through a narrow mountain pass could be considered a migratory bottleneck.

Migratory bottlenecks located in areas with abundant human structures (*e.g.* wind farm turbines, power lines) could represent a potential source of mortality for migratory species. Due to high wind speeds in the area, the eastern foothills are currently being developed for wind energy production (Environment Canada 2003). Previous studies have shown that wind farm facilities are a source of mortality for migratory birds. Wind farms in Buffalo Ridge (Minnesota) and Altamount Pass (California) showed an average of approximately 12

and 17.5 passerine casualties per year, respectively (Johnson *et al.* 2002; Smallwood and Thelander 2008). Although these numbers suggest a relatively low collision risk per wind farm, several of these facilities placed between nesting and wintering areas could act as a significant barrier to migration (Drewitt and Langston 2006). As birds will encounter several wind farms (plus several other man-made facilities) during migration, a barrier effect may be created due to these structures either compounding the collision risk, or by displacing migrants from their usual migratory route to a less efficient one, thereby altering fitness and increasing energy costs.

In order to have a better understanding of the migratory populations of songbirds in western Canada, one can study a representative species that could be compared with species using similar migratory corridors. The White-throated Sparrow (*Zonotrichia albicollis*: Emberizidae) was chosen as a representative species because it is a common North American short distance migrant with a widespread distribution. It breeds mainly east of the Rocky Mountains (Campbell *et al.* 2001; Mazerolle and Hobson 2007), but a population located in the Central Interior of British Columbia (west from the Rockies), that is not included in most species breeding range maps also exists (*e.g.,* Sibley 2000). The genetic relationship of the Central Interior population to those breeding east of the Rockies is unknown; however, preliminary data of song structure indicates some degree of differentiation (unpublished data, Mesias, V., Otter, K., Mora, M., Ramsay, S., and Murray, B.). There has been much speculation around the migratory behaviour of this population. It has been suggested by several authors (*e.g.,* Campbell *et al.* 2001; Wythe 1938) that individuals from this population migrate to the disjunct south-western overwintering area of California and

Oregon instead of to the main overwintering area in the south-eastern United States (Figure 1.1). The migratory routes used by populations on both sides of the Rocky Mountains are still undetermined. It is also unknown if these birds cross the Rocky Mountains at any point through the several mountain passes under consideration for wind development.

The main objective of this study was to examine the genetic relationship and migratory behaviour of the White-throated Sparrow populations in northern BC. By examining the genetic relationships of populations on either side of the presumed migratory divide the degree of spatial genetic structure was assessed. Differences in migratory routes were inferred through the analysis of population specific markers in birds collected at breeding locations and during the autumn migration. Deuterium stable isotopes were used to infer differences in migratory routes and to identify potential wintering areas while molecular markers were used to attempt to genetically assign migratory individuals.

Understanding the spatial genetic variation and migratory behaviour of White-throated Sparrow populations will provide useful information for proactive conservation plans. These include: the amount of genetic differentiation that exists between populations, an understanding of how each population could be affected if a migratory route is disrupted or altered, and an indication of how this disruption could affect gene flow between populations. This information may lead to a better understanding of the implications of human development on species that use corridors that cross migratory divides.

Figure 1.1 Classical Distribution of breeding (blue) and wintering (red) range of the White-throated Sparrow (*Zonotrichia albicollis*) in North America. The non-migratory range is shown in purple. The red dot indicates the Central Interior BC population which it not shown in the classical distribution map. Black arrows represent two potential wintering areas used by the Central Interior population. The distribution map was constructed with ArcMaps with layers of passerine distribution ranges obtained from NatureServe (Ridgely *et al.* 2007).

1.2 WHITE-THROATED SPARROW

The White-throated Sparrow (*Zonotrichia albicollis*: Emberizidae) is a common North American short distance migrant with a widespread distribution, mainly east of the Rocky Mountains (Campbell *et al.* 2001; Mazerolle and Hobson 2007). This species exhibits two distinct types of plumages or morphs, white and tan, which originate from a chromosomal polymorphism resulting from a pericentric inversion on the second chromosome (Tuttle 2003). White morph birds are usually heterozygous for the inversion, while tan morph birds are homozygous non-carriers (Tuttle 2003). Phenotypically, both morphs differ from each other based on the brightness in the median and superciliary crown stripes (Tuttle 2003; Campbell *et al.* 2001). Additionally, polymorphisms coincide with behavioural differences. For instance, white morph birds tend to be more aggressive with less parental care than tan morph birds (Tuttle 2003). Both morphs mate disassortatively (white males mate with tan females and *vice versa*). The maintenance of the morph polymorphism found within populations has been attributed to disassortative mating (Tuttle 2003; Campbell *et al.* 2001; Knapton *et al.*1984). Even though one of the two morphs has been reported to be in higher proportion in some populations, overall both morphs are equally represented through the entire breeding range (Falls and Kopachena 2010).

Two hypotheses have been proposed to explain why this negative assortative mating has been maintained (Falls and Kopachena 2010). First, there is a possibility that homozygote individuals for the inversion are selected against as many deleterious mutations may have accumulated in the inverted region. Second, negative assortative mating could be maintained by a combination of different strategies. For instance, white birds tend to be more territorial while tan birds engage in higher levels of parental care. In this case, both strategies could

complement each other resulting in a higher fitness for mixed couples than for non-mixed couples that are very territorial but invest little in parental care or *vice ver*sa.

White-throated Sparrow breeding populations are spread throughout large regions of Canada (Figure 1.1). Historically the distribution was believed to extend east from the slopes of the Rocky Mountains to the Atlantic coast, and north into large portions of the Yukon-Northwest Territories (Campbell *et al.* 2001). However, in 1919, breeding individuals were reported west of the Rocky Mountains in the Central Interior of British Columbia (Campbell *et al.* 2001). The origin of this breeding population is unknown, and it currently extends from the town of Mackenzie in the north, to Quesnel in the south, and to Houston, and possibly the Kispiox valley, in the west (Campbell *et al.* 2001; Wythe 1938). No studies have analyzed the genetic relationship of this population to those found east of the Rocky Mountains. An analysis of song however found population differentiation based on discriminant function analysis (unpublished data, Mesias, V., Otter, K., Mora, M., Ramsay, S., and Murray, B.). This differentiation was found to be higher when individuals were grouped per region (Central Interior BC, Peace River Region, Alberta) than as sample area. Additionally, misclassified individuals were assigned mostly to geographically close groups, suggesting higher contact between the Peace River Region and Central Interior than between Peace River Region and Alberta or Central Interior and Alberta (unpublished data, Mesias, V., Otter, K., Mora, M., Ramsay, S., and Murray, B.).

Wintering grounds of White-throated Sparrows have been reported mainly in the east and southeast United States, from Minnesota to Maine in the north, along the Gulf of Mexico from Texas to Florida in the south (Campbell *et al.* 2001). This wintering range has been extended according to observational and banding information that reported the presence of this species in California (Figure 1.1). Initially noted in the first records available in 1888 (Wythe 1938), these reports were first considered "accidental". However, with continued and increased reports in this area, authors like Whyte (1938) began to suspect that thousands of birds could be wintering along the Pacific coast. Reports of wintering sparrows have also been found in the coastal states north of California (*i.e.,* Oregon and Washington). Wythe (1938) and Campbell *et al.* (2001) include all three states, as well as parts of southwestern British Columbia, as part of the White-throated Sparrow wintering range. Although the exact breeding location of these wintering coastal sparrows still needs to be determined, it has been speculated that the sparrows breeding in the Central Interior of British Columbia are the most likely source of sparrows wintering in this location (Wythe 1938).

Compared with other wintering locations, White-throated Sparrows wintering in the Pacific coastal region have been reported to be rare but regular from Oregon to the Mexican border (Kucera 2008). Banding records of recaptures, obtained through literature searches and with the collaboration of the USGS Patuxent Wildlife Research Center Bird Banding Laboratory, as well as the110th Annual Christmas Bird Count, seem to indicate that these birds are found mainly in coastal areas, or centered around northern California (Kucera 2008; Garrison 2008; Wythe 1938; National Audubon Society 2010) (Figure 1.2).

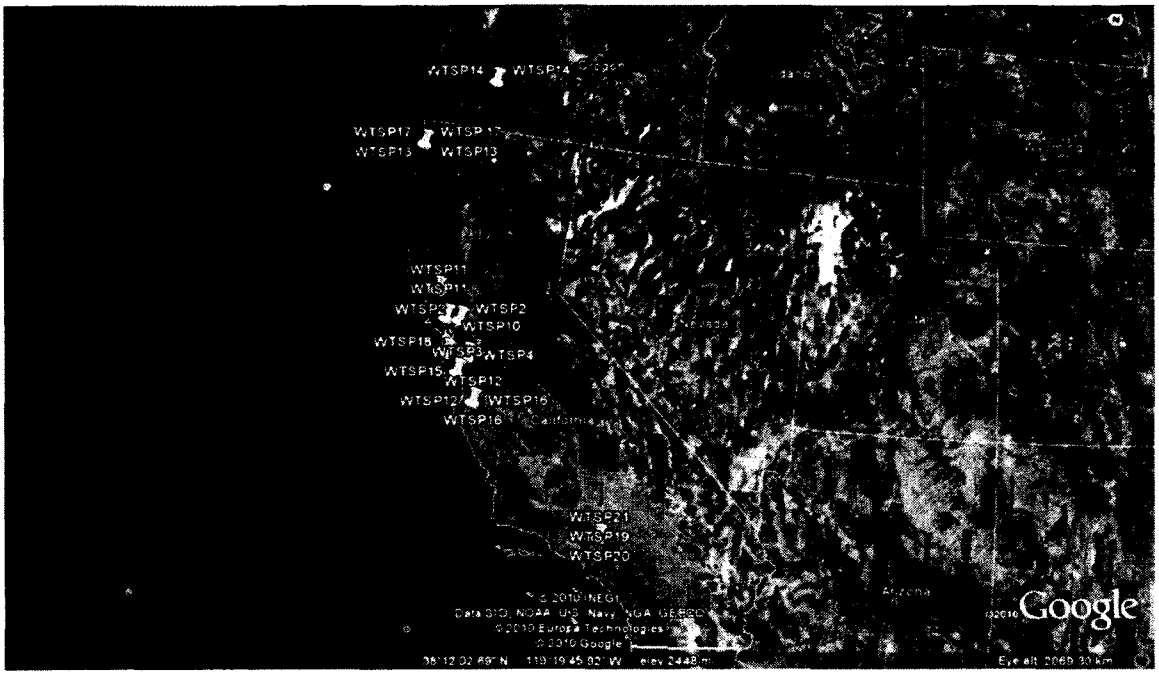


Figure 1.2 Banding recapture records in California obtained with the collaboration of the USGS Patuxent Wildlife Research Center Bird Banding Laboratory (http://www.pwrc.usgs.gov/bbl/). The map was constructed in Google Earth v.5.2.

1.3 OVERVIEW OF IMPORTANT MARKERS USED TO DELINEATE MIGRATORY POPULATIONS

Stable isotopes, molecular markers and banding recaptures are the most commonly used markers to study migratory connectivity between breeding and wintering populations. Each technique has its own pros and cons (Coiffait *et al.* 2009); however, none of them has been shown to be powerful enough to answer all the questions about bird migration. For this reason, several studies have used different combinations of techniques to understand migratory connectivity (*e.g.,* Boulet and Gibbs 2006; Clegg *et al.* 2003; Mazerolle and Hobson 2007; Norris *et al.* 2006).

Analysis of stable isotopes is a useful technique to study migratory connectivity of bird populations. The strength of this technique relies on the fact that feathers only grow during a short period of time, after which they become inert. This technique measures isotopes, ingested with food or from the environment, that accumulate in certain tissues (like feathers or claws). Isotope patterns are therefore signatures of the location where feather molting occurred, which generally happens in the breeding or wintering territories (Farmer *et al.* 2008).

The most important disadvantages associated with stable isotopes (compared with other methodologies) are the effects of climatic variation and local environmental factors on the observed ratios. Hydrogen/deuterium presents a latitudinal gradient based on precipitation and altitude, so its utility as a marker depends also on the resolution of environmental isotope

maps available (Clegg *et al.* 2003). Isotope maps, however are based on long term averages. Year to year variation has been attributed to the amount of hydrogen exchanged between the feather and ambient water vapour in a given year (Mazerolle *et al.* 2005). To compensate for yearly variation, a discrimination factor is usually added to the predicted precipitation isotope ratios (Mazerolle *et al.* 2005). In addition to seasonal climatic changes in precipitation patterns, local anthropogenic factors, causing differences in moisture regimes, such as logging, can add variability to isotopes ratios (Coiffait *et al.* 2009). Other factors that can also increase local variability are the differences in proportions of isotopes absorbed, isotope fractionation, among organisms caused by different biological, physical or chemical processes (UGSS 2012). Isotopic fractionation can have great influence on the variability of isotope markers. For instance, differences in the diet (prey sources) between juveniles and adults have been suggested as a major cause of local variation in isotopic ratios of hydrogen (Langin et al. 2007).

Another important tool for migratory connectivity studies are molecular markers. This diverse group of markers has proven to be very useful in differentiating east versus west populations that are normally located near a migratory divide (*e.g.*, Zink 1994; Zink 2008; Lecomte *et al.* 2009; Perez-Tris *et al.* 2004). Nuclear markers such as microsatellites (*e.g.*, Burg and Croxall 2001) and mitochondrial DNA sequences (*e.g.*, Perez-Tris *et al.* 2004) are the two techniques most widely used for population genetic analysis.

Both of these techniques have their own advantages. On one hand, neutral microsatellites are codominant markers that can be very useful to determine population structure and genetic diversity due to their high mutation rate (Clegg *et al.* 2003). On the other hand, mitochondrial markers do not undergo recombination, and because of their relatively lower effective population size and maternal inheritance, can be very useful when studying populations that have differentiated recently in time (Zink 2008).

The main disadvantage of genetic markers in connectivity studies is the dependence on finding a strong differentiation between populations. The degree of population structure observed depends on barriers to reproduction and on the time of divergence between populations. Genetic differentiation is an important prerequisite to effectively use a population assignment method to infer the breeding location of individuals collected along migratory routes or on wintering grounds. In order to find this differentiation, it may be necessary to develop a high number of markers, which can be difficult and time consuming in the case of non-model species. Additionally, finding population structure can be problematic in cases where avian species originate from a recent expansion from a single refugium (*e.g.,* Davis *et al.* 2006).

Other markers widely used in connectivity studies are leg/neck band recoveries. This is a cost-effective, widely-used method. Throughout North America, a large network of banding stations exists to aid in the study of bird migration and conservation. The biggest limitation with this technique is that, even in the most abundant species, low numbers of recaptures are

recovered (Mazerolle *et al.* 2005; Butler *et al.*1996). However in spite of this limitation, several cases have shown that leg band recoveries provide useful information finding migratory differentiation when other techniques fail. For instance, Mazerolle *et al.*(2005), used hydrogen isotopes from tail and head feathers (collected in Delta Marsh banding station) in order to determine the breeding and wintering areas of White-throated Sparrows. In their study, stable isotope analysis was uninformative between estimated breeding and wintering areas (head and tail feather isotope ratios), while leg banding records showed that birds from breeding populations from western Canada (Alberta, Manitoba, Saskatchewan and British Columbia) wintered in more westerly locations than birds in central (Ontario and Quebec) and eastern (New Brunswick, Nova Scotia, Prince Edward Island, Newfoundland, and Labrador) Canada.

In many cases, leg band recoveries are used to complement information from other markers. For example, Smith and colleagues (2003) used stable isotopes and leg band recoveries to study the spatial and temporal patterns of migration in sharp-shinned hawks. The stable isotope analysis data showed that early migrants passing through the migration site were from lower latitudes, while leg-band information indicated that these early migrants winter further south than birds passing later. These combined data suggest that sharp-shinned hawks use a chain migration pattern instead of leap-frog migration and illustrates the power of multiple markers to gain a full understanding of migratory behaviour.

1.4 MIGRATORY FLYWAYS AND BANDING RECOVERIES

The migratory connectivity of White-throated Sparrows has been examined in only a few studies. Deuterium stable isotope samples from the Delta Marsh banding station in Manitoba, located in the Mississippi flyway, were used to determine the southeastern United States as the main wintering area for the birds passing through this migratory station (Mazerolle et al. 2005). This study was unable to find differentiation between estimated breeding (measured by tail feather isotope ratio) and wintering (measured by head feather isotope ratio) isotopic ratios. Banding recoveries, however, showed a slight east/west differentiation in wintering areas used by western (Northeastern British Columbia, Alberta and Saskatchewan) and the rest of the eastern breeding populations (Mazerolle *et al.* 2005). It is important, however, to state that this study, as well as others, has not been performed on birds breeding in the far western portions of the range, specifically the Central Interior population of BC.

It has long been speculated that there is a link between the western wintering distribution (California and Texas) and the western breeding distribution (Central Interior British Columbia and western Alberta/Peace River Region). Wythe (1938) hypothesised that sparrows breeding at the western limit of the range (described until 1938, *i.e.* Alberta/Peace River Region) could be wintering in Texas (possibly using the Central flyway). Additionally, he suggested that birds breeding in the Central Interior could be using a Rocky Mountain passage to cross the Rocky Mountains and then head southwest from Alberta to California (using for instance the Columbia River flyway). No studies, however, have provided detailed information on migratory routes used by sparrows breeding at the western distribution of the species range that would allow an examination of these hypotheses.

The Yellowhead, Peace River and Pine Passes are described by Wythe (1938) as three possible passages used by White-throated Sparrows for fall migration. The Yellowhead Pass elevation is 975 m, the Peace River 600 m and the Pine Pass has an elevation of 869 m above sea level. Wythe (1938) suggested the Pine Pass/Peace River as possible passages used by the White-throated Sparrows that founded the first breeding population in Central Interior BC. In this hypothesis, White-throated Sparrows could have arrived when birds (normally breeding east of the Rockies) deflected from their usual migratory path, and followed the Peace River system or Pine Pass until they found suitable habitats in the Central Interior region (Wythe 1938). This hypothesis would predict a close genetic relationship between the populations on either side of the continental divide and a shared post-glacial history. It is also possible that birds in the Central Interior represent a much older population with an independent postglacial history, i.e., have separate refugia. Although evidence of breeding was first described in 1919, this date corresponds with the major influx of European settlement in the area. Historic breeding populations may have simple gone unrecorded until this date.

1.5 BANDING STATION INFORMATION

Another important source of information for understanding migratory behaviour is the banding records from banding stations across western Canada. From the banding stations that are located in BC and Alberta, eight collaborated with the present study. Five of these

stations are located in British Columbia (Mackenzie, Tattlayoko, Revelstoke, Rocky Point, Vaseux Lake) and two in Alberta (Lesser Slave lake and Beaverhill).

As expected from the species distribution maps, the Alberta banding stations reported the highest numbers of White-throated Sparrows during the Autumn migration. Beaverhill reports a total of 110 White-throated Sparrows (from 1997-2006) during the fall, and an average of 11 birds per fall (Priestley 2007), while Lesser Slave Lake reports a total of 161 White-throated Sparrows (from 2005-2008) during the fall, and an average of 40.25 birds per fall (Krikun 2005; 2006; 2007; 2008). White-throated Sparrows are regularly reported in these banding stations in the top 10 list of species captured (Krikun 2005; 2006; 2007; 2008; Priestley 2007).

The Mackenzie Nature Observatory reported the highest numbers of White-throated Sparrows in British Columbia. From 1995 -2009 this station reported a total of 170 White-throated Sparrows, with an average of 11.33 birds per year each fall (note, reports from 1995-1997 come from a smaller number of nets used) (Mackenzie Nature Observatory 2009). Banding stations in the southern part of BC reported fewer numbers of sparrows. For instance, Rocky Point Bird Observatory reported only 38 banded/45 observed White-throated Sparrows from 1994 to 2009 (reports in 1998 and 2007 were not available) (Melcer and Nightingale 2009; David 2006; 2008; Jantunen 2003; 2004; Gibson 2002; Derbyshine 1999; 2000). Only 3.5 birds per year where observed during this range of time with most individuals banded between mid-September to late-October.

Other stations in the south part of the province, such as Tatlayoko, Revelstoke and Vaseux Lake Bird Observatories, rarely report captures of White-throated Sparrows. Tatlayoko, for instance, had an average of one bird banded (or observed) per year, from 2006 to 2009 (Ogle 2008; 2009a; 2009b). Vaseux Lake reports the same average from 2002 to 2009; however, their captures year-to-year are more inconsistent than Tatlayoko with a high of 3 sparrows captured in 2005 and a low of no sparrows captured in 2004, 2006, and 2007 (eBird Canada 2010).

1.6 RESEARCH OBJECTIVES AND THESIS ORGANIZATION

The main objective of the present study was to investigate the genetic and migratory differences among White-throated Sparrow populations in western Canada. This information is needed to address hypotheses on the location of a migratory divide and on population specific migration routes that, in the context of ongoing development, can ultimately be used for proactive management. To do this, the differential use of migratory routes was inferred by analyzing the migratory connectivity between breeding populations and overwintering areas (Chapter 2), as well as the spatial genetic structure of breeding populations in Western Canada (Chapter 3).

Chapter two compares natural ratios of deuterium in White-throated Sparrow head and tail feathers (δD_f) with the latitudinal gradient displayed by deuterium isotope concentrations in precipitation (δD_p) in order to identify wintering grounds used by breeding populations in

British Columbia on either side of the continental divide. Head feathers δD_f were used to infer the wintering grounds used by birds from known breeding populations, while tail feathers δD_f were used to infer breeding areas used by migratory sparrows in order to infer migratory routes used by those birds.

Chapter three uses neutral microsatellite markers and mitochondrial DNA sequences to determine the amount of genetic differentiation among breeding populations of White-throated Sparrows in western Canada. This genetic structure was then used to attempt to assign migratory individuals captured with the collaboration of migratory banding stations across BC and Alberta. This technique could then allow us to establish routes of importance for many populations of White-throated Sparrows and help determine how different populations of these birds could be contributing to the mixed genetic stock found in each migratory corridor.

Chapter four is a synthesis of the above information that indicates the utility of these techniques for proactive management of migratory bird species. This chapter also performs an assessment of the techniques used, describing their applicability to other species and how they can be improved. Additionally, this chapter shows how the present study contributed to the understanding of the migratory behaviour of White-throated Sparrows and what are the environmental and management implications that could be estimated based on the information obtained.

CHAPTER 2

DETERMINATION OF MIGRATORY CONNECTIVITY AND MIGRATORY PATTERNS USED BY WHITE-THROATED SPARROWS IN WESTERN CANADA USING DEUTERIUM STABLE ISOTOPES

2.1 INTRODUCTION

Bird migration and the connectivity between breeding and wintering populations has been a central area of interest in ecological and evolutionary studies. Detailed knowledge of the use of migratory corridors and wintering areas by songbirds is important for understanding both their ecology as well as in the development of appropriate conservation plans for maintaining breeding populations. Such plans rely on an understanding of the connectivity between summer/winter grounds of individual populations and the potential impact that human-made structures may have as a result of altering or disrupting migratory routes. However, due to the inherent difficulty of tracking birds during migration, the study of migratory connectivity has been severely obstructed (Webster *et al.* 2002).

The Peace River Region of British Columbia is one area of potential importance for bird migration and which is situated in a site of increasing human development (*i.e.,* wind energy). The Peace River Region is located on the east side of the Continental Divide of the Americas, marked in this region by the presence of the Rocky Mountains. This region of the Continental Divide has also been associated with an important migratory divide for several avian species (Dunn *et al.* 2006). This region also has several passes that connect both sides

of the divide that are used by migratory birds. These passes are migratory bottlenecks for birds crossing both sides of the mountains. It is not known how human development in this area might affect migration. Depending on population specific migrations routes, bottlenecks could affect one or both of the populations on either side of the migratory divide.

The White-throated Sparrow (*Zonotrichia albicollis*) is a common North American short distance migrant generally found east of the Continental Divide. Differences in song structure have been noted for a breeding population located west of the divide in the Central Interior of BC to those located east of the divide (unpublished data, Mesias, V., Otter, K., Mora, M., Ramsay, S., and Murray B.). It has also been proposed that the Central Interior population has a different migratory route and overwintering area than those found east of the divide (Whyte 1938; Campbell *et al.* 2001). Details on population specific migratory routes, however, are lacking and hypotheses of migratory connectivity have yet to be tested. Understanding population specific migratory connectivity and routes on both sides of the Continental Divide (also possibly a migratory divide for this species) is crucial in order to identify how these populations could be affected by human development.

Unlike to more traditional markers, such as leg/neck banding, data collection is relatively rapid and does not suffer from the limitations associated with the low number of band recoveries (Coiffait *et al.* 2009). Stable isotopes are variants of individual atomic elements, which differ in the number of neutrons and therefore have unique atomic masses (Coiffait *et al.* 2009). The relative proportions of isotope that accumulate in growing tissue vary

according to the environment and diet. In birds, the use of isotopes to identify wintering or breeding habitats has exploited the fact that specific body tissues, such as feathers or claws, incorporate different isotope signatures from the environment (*e.g.* rain or diet) during the period of their growth. These tissues then become metabolically inert, preventing the isotopic signatures from fluctuating over time (Mazerolle *et al.* 2005). These characteristics have been very useful for determining animal movements across a landscape, especially across latitudinal migratory patterns (*e.g.,* Clegg *et al.* 2003; Smith *et al.* 2003; Wassenaar and Hobson 2001; Hobson and Wassenaar 1997).

Hydrogen/Deuterium has been one of the most successful isotopes in bird studies, showing a clear latitudinal differentiation in several studies (*e.g.,* Mazerolle *et al.* 2005; Clegg *et al.* 2003; Smith *et al.* 2003; Wassenaar and Hobson 2001; Hobson and Wassenaar 1997). Hydrogen isotopes (or deuterium δD) have also been shown to be associated with rainfall patterns and other environmental variables. Levels of Deuterium seem to follow a latitudinal pattern decreasing at higher latitudes, elevations, and towards the continental interior (Clegg *et al.* 2003). However, temporal changes in climatic conditions and diet of birds can add variation to the observed isotope ratios (Wassenaar and Hobson 2006).

The discrimination power of this element depends on the resolution of the environmental isotope map available (Clegg *et al.* 2003). These environmental maps are based on a 40-year average of both geographical and climatological isotope information available from the Global Network for Isotopes in Precipitation (GNIP) database. These models then use a

"kriging" statistical procedure to interpolate isotope data of unknown locations from known locations. In this method, a georeferenced isotope precipitation profile (δD_p) map is used to infer the location where tissue growth occurred based on the tissue, *i.e.,* feather, isotope profile (δD_f) (Meehan *et al.* 2004).

Deuterium stable isotopes are particularly useful in White-throated Sparrows because these birds have two differential moults that happen during different parts of the season. The crown and tail feathers of this sparrow have different periods of growth that provide isotopic signatures of the breeding and winter grounds, respectively. In the first moult (on breeding grounds before fall migration) all feathers are replaced, while at the second moult (on wintering grounds before spring migration) only the body feathers in the head region are replaced (Mazerolle *et al.* 2005). Although this differential moult pattern is not present in all avian species, in the present study this characteristic was useful to determine wintering territories used by breeding individuals as well as to determine potential breeding areas used by migratory individuals.

The first objective of this study was to use the head feather δD_f to establish the wintering areas used by western Canadian populations of White-throated Sparrows on both sides of the Continental Divide (*i.e.,* Central Interior BC and Peace River Region). The second objective was to use the tail feather δD_f to estimate the most probable breeding grounds used by migratory individuals sampled at stopover sites and banding stations across British Columbia

and Alberta. These studies will provide evidence on the presence of a migratory divide and locations of migratory bottlenecks in northern British Columbia as well as the potential impact that human-made structures can have on the disruption of migratory routes of White-throated Sparrows. Elucidation of population's specific migratory routes and wintering areas used by White-throated Sparrows are vital in order to delineate future conservation projects in which important areas for bird migration could be conserved, not only for these songbirds, but also for other species using similar migratory corridors.

2.2 METHODOLOGY

2.2.1 STUDY AREA AND SAMPLE COLLECTION

Standard mist-net technique was used to capture White-throated Sparrows from breeding and migratory locations (Figure 2.1). For most breeding birds captured (May2009- June 2010) in Prince George (n = 20) (19 tail and 18 head) and Dawson Creek (n = 27) (26 tail and 26 head) both tail and head feathers were analyzed. In addition, tail samples from three breeding individuals (collected May 2009) from Sikanni River (most northern location) were included for calibration purposes. Seven banding stations across British Columbia (BC) and Alberta (AB) collaborated to sample migratory White-throated Sparrows (July – Sept 2009). The frequency of White-throated Sparrows sampled varied according to the banding station; with no sparrows being captured at Vaseux Lake, Tatlayoko, and Revelstoke, three samples from Rocky Point, nine from Mugaha Marsh, nine from Lesser Slave Lake and seven from Beaverhill banding station.

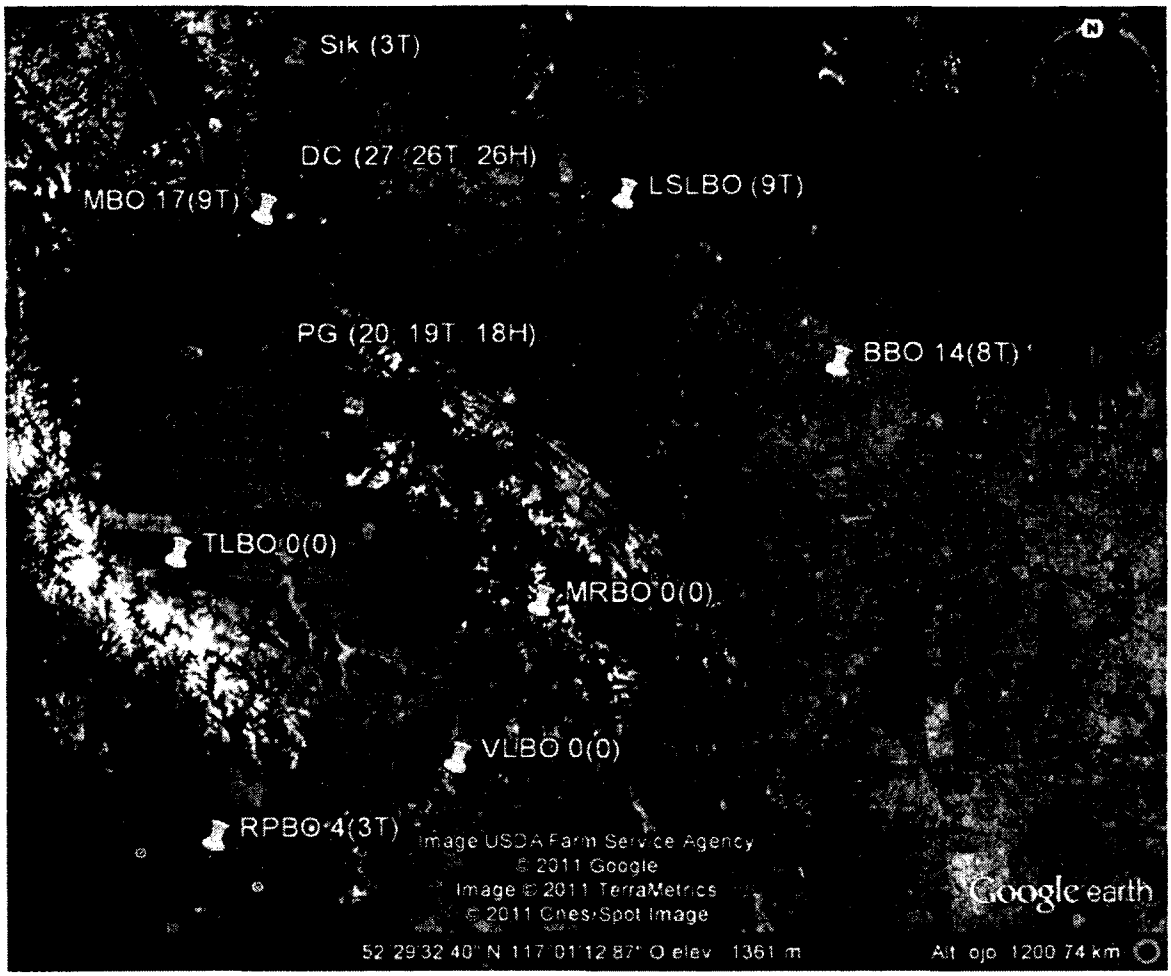


Figure 2.1 Map of sampling locations included in stable isotope analysis. Prince George (PG), Dawson Creek (DC), Sikanni (Sik), Mugaha Marsh (MBO), Rocky Point (RPBO), Vaseux Lake (VLBO), Mount Revelstoke (MRBO), Tattlayoko (TLBO), Lesser Slave Lake (LSLBO) and Beaverhill Bird Observatory (BBO) were included. Sampling locations west from the continental divide were grouped into the Central Interior population (in blue), and east from the divide were grouped as the Peace River Region population (in green). Sampling locations collected during fall migratory season in collaboration with banding station in BC and AB (in yellow) was also included. The number outside the parenthesis represents the amount of sparrows caught during the fall season of 2009 (if available).The first number inside parenthesis represent the number of samples obtained from each location, Second and third numbers represent the amount of tail (T) and head (H) samples analyzed per location. The map was constructed in Google Earth v.5.2.

2.2.2 SAMPLE PREPARATION AND ANALYSIS

Tail and feather samples were subjected to three rounds of cleaning using a 2:1 chloroform:methanol solution, rinsed with MilliQ H_2O, and air-dried for 24 hours under the fumehood (Wassenaar and Hobson 2006; Mazerolle *et al.* 2005). Entire head feathers and the terminal veins of the tail feathers were sub-sampled in the same location to avoid inconsistent periods of feather growth (Wassenaar and Hobson 2006). Subsamples were weighed until they reached an optimum weight (0.1 - 0.3 mg) and then packed into silver capsules (Isomass Scientific Inc). Silver capsules containing the feather samples were crushed into small balls and inserted in a 96-well PCR tray. Non-exchangable Hydrogen (δD_f) of feathers was analyzed using a Hekatech HT Oxygen Analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the University of California, Davis. The fraction of exchangeable hydrogen between feathers and environment was corrected using keratin standards as described by Wassenaar and Hobson (2003). Deuterium concentration was expressed in units per ml (‰) relative to the international standard V-SMOW (Vienna Standard Mean Ocean Water).

To estimate the migratory areas used by White-throated Sparrows breeding populations, non-exchangeable feather deuterium isotope ratio values (δD_f) were compared with the precipitation deuterium isotope ratio values (δD_p) for North America. However, δD_f values are often different from δD_p values [because of different variables, such as, fractionation factors or the percentage of Hydrogen that is exchangeable with the environment (Farmer *et al.* 2008)].

Forty per cent of the feather hydrogen is potentially exchangeable with the environment (Hobson and Wassenaar 1997). To adjust for amount of hydrogen exchanged with the environment, a correction factor was used to modify the mean growing season precipitation (δD_p) map of Meehan *et al.* (2004) (Figure 2.2). This map is based on a 40-year average of geographical (sometimes altitudinal data) and climatologically isotope information available at the Global Network for Isotopes in Precipitation (GNIP) database. To estimate the correction factor to be used, δD_f values of tail feathers from individuals captured at known breeding locations (Prince George, Dawson Creek and Sikanni) were compared with predicted precipitation isotopes for those locations. Based on these comparisons, an adjustment factor -30‰ was used to calibrate δD_p precipitation values. Predicted precipitation isotopes were obtained using the Online Isotopes in Precipitation Calculator OIPC v2.2 (Bowen G.J 2011). The modified Meehan *et al.* (2004) GIS map layer was combined with a White-throated Sparrow range layer available on the NatureServe web site (Ridgely *et al.* 2007) in order to estimate isotope values for breeding (tail feathers) and wintering (head feathers) areas. These values were compared to observed δD_f ratio of the White-throated Sparrow feathers.

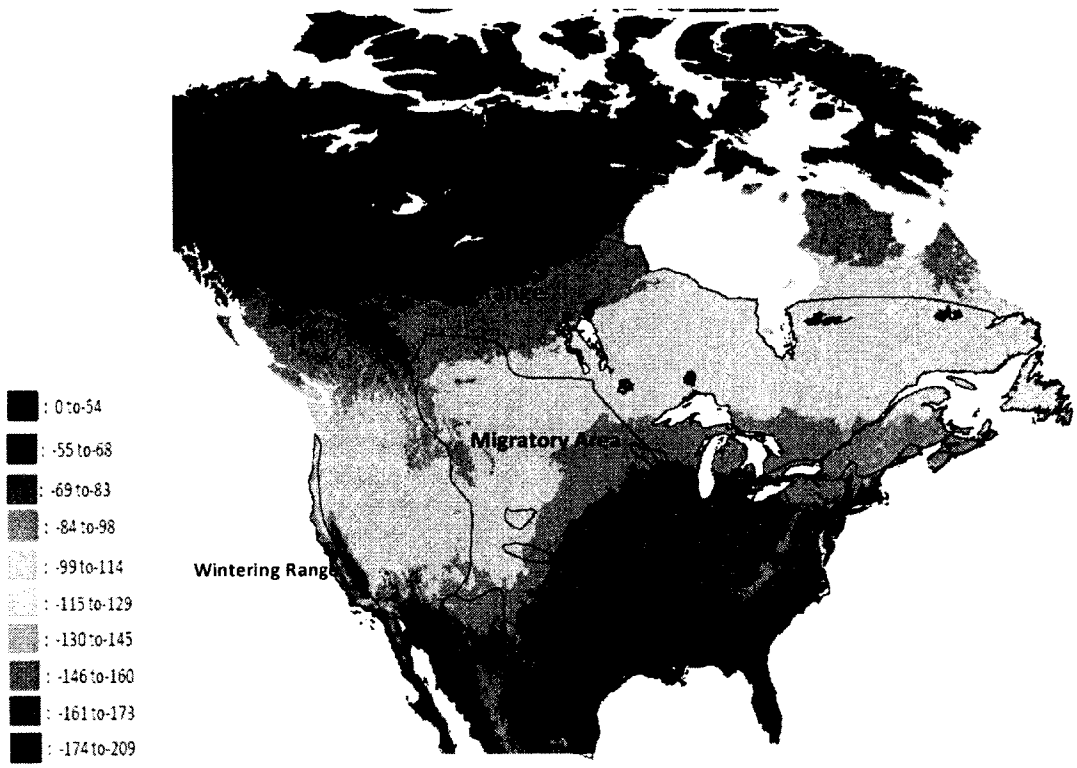


Figure 2.2 Stable Isotope precipitation GIS map (δD_p) modified from Meehan *et al.* (2004). Isotope relief map contour areas from isotope environmental map were modified with adjustment factor of -30 ‰ to compensate the Hydrogen exchange of δD_f. A layer showing the White-throated Sparrow range was added; obtained from NatureServe (Ridgely *et al.* 2007). GIS map obtained with permission of Tim Meehan (December 20, 2010).

2.2.3 STATISTICAL ANALYSIS

All statistical analyses were done with SPSS v.18.0 with and without outliers. Outliers were determined using a stem-and-leaf plot and according to reported wintering and breeding ranges in Western Canada (>-130 ‰ δD_f for wintering samples and < -131 ‰ δD_f for breeding samples). Once outliers were detected they were removed for all subsequent analysis. Descriptive statistics (Mean, Standard Deviation and Confidence Intervals) were calculated and plotted. Frequency distribution of tail and head feathers δD_f values (‰) were plotted in a bar graph against corrected δD_p ranges (based on Meehan *et al.* 2004 precipitation map). Normality of samples for each sampling location was assessed using a Kolmogorov-Smirnov test. Head feather isotope ratios were analyzed with parametric one-way ANOVA and t-tests.

To test statistical differences of tail feather isotope ratios, an initial comparison between all locations was done using Kruskal-Wallis tests. After this analysis, pairwise comparisons between Central Interior, Peace River Region and the banding stations were analyzed using non-parametric Mann-Whitney-U test. Additionally, in order to test if the date when birds were banded had any influence on the isotope ratios, ANCOVA analysis of banding stations data with date as a covariate was conducted.

2.3 RESULTS

2.3.1 DISTRIBUTION AND OUTLIERS

Most stable isotopes data from head and tail samples fit a normal distribution ($P>0.5$) (Kolmogorov-Smirnov for one sample test). The only sample location that showed significant deviation from a normal distribution was the tail-feather samples from the Peace River Region ($0.01> P <0.05$). As a result of this deviation from normality, tail-feather samples were analyzed using non-parametric statistics while head-feather samples were analyzed with parametric (ANOVA) statistics.

Analysis of tail samples showed three values of δD_f values (-85‰, -89‰ and -130‰) as clear outliers (Table 2.1a, b). These values lie outside of the values noted for the breeding distribution of White-throated Sparrows in western North America. Head-feather analysis failed to show statistical outliers; however, as four of the lowest values (from -137.2‰ to -145.9‰) clearly mapped outside of the wintering area, they were considered as outliers (Table 2.1c). Statistical analyses were done with and without outliers. However, as excluding outliers from analysis did not significantly change the outcome of the study all the analysis shown in the present study was done without outliers.

Table 2.1 A) Five highest and lowest δD_f (‰) values of Central Interior tail feather samples. B) Five highest and lowest δD_f (‰) values of Peace River Region tail feather samples. C) Five highest and lowest δD_f (‰) values of head feather samples of all regions.

A)

	Relative Rank	Individual	δD_f (‰)	Included or Excluded
	1	Zoal-ja174	-89.8	Excluded
	2	Zoal-ja177	-137.9	Excluded
Highest	3	Zoal-ja176	-138	Included
	4	Zoal-ja170	-139.2	Included
	5	Zoal-ja171	-139.6	Included
	5	Zoal-ja183	-151	Included
	4	Zoal-ja173	-151.4	Included
Lowest	3	Zoal-ja175	-152.3	Included
	2	Zoal-ja188	-156.7	Included
	1	Zoal-ja182	-156.8	Included

B)

	Relative Rank	Individual	δD_f (‰)	Included or Excluded
	1	Zoal-jd157	-85.1	Excluded
	2	Zoal-jd147	-130	Excluded
Highest	3	Zoal-jd145	-138.3	Included
	4	Zoal-jd156	-147.5	Included
	5	Zoal-jd150	-148.6	Included
	5	Zoal-jd155	-162.2	Included
	4	Zoal-jd154	-163.6	Included
Lowest	3	Zoal-jd137	-164.3	Included
	2	Zoal-jd138	-165.6	Included
	1	Zoal-jd160	-167.6	Included

C)

	Relative Rank	Individual	δDf (‰)	Included or Excluded
	1	Zoal-jd141	-54.3	Included
	2	Zoal-jd153	-55.2	Included
Highest	3	Zoal-jd160	-58.7	Included
	4	Zoal-jd156	-59.1	Included
	5	Zoal-ja178	-60.4	Included
	4	Zoal-ja173	-123.2	Included
	3	Zoal-jd148	-137.2	Excluded
Lowest	2	Zoal-jd060	-138	Excluded
	1	Zoal-ja188	-138.7	Excluded
	5	Zoal-jd146	-145.9	Excluded

2.3.2 HEAD FEATHER SAMPLE ANALYSIS

Head feather δD_f (‰) values for each sampling location were binned (Figure 2.3) according to the relief map countour lines (Figure 2.2). The Central Interior samples δD_f (‰) values were mainly distributed (~40%) between -69 – -83 ‰, while the remaining samples were equally distributed in a lower percentage across four other areas (Figure 2.3). The Peace River Region had δD_f (‰) values distributed across five different relief map contour areas, with higher percentages (~30%) between -69 – -83 ‰ and -99 – -114 ‰ (Figure 2.3). Head-feather samples showed a high standard deviation, which was twice as much as the standard deviation obtained from tail-feather samples. Central Interior head samples had a mean δD_f (‰) and standard deviation (-84.12±18.16 ‰) lower than Peace River Region (-90.33±21.46 ‰). No statistical differences were detected using ANOVA (F: 0.931, P: 0.341) or T-tests (F: 1.47, P: 0.233). Confidence intervals at 95% of head feather samples were also much greater than tail feather samples (Figure 2.4).

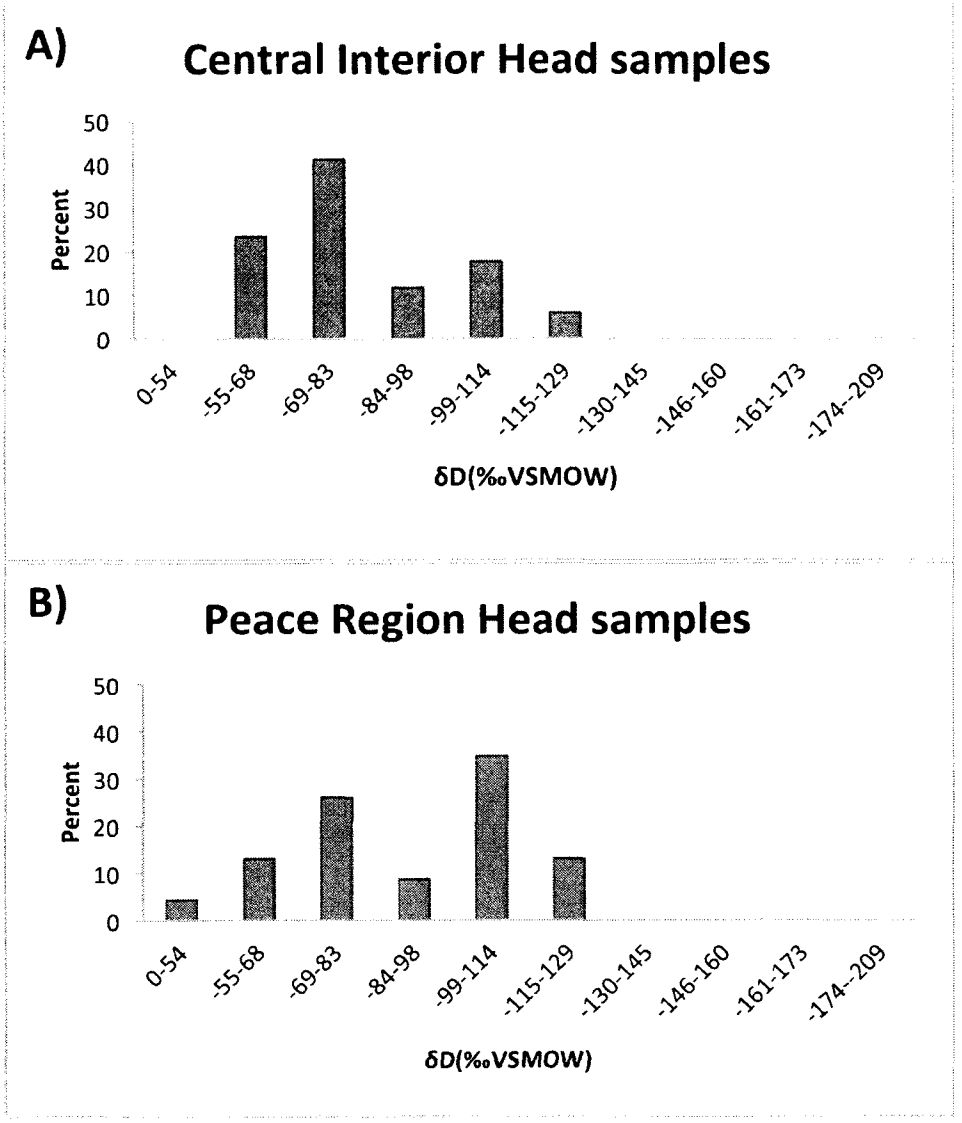


Figure 2.3 Percentage of δD_f (‰) from head samples (without outliers) binned based on the δD_p (‰) relief contour areas from the modified Meehan *et al.* (2004) isotope environmental map (Figure 2.2). A) Central Interior, B) Peace River Region

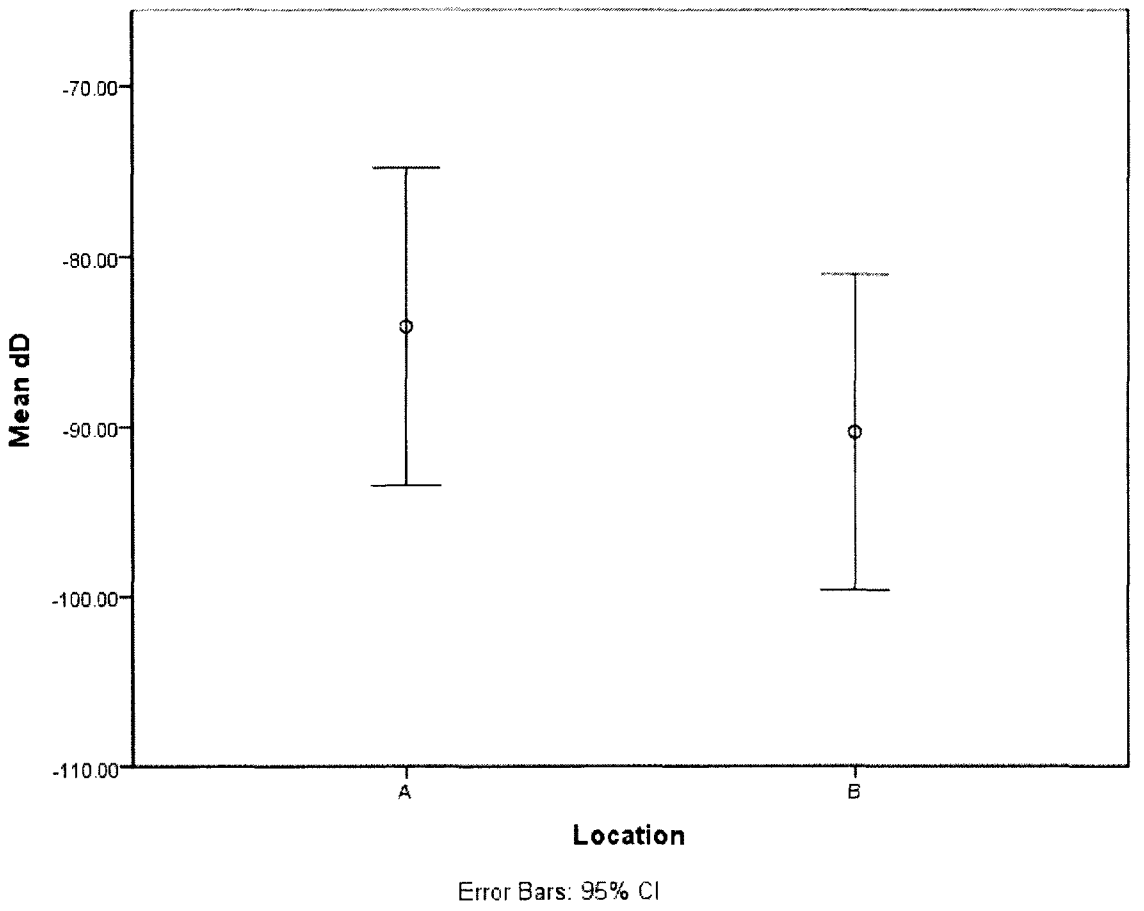
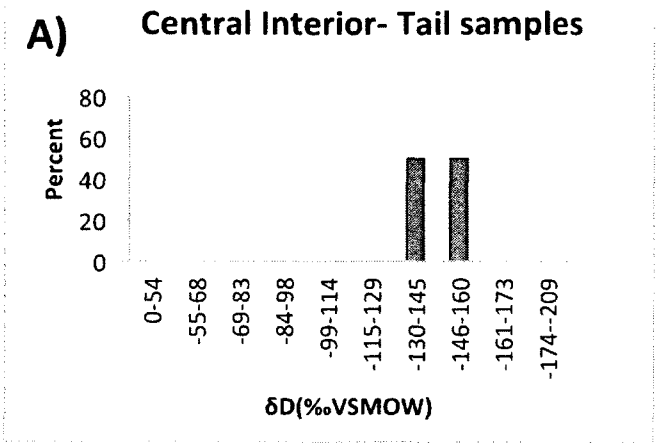


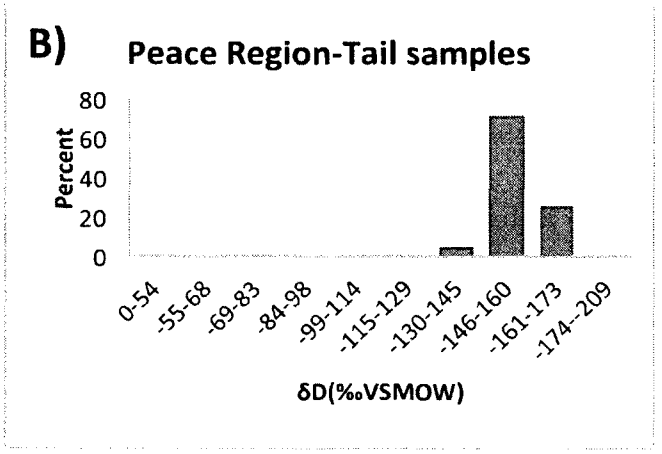
Figure 2.4 Mean δD_f (‰) values of head samples with Confidence Intervals 95% (95% CI) bars. A) Central Interior and B) Peace River Region locations are shown.

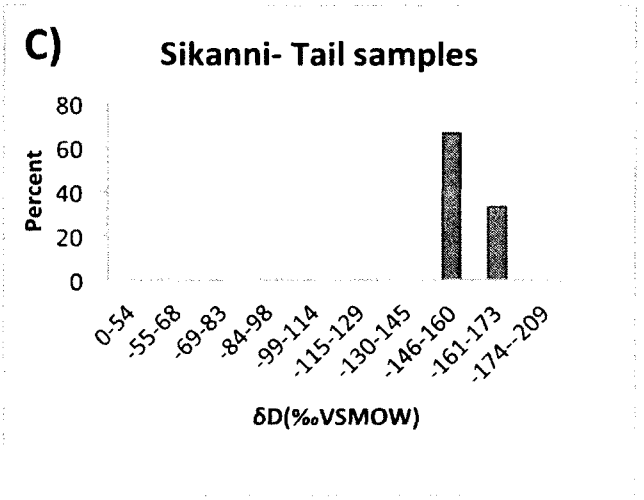
2.3.3 TAIL FEATHER SAMPLE ANALYSIS

To assess the distribution of data, tail feather δD_f (‰) values were also compared according to the δD_p (‰) value relief areas (Figure 2.5) from the Meehan *et al.* (2004) isotope environmental map (Figure 2.2). All Central Interior sample δD_f (‰) values were distributed between -130 ‰ – -160 ‰ zones (Figure 2.5A). In contrast the Peace River and Sikanni samples were distributed primarily in the -146 – -173 ‰ zones (Figure 2.5, B+C). Tail isotope values distribution from Lesser Slave Lake (LSLBO), Beaverhill (BBO) and Mackenzie (MBO) samples were mostly spread between -146 – -160‰, and -161 – -173‰ while Rocky Point (RPBO) distributed in the -146 –-160‰ zone (Figure 2.5, D-G).

Breeding Locations







Migratory Locations

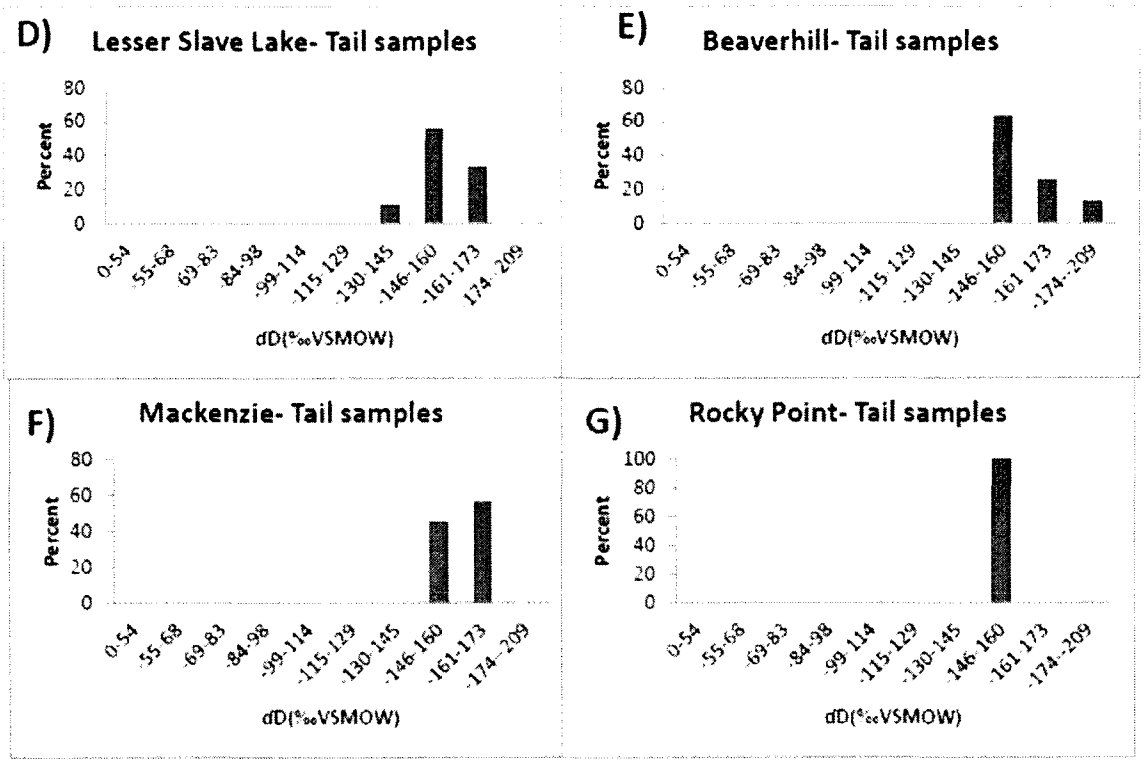


Figure 2.5 Percentage of δD_f (‰) from tail feather samples (outliers included) binned based on the δD_p (‰) relief contour areas from the Meehan *et al.* (2004) isotope environmental map (modified with -30 ‰ adjustment). Locations: A) Central Interior, B) The Peace River Region, C) Sikanni River, D) Lesser Slave Lake bird observatory, E) Beaverhill bird observatory, F) Mackenzie bird observatory, and G) Rocky Point bird observatory are shown.

Samples collected from known breeding locations (Central Interior, Peace River Region and Sikanni) show mean values that agree with the expected latitudinal pattern, with mean δD_f (‰) values that decreased (*i.e.,* became more negative) as their latitudinal location increased (Figure 2.6). The Central Interior showed the highest mean (-146.04 ±6.29 ‰ SD) compared with the Peace River Region (-154.8 ±7.01 ‰ SD) and Sikanni (-158.63 ±2.87 ‰ SD). The Rocky Point Bird Observatory (RPBO) (-151.77 ±3.6 ‰ SD) mean value was intermediate between Peace River Region and Central Interior while the Mackenzie Bird Observatory mean (-159.95 ±4.56 ‰ SD) was very close to Sikanni River. The Alberta banding stations showed mean values within this range; however, their standard deviation and 95% CI were bigger than the rest of samples. Lesser Slave Lake had a higher mean value (-154.67 ±9.49 ‰SD) compared with Beaverhill mean (-158.08 ±8.74 ‰ SD).

Initial comparison using Kruskal-Wallis test (Non-parametric alternative to ANOVA) showed highly significant differences ($P \leq 0.01$) between means among populations. Pairwise comparisons using Mann-Whitney-U of Central Interior showed significant differences versus Peace River Region, Sikanni, Mackenzie and Beaverhill, and Lesser Slave Lake (Figure 2.6). On the other hand, The Peace River Region showed significant differences only against the Central Interior and Mackenzie. ANCOVA analysis showed no significant differences ($P > 0.5$) between banding stations isotope ratios with collection date as a covariant.

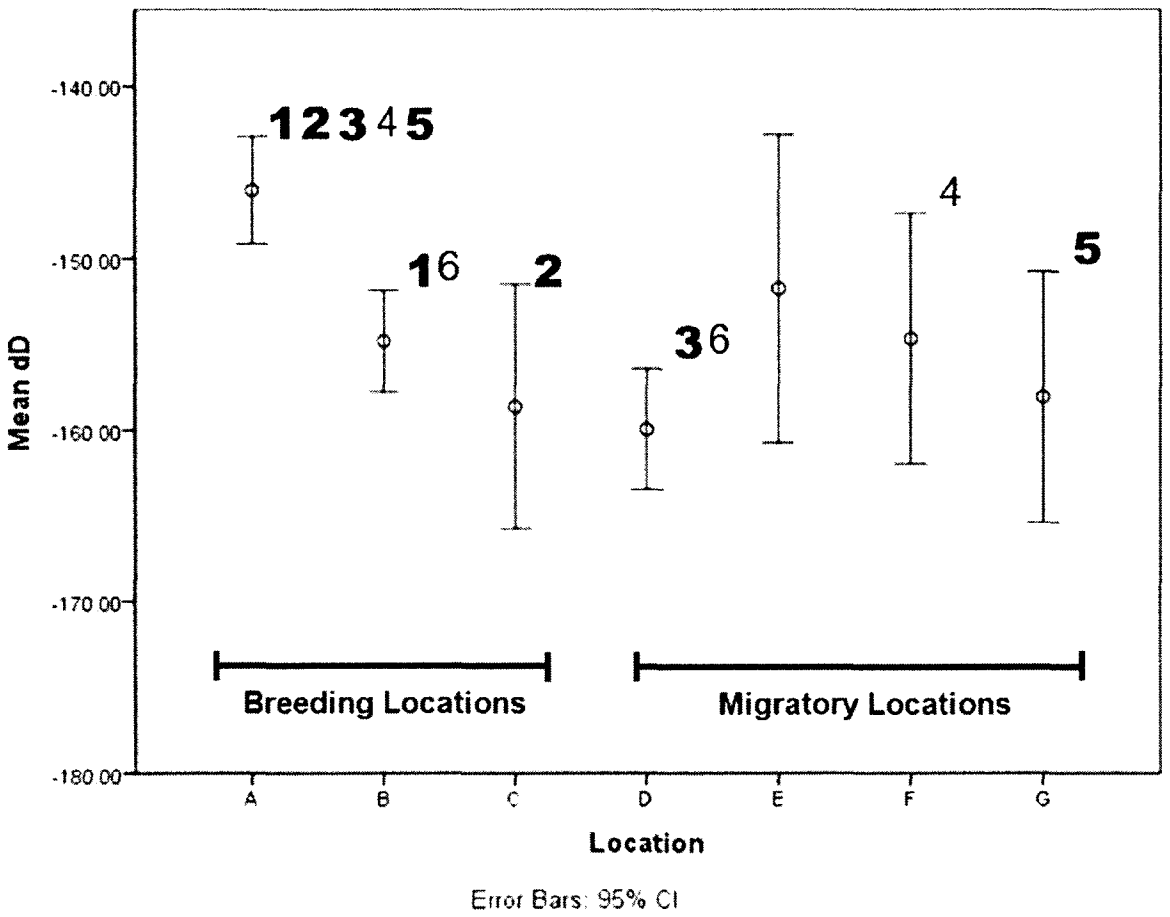


Figure 2.6 Mean δD_f (‰) values of tail samples with Confidence Intervals 95% (95%CI). Breeding locations: A) Central Interior, B) Peace River Region, C) Sikanni, as well as migratory locations: D) Mackenzie, E) Rocky Point, F) Lesser Slave Lake, G) Beaverhill are shown. Bold numbers indicate highly significant differences ($P \leq 0.01$) in pairwise comparisons (using Mann Whitney-U), while smaller font not-bold numbers indicate significant differences ($P \leq 0.05$).

2.4 DISCUSSION

2.4.1 DETERMINATION OF WINTERING TERRITORIES BASED ON HEAD FEATHER DEUTERIUM ISOTOPES

Head feathers isotope values (δD_f) presented a greater range of values compared with tail feather isotope values. This variability may reflect the higher complexity of the isotope δD_p relief patterns at the wintering range than at the breeding range and/or may be further influenced by the differential migration by males and females. Differential migration of both sexes, where females migrate longer (have more positive δD_f values) than males has been noted in White-throated Sparrows (Mazerolle and Hobson 2007) and could partially explain the wide distribution of head-feather samples. Differential migration could not be fully tested in this study because in many samples the sex of the bird could not be determined in the field. An alternative to avoid the difficulties of visual determination of sex in sparrows would be using molecular techniques for sex discrimination (such as the CHD gene amplification) for more detailed examination of sex specific migratory patterns (Dubiec and Zagalska-Neubauer 2006). However if present, the effect of sex specific migration patterns would appear minimal as when the small number of known males were tested statistically (not shown) no differences were observed with the overall mean.

The timing of moults can also influence the range of values observed. If a proportion of the birds moult during migration then the range of values might not only reflect the isotopic signal of wintering sites but those of stopover locations. This effect, however, was not noted in previous studies of White-throated Sparrows, which have shown that less than 4% of

individuals had not completed their prealternate moult when banded at spring migration monitoring banding stations (*e.g.,* Mazerolle *et al.* 2005). Additionally, head feather samples compared with claws and blood samples were reported as the most accurate and consistent samples used for isotopes studies (Mazerolle and Hobson 2005).

Head feather stable isotope values did not show significant differences between the Peace River Region and Central Interior populations. Both populations, however, had higher isotopic signatures than what were expected if they would be wintering in the southeastern US (Figure 2.2). The southeastern US (east of Great Plains) has been described as the main wintering ground used by White-throated Sparrows (Campbell *et al.* 2001; Falls and Kopachena 2010). Stable isotope analysis has also identified this area as the main wintering ground (expected δD_f between 0 and ~68‰) used by migratory White-throated Sparrows captured at the centrally located Delta Marsh (Manitoba) banding station (Mazerolle *et al.* 2005). In contrast, the mean value of head feather δD_f observed in Central Interior and Peace River Region populations fit with the upper limit of the wintering range, limiting possible wintering areas used by these populations to three locations: The Pacific coast of the United States, New Mexico-Arizona and Colorado/Kansas (Figure 2.7).

Previous studies have suggested that the Pacific coast is the most likely wintering area for breeding population of the Central Interior (Campbell *et al.* 2001; Wythe 1938). However, due to the overlap in expected isotope values we cannot differentiate among the Pacific coast

or the New Mexico-Arizona and Colorado/Kansas areas. In the Pacific wintering area, most White-throated Sparrows have been observed in the northern California coastal areas (*e.g.* San Francisco); although sparrows have been observed wintering in Washington and Oregon coasts and southeastern Vancouver Island (Kucera 2008; Garrison 2008; Wythe 1938; National Audubon Society 2010) (Figure 1.2). Very few birds have been observed wintering in the Sonora desert and at higher altitude in the Sierra Nevada Mountains (National Audubon Society 2010). This distribution predicts that the birds wintering along the Pacific coast should have a narrower isotope profile than birds wintering at the New Mexico/Arizona and Colorado/Kansas area (Figure 2.7). The Central Interior population does have a narrower isotope distribution than the Peace River Region, suggesting that this population could be wintering in the Pacific coastal region (Figure 2.3).

The most parsimonious hypothesis for wintering areas used by the Peace River Region population would be that these birds are migrating to the New Mexico-Arizona and Colorado/Kansas region. Because of the proximity to the southern Rocky Mountains (altitude effect on δD_p), these two regions present a wider range of isotope values (Figure 2.7), which seems to fit the pattern observed in the isotope distribution present at this population (Figure 2.3). Note also that if at least some of these birds are wintering in more southern locations (*i.e.* northwest Texas), it would also explain the higher variance in this sample location.

White-throated Sparrows band recapture information suggests an east-west segregation at wintering grounds of White-throated Sparrows (Mazerolle *et al.* 2005). The δD_f values of

head feathers of western Canadian populations obtained in the present study (Figure 2.4), as well as those examined in Delta Marsh Manitoba (Mazerolle *et al.* 2005), are congruent with an east-west segregation on the wintering grounds, in which eastern birds migrate towards southeast US, and western birds (Central interior and Peace River Region) migrate to either the Pacific west coast or to New Mexico/Arizona and Colorado/Kansas areas. These data suggest a general trend of parallel migration in North America and is consistent with the connectivity of the Central Interior with the Pacific coast and the Peace River Region with the New Mexico/Arizona and Colorado/Kansas areas.

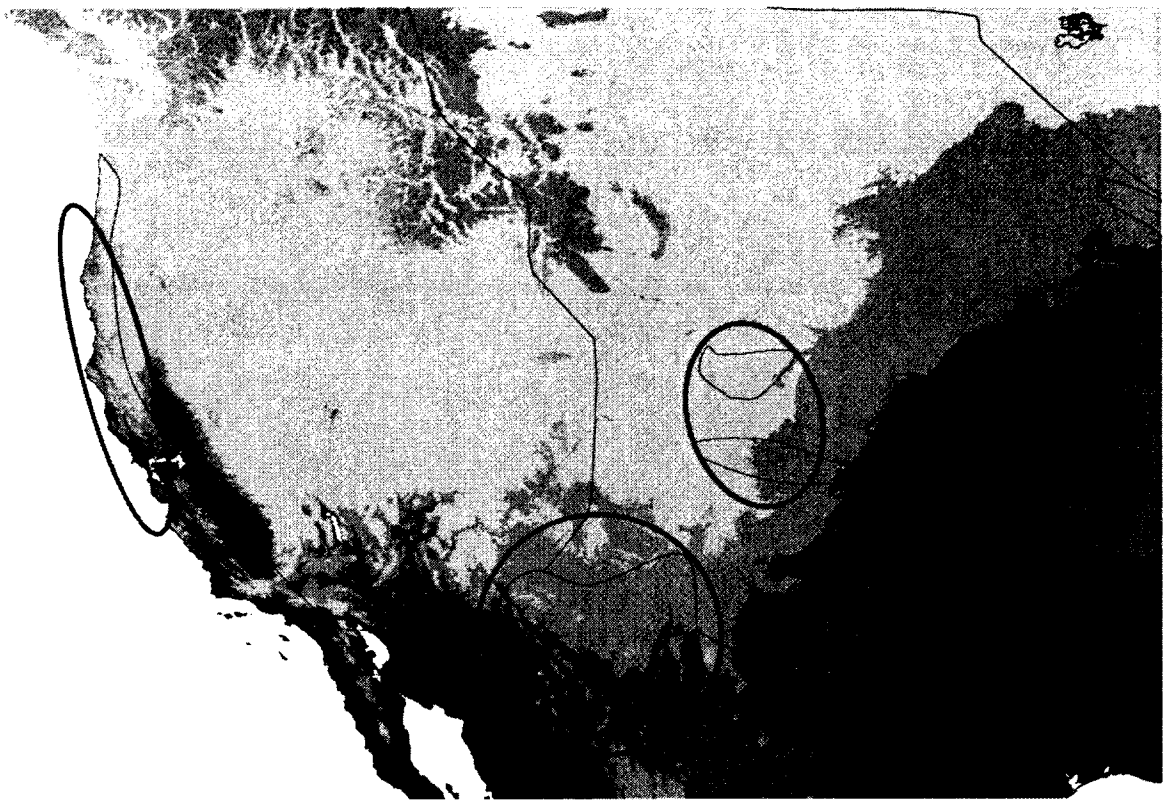


Figure 2.7 Stable Isotope precipitation GIS map (δD_p) modified from Meehan *et al.* (2004). Isotope relief patterns were modified with adjustment factor of -30 ‰ to compensate the hydrogen exchange of δD_f. A layer of showing the White-throated Sparrow range was obtained from NatureServe (Ridgely *et al.* 2007), and superimposed to the map. Blue circles show the probable wintering sites located east from the Rockies and the red circle shows the possible wintering area west from the Rockies. GIS map obtained with permission of Tim Meehan (December 20, 2010)

2.4.2 DETERMINATION OF BREEDING TERRITORIES BASED ON TAIL FEATHER DEUTERIUM ISOTOPES

Analysis of tail-feather isotopes showed significant statistical differences among breeding sample locations. These differences were maintained when outliers were included, showing that extreme values on the distribution did not affect the results. Notably, significant statistical differences were seen between the Peace River Region and Central Interior populations. This distribution is expected and is consistent with the predicted geographical distribution of isotope values for each sampling location (Figure 2.2), where the mean value in Central Interior (-146 ±6.22 ‰ SD) is 8‰ higher than the Peace River Region (-154 ±7.01 ‰ SD) and 12‰ higher than Sikanni River is (-158 ±2.87 ‰ SD) (Figure 2.6). The 95% confidence intervals of Central Interior and Peace River Regions do not overlap, reflecting a high degree of confidence in assigning samples to either of these locations. This lack of overlap in confidence intervals is unexpected as a high degree of variance associated with within-population estimates has been recorded for some species (Langin *et al.* 2007). For instance, Farmer and colleagues (2008) estimated that it is necessary to have a difference of 31‰ in order to assign confidently two samples as coming from distinct latitudes. However, the confidence intervals at 95% values from Central Interior and Peace River Region showed that the 31‰ value suggested by Farmer and colleagues (2008) might not apply for these populations.

The banding stations in Alberta are important for the present study as they potentially capture White-throated Sparrow migrants using either the Central, Mississippi, or eastern Pacific

(Columbia River) flyways. Lesser Slave Lake Bird Observatory, located on the eastern shore of Lesser Slave Lake and, Beaverhill Bird Observatory, located 80 km southeast of Edmonton, is situated south of the sampled breeding locations, on the eastern side of the Continental Divide. Located at a convergence of the Mississippi, Central and eastern Pacific flyways (Krikun and Holroyd 2001) these banding stations have a high likelihood of capturing samples from the breeding locations tested if they are using a migratory path east of the Rocky Mountains.

Tail feather isotopes of birds from Lesser Slave Lake and Beaverhill banding stations showed significant differences with the Central Interior suggesting that sparrows from this population are not being detected at these station (Figure 2.6). On the other hand, tail feather isotope ratio at these two banding stations showed no statistical differences with the Peace River Region. This is consistent with the Peace River Region birds are being detected at Beaverhill and Lesser Slave Lake. However, it is important to consider that other breeding locations (not sampled) will have similar isotope signatures and that the higher standard deviation (compared with the rest of locations) observed at both of these banding stations suggests that birds from more than one population are most likely being captured at these locations. The observed isotope signatures suggest that populations north of the Peace River Region are also being captured at these locations, since both banding stations sampled a number of birds from the -146 – -160 ‰ zone (Figure 2.2).

This data is consistent with the hypothesis that birds breeding in the Peace River region are using a Central migratory pathway to over wintering areas in New Mexico/Arizona and Colorado/Kansas areas. Although the use of the eastern Pacific flyway, crossing the Rockies at the Columbia River and proceeding to the Pacific coast area, cannot be rejected, the use of a northern Rocky Mountain pass seems unlikely. Data from Mugaha Marsh Banding Station also suggests that birds from the Peace River Region are not crossing through the Peace River Pass.

Mugaha Marsh Banding Station is located 14km northwest of Mackenzie, BC, east of the Parsnip Reach of the Williston reservoir (Mackenzie Nature Observatory 2011) and may potentially capture birds using the two major mountain passes in this area, the Peace River and Pine Pass. The Williston reservoir is the impoundment of the Peace River watershed from the WAC Bennett Dam in the Peace Canyon, which cuts through the Rocky Mountains (600 m elevation), into the north and south arms west of the Rockies that extend along the former tributaries that flowed along in the Rocky Mountain trench. This banding station is also located 68 km from the entrance to the second major pass through the Rockies in this region, the Pine Pass. The Pine Pass (874 m elevation) has the distinction over other passages that it is the only one that cuts the Rockies in a transversal orientation (following the migratory direction).

The Mugaha Marsh station has the highest amount of captures of White-throated Sparrows in British Columbia, with an average of 16.18 birds per year (1995 - 2011) and a high peak of

78 captures in the 2011 (Mackenzie Nature Observatory 2009). Because of being strategically located in the Rocky Mountains trench, Mugaha Marsh Banding Station is important for detecting birds that are using the Peace River Pass. Additionally this station is located near to the migratory divide for many bird species such as the Yellow-rumped Warbler (*Dendroica coronata*), Blackpoll Warbler (*Dendroica striata*), American Redstart (*Setophaga ruticilla*), Northern Waterthrush (*Seiurus novaboracensis*) and Wilson's Warbler (*Wilsonia pusilla*) (Dunn *et al.* 2006).

Tail feather isotopes samples from this banding station showed a different distribution from the rest of the banding stations. The distribution of samples at this station presented a high percentage of samples (~60%, 5/9) in the -161 – -173‰ zone. This zone corresponds to the northern BC-Yukon border region (Figure 2.2). The high mean and distribution of δD_f values at Mugaha Bird Observatory suggests that birds caught at this location could be either coming from a population north of the Peace River Region (east of the Rockies, *e.g.*, Sikanni) or from a population north of Mackenzie (west of the Rockies, *e.g.*, northern Williston Lake/Finlay River or even south-eastern Yukon following Liard river watershed). The lower standard error (CI 95%) (compared with the rest of banding locations) observed at this banding station may indicate that birds banded at this location come from a more narrow distribution than the Alberta banding stations.

The range of values observed at Mugaha Marsh are significantly different from those observed in the Central Interior and Peace River breeding locations. Despite being located

on the western side of the Continental Divide, birds banded at this station have isotopic values that represent a higher latitudinal range than for both breeding locations. No evidence has been reported that the Central Interior population extends to such high latitudes along the west side of the Rocky Mountains. These data indicates that a third breeding population is potentially using the Peace River area during migration.

The Rocky Point banding station also regularly captures White-throated Sparrows in British Columbia. With an average of 3 White-throated Sparrows each year (1994 to 2009), most banded between mid-September to late-October (Melcer and Nightingale 2009; David 2006, 2008; Jantunen 2003, 2004; Gibson 2002; Derbyshine 1999, 2000), the number of recaptures at this station is lower than Mugaha Marsh. Because of being located at the south end of Vancouver Island, this station represents an important Pacific flyway sampling location. The mean δD_f ratio of migrants banded at Rocky Point banding station (-151.77 ±3.6 ‰ SD, n = 3), as well as the lack of statistical differences with birds from Central Interior and Peace River Region suggest that birds from either Central Interior or Peace River Region breeding populations could be passing through this station and, as a result, using the Pacific Flyway. Given the small sample size, no definite conclusion can be drawn, although the low numbers of White-throated Sparrows annually banded at this station suggest that this is not a major migration route. The inclusion of other Pacific flyway banding stations (such as the Delta Marsh in Vancouver, not included in this study) may be more useful locations that could help in the understanding of the migratory behaviour of Central Interior and Peace River Region populations.

In summary, the results indicate that the populations breeding in the Central Interior and Peace River are overwintering in the western area of the wintering range. The results are also consistent with most parsimonious hypotheses of migration, *i.e.*, that the Central Interior population overwinters in Pacific coast while the Peace River population overwinters in the New Mexico-Arizona and Colorado/Kansas area. Alternate hypotheses involving combinations of these breeding locations and wintering areas however could not be ruled out. Partial information on migratory routes was also obtained. Banding station data is consistent with birds from the Peace River region using the flyway east of the Continental Divide. At the same, time this data did not support use of this migratory corridor by the Central Interior population.

A number of possible migratory pathways may be used by the Central Interior population. One possible migratory route would be that the sparrows are crossing through the Pine Pass and then following an undetected route, such as along the eastern foothills of the Rockies, before heading to their wintering destination. Another possibility is that Central Interior sparrows fly south following the Fraser River and then proceed down the coast. Other western inland routes seems to be unlikely as the Mount Revelstoke, Tattlayoko and Vaseux Lake banding stations rarely report White-throated Sparrow captures (eBird Canada 2010, Ogle 2008; 2009a; 2009b). Obtaining samples from additional banding stations in BC, *e.g.*, Delta Marsh, and Alberta, *e.g.*, Inglewood, as well as from strategically placed locations will be needed to provide the crucial evidence to support or refute these possible routes. It is also

recommended that both head and tail feathers be collected from all migratory samples so that both possible breeding and wintering locations can be linked to migratory samples.

Based on the ability to successfully differentiate breeding populations on either side of the Continental Divide using tail-feather isotopes, one alternative study that would help understanding migratory behaviour would be to sample tail and head feathers from birds wintering along the Pacific coast or New Mexico-Arizona and Colorado/Kansas areas. These isotopic ratios could then be compared to the present study in order to infer breeding populations. A similar technique has been applied successfully before to other species, such as Swainson's Thrush (*Catharus ustulatus*) and has shown to be powerful enough to differentiate the origin of wintering individuals (Kelly *et al.* 2005)

Elucidating the complexity of migratory routes in western Canada is crucial to understand the effects that local disruptions could have on White-throated Sparrow populations. These effects could be important as birds fly through migratory bottlenecks when they cross the Rocky Mountains. However, without detailed information on the migratory routes of western Canadian White-throated Sparrow populations, it would be very difficult to determine which population could be affected by a disruption on a migratory route and what would be the repercussions of these disruption events.

CHAPTER 3

GENETIC DIFFERENTIATION ANALYSIS OF WESTERN CANADA WHITE-THROATED SPARROW POPULATIONS AND GENETIC ASSIGNMENT OF MIGRATORY INDIVIDUALS

3.1 INTRODUCTION

Understanding genetic structure or differentiation of breeding populations along a migratory divide has been an important aspect of many migratory connectivity studies (*i.e.,* Clegg *et al.* 2003, Kelly and Hutto 2005, Ruegg and Smith 2002, Davis *et al.* 2006). These studies have used genetic differences among breeding populations to identify discrete migratory groups, which can confirm the presence of a migratory divide. The amount of genetic divergence among populations however, is related to a number of factors including population/evolutionary history, the presence of barriers to gene flow, and the resolution of the genetic marker system used.

Population history (response to glaciation and post-glaciation events) has a strong influence on the population structure of avian populations as well as the evolution of migratory routes. Many cases of migratory and genetic differentiation among populations appear to be a result of the independent evolution of populations that have originated from separate refugia (and then have come into secondary contact) after a process of post-glacial range expansion.(*e.g., Sylvia atricapilla,* Perez-Tris *et al.* 2004*; Dendroica petechia,* Boulet and Gibbs 2006).

Additionally, as in Blackcaps a population bottleneck prior to post-glaciation expansion can affect the genetic differentiation among populations (Perez-Tris *et al.* 2004). In contrast, a lack of differentiation, as found in Black-throated Blue Warbler (*Dendroica caerulescens*), is usually attributed to a recent expansion from a single refugium (Davis *et al.* 2006).

Demographic events and population history can not entirely explain how differences in migratory behaviour are maintained. Genetic and migratory differences can promote reproductive isolation by pre-zygotic and post-zygotic mechanisms and restrict gene flow after populations meet in a secondary contact zone (Irwin and Irwin 2005; Toews and Irwin 2008). For example, the Greenish Warbler (*Phylloscopus trochiloides*) (Irwin and Irwin 2005) and Yellow-rumped Warbler (*Dendroica coronata*) (Brelsford and Irwin 2009) have shown differentiation enhanced by a selective pressure against hybrid individuals. In these cases, secondary contact between populations with different migratory strategies resulted in hybrid individuals that exhibit a sub-optimal migratory strategy with a lower fitness than either parental lineage. This lower fitness could be influenced by the presence of an ecological or geographical barrier complicating migration of hybrids, as is the case in the Greenish Warbler (*Phylloscopus trochiloides*) where the barrier is the Tibetan Plateau (Irwin and Irwin 2005).

In many cases, genetic differentiation follows a geographical pattern. This pattern can be influenced by a geographical barrier or by the dispersal ability of the organism. Being able to differentiate between two or more populations along a geographical divide is important as

it may indicate that these populations use different migration corridors or wintering areas (*e.g.,* Clegg et al. 2003; Kelly and Hutto 2005; Irwin and Irwin 2005). The Rocky Mountains, which form the main geographic feature of the Continental Divide, are an east/west genetic and migratory divide for breeding populations of many avian species (Boulet and Gibbs 2006; Kelly and Hutto 2005). The White-throated Sparrow (Zonotrichia albicollis) has breeding populations on either side of the Rocky Mountains. Differences in song structure (unpublished data, Mesias, V., Otter, K., Mora, M., Ramsay, S., and Murray, B.) and, possibly, migration strategies have been noted between populations west of the Rocky Mountains in the Central Interior of BC and those east of the Rockies in the Peace River region (Chapter 2). The degree of genetic differentiation between these populations is unknown.

In spite of the fact that bird species tend to have lower levels of genetic differentiation than other vertebrates, probably because of their higher mobility and larger population sizes (Winker *et al.* 2000), many studies using a variety of molecular markers have found genetic differentiation among populations of the same species (*e.g.,* Boulet and Gibbs 2006; Clegg *et al.* 2003; Lecomte *et al.* 2009; Helbig *et al.* 1995; Winker *et al.* 2000). Different markers have distinct strengths that, when combined, can provide a more complete picture of the spatial genetic variation within a species. For instance, mitochondrial DNA is a maternally inherited marker which does not undergo recombination and has a higher substitution rate than corresponding sequences in the nuclear genome, making it an effective marker to study populations that have recently diverged (Zink and Barrowclough 2008) and for phylogeographic studies (Avise *et al.* 1998). Microsatellites, on the other hand, are nuclear

codominant markers that usually produce unique individual genotypes that are used for a range of applications, from paternity or forensic analysis to studying population or species relationships.

The primary objective of the present study was to determine the genetic differentiation of White-throated Sparrow breeding populations distributed on both sides of the likely migratory divide (the Rocky Mountains). Eight neutral microsatellite markers as well as a partial sequence (500bp) of the mitochondrial *Cytochrome Oxidase Subunit I (COI)* gene were assessed. The combination of these markers is useful not only to determine the presence or absence of structure/differentiation between White-throated Sparrow populations, but also to help us infer historical demographic events (*i.e.*, the use of a single refugium or multiple refugia) that could influence genetic differentiation and migratory behaviour. The second objective, if evidence of genetic differentiation is noted, is to directly study migratory behaviour through the genetic assignment of migratory individuals, banded along important migratory routes in western Canada, to their likely breeding grounds.

3.2 METHODOLOGY

3.2.1 STUDY AREA AND SAMPLE COLLECTION

Based on White-throated Sparrow habitat preferences, sampling locations were located in shrub-dominated habitats (*i.e.,* clearcuts, forest edges, oil and gas lines, and ATV trails) (Figure 3.1). One hundred and five feather samples from breeding birds were collected during the months of May-July of 2009-2010 across 3 regions in the Central Interior of British Columbia (BC), Canada; however, from these samples only one hundred and one samples were successfully used: Prince George (23), John Prince Research Forest (4) and MacLeod Lake (12), and 4 locations in Peace River Region of BC: Moberly Lake (11), Tumbler Ridge (11), Dawson Creek (37), and Sikanni River (3) (Figure 3.2). Additionally, 20 blood samples from Algonquin Provincial Park (Ontario) and 20 blood samples from Prince George (43 in total for this location) were obtained in collaboration with Dr. Scott Ramsay (collected between 2004-2009).

Sixty-seven samples from fall migratory birds were obtained in August-September of 2009. Seven banding stations across British Columbia (BC) and Alberta (AB) collaborated to sample migratory White-throated Sparrows: Rocky Point Bird Observatory (3), Mugaha Marsh Bird Observatory (9), Lesser Slave Lake Bird Observatory (9), Beaverhill Bird Observatory (8), Tattlayoko Bird observatory (0), Vaseux Lake Bird Observatory (0) and Revelstoke Bird Observatory (0). Additionally (38) were collected in Dawson Creek in the month of September 2009 (Figure 3.3).

White-throated Sparrows from breeding populations (and from Dawson Creek in the fall) were captures using active mist-nest techniques. Birds were attracted to the nest with seeds (fall) or using a playback (summer). Migratory samples from banding stations were captured using passive mist-net technique. Two feather samples from external tail rectrices were obtained from each bird prior to their release. Feathers were transported in hermetic Ziploc bags that were labelled detailing: date, banding location, species, CWS number, and individual code.

Figure 3.1 Example of shrub-dominated habitat in Pouce Coupe (BC) where breeding and migratory birds were collected. Birds were attracted to the nest with seeds (autumn) or using a playback (summer).

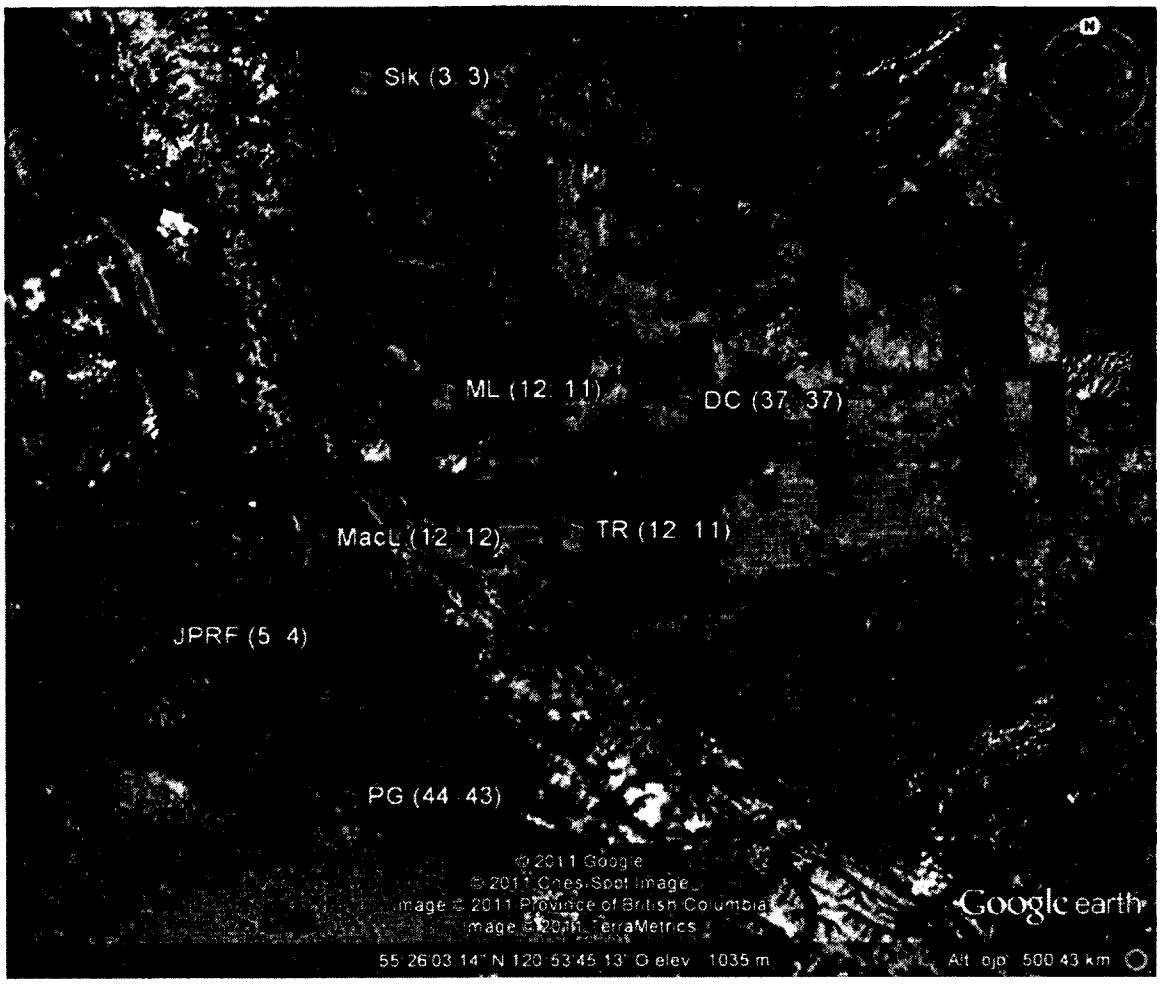


Figure 3.2 Map of sampling locations from collected during breeding season. Prince George (PG), John Prince Research Forest (JPRF), MacLeod Lake (MacL), Tumbler Ridge (TR), Moberly Lake (ML), Dawson Creek (DC), and Sikanni (Sik) were included. Sampling locations west from the continental divide were grouped into the Central Interior population (in blue), and east from the divide were grouped as the Peace River Region population (in green). The first number in parenthesis represents the total amount of individuals collected per location; the second number represents the amount of individuals that were successfully genotyped for microsatellite analysis. Ontario samples were not included in map. The map was constructed with Google Earth v.5.2.

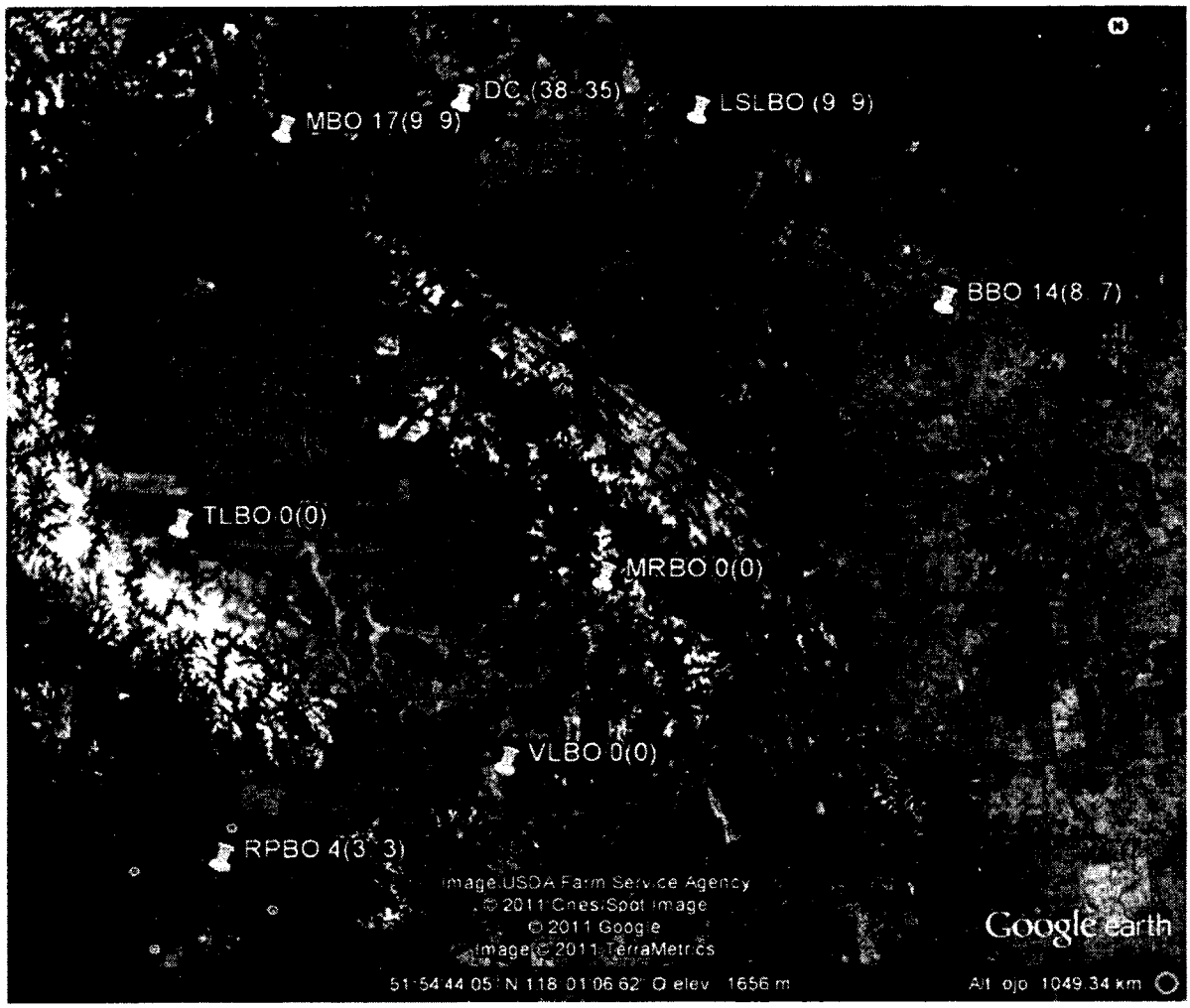


Figure 3.3 Map of sampling locations collected during the fall migratory season in collaboration with banding station in BC and AB. Mugaha Marsh (MBO), Rocky Point (RPBO), Tattlayoko (TLBO), Vaseux Lake (VLBO), Mount Revelstoke (MRBO), Dawson Creek-migratory (DC), Lesser Slave Lake (LSLBO) and Beaverhill Bird Observatory (BBO) were included. The number outside the parenthesis represents the amount of sparrows caught during the fall season of 2009 (if available). The first number inside parenthesis represents the total amount of individuals collected per location; the second number represents the amount of individuals that were successfully genotyped for microsatellite analysis. The map was constructed with Google Earth v.5.2.

3.2.2 GENOMIC DNA EXTRACTION

Studies have indicated that blood sampling has the potential to lower survival probability of birds during the first year after sampling (Brown and Brown 2010). In order to decrease bird stress, mortality and simplify sampling, the terminal portion of feathers was used to obtain genomic DNA instead of blood. Feather tip samples (with the blood pulp included) were stored at -30°C in Lysis Buffer (50mM Tris-HCL, pH 8, 20mM EDTA, and 2 % SDS) prior to DNA extracted using a modified phenol/chloroform/isoamyl alcohol (25:24:1) procedure (Bello *et al.* 2001)

3.2.3 MICROSATELLITE AMPLIFICATION

Genomic DNA samples were diluted 1:10 in Nuclease free H_2O before use to reduce the effects of possible contaminants. Twenty five microsatellite primers described for different avian species were screened in order to find suitable polymorphic primers, from which eight primers were chosen (Table 3.1) (Petren 1998; Dawson *et al.* 1997; Poesel *et al.* 2009). An M13-tailed dye-labelled primers technique was used for PCR amplification and fragment analysis (Ganache *et al.* 2001). For the first five primers (Zole-A08, Zole-B01, Zole-B03, Zole-A02, Zole-C06), 3 µl of DNA (1:10) were added to 22 µl of Mastermix containing 1X buffer (Invitrogen), 0.2mM dNTP's (each), 12.5µg of BSA, 3mM $MgCl_2$, 0.26 µM of reverse, 0.26 µM of M13 dye-labeled primer, 0.13 µM of M13-tailed Forward primer (Table 3.1), and one Unit of *Platinum Taq* (Invitrogen). The amplification cycle was 94°C for 5 minutes, followed by 12 cycles of 94°C for 30 seconds, 56°C (decreased 0.5°C per cycle) for

40 seconds, 72°C for 40 seconds, then 28 cycles of 94°C for 30 seconds, 50°C for 40 seconds, 72°C for 40 seconds and a final extension of 72°C for 10 minutes.

For the remaining three primers (Zole-C11, Zole-C07, Zole-F11), a preamplification method was used before the amplification with the M13 dye-labelled primer. The preamplification contained 3 µl of DNA 1:10 added to a 22 µl of Mastermix containing 1X buffer (Invitrogen), 0.2mM dNTP's (each), 12.5µg of BSA, 2mM $MgCl_2$, 0.26 µM of untailed forward and reverse primers and 1 Unit of *Platinum Taq* (Invitrogen). The amplification cycle was 94°C for 5 minutes, followed by 40 cycles of 94°C for 30 seconds, 58°C for 40 seconds, 72°C for 40 seconds, and a final extension of 72°C for 10 minutes. One microlitter of DNA from the preamplification was added to 24 µl of Mastermix, as above, containing M13 dye-labeled primer, 0.13 µM of M13-tailed Forward primer (Table 3.1), and 1 Unit of *Platinum Taq* (Invitrogen). The same amplification protocol as for the preamplification was used with the exception that a 50°C annealing temperature was used instead of 58°C.

3.2.4 MICROSATELLITE DATA ANALYSIS

Microsatellite markers were sized using CEQ8000 Genetic Analysis system (Beckman Coulter) and scored using the Fragment Analysis Module (400 bp ladder, Cubic model). Only fragments located within the approximate size range described by Poesel *et al.* (2009) were scored. Null alleles were minimized by re-amplifying samples that showed low quality signal (Segelbacher 2002).

Table 3.1. White-crowned sparrow (*Zonotrichia leucophrys*) microsatellite primers (5'- 3') that showed polymorphism in White-throated Sparrow (*Zonotrichia albicollis*) (Poesel *et al.* 2009). All primers span a tetranucleotide repeat. The size range observed in White-crowned sparrows in base pairs (Poesel *et al.* 2009) is shown.

Marker	Forward*	Reverse	Size (Bp)
Zole_A08	ACCCAAAGTGCAAATCCCATC	ACAAAGTCCCGTTTTCCTTGC	252-288
Zole_B01	GGACTGTGTTTCACTTCCTATC	ACAGATGTTGCATTGCGG	250-304
Zole_B03	GCCAAACTCAGTGACCTGC	AGTTCCTGCACGGTTCTTC	222-278
Zole_A02	GCAGCCATTTTGCTGTCATTC	CCATCTGTCTGTCTTTCTGTCTG	160-294
Zole_C06	CCAGCCTGATTTCCCATGC	TGTTGAGCATCTCTGGAGG	202-252
Zole_C07	TGCCAGCAACTCTGCCTC	TGAGCTTCCAGCCCTTCAG	188-280
Zole_C11	TCCATGCTTCTGAACTGCC	ACACCTGCTTTTCCTGACTG	164-200
Zole_F11	AACCAAGCCACCACAATGC	GACAGGCACTAGGATGGGAG	205-291

* an M13 sequence (TGTAAAACGACGGCCAG) was added 5' of each Forward primer

Samples from breeding populations were grouped according to their geographical location (Central Interior, Peace River Region, and Ontario) into three classes for the genotypic analysis. Analysis of Molecular Variance (AMOVA) and pairwise Rst comparisons were performed using Arlequin v.3.1 (Microsatellite data type; Distance method: Sum of Squared size differences Rst; 1000 permutations). Population structure was analyzed using STRUCTURE v.2.3. Admixture and no-admixture models were used with correlated allele frequencies (length of burnin period: 100000; number of MCMC reps after burnin: 50000). Ten runs per each number of populations (k) examined (k = 1-7) were used in order to assume the correct number of populations. TESS v.2.3 was also used in order to include a geographical distance matrix based on latitude and longitude coordinates into the population structure estimation. For TESS a without admixture model was used with 100 runs per k (k = 2-8), a burnin of 10000 and a running period of 50000.

Samples from migratory individuals were grouped according to sampling location. Potential breeding populations were assigned or excluded using the program Geneclass2 (Piry *et al.* 2004). The Rannala and Mountain (1997) Bayesian method was used as the criteria of population probability. A Monte Carlo resampling simulation using the Paetkau *et al.* (2004) simulation algorithm was used to identify the probability of individuals to be excluded from a given population. In order to test confidence in the assignment, all breeding individuals were assigned to breeding population samples.

3.2.5 MITOCHONDRIAL DNA AMPLIFICATION

A 650 base pair (bp) fragment of the *Cytochrome Oxidase subunit I*(*COI*) gene was amplified. Three microliter of 1:10 diluted DNA was added to a 47 µl PCR mastermix containing 5%Trehalose, 1X buffer (Invitrogen), 0.2mM dNTP's (each), 2.5mM $MgCl_2$, 0.2µM of M13-tailed primer cocktails (Table 3.2), and 0.6 Units of *Platinum Taq* (Invitrogen). The amplification cycle was 94°C for 4 minutes, followed by 40 cycles of 94°C for 30 seconds, 52°C for 40 seconds, 72°C for 1 minute, and final extension of 72°C for 10 minutes.

Table 3.2 PCR primers and cocktail combinations (Wong and Hanner 2008) used in amplification of mitochondrial COI gene from White-throated Sparrows.

Name	Conc.	Primer
COI-FW (cocktail)		
VF2_t1	0.1μM	TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC
FishF2_t1	0.1μM	TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC
COI-RV (cocktail)		
FishR2_t1	0.1μM	CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA
FR1d_t1	0.1μM	CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA
Zoal_COIF*	0.2 μM	TGTAAAACGACGGCCAGGTACCGCCCTAAGCCTTCTC

* This primer was designed using PRIMER 3 (Rozen *et al.*2000)

Primers Zoal_COI_M13 and COI RV were used to amplify 48 samples that failed using the first two cocktails of primers

3.2.6 MITOCHONDRIAL DNA DATA ANALYSIS

Mitochondrial *COI* gene fragments were sequenced using a CEQ8000 Genetic Analysis system (Beckman Coulter) or an ABI 3130xl Genetic Analyzer (FADSS facility of the University of British Columbia-Okanagan). Sequence chromatograms were edited in program Sequencher 4.2 (Gene Codes, Ann Arbor, MI). Haplotype sequences were aligned using Mega v4.0 (Tamura *et al.* 2007) and variables sites only were exported to TCS v1.21 (Clement *et al.* 2000) in order to avoid inconsistence with missing information while building a haplotype network tree. Additionally, AMOVA and haplotype frequencies were calculated using Arlequin v.3.1 (data type DNA; 1000 permutations).

3.3 RESULTS

3.3.1 MICROSATELLITE BREEDING SAMPLES ANALYSIS

Most of the microsatellites were within Hardy-Weinberg (H-W) expectations, with the exception of Zole C11, Zole-F11 and Zole-C07 that were out of H-W equilibrium in one or two regions (Table 3.3). However, the preamplification method used in these microsatellites could increase the probability of null alleles altering the heterocigozity ratio. The effect of null alleles is also evidenced in the excess of homozygotes observed in microsatellites out of H-W equilibrium (Table 3.3). Extracted DNA yield from feather samples ranged from 0.15 to 10.35 μg.

Eight polymorphic microsatellite markers were used to genotype 145 samples from breeding locations. In total 155 alleles were obtained from all loci: Zole-A08 (12), Zole-B01 (21), Zole-B03 (20), Zole-A02 (19), Zole-C06 (18), Zole-C11 (14), Zole-C07 (32), Zole-F11 (19). Fifty-four private alleles were observed in White-throated Sparrow breeding populations (Table 3.3). The Peace River Region contained the highest number of private (29) as well as total alleles (129), followed by the Central Interior region (115 total and 15 private alleles) and Ontario (84 total and 10 private alleles).

AMOVA analysis (under Rst-like model) showed non-significant results ($P > 0.05$) with 1% of variation (Rst: 0.01031) explained among populations (Central Interior, Peace River Region, and Ontario) (Table 2.4). However, Pairwise Rst comparisons showed significant

differences ($P < 0.05$) between Central Interior and Peace River Regions (Rst: 0.02253), but non-significant ($P > 0.05$) between Central Interior and Ontario regions (Rst: -0.01452) (Table 3.5) and between Ontario and Peace River Region (Rst: -0.00255).

Under a number of different models (*e.g.,* Fst-like) the statistical results do not change even though the values change. Overall AMOVA still shows non-significant results ($P > 0.05$) but with a lower percentage of variation and Fst (Fst: 0.0028). Pairwise Fst comparisons showed similar results showing shallow non-significant results between Central Interior and Peace River Regions (Fst: 0.003) and all other comparisons.

The symmetric proportion of samples assigned to each population showed that there is no structure in the samples analyzed (Pritchard *et al.*2007) with the program STRUCTURE which does not take in account geographical distances (Figure 3.4a). When the Estimated Logarithm Probability of Data [LnP(D)] was plotted against each estimated population (K), the curve does not plateau, also suggesting a lack of genetic structure (Figure 3.5a). This same pattern was observed in program TESS (which uses geographical distances) when the Deviance Information Criterion (DIC) was plotted against each K (Figure 3.4b, 3.5b).

Table 3.3. Amplification size (bp), number of alleles (Na), observed (Ho) and expected (He) heterozygosity, and number of private alleles (Pa) found for all eight microsatellite loci in each region: Central Interior, Peace River Region and Ontario. Heterozygosity values in bold represent significant differences after Bonferroni correction between Ho and He.

		Central Interior				Peace River Region				Ontario			
Marker	**Size (bp)**	**Na**	**Ho**	**He**	**Pa**	**Na**	**Ho**	**He**	**Pa**	**Na**	**Ho**	**He**	**Pa**
Zole-A08	252-286	9	0.71	0.78	1	9	0.64	0.72	1	8	0.75	0.77	2
Zole-B01	252-304	17	0.95	0.88	0	20	0.92	0.91	4	12	1	0.9	0
Zole-B03	222-270	11	0.88	0.83	1	19	0.87	0.86	9	9	0.95	0.85	0
Zole-A02	160-204	12	0.88	0.87	0	19	0.79	0.9	6	8	0.75	0.85	1
Zole-C06	212-248	15	0.83	0.87	1	15	0.92	0.87	2	9	0.85	0.86	1
Zole-C11	180-200	13	**0.65**	**0.85**	5	9	0.87	0.8	1	7	0.9	0.84	0
Zole-C07	192-280	23	**0.61**	**0.95**	3	24	**0.79**	**0.95**	2	21	0.85	0.96	5
Zole-F11	205-291	15	0.81	0.87	4	14	**0.72**	**0.86**	4	10	0.8	0.88	1
Total		115			15	129			29	84			10

Table 3.4 AMOVA from breeding population samples. Source of variation, degree of freedom (d.f), sum of squares and Percentage of Variation are shown. Distance method: Sum of Squared size differences Rst; 1000 permutations.

Source of Variation	**d.f.**	**Sum of Squares**	**Percentage of Variation**
Among populations	2	3555.133	1.03
Within populations	281	262410.628	98.97
Total	283	265965.761	
RST : 0.01031			
P-value : 0.1417 ± 0.0089			

Table 3.5 Pairwise Fst Population Comparison between Central Interior (CI), Peace River Region (PR) and Ontario (ONT). Distance Method used: Sum of squared size differences (Rst). Significance (P-values) is shown in parenthesis next to Fst value.

	Central Interior	**Peace River Region**	**Ontario**
Central Interior	0		
Peace River Region	0.0225 (**0.0107**)	0	
Ontario	-0.0145 (0.9385)	-0.0026 (0.5615)	0

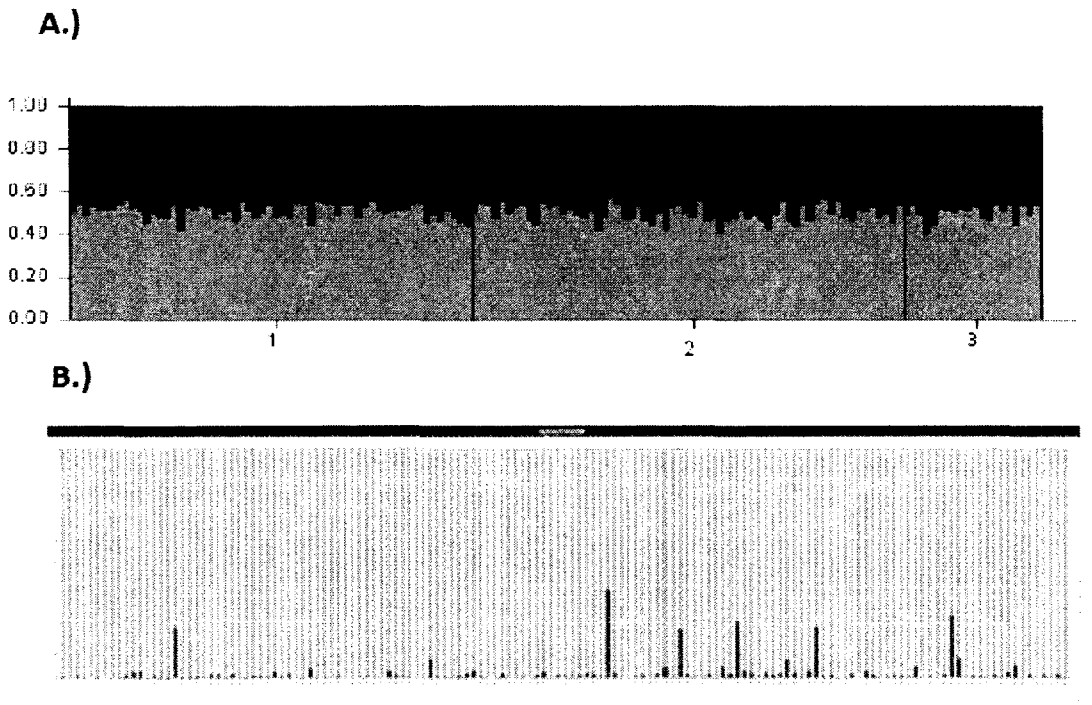


Figure 3.4 A) Example of one of the 10 runs of Structure Bar plot with k = 2. Color lines represent probability of regions (1. Central Interior, 2. Peace River Region, 3. Ontario) to be assigned to a population k. B) Example of Tess bar plot of one of the 100 runs with K = 2, Bars represent probability of an individual sample to be assigned to a population. Green bars indicate that all individuals were assigned to the same population.

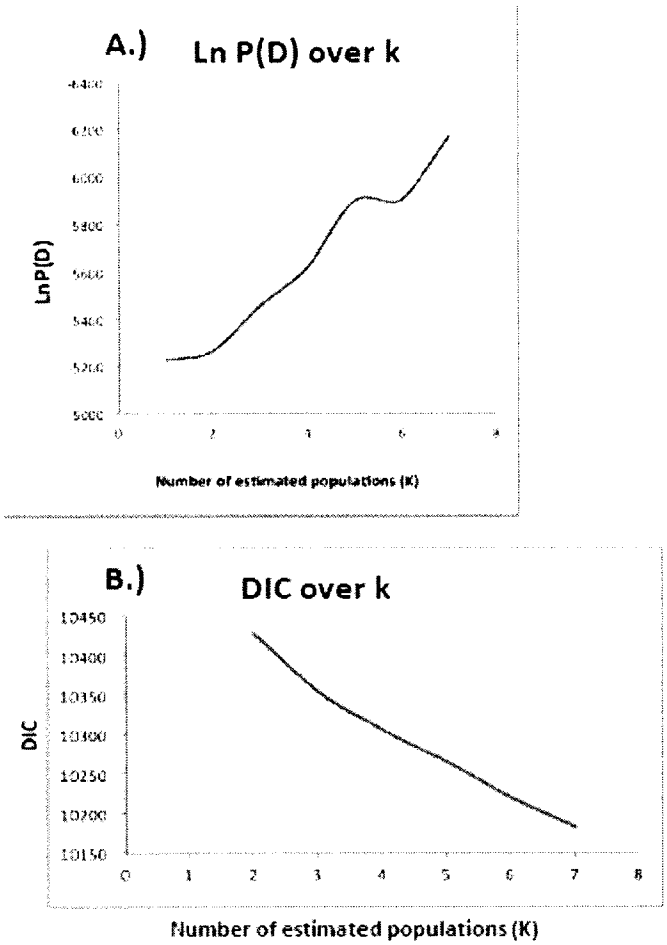


Figure 3.5 A.) Estimated Logarithm Probability of Data (LnP(D)) plotted against each estimated population (K), calculated with STRUCTURE. B.) Deviance Information Criterion (DIC) plotted against each estimated population (K), calculated with TESS.

3.3.2. MICROSATELLITE MIGRATORY SAMPLES ANALYSIS

The self-assigning test using Geneclass2 showed that 50.7% of samples were assigned correctly to their breeding population (Table 3.6). John Prince and McLeod Lake, in spite of being located within Central Interior were mostly assigned to the Peace River Region. Similarly, more samples from Algoquin Park (ON) were assigned to Central Interior and the Peace River Region than to Ontario.

Assignment test of migratory locations to breeding populations showed that for all locations samples were assigned to all three breeding locations. More samples from Mugaha Marsh and Rocky Point were assigned to the Central Interior while more samples from Lesser Slave Lake and Dawson Creek (migratory) were assigned to the Peace River Region (Table 3.7). For few samples the probability of assignment to a population was rejected ($P < 0.05$) (Table 3.7). Notably, Mugaha Marsh showed the greatest amount of population rejection. Of interest, one breeding individual collected in Dawson Creek (Peace River Region) was recaptured the next year from a migratory flock captured at on the same location.

Table 3.6 Self-assignment of sampling locations during breeding season to their breeding regions using Geneclass2. The first number (outside the parenthesis) represents the number of individuals assigned with highest probability to each breeding region. In parenthesis is represented the number of individuals that were statistically rejected ($P < 0.05$) as being part of this location (using MonteCarlo resampling and Paetkau *et al.* 2004 simulation algorithm).

	Central Interior			Peace River Region				Ontario
	Prince George	John Prince	McLeod Lake	Moberly Lake	Dawson Creek	Tumbler Ridge	Sikanni River	Algonquin Park
Central Interior	26(2)	0(0)	2(0)	4(0)	5(2)	4(1)	2(0)	7(1)
Peace River Region	11(1)	4(0)	10(0)	6(0)	25(0)	6(0)	0(0)	7(0)
Ontario	6(3)	0(0)	0(1)	1(1)	4(5)	1(0)	1(0)	5(1)
Excluded from all locations	0	0	0	0	3	0	0	1

Table 3.7 Assignment of individuals from migratory sampling locations (Mugaha Marsh, Lesser Slave, Beaverhill, Rocky Point and Dawson Creek-migratory)to the three breeding regions (Central Interior, Peace River Region and Ontario) using Geneclass2. The first number (outside the parenthesis) represents the number of individuals assigned with highest probability to each breeding region. In parenthesis is represented the number of individuals that were statistically rejected ($P < 0.05$) as being part of this location (using MonteCarlo resampling and Paetkau *et al.* 2004 simulation algorithm).

	Mugaha Marsh	Rocky Point	Lesser Slave	Beaverhill	Dawson Creek (mig)
Central Interior	4(2)	2(0)	3(0)	2(0)	7(2)
Peace River Region	2(3)	1(0)	5(0)	2(0)	18(0)
Ontario	2(2)	0(0)	1(0)	1(0)	6(0)
Excluded from all locations	1	0	0	2	4

3.3.3 MITOCHONDRIAL DNA RESULTS

One hundred and twenty nine *COI* sequences (461bp with 17 variable sites) from three regions, Central Interior (56), Peace River Region (54) and Ontario (19), were determined in the present study (Table 3.8). In total, 19 haplotypes were found with four haplotypes (A, B, C, and K) found more than once (Table 3.9). A statistical parsimony network tree showed a star-like arrangement with the most frequent haplotype in all locations, B (68-83%), found in the center. Most haplotypes (except for haplotype J and R) differ from B by only one mutation (Figure 3.8). Haplotype C was also present in all three locations while haplotype K was found in the Central Interior and Ontario and haplotype A found in the Central Interior and the Peace River Region (Table 3.9, Figure 3.6).

AMOVA results for mtDNA haplotypes showed similar results to the nuclear microsatellite markers with non-significant differences among the three locations ($Fst = 0.01$, $P > 0.05$) (Table 3.10). Pairwise Fst comparisons also showed non-significant differences between all sampling locations (Table 3.11).

Table 3.8 Number of mtDNA haplotypes per location and number of unique haplotypes as well as number of sequences

	Central Interior	Peace River Region	Ontario
Haplotype number	8	10	7
Unique haplotypes	4	7	4
Number of sequences	54	53	19

Table 3.9 Frequency of shared haplotypes per sampling location. Haplotypes name was stated with letters (A, B, C, and K). Central Interior, Peace River Region and Ontario populations were included.

	Frequency of shared haplotypes		
	Central Interior	Peace River Region	Ontario
A	0.070	0.150	0
B	0.820	0.700	0.680
C	0.018	0.019	0.050
K	0.018	0	0.050

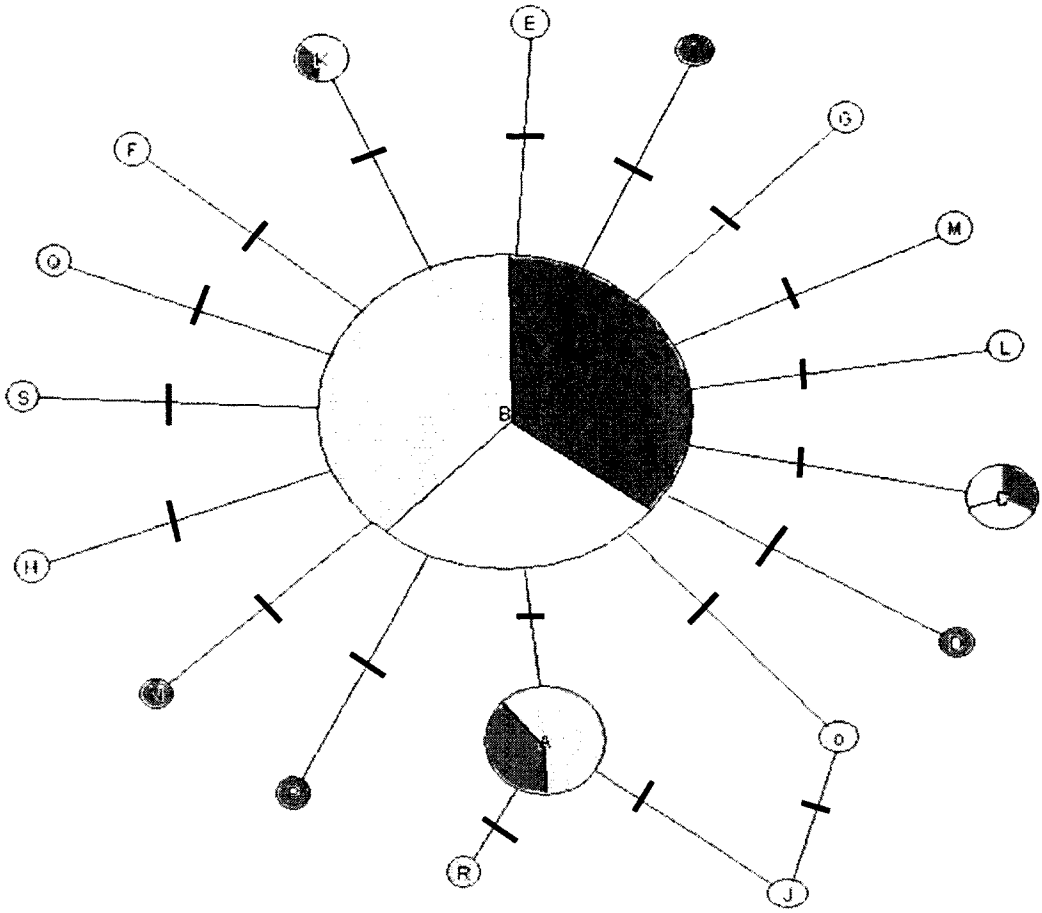


Figure 3.6 Statistical Parsimony tree of mtDNA haplotypes based on 17 variable sites of a 461 bp *COI* gene. Circle circumference is proportional with haplotype frequency. Colour represents the population where haplotypes are found: Central Interior (red), Peace River Region (blue) and Ontario (white). Bars represent number of nucleotide changes between haplotypes.

Table 3.10 AMOVA from mtDNA samples. Source of variation, degree of freedom (d.f), sum of squares and Percentage of Variation, and Fst are shown (1000 permutations).

Source of Variation	d.f.	Sum of Squares	Percentage of Variation
Among populations	2	0.596	1.01
Within populations	126	26.768	98.99
Total	128	27.364	
FST : 0.0101			
P-value : 0.1975 ±0.0121			

Table 3.11 Pairwise Population Comparison of mtDNA haplotypes Fst between Central Interior, Peace River Region and Ontario. Statistical significance (P-value) are shown in parenthesis.

	Central Interior	Peace River Region	Ontario
Central Interior	0		
Peace River Region	0.0110 (0.17)	0	
Ontario	0.0157 (0.14)	0.0039 (0.32)	0

3.4 DISCUSSION

3.4.1 POPULATION HISTORY OF CENTRAL INTERIOR WHITE-THROATED SPARROW POPULATIONS

Neutral nuclear markers as well as mitochondrial *COI* sequences showed little evidence for genetic structure among the sparrow breeding locations tested. Pairwise differences were noted between the Central Interior and the Peace River Region, but these values were small, ($Rst \leq 0.0225$). This lack of differentiation is reflected in the results from Bayesian assignment techniques (TESS and STRUCTURE) where no evidence of population structure was observed (Figure 3.4, 3.5). This low differentiation precludes the assignment of migratory individuals to breeding populations, but it provides important evidence on the historical and evolutionary history of Central Interior White-throated Sparrow population.

The lack of differentiation and low Rst/Fst values found with microsatellite and mitochondrial data suggests either high gene flow among all populations or a recent range expansion into the Central Interior by eastern populations (although both events are not exclusive from each other). The paraphyletic star-like *COI* tree pattern (Figure 3.6) is also consistent with range expansion in which a mutation-drift equilibrium has not been reached (Beebee and Rowe 2008). This same pattern has been observed in other passerine species such as the Black-throated Blue Warbler where using both microsatellite and mitochondrial markers, no populations differences were observed despite migratory and phenotypic differences (Davis *et al.* 2006). Davis and colleagues suggested that the star like phylogeny and lack of genetic differences indicates that populations expanded from a single glacial

refugium in the Pleistocene, and further that migratory and phenotypic differences appeared more recently. Other birds, such as the prairie warbler (*Dendroica discolor*) and the chipping sparrow (*Spizella passerina*), also have similar star-like mtDNA trees (even though the prairie warbler showed a significant genetic differentiation between subspecies *D. d. dicolor* and *D. d. paludicola*) suggesting a rapid post-glacial expansion during Pleistocene followed by a subsequent differentiation of migratory behaviour (Buerkle 1999; Mila *et al.* 2006).

Consistent with evidence of a single Pleistocene refugium, Whyte (1938) suggested that the Central Interior population arose from an expansion from populations along the eastern side of the Rocky Mountains, crossing west through lower altitude mountain passes. The Pine Pass and the Peace River have been suggested as the most probable routes of these passages, as birds probably were deflected from their migratory routes following the Peace River system until they found suitable territories west of the Rocky Mountains (Wythe 1938).The exact date of the establishment of the Central Interior population is unknown. The first historical records from the Central Interior are from around 1925 (Wythe 1938); however it is important to consider that banding information prior to the first influx of European settlers into the Central Interior in 1850's (Stevenson *et al.* 2011) is practically non-existent. Based on available information, a likely hypothesis would be that White-throated Sparrows have been breeding in the Central Interior in low numbers for at least a century, and that recently population numbers have increased as a result of the rise of the forest industry (around 1960's) which significantly modified the landscape creating more suitable habitat.

Despite evidence of westward range expansion, the presence of a geographical barrier such as the Rocky Mountains is expected to act as a barrier to gene flow between the Central Interior and Peace River Region. Interestingly, pairwise AMOVA of microsatellite results showed a statistical difference between the Peace River Region and Central Interior, but no differences between the former two and the Ontario population (Table 3.5). Although these results may indicate that the Rocky Mountains are acting as a barrier to gene flow between Central Interior and the Peace River Region, the overall genetic data do not follow what was expected according to an isolation-by-distance model. A range wide lack of isolation-by-distance would support the recent expansion hypothesis with high levels of gene flow. However, with only three sampling points conclusions are tentative and would require many more sampling locations in order to confirm a lack of isolation-by-distance among White-throated Sparrow populations.

Weak population structure (at drift-dispersal equilibrium) is usually considered to be the result of high dispersal and gene flow (Lecomte *et al.* 2009). This lack of structure would be expected in many migratory bird species where dispersal and effective population sizes are higher than in other vertebrates (Winker *et al.* 2000). However differences in song structure (unpublished data, Mesias, V., Otter, K., Mora, M., Ramsay, S., and Murray, B.) the small, but significant, statistical genetic difference between Peace River Region and Central Interior and the possible migratory differences (Chapter2) suggest that populations on both sides of the Rocky Mountains could be in the early stages of differentiation.

3.4.2 POPULATION STRUCTURE AND GENETIC ASSIGNMENT OF WHITE-THROATED SPARROW POPULATIONS

To test confidence of the assignment technique used in the present study, breeding individuals were assigned back to the breeding populations sampled (Table 3.6). Consistent with the general lack of structure, this technique showed that only 40-50% of samples were assigned correctly to their breeding locations and that there were very few population exclusions. In spite of the relatively low percentage of samples assigned correctly in the present study, this percentage was slightly higher than what was expected under simulation studies (from 25 to 30%) for an overall Fst of 0.01 (with 10 loci and 30 samples per population) (Cornuet *et al.* 1999).

The self-assignment test showed that individuals from breeding sample locations located east of the Continental Divide were mostly assigned to the Peace River Region. The only exceptions were Sikanni that was assigned mostly to the Central Interior (although it only has a sample size of 3 individuals) and Ontario that was mostly assigned to the Central Interior and the Peace River Region. Sampling locations west of the Continental Divide had two different scenarios: Prince George which was assigned mostly to the Central Interior, and the John Prince Research Forest and MacLeod Lake that were assigned mostly to the Peace River Region. However, it is hard to make any conclusions based on this test because of the low genetic structure and different sampling sizes among locations.

Samples originating from the different migratory stations, *i.e.,* Mugaha Marsh, Lesser Slave Lake, Rocky Point and Beaverhill, did not show a clear preference of assignment of individuals to any of the sampled breeding populations (Table 3.6). The lack of genetic structure has confounded the assignment of migratory individuals. It is important to state that this likelihood technique only calculates a probability of a sample belonging to a reference population. Analysis of tail feather isotopes signatures (Chapter 2) suggests that at least some of the migratory samples at these locations have values outside the range observed in the breeding populations indicating that they could be breeding in populations not sampled. The Mugaha banding station, although west of the Rockies had a distinct isotope signature from the Central Interior breeding birds. These migratory samples also showed the greatest amount of exclusions for the breeding populations tested. The combined results support the hypothesis that this station is capturing migrants breeding outside, presumable north, of the breeding locations sampled in this study

Migratory birds were also captured in Dawson Creek where 18 out of 35 migratory birds were assigned most likely to the Peace River Region breeding population (Table 3.7). This result was expected since this sampling location of migratory birds was also used during the breeding season and it is the closest to migratory stopover location sampled to the breeding locations included in this study. For this reason, it is very possible that at least part of the migratory flock sampled came from breeding territories near that sampled stopover site. This was confirmed by a recapture of an individual (code: Zoal-jd163/Zoal-id134) sampled two times in the same area, the first one with the migratory flock on the fall of 2008 and the second time in his breeding territory in the summer of 2009.

Lack of resolution in assignment tests caused by a poor population structure could be resolved by using additional markers. such as selective markers that are able to detect recent differentiation events at a genetic level. Selective markers have been used in other studies (*i.e.,* Bredford and Irwin 2009) to detect hybrid zones in avian species and could provide enough resolution to find the genetic differentiation necessary in order to effectively use assignment tests to elucidate migratory behaviour of White-throated Sparrows.

In conclusion, results from microsatellite and mitochondrial data of this study could not determine the presence or absence of a migratory divide in western Canadian White-throated Sparrow populations. However, they provide evidence that suggests that the breeding populations studied arose from an expansion from single glacial refugium. Additionally, shallow but significant genetic differences, plus differences in song structure (unpublished data, Mesias, V., Mora, M., Ramsay, S., and Murray, B, and Otter, K.) between the Central Interior and Peace River Region suggest that both populations could be in an early stage of differentiation. This early stage of differentiation implies that determining the migratory behaviour of western Canadian White-throated Sparrows using genetic markers will require additional (locally adaptive) markers. Further the results of the Mugaha banding station indicate that additional breeding populations are migrating through Northern BC and point for the need of sampling a wider range of breeding locations in Western Canada.

GENERAL DISCUSSION

4.1 MIGRATORY CONNECTIVITY BETWEEN BREEDING AND MIGRATORY POPULATIONS

The aim of this study was to use genetic and stable isotope information in order to determine the migratory corridors and wintering areas used by western Canadian White-throated Sparrow populations. Elucidating the migratory connectivity of these populations will provide key information that could be applied to enhance conservation projects that aim to preserve ecological areas that are important for migratory species. Determining ecologically important areas of migratory birds is very important because if one of these areas is affected by human related activity, more than one breeding population could be threatened.

One of the objectives of the present study was to elucidate wintering areas used by the Central Interior BC and Peace River Region populations. Deuterium stable isotopes did not completely elucidate wintering areas of these two populations; however, head feathers stable isotopes successfully narrowed down the tentative wintering areas to the south-western coast (California, Oregon, Washington) or to the northern isotopic limit of the eastern wintering range (New Mexico/Arizona or Colorado/Kansas) (Figure 2.7). Samples from head feathers isotopes did not show significant differences [but situated the δD_f mean value in the higher part of the wintering range (Figure 2.4 A, B)]. These values showed differences with other studies (*e.g.*, Mazerolle *et al.* 2005) in which birds were wintering at the south-eastern part of the wintering range.

Another objective of this study was to investigate migratory differences among White-throated Sparrow populations located on either side of the continental divide in western Canada. It was not possible to determine exactly the migratory routes being used by each breeding population, but the significant differences between tail-feathers samples of Central Interior and all of the rest of banding stations imply that Central Interior sparrows are not using routes that correspond to the geographic location of any of those stations (except maybe Rocky Point). This suggests that sparrows from Central Interior might not be crossing the Rocky Mountains during fall migration (Figure 2.6). Still, another possibility is that Central Interior birds could be crossing the mountains, but using an undetected pathway; for this reason it would be important to extend the sampling in the future to other areas that could be important for migration.

A lack of differences between Peace River Region and Beaverhill and Lesser Slave Lake banding stations suggest that birds from these breeding populations are not crossing the Rocky Mountains during fall migration (Figure 2.6). The most parsimonious explanation would be that these birds are using the Central flyway to a wintering destination close to New Mexico/Arizona or Colorado/Kansas area. However, there is still a possibility that those birds could be using another migratory route like the Columbia River route to California or the Mississippi flyway to Texas. However, most of the data used in this study is based on the information gathered during the fall migration, and data collected during spring migration might also help to determine the migratory behaviour of these sparrows.

The banding station with the highest number of White-throated Sparrow captures west of the Continental Divide is Mugaha Marsh. Even though it is located within the Central Interior, this banding station seems to be capturing birds from higher latitudes than any of the breeding populations sampled. This is evidenced by the northern isotopic distribution of the tail feather isotopes found in sparrows banded at this station (Figure 2.6). These isotopic ratios are statistically different from Central Interior and the Peace River Region, suggesting that sparrows from these breeding populations are not being detected at this banding station.

This evidence suggests the importance of the Mackenzie area for migration, because instead of capturing birds from Central Interior or the Peace River Region during the fall, the banding station apparently receives sparrows from higher latitudes which then could be using low altitude passages (*e.g.,* Pine Pass) to cross the Rocky Mountains to their wintering destinations. From there, they may follow more direct routes (*e.g.* the Fraser River) to the Pacific coast. As the area on the opposite side of the Continental Divide from Mackenzie is proposed for intense wind and other energy development, evidence suggesting this region may be a confluence of migratory corridors is important.

4.2 UNIQUE NATURE OF CENTRAL INTERIOR

Molecular Genetic markers (Chapter 3) have been very useful to find genetic differentiation in migratory connectivity and behaviour studies (*e.g.,* Boulet and Gibbs 2006; Clegg *et al.* 2003; Lecomte *et al.* 2009; Winker *et al.* 2000; Helbig *et al.* 1995). However, in the present

study no population structure was found using neutral microsatellites and the partial coding sequence of the mitochondrial *Cytochrome Oxidase I* gene (Table 3.4, 3.10, Figure 3.4).

Mitochondrial DNA data showed a star-like haplotype tree that lacked reciprocal monophyly. This tree, as well as the lack of overall structure found in microsatellite data suggests that White-throated Sparrow populations originated from single glacial refugia, followed by a range expansion to the Central Interior from an Eastern population. Similar results have been obtained in other avian passerine species such as the Black-throated Blue Warbler, in which lack of differentiation was attributed to similar phenomenon (Davis *et al.* 2006). On the other hand, birds which were originated from two or more refugia (*e.g.,* Yellow Warbler) showed clear genetic differentiation along an East/West axis (Boulet and Gibbs 2006).

The question that remains unresolved is how recent is this vicariant event? The complexity of the haplotype tree and the number of rare alleles/haplotypes (Table 3.3, 3.7) obtained in the Central Interior seems to suggest that this population could be older than what was suggested from the first records of White-throated Sparrows in the province (Whyte 1938). Additionally, song analysis showed some degree of differentiation in song structure between western Canadian populations (unpublished data, Mesias, V., Otter, K., Mora, M., Ramsay, S., and Murray, B.) and these differences reflect that the vicariant event could be old enough for song differences to become established in these populations.

4.3 ENVIRONMENTAL/MANAGMENT IMPLICATIONS

As wind power industry keeps growing as an important source of energy in many countries, concerns about the environmental impacts that this technology could have are increasing. Studies in birds have shown that in spite of the fact that wind farms have less impact in terms of bird casualties than other man-made structures like power lines (Johnson *et al.* 2002), a high number of birds can be affected within certain wind farm locations. For instance, significant numbers of Gryphon Vulture (*Gyps fulvus*) casualties have been reported at the Strait of Gibraltar, because installations seems to be located at a migratory bottleneck (*e.g.,* De Lucas *et al.* 2008; Bildstein *et al.* 2009). Wind farms located at these migratory bottlenecks have affected raptors more than any other group of birds (*e.g.,* Madders and Whitfield 2006), however, possibly in a lower degree, passerines have also shown to be affected by wind power structures during migration (Johnson *et al.* 2002).

In the case of the present study, the effect that cumulative effect of multiple wind farm facilities located at a migratory bottleneck for the western White-throated Sparrow populations, as well as potentially other song birds could be important. Due to the high average wind speed in the area, there is increasing interest in the Peace River Region to develop wind power projects (BC Hydro 2009). Migrants crossing the Rocky Mountains could be funnelled into narrow passes, and this funnelling might concentrate movement to areas that overlap with wind farm development. Birds tend to lower their flying height during migration in locations like coast-lines and when crossing a ridge (Drewitt and Langston 2006), so migrants flying through the Pine Pass (or the Peace River) to eastern or western

wintering locations could be affected by potential wind power projects in the Peace River Region (plus several other wind farm projects in the United States).

An extensive expansion of wind farm projects in Peace River Region could represent a significant impact on a disjunct population like the Central Interior. This impact would include a decrease on the gene flow between this population and the rest of the species' range (creating genetic isolation). However, it is hard to assess if Central Interior population would be affected by wind farms, because even though stable isotope evidence suggests that Central Interior birds might not be crossing the Rocky Mountains during the fall; this cannot be demonstrated with the data available so far. Additionally, Central Interior sparrows could still be funnelled into several wind farm projects while they cross the mountains in spring migration to northern breeding grounds.

Estimating the impact that the cumulative effect of wind farm projects could have on migratory bird populations will be a difficult task, because as the White-throated Sparrow illustrate more than one population is likely to be affected. For instance, the Central Interior and Peace River Region are two likely populations to be affected by a wind farm project expansion. However, stable isotope results also suggest that Mugaha Marsh is banding birds from northern latitudes. These northern populations could be also affected by a sudden expansion in the wind power projects in the Peace River Region, because these birds could be funnelled into the area before or after crossing the mountains into the Mackenzie area during the fall (and possibly spring) migration.

4.4 IMPROVEMENTS TO THE TECHNIQUE

In terms of efficiency, deuterium stable isotopes were effective in showing latitudinal discrimination. This is evident from feathers of White-throated Sparrows from the Central Interior and Peace River Region which indicate that these birds winter at latitudes with a higher isotopic signature than was obtained in previous studies (*i.e.,* Mazerolle *et al.* 2005) for the rest of the distribution. However, deuterium isotopes were not able to discriminate wintering areas (Pacific South-west coast and New-Mexico/Arizona or Colorado/Kansas) at a longitudinal level.

Other studies, such as Kelly and colleagues (2005), have used a combination of techniques to study connectivity between breeding and wintering areas at a longitudinal level (*i.e.,* Coastal vs. Inland birds). These techniques include combining deuterium with sulphur isotopes and mitochondrial DNA data. Even though sulphur isotopes did not show differentiation by itself, it significantly increased the resolution of the technique when both isotopes were combined in a discriminant function analysis.

Several other isotopes could be effectively used to complement the present study. Carbon isotopes have been effective in studying wintering habitat via reflecting the abundance of C3 versus C4 plants (Bearhop *et al.* 2004; Chamberlain et al. 2000; Pain *et al.* 2004; Yohannes *et al.* 2005). This element, as well as nitrogen, has been used successfully to find differentiation along a migratory divide between two subspecies of the Willow warbler (*Phylloscopus trochilus trochilus* and *Phylloscopus trochilus acredula*) (Chamberlain *et al.*

2000). Other studies such as Chambelain *et al.* (1997) have not only found that Carbon and Nitrogen are useful for discriminating breeding areas of migratory populations, but have also used strontium isotopes to find East/West differentiation.

The latitudinal discrimination of tail feather isotopes on breeding populations could be used in order to infer breeding territories of migrants captured at their wintering areas. Other studies such as Kelly and colleagues (2005) have successfully used feathers from Swainson's Thrush (*Catharus ustulatus*) migrants in order to infer breeding territories using deuterium isotopes and mitochondrial haplotypes. A similar strategy could be employed to complement this study, in which feathers from wintering areas such as California, Oregon, New Mexico Arizona, Colorado and Kansas could be sampled, and tail feather isotopes could then be compared to the data from the present study.

Another way to increase the efficiency of the technique used in the present study is to improve the resolution of the genetic markers. Increasing the resolution of genetic markers is very important in order to delineate the population structure of White-throated Sparrow breeding populations. Finding population differentiation with genetic markers could be a challenging task, as in many cases, it is necessary to develop high number of markers, which can be difficult and time consuming in the case of non-model species (as the White-throated Sparrow). Additionally, avian species that have originated from a single refugium and have recently separated or high levels of gene flow could present low levels of differentiation between populations (*e.g.*, Davis *et al.* 2006).

Increasing the number of microsatellite markers may not increase the resolution significantly. Adaptive markers have been shown to work effectively in cases where other markers have failed to find genetic differentiation. For instance, Brelsford and Irwin (2009) found genetic differences in two possibly adaptive markers (one autosomal and one sex-linked) with fixed differences across a hybrid-zone in the Yellow-rumped warbler, when little differentiation previously was reported using mtDNA markers.

Other markers such as Exon-Primed Intron-Crossing (EPIC) markers and Expressed Sequence Tags (EST)-linked microsatellites have become available thanks to the increasing amount of information on bird genomic projects. The main advantage of EPIC markers is that they are highly variable because they have target intronic regions which are flanked by conserved exonic regions (Thomson *et al.* 2010). A high number of these markers have been developed for bird population studies because of their advantages (*e.g.,* Backstrom *et al.* 2008). For instance, besides been highly variable, they are also conserved at primer regions and can provide adaptive information via hitchhiking of close gene regions (Thomson *et al.* 2010).

On the other hand, EST-linked microsatellites are markers that can be more useful than EPIC's for adaptive population studies; this is because the microsatellite allele variation of these markers can be located within the transcript region and have an important effect in the protein coding sequence. Additionally, a high number of these markers have been described

in passerine species, most of which have been successfully transferred between different avian species (Dawson *et al.* 2010; Karaiskou *et al.* 2008).

4.5 TECHNIQUE ASSESMENT

Combining genetic data from mitochondrial and microsatellite markers with deuterium stable isotopes was useful for determining the population history and inferring the migratory routes in order to locate the presence of a migratory divide in White-throated Sparrows, however, the technique presented certain limitations that can be optimized to increase the resolution of this methodology. Several changes can be suggested for future projects in order to optimize these techniques including a fully integrated sampling strategy with additional sampling locations during both the breeding season, as well as, fall/spring migratory seasons to supplement banding station information.

Lack of population structure in molecular genetic markers did not allow us to confidently estimate the origin of migratory individuals banded in western Canada. Adding more samples from other breeding populations would be very important to clarify if this lack of population structure is extended to all the breeding range or if there is a regional genetic structure that is not been detected. Understanding the genetic structure and the amount of gene flow between breeding populations of White-throated Sparrow can be very important to understand migratory behaviour since migration has a strong genetic component.

Besides the lack of structure obtained with genetic data, the significant differences observed on the tail feather isotopes ratios δD_f of Central Interior and the Peace River Region are promising, as they can be used in the future to estimate the breeding territories of migrants captured at their migratory and wintering grounds. Sampling birds at wintering and migratory grounds could be a great strategy to implement to optimize the techniques used in this study. This could be done by either choosing sampling sites that could complement migratory routes banded by banding stations (*e.g.,* sampling birds in Quesnel that are flying down Fraser River or in Washington State (US) flying down the Columbia River drainage).

Sampling birds from additional breeding locations could be also important to solve questions regarding genetic or demographic effects, such as isolation-by-distance, or bottleneck effects that could explain the genetic distribution. One alternative to study these effects would be sampling locations following one or two transects that cover both sites of the geographical divide. These two transects could be useful to determine if there is a general isolation-by-distance pattern across the species range or if there is a point which forms a genetic divide between the Central Interior and eastern populations.

Another improvement to the study would be using an integrated sampling strategy in which head feather samples are collected not only from breeding populations but also from migratory birds. Unfortunately in the present study this was not the case as only head feather samples of breeding individuals were taken (Table 4.1). Results, such as the tail feather

isotope signal of Mugaha Marsh migrants, indicate the importance of sampling head feathers from migrants at banding stations in future studies.

Table 4.1 Summary of number of samples that were analyzed from each location for all the markers: Tail and Head feathers isotopes δD_f, Microsatellite, and Mitochondrial DNA.

Regions	Breeding Locations	Samples Collected	Tail feather δD_f	Head feather δD_f	Microsat	mtDNA
Central Interior	Prince George	44	19	18	43	40
	John Prince	5	-	-	4	4
	McLeod Lake	12	-	-	12	10
CI Totals		**61**	**19**	**18**	**59**	**54**
Peace River Region	Dawson Creek	37	26	26	37	32
	Moberly Lake	12	-	-	11	9
	Tumbler Ridge	12	-	-	11	10
	Sikanni River	3	3	-	3	2
PR Totals		**64**	**29**	**26**	**62**	**53**
Ontario	Ontario	20	-	-	20	19
Totals		**145**			**141**	**126**
	Migratory Locations					
	Mugaha Marsh	9	9	-	9	-
	Rocky Point	3	3	-	3	-
	Lesser Slave	9	9	-	9	-
	Beaverhill	8	7	-	7	-
	Dawson Creek	38	-	-	35	-
Totals		**67**			**63**	

In conclusion, the strategy of the present study of combining genetic data from mitochondrial and microsatellite markers with deuterium stable isotopes showed that White-throated Sparrow has a good potential for being an indicator species in migratory behaviour studies. Results obtained were successful in providing evidence to determine the population history and migratory connectivity of breeding and wintering populations of White-throated Sparrows. While the genetic data provided evidence of a recent expansion from single glacial refugia, head feather isotopes suggested that Central Interior and the Peace River Region are not following migratory routes to south-eastern wintering grounds. I suggest that both populations could be following an east/west migration pattern where Central Interior population could be migrating to the south-west Pacific Coast and the sparrows from the Peace River Region to the New Mexico/Arizona or Colorado/Kansas area.

Improving the resolution of the molecular and isotopic markers as well as optimizing the sampling strategy could be a very successful tool to study the connectivity between breeding and wintering populations of migratory species. Preferentially, species with and east/west distribution that originated from different glacial refugia are recommended if this technique is going to be implemented. If that is not the case, local adaptive markers and a fully integrated sampling strategy could be applied. In summary, this technique showed that White-throated Sparrow has potential to be a good indicator species for proactive conservation studies on migratory connectivity of avian species, but that more work needs to be done before applying this strategy in this and other species.

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APPENDIX 1 RAW DATA SUMMARY

Table A1.1 Raw data of breeding individuals including: Alleles of eight neutral microsatellites, mtDNA *Cytochrome Oxidase I* haplotypes, deuterium stable isotopes δD_f (‰) for tail and head feather samples. GPS coordinates were taken per sampling location.

				Microsatellites								MtDNA	Stable Isotopes		Additional Info
Sample ID	Location	UTM	Collection Date	A08	B01	B03	A02	C06	C11	C07	F11	Haplotype	Tail δD_f (‰)	Head δD_f (‰)	Song recorded
4-01	Prince George	-	-	272	284	254	182	232	180	224	261	B	?	?	-
				272	294	262	190	236	188	224	269				
4-02	Prince George	-	-	264	272	246	178	224	182	256	254	B	?	?	-
				280	294	262	194	252	182	256	265				
4-03	Prince George	-	-	268	268	258	190	232	190	240	249	B	?	?	-
				280	280	262	190	236	194	240	257				
4-04	Prince George	-	-	268	280	254	174	224	188	224	291	B	?	?	-
				268	294	258	194	224	188	248	291				
4-09	Prince George	-	-	268	284	258	178	232	194	256	249	B	?	?	-
				280	288	258	186	236	194	256	253				
4-10	Prince George	-	-	272	252	254	182	216	180	216	253	B	?	?	-
				272	282	262	190	232	194	260	261				
4-11	Prince George	-	-	268	256	246	174	224	184	216	249	B	?	?	-
				272	294	254	174	236	194	260	249				
4-12	Prince George	-	-	276	268	250	190	224	180	220	253	B	?	?	-
				284	284	258	198	228	196	220	277				
4-17	Prince George	-	-	272	288	250	170	224	180	276	245	A	-	-	-
				272	294	258	170	232	184	276	249				
4-18	Prince George	-	-	276	252	250	174	236	188	232	241	B	?	?	-
				276	288	254	194	240	194	232	257				
4-19	Prince George	-	-	272	276	246	182	216	194	236	245	B	?	?	-
				276	276	254	186	224	200	240	261				

Table A1.1 Continued

				Microsatellites								MtDNA	Stable Isotopes		Additional Info
Sample ID	Location	UTM	Collection Date	A08	B01	B03	A02	C06	C11	C07	F11	Haplotype	Tail δD_f (‰)	Head δD_f (‰)	Song recorded
4-20	Prince George	-	-	272	280	246	174	216	180	224	253	A	?	?	-
				272	294	258	182	232	196	224	269				
4-21	Prince George	-		276	280	242	186	220	188	248	253	I	?	?	-
				284	284	254	198	224	194	248	253				
4-26	Prince George	-	-	272	264	254	182	206	188	248	253	A	-	-	-
				276	268	262	186	206	192	276	253				
4-27	Prince George	-	-	264	272	254	174	232	180	236	245	B	?	?	-
				276	288	258	182	240	188	276	249				
4-33	Prince George	-	-	272	280	246	188	228	190	256	261	B	-	-	-
				272	294	250	194	232	194	276	265				
4-34	Prince George	-	-	276	288	250	186	224	180	205	249	B	?	?	-
				280	290	258	194	232	194	224	261				
4-35	Prince George	-	-	268	284	258	190	232	184	225	257	B	?	?	-
				272	294	266	194	236	184	232	257				
4-46	Prince George	-	-	260	260	254	182	216	184	208	249	B	?	?	-
				260	294	258	190	228	184	234	257				
4-52	Prince George	-	-	260	268	258	182	228	180	232	249	B	?	?	-
				280	294	266	190	228	180	248	253				
Zoal-ia008	Prince George	10U 0512078 E	26-May-09	272	264	250	174	216	194	240	245	B	?	?	-
		5971972 N		276	280	258	186	232	196	240	261				
Zoal-ia009	Prince George	10U 0512002 E	27-May-09	276	268	254	174	232	180	260	265	B	?	?	-
		5971738 N		276	294	262	182	232	188	264	273				
Zoal-ia010	Prince George	10U 0512078 E	26-May-09	268	264	246	178	224	194	252	253	B	?	?	-
		5971972 N		280	288	258	186	240	196	252	261				
Zoal-ja164	Prince George	-	-	276	280	242	182	216	184	232	269	B	-148.7	-77.6	-
				276	294	250	194	232	184	252	273				

Table A1.1 Continued

				Microsatellites								MtDNA	Stable Isotopes		Additional Info
Sample ID	Location	UTM	Collection Date	A08	B01	B03	A02	C06	C11	C07	F11	Haplotype	Tail δD_f (‰)	Head δD_f (‰)	Song recorded
Zoal-ja165	Prince George	-	-	272	276	258	182	232	172	240	265	?	-150.9	-110.6	-
				276	298	266	198	232	196	240	269				
Zoal-ja166	Prince George	-	-	264	284	254	178	220	180	?	253	B	-142.8	-107.6	-
				272	294	258	186	228	180	?	261				
Zoal-ja167	Prince George	-	-	268	280	254	190	220	180	228	249	B	-140.1	-91.5	-
				276	294	270	190	224	194	234	261				
Zoal-ja168	Prince George	-	-	272	252	254	190	212	180	208	249	B	-141.9	-65.2	-
				280	294	262	194	236	194	236	269				
Zoal-ja169	Prince George	-	-	280	284	232	166	232	180	232	245	B	-148.2	-95.6	-
				284	294	258	182	236	180	252	253				
Zoal-ja170	Prince George	-	-	272	284	250	186	224	184	232	249	B	-139.2	-79.3	-
				284	294	250	190	232	184	252	273				
Zoal-ja171	Prince George	-	-	276	272	246	182	228	168	228	?	B	-139.6	-77.2	-
				276	290	254	186	240	168	228	?				
Zoal-ja172	Prince George	-	-	264	260	254	186	212	184	232	241	B	-	-	-
				272	294	254	190	220	184	244	245				
Zoal-ja173	Prince George	-	-	268	288	250	178	216	180	216	205	B	-151.4	-123.2	-
				276	294	254	194	236	188	248	205				
Zoal-ja174	Prince George	10 U 0511144 E	30-May-10	268	264	242	182	232	180	228	253	B	-89.8	-73.6	-
		5971689 N		272	294	258	194	240	180	228	253				
Zoal-ja175	Prince George	10 U 0511144 E	30-May-10	272	294	242	178	228	180	216	241	B	-152.3	-64	-
		5971689 N		272	294	262	186	228	180	252	253				
Zoal-ja176	Prince George	10 U 0511144 E	31-May-10	?	268	258	178	228	178	188	261	K	-138	-76.7	-
		5971689 N		?	294	266	178	228	178	272	265				

Table A1.1 Continued

				Microsatellites								MtDNA	Stable Isotopes		Additional Info
Sample ID	Location	UTM	Collection Date	A08	B01	B03	A02	C06	C11	C07	F11	Haplotype	Tail δD_f (‰)	Head δD_f (‰)	Song recorded
Zoal-ja177	Prince George	10 U 0511144 E	30-May-10	272	280	242	178	224	180	228	241	O	-137.9	-102.2	-
		5971689 N		272	294	258	182	228	184	228	249				
Zoal-ja178	Prince George	10 U 0511144 E	31-May-10	272	280	242	186	232	180	248	249	B	-142.1	-60.4	-
		5971689 N		276	290	250	198	232	180	264	261				
Zoal-ja179	Prince George	10 U 0511144 E	31-May-10	268	276	258	182	228	180	188	249	D	-142.7	?	-
		5971689 N		280	276	262	186	232	180	220	257				
Zoal-ja180	Prince George	10 U 0511144 E	31-May-10	276	264	258	198	236	192	?	249	N	-148.5	-65.7	-
		5971689 N		276	290	262	202	236	192	?	253				
Zoal-ja181	Prince George	10 U 0511144 E	31-May-10	268	290	246	182	220	?	?	257	B	?	-81.3	-
		5971689 N		276	294	258	186	228	?	?	257				
Zoal-ja182	Prince George	10 U 0511144 E	30-May-10	272	272	254	182	220	188	?	253	?	-156.8	?	-
		5971689 N		276	280	262	198	228	192	?	257				
Zoal-ja183	Prince George	10 U 0511144 E	30-May-10	272	272	262	178	228	?	?	249	?	-151	-78.3	-
		5971689 N		280	290	266	180	232	?	?	273				
Zoal-ja188	Prince George	10 U 0511144 E	30-May-10	?	?	?	?	?	?	?	?	?	-156.7	-138.7	-
		5971689 N		?	?	?	?	?	?	?	?				
Zoal-ib025	John Prince Forest	10U 0409749 E	17-Jun-09	272	272	246	182	214	184	236	249	B	-	-	-
		6055679 N		286	280	254	190	224	192	236	261				
Zoal-ib028	John Prince Forest	10U 0416594 E	19-Jun-09	280	264	254	178	232	184	220	245	B	-	-	-
		6052975 N		280	272	258	194	248	188	248	261				
Zoal-ib045	John Prince Forest	10U 0416594 E	19-Jun-09	280	280	246	194	224	188	212	249	B	-	-	Yes
		6052975 N		284	294	254	198	236	192	244	261				
Zoal-ib055	John Prince Forest	10U 0416594 E	19-Jun-09	268	280	254	186	232	188	268	249	C	-	-	-
		6052975 N		280	284	254	186	236	192	268	261				

Table A1.1 Continued

				Microsatellites								MtDNA	Stable Isotopes		Additional Info
Sample ID	Location	UTM	Collection Date	A08	B01	B03	A02	C06	C11	C07	F11	Haplotype	Tail δD_f (‰)	Head δD_f (‰)	Song recorded
	MacLeod Lake	10U 0513725 E	26-Jun-09	272	264	254	182	228	180	220	237		-	-	
Zoal-ih019		6067293 N		280	294	266	186	228	192	234	261	B			Yes
	MacLeod Lake	10U 0513725 E	26-Jun-09	272	284	257	174	210	184	224	249		-	-	
Zoal-ih020		6067293 N		276	294	262	182	236	192	224	257	B			-
	MacLeod Lake	10U 0498878 E	26-Jun-09	268	276	254	186	228	180	188	249		-	-	
Zoal-ih029		6108321 N		272	300	254	194	236	192	252	261	B			-
	MacLeod Lake	10U 0500490 E	29-Jun-09	268	276	238	178	240	180	224	253		-	-	
Zoal-ih031		6114644 N		282	294	266	190	245	196	240	269	B			-
	MacLeod Lake	10U 0500490 E	29-Jun-09	268	284	246	182	216	188	260	253		-	-	
Zoal-ih032		6114644 N		276	296	250	182	224	192	260	253	?			Yes
	MacLeod Lake	10U 0500490 E	29-Jun-09	272	252	250	170	212	192	264	249		-	-	
Zoal-ih033		6114644 N		272	292	254	178	236	192	268	261	B			Yes
	MacLeod Lake	10U 0500490 E	29-Jun-09	272	268	254	180	228	180	220	257		-	-	
Zoal-ih034		6114644 N		276	294	254	198	240	192	240	265	A			Yes
	MacLeod Lake	10U 0498878 E	26-Jun-09	272	264	250	178	220	188	212	245		-	-	
Zoal-ih046		6108321 N		280	280	254	186	228	192	240	245	B			-
	MacLeod Lake	10U 0508224 E	28-Jun-09	272	268	254	178	228	192	248	257		-	-	
Zoal-ih047		6078477 N		276	276	254	194	236	192	248	265	B			Yes
	MacLeod Lake	10U 0513725 E	26-Jun-09	276	292	254	174	216	188	264	253		-	-	
Zoal-ih052		6067293 N		280	294	262	182	220	192	270	253	B			-
	MacLeod Lake	10U 0498993 E	26-Jun-09	276	260	250	182	220	176	212	249		-	-	
Zoal-ih053		6108354 N		280	294	258	190	232	188	212	287	B			-
	MacLeod Lake	10U 0498878 E	28-Jun-09	272	260	238	190	208	176	204	249		-	-	
Zoal-ih054		6108321 N		272	284	254	194	228	192	244	253	?			Yes

Table A1.1 Continued

				Microsatellites								MtDNA	Stable Isotopes		Additional Info
Sample ID	Location	UTM	Collection Date	A08	B01	B03	A02	C06	C11	C07	F11	Haplotype	Tail δD_f (‰)	Head δD_f (‰)	Song recorded
Zoal-ic012	Moberly Lake	10U 0588005 E	31-May-09	272	294	262	182	228	180	240	249	A	-	-	Yes
		6181234 N		272	296	262	198	232	188	240	253				
Zoal-ic013	Moberly Lake	10U 0571140 E	31-May-09	264	288	250	186	224	184	248	241	?	-	-	-
		6182770 N		276	288	254	198	228	192	252	249				
Zoal-ic014	Moberly Lake	10U 0588005 E	01-Jun-09	280	264	254	182	224	184	224	249	H	-	-	-
		6181234 N		280	294	266	186	236	192	236	253				
Zoal-ic018	Moberly Lake	10U 0571140 E	30-May-09	272	268	254	186	224	176	248	249	B	-	-	-
		6182770 N		280	298	268	194	236	192	280	249				
Zoal-ic039	Moberly Lake	10U 0571140 E	30-May-09	276	280	246	182	220	180	248	241	B	-	-	-
		6182770 N		276	294	256	190	232	188	248	261				
Zoal-ic040	Moberly Lake	10U 0571140 E	31-May-09	280	268	254	174	228	176	248	213	B	-	-	-
		6182770 N		280	268	262	182	228	192	268	213				
Zoal-ic048	Moberly Lake	10U 0571140 E	30-May-09	272	280	246	170	224	180	240	245	B	-	-	Yes
		6182770 N		280	294	250	186	232	192	240	269				
Zoal-ic049	Moberly Lake	10U 0571140 E	30-May-09	272	280	258	182	236	192	228	257	B	-	-	-
		6182770 N		272	284	266	190	248	192	228	261				
Zoal-ic050	Moberly Lake	10U 0571140 E	30-May-09	276	272	246	170	232	188	232	237	B	-	-	Yes
		6182770 N		280	298	254	204	236	188	244	257				
Zoal-ic058	Moberly Lake	10U 0571140 E	31-May-09	272	272	250	182	212	192	248	257	B	-	-	-
		6182770 N		276	276	254	186	248	192	272	261				
Zoal-ic065	Moberly Lake	10U 0588005 E	29-May-09	272	264	250	190	228	188	208	249	?	-	-	-
		6181234 N		272	284	262	198	228	192	260	249				
Zoal-id007	Dawson Creek	10U 0680870 E	02-Jun-09	272	280	262	170	228	164	244	233	B	-	-	-
		6176372 N		280	294	262	194	232	192	256	253				

Table A1.1 Continued

				Microsatellites								MtDNA	Stable Isotopes		Additional Info
Sample ID	Location	UTM	Collection Date	A08	B01	B03	A02	C06	C11	C07	F11	Haplotype	Tail δD_f (‰)	Head δD_f (‰)	Song recorded
Zoal-id015	Dawson Creek	10U 0680708 E	03-Jun-09	276	268	?	178	224	192	200	245	B	-	-	-
		6177208 N		276	284	?	180	228	196	228	261				
Zoal-ie017	Dawson Creek	10U 0668852 E	04-Jun-09	272	272	246	178	216	184	244	249	A	-	-	-
		6176114 N		272	290	250	178	224	192	276	253				
Zoal-ie027	Dawson Creek	10U 0668852 E	04-Jun-09	286	280	246	186	232	180	252	239	B	-	-	-
		6176114 N		288	290	254	198	248	196	268	239				
Zoal-ie030	Dawson Creek	10U 0668852 E	04-Jun-09	280	272	254	166	216	188	228	261	B	-	-	Yes
		6176114 N		280	304	262	178	236	192	236	261				
Zoal-id038	Dawson Creek	10U 0680870 E	02-Jun-09	272	268	254	190	232	188	252	249	?	-153.5	?	-
		6176372 N	06-Jun-10	276	272	254	194	236	192	268	253				
Zoal-ie044	Dawson Creek	10U 0668852 E	04-Jun-09	264	280	246	178	220	184	220	257	B	-	-	-
		6176114 N		280	294	258	186	228	188	236	257				
Zoal-ie051	Dawson Creek	10U 0668852 E	04-Jun-09	272	280	254	178	224	180	224	253	B	-	-	-
		6176114 N		272	294	262	186	228	180	240	269				
Zoal-ie060	Dawson Creek	10U 0668852 E	04-Jun-09	264	252	250	182	220	188	228	249	L	-151.9	-138	-
		6176114 N	05-Jun-10	272	276	274	190	236	192	232	269				
Zoal-id062	Dawson Creek	10U 0680809 E	05-Jun-09	272	252	246	194	224	192	272	245	B	-	-	-
		6176912 N		280	260	258	198	228	196	272	253				
Zoal-ie064	Dawson Creek	10U 0668852 E	04-Jun-09	272	250	254	186	232	184	236	257	B	-	-	-
		6176114 N		276	284	254	190	236	192	272	257				
Zoal-ie134/Zoal-jd163	Dawson Creek	10U 0680721 E	07-Sep-09	268	276	256	194	224	184	244	241	B	-	-	-
		6177627 N	05-Jun-10	272	294	270	198	232	188	276	249				
Zoal-je136	Dawson Creek	10U 0680721 E	05-Jun-10	272	272	250	186	224	188	224	245	R	-154.3	-116.8	-
		6177627 N		276	276	254	190	228	200	256	253				

Table A1.1 Continued

				Microsatellites								MtDNA	Stable Isotopes		Additional Info
Sample ID	Location	UTM	Collection Date	A08	B01	B03	A02	C06	C11	C07	F11	Haplotype	Tail δD_f (‰)	Head δD_f (‰)	Song recorded
Zoal-je137	Dawson Creek	10U 0680721 E	05-Jun-10	272	288	236	178	216	176	252	253	?	-164.3	-82.6	-
		6177627 N		280	290	250	194	240	192	264	253				
Zoal-je138	Dawson Creek	10U 0680721 E	05-Jun-10	272	280	250	162	228	188	234	241	B	-165.6	-117.1	-
		6177627 N		272	284	266	162	248	192	252	241				
Zoal-je139	Dawson Creek	10U 0680721 E	05-Jun-10	268	284	242	182	216	184	220	245	B	?	-81.9	-
		6177627 N		276	294	246	186	244	188	264	249				
Zoal-je140	Dawson Creek	10U 0668826 E	05-Jun-10	264	260	?	182	220	188	232	225	B	-150.3	-101.4	-
		6176112 N		272	272	?	294	244	192	248	273				
Zoal-je141	Dawson Creek	10U 0668826 E	05-Jun-10	272	268	226	170	232	180	240	249	A	-161.5	-54.3	-
		6176112 N		272	294	254	194	236	184	240	265				
Zoal-jd142	Dawson Creek	10U 0680833 E	06-Jun-10	268	268	246	182	224	192	240	241	M	-160.3	-105.5	-
		6176357 N		268	272	246	182	232	196	248	249				
Zoal-jd143	Dawson Creek	10U 0680833 E	06-Jun-10	276	292	230	182	228	180	244	245	B	-149.9	-102.7	-
		6176357 N		276	294	254	194	232	192	244	261				
Zoal-je144	Dawson Creek	10U 0680833 E	06-Jun-10	276	260	230	182	220	184	240	249	A	-150.6	-111.6	-
		6176357 N		280	280	238	190	224	188	264	253				
Zoal-je145	Dawson Creek	10U 0680833 E	06-Jun-10	272	264	246	178	224	184	224	257	B	-138.3	-68.7	-
		6176357 N		280	280	262	190	232	188	228	257				
Zoal-je146	Dawson Creek	10U 0668675 E	07-Jun-10	272	268	234	162	232	196	256	253	?	-148.7	-145.9	-
		6175712 N		276	280	254	190	240	200	256	253				
Zoal-je147	Dawson Creek	10U 0668675 E	07-Jun-10	276	268	246	162	224	188	228	249	?	-130	-109.6	-
		6175712 N		284	284	254	166	224	196	260	257				
Zoal-je148	Dawson Creek	10U 0668675 E	07-Jun-10	272	280	222	194	214	188	207	?	C	-148.9	-137.2	-
		6175712 N		272	280	226	202	232	192	216	?				

Table A1.1 Continued

				Microsatellites								MtDNA	Stable Isotopes		Additional Info
Sample ID	Location	UTM	Collection Date	A08	B01	B03	A02	C06	C11	C07	F11	Haplotype	Tail δD_f (‰)	Head δD_f (‰)	Song recorded
Zoal-je149	Dawson Creek	10U 0668675 E	07-Jun-10	268	268	246	177	224	188	252	253	S	-154.2	-89.9	-
		6175712 N		272	282	250	177	236	192	252	253				
Zoal-je150	Dawson Creek	10U 0668675 E	07-Jun-10	276	282	254	170	228	188	232	245	B	-148.6	-104	-
		6175712 N		284	282	258	186	232	192	272	253				
Zoal-je151	Dawson Creek	10U 0665804 E	07-Jun-10	280	288	262	194	228	176	212	253	B	-156.1	-75	-
		6173116 N		280	294	262	194	232	188	228	253				
Zoal-je152	Dawson Creek	10U 0665804 E	07-Jun-10	268	276	254	170	228	184	?	245	B	-156.8	-106.8	-
		6173116 N		272	288	278	178	244	192	?	257				
Zoal-je153	Dawson Creek	10U 0665804 E	07-Jun-10	272	276	254	?	224	188	200	249	?	-157.7	-55.2	-
		6173116 N		284	280	258	?	240	200	244	257				
Zoal-je154	Dawson Creek	10U 0665804 E	07-Jun-10	272	272	254	180	224	184	192	245	B	-163.6	-121.1	-
		6173116 N		276	294	270	188	232	188	252	245				
Zoal-je155	Dawson Creek	10U 0665804 E	07-Jun-10	272	256	254	180	230	188	264	241	B	-162.2	-81.6	-
		6173116 N		272	294	258	180	244	196	272	261				
Zoal-je156	Dawson Creek	10U 0665804 E	08-Jun-10	272	272	254	166	210	180	240	249	B	-147.5	-59.1	-
		6173116 N		276	294	258	202	232	196	244	253				
Zoal-je157	Dawson Creek	10U 0665804 E	08-Jun-10	272	264	242	194	228	180	240	253	A	-85.1	-103.9	-
		6173116 N		272	294	258	194	232	188	244	261				
Zoal-je158	Dawson Creek	10U 0665804 E	08-Jun-10	264	268	252	178	210	184	224	249	B	-153.6	-97.9	-
		6173116 N		272	294	270	190	240	188	252	253				
Zoal-je159	Dawson Creek	10U 0665804 E	08-Jun-10	276	280	254	172	202	192	260	249	A	-149.4	-72.3	-
		6173116 N		276	288	258	172	248	196	260	257				
Zoal-je160	Dawson Creek	10U 0665804 E	08-Jun-10	264	272	262	186	220	184	220	241	B	-167.6	-58.7	-
		6173116 N		272	294	262	186	236	188	220	249				

Table A1.1 Continued

				Microsatellites								MtDNA	Stable Isotopes		Additional Info
Sample ID	Location	UTM	Collection Date	A08	B01	B03	A02	C06	C11	C07	F11	Haplotype	Tail δD_f (‰)	Head δD_f (‰)	Song recorded
Zoal-if016	Sikanni River	10U 0523139 E	08-Jun-09	264	256	250	182	216	188	244	257	A	-	-	-
		6327484 N		264	294	258	198	236	188	276	265				
Zoal-if021	Sikanni River	10U 0523139 E	09-Jun-09	272	276	250	178	228	180	258	261	B	-	-	Yes
		6327484 N		272	294	254	178	232	192	268	261				
Zoal-if043	Sikanni River	10U 0523139 E	07-Jun-09	268	268	250	178	232	180	249	257	?	-	-	-
		6327484 N		272	294	262	178	236	188	256	261				
Zoal-ig006	Tumbler Ridge	10U 0631034 E	12-Jun-09	272	264	270	162	232	?	246	261	O	-	-	Yes
		6106110 N		276	264	274	194	232	?	252	261				
Zoal-ig022	Tumbler Ridge	10U 0625243 E	13-Jun-09	272	256	254	182	228	188	224	253	?	-	-	-
		6109316 N		280	266	266	190	252	188	224	257				
Zoal-ig023	Tumbler Ridge	10U 0625243 E	13-Jun-09	272	284	254	182	224	180	260	257	B	-	-	-
		6109316 N		272	294	254	186	224	196	272	261				
Zoal-ig024	Tumbler Ridge	10U 0626343 E	13-Jun-09	276	276	254	182	240	180	220	253	B	-	-	Yes
		6110423 N		280	294	258	190	244	196	268	253				
Zoal-ig026	Tumbler Ridge	10U 0625243 E	13-Jun-09	272	272	254	174	216	188	232	245	Q	-	-	-
		6109316 N		276	280	262	182	220	192	232	261				
Zoal-ig042	Tumbler Ridge	10U 0631034 E	12-Jun-09	272	268	258	178	224	192	232	?	A	-	-	-
		6106110 N		276	292	262	178	232	200	256	?				
Zoal-ig056	Tumbler Ridge	10U 0631034 E	12-Jun-09	264	280	246	174	220	180	236	233	B	-	-	Yes
		6106110 N		276	290	258	194	224	188	264	249				
Zoal-ig057	Tumbler Ridge	10U 0631034 E	12-Jun-09	272	280	254	186	236	180	224	253	B	-	-	Yes
		6106110 N		280	286	258	186	240	192	272	261				
Zoal-ig059	Tumbler Ridge	10U 0614788 E	10-Jun-09	260	264	254	160	216	188	236	249	?	-	-	-
		6126559 N		272	276	258	186	232	188	252	253				

Table A1.1 Continued

				Microsatellites								MtDNA	Stable Isotopes		Additional Info
Sample ID	Location	UTM	Collection Date	A08	B01	B03	A02	C06	C11	C07	F11	Haplotype	*Tail* δD_f (‰)	*Head* δD_f (‰)	Song recorded
Zoal-ig061	Tumbler Ridge	10U 0614788 E	10-Jun-09	272	264	246	168	216	180	268	253	B	-	-	-
		6126559 N		272	294	250	180	228	192	280	261				
Zoal-ig063	Tumbler Ridge	10U 0625243 E	13-Jun-09	272	268	254	182	224	172	224	245	B	-	-	Yes
		6109316 N		280	288	256	186	228	192	228	253				
Zoal-ig066	Tumbler Ridge	10U 0625243 E	13-Jun-09	272	284	250	182	220	192	232	245	B	-	-	-
		6109316 N		280	288	274	194	224	192	248	249				
5-24	Ontario	-	-	272	260	258	182	220	176	192	261	B	-	-	-
				284	290	262	194	228	196	252	265				
7-06	Ontario	-	-	252	284	258	186	220	188	256	253	G	-	-	-
				280	288	262	190	220	196	268	257				
8-52	Ontario	-	-	280	276	242	174	224	180	256	245	F	-	-	-
				280	294	250	186	224	196	268	253				
8-55	Ontario	-	-	272	276	242	190	232	184	236	241	B	-	-	-
				276	294	262	194	236	184	252	257				
9-05	Ontario	-	-	268	288	258	186	232	192	220	253	B	-	-	-
				276	290	262	186	240	196	276	257				
9-81	Ontario	-	-	272	272	254	182	208	180	200	245	B	-	-	-
				280	284	258	194	240	192	218	257				
9-82	Ontario	-	-	272	284	254	178	224	188	232	257	C	-	-	-
				276	288	258	178	224	188	252	273				
9-84	Ontario	-	-	276	280	250	178	224	184	254	261	B	-	-	-
				280	284	266	182	228	188	276	261				
9-85	Ontario	-	-	272	252	254	178	228	192	224	257	B	-	-	-
				276	268	258	186	232	196	272	265				

Table A1.1 Continued

				Microsatellites								MtDNA	Stable Isotopes		Additional Info
Sample ID	Location	UTM	Collection Date	A08	B01	B03	A02	C06	C11	C07	F11	Haplotype	Tail δD_f (‰)	Head δD_f (‰)	Song recorded
9-87	Ontario	-	-	272	288	246	178	224	188	200	249	B	-	-	-
				272	292	258	178	232	196	200	249				
9-89	Ontario	-	-	268	252	250	182	228	184	236	249	B	-	-	-
				272	290	254	194	236	188	252	249				
9-90	Ontario	-	-	272	276	250	174	220	188	228	245	B	-	-	-
				272	294	258	174	232	192	264	261				
9-91	Ontario	-	-	256	276	254	182	220	180	242	245	J	-	-	-
				268	294	266	194	228	188	242	249				
9-93	Ontario	-	-	268	272	254	186	216	176	192	245	K	-	-	-
				272	294	268	186	232	188	236	249				
9-94	Ontario	-	-	272	268	262	182	234	180	228	221	E	-	-	-
				276	294	268	184	240	188	236	253				
9-95	Ontario	-	-	268	280	246	178	224	176	258	253	B	-	-	-
				272	294	250	186	240	180	264	257				
9-96	Ontario	-	-	272	284	245	174	224	184	240	245	B	-	-	-
				272	288	258	190	232	196	276	257				
9-99	Ontario	-	-	268	276	258	186	234	172	226	269	B	-	-	-
				272	284	268	190	240	188	278	269				
9-100	Ontario	-	-	272	276	258	182	228	180	248	249	?	-	-	-
				276	300	262	198	232	184	256	261				
9-101	Ontario	-	-	264	276	254	182	232	184	220	221	B	-	-	-
				264	294	254	186	240	192	220	249				

Table A1.2 Raw data of migratory individuals including: Alleles of eight neutral microsatellites, Deuterium Stable Isotopes δDf (‰) of tail feather samples.

Sample ID	Location	UTM	Collection Date	A08	B01	B03	A02	C06	C11	C07	F11	Tail δD_f (‰)
Zoal-ij097	Mugaha Marsh	-	29-Aug-09	268	272	250	186	232	188	248	249	-161.2
				276	272	254	194	232	192	260	265	
Zoal-ij098	Mugaha Marsh	-	29-Aug-09	268	264	270	186	232	188	248	253	-160.8
				272	294	274	190	240	192	272	257	
Zoal-ij099	Mugaha Marsh	-	29-Aug-09	272	292	258	190	228	188	228	249	-168.2
				276	294	266	194	228	192	240	265	
Zoal-ij100	Mugaha Marsh	-	01-Sep-09	272	284	250	182	214	180	234	?	-163
				276	288	270	194	220	190	254	?	
Zoal-ij101	Mugaha Marsh	-	09-Sep-09	260	284	254	170	228	196	254	261	-153.8
				264	288	242	174	228	196	266	265	
Zoal-ij102	Mugaha Marsh	-	13-Sep-09	272	268	258	194	228	180	228	249	-154
				276	290	258	194	232	192	240	257	
Zoal-ij103	Mugaha Marsh	-	14-Sep-09	268	284	250	178	220	180	242	237	-162.4
				280	288	260	186	228	186	266	261	
Zoal-ij104	Mugaha Marsh	-	16-Sep-09	276	264	238	182	216	176	244	241	-157.5
				280	268	250	194	224	196	272	245	
Zoal-ij105	Mugaha Marsh	-	18-Sep-09	272	268	258	?	232	194	250	243	-158.7
				280	290	262	?	240	194	282	251	
Zoal-io106	Lesser Slave Lake	-	16-Sep-09	280	268	254	182	232	188	?	261	-142.8
				284	298	258	186	236	188	?	269	
Zoal-io107	Lesser Slave Lake	-	22-Aug-09	272	268	254	170	216	184	236	249	-163.9
				280	284	266	182	224	192	248	261	
Zoal-io108	Lesser Slave Lake	-	24-Aug-09	276	254	254	174	228	184	240	257	-151.1
				280	294	262	190	228	192	272	257	
Zoal-io109	Lesser Slave Lake	-	28-Aug-09	272	288	258	186	232	188	236	245	-148.8
				272	294	258	194	236	192	236	253	
Zoal-io110	Lesser Slave Lake	-	16-Sep-09	272	284	254	174	244	180	?	249	-149
				280	292	254	174	244	184	?	261	
Zoal-io111	Lesser Slave Lake	-	16-Sep-09	268	288	258	182	228	180	220	253	-154
				272	294	258	182	252	184	232	261	
Zoal-io112	Lesser Slave Lake	-	16-Sep-09	268	280	262	178	240	188	212	249	-148.7
				280	294	274	186	252	188	260	261	

Table A1.2 Continued

Sample ID	Location	UTM	Collection Date	Microsatellite								Stable Isotopes
				A08	B01	B03	A02	C06	C11	C07	F11	Tail δD_f (‰)
Zoal-io113	Lesser Slave Lake	-	16-Sep-09	268	280	238	170	220	188	208	257	-173.2
				272	284	254	186	224	192	232	273	
Zoal-io114	Lesser Slave Lake	-	12-Sep-09	276	288	262	182	232	180	228	245	-160.6
				280	294	266	198	236	190	228	249	
Zoal-ik115	Beaverhill	-	16-Sep-09	268	276	254	174	232	172	228	245	-149.5
				272	294	258	194	240	192	252	249	
Zoal-ik116	Beaverhill	-	16-Sep-09	272	284	258	182	216	188	216	249	-158.7
				272	288	270	186	228	196	252	257	
Zoal-ik117	Beaverhill	-	16-Sep-09	274	258	248	194	220	184	?	253	-175.7
				274	288	252	202	236	192	?	253	
Zoal-ik118	Beaverhill	-	16-Sep-09	280	284	254	184	228	192	216	249	-152.2
				280	284	258	194	232	192	264	265	
Zoal-ik120	Beaverhill	-	21-Sep-09	260	288	246	182	228	184	232	253	-163.2
				268	292	262	186	228	188	240	265	
Zoal-ik121	Beaverhill	-	21-Sep-09	268	260	258	194	228	192	240	245	-161.2
				268	264	262	194	232	192	272	249	
Zoal-ik122	Beaverhill	-	21-Sep-09	276	254	268	178	232	188	204	253	-150.2
				280	254	288	202	236	192	276	253	
Zoal-im123	Rocky Point	-	01-Oct-09	276	252	254	178	220	190	236	249	-151.5
				276	268	258	202	224	190	260	257	
Zoal-im124	Rocky Point	-	30-Sep-09	272	290	234	198	220	184	236	233	-148.3
				280	294	254	198	232	188	240	261	
Zoal-im125	Rocky Point	-	26-Sep-09	272	272	246	186	216	180	222	249	-155.5
				280	294	250	198	224	190	252	253	
Zoal-id068	Dawson Creek	10U 0680970 E	08-Sep-09	268	288	254	182	214	182	254	261	-
		6177181 N		276	294	258	194	220	190	266	265	
Zoal-id070	Dawson Creek	10U 0680970 E	08-Sep-09	276	294	254	186	224	172	272	249	-
		6177181 N		280	304	258	190	232	188	272	261	
Zoal-id071	Dawson Creek	10U 0680970 E	08-Sep-09	272	276	238	178	224	176	224	245	-
		6177181 N		280	290	250	194	224	184	244	269	
Zoal-id072	Dawson Creek	10U 0680970 E	08-Sep-09	272	280	258	168	224	176	244	245	-
		6177181 N		272	282	262	194	236	188	260	253	
Zoal-id073	Dawson Creek	10U 0680970 E	08-Sep-09	264	276	254	178	232	172	228	249	-
		6177181 N		276	294	254	186	232	192	240	249	

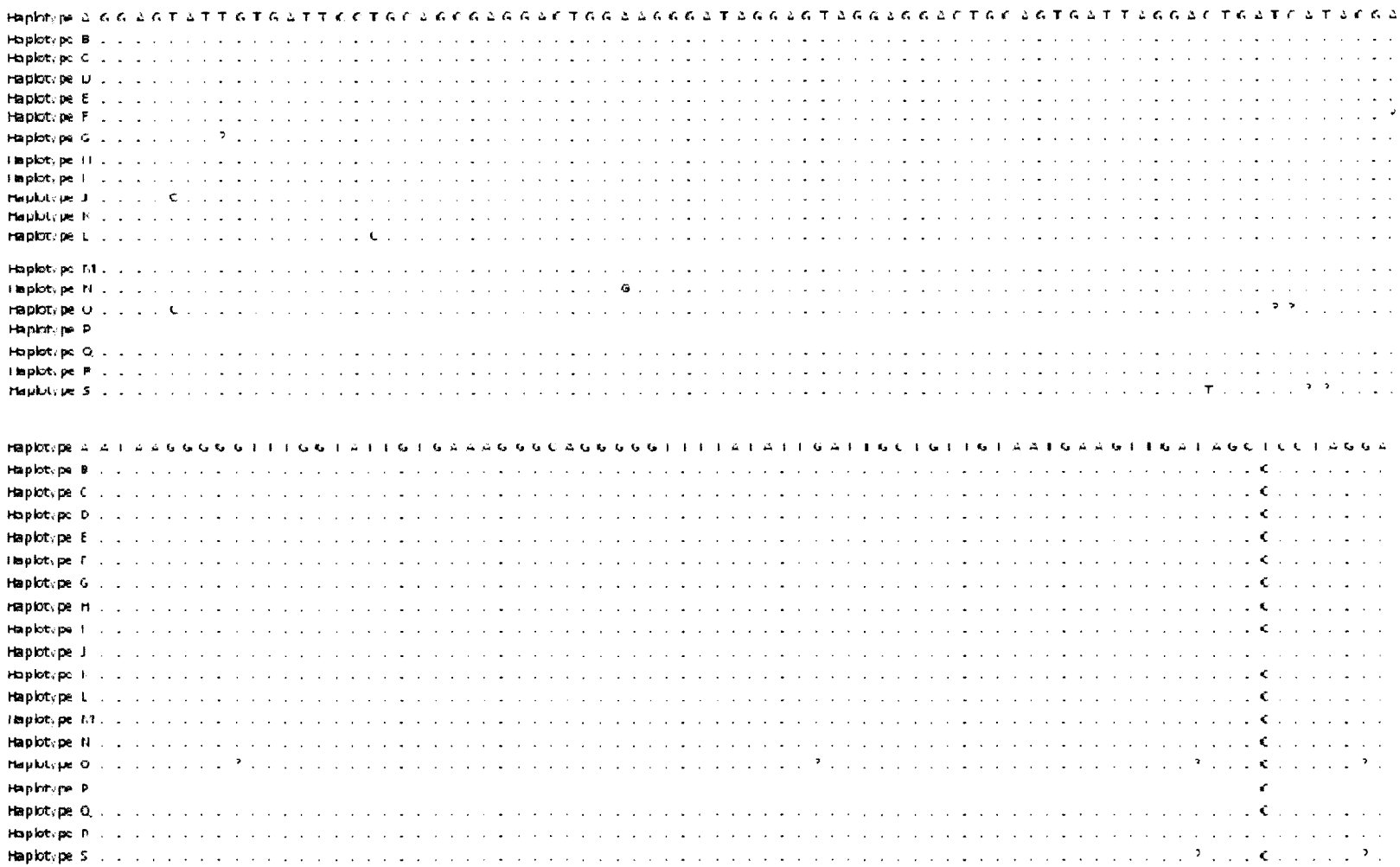
Table A1.2 Continued

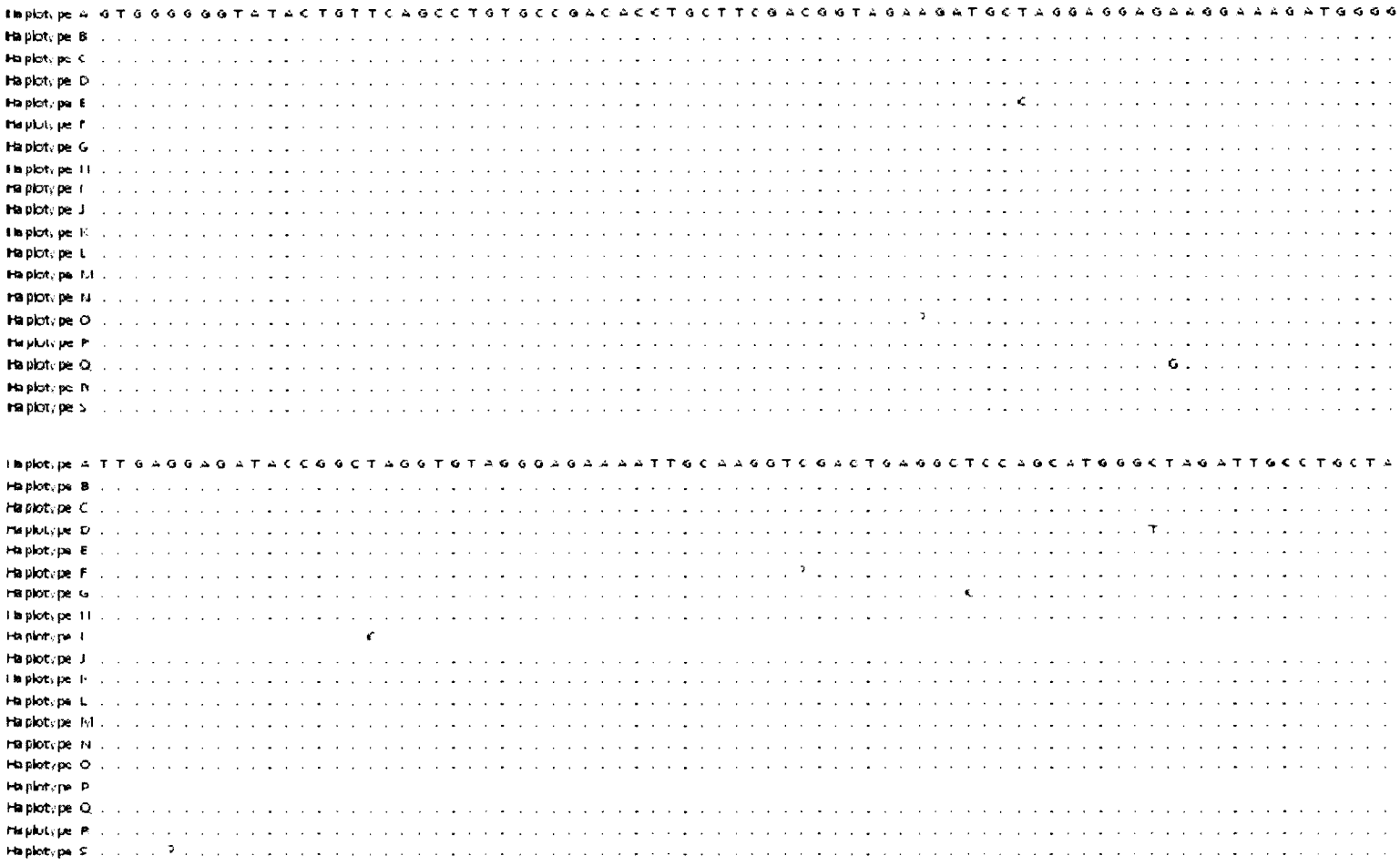
				Microsatellite								Stable Isotopes
Sample ID	Location	UTM	Collection Date	A08	B01	B03	A02	C06	C11	C07	F11	Tail δD_f (‰)
Zoal-id074	Dawson Creek	10U 0680970 E	08-Sep-09	266	234	250	190	198	176	256	253	-
		6177181 N		274	240	254	194	228	192	256	269	
Zoal-id075	Dawson Creek	10U 0680970 E	08-Sep-09	264	276	254	186	222	188	248	249	-
		6177181 N		276	294	254	190	236	198	248	249	
Zoal-id076	Dawson Creek	10U 0680970 E	08-Sep-09	272	268	254	178	224	176	224	249	-
		6177181 N		276	294	266	198	228	192	236	249	
Zoal-id077	Dawson Creek	10U 0680970 E	08-Sep-09	264	276	254	182	228	196	192	249	-
		6177181 N		276	294	254	198	232	200	248	253	
Zoal-id079	Dawson Creek	10U 0680970 E	09-Sep-09	272	284	250	178	226	196	228	245	-
		6177181 N		272	284	262	186	232	200	240	257	
Zoal-id080	Dawson Creek	10U 0680970 E	09-Sep-09	?	?	256	?	?	188	252	249	-
		6177181 N		?	?	256	?	?	188	256	249	
Zoal-id081	Dawson Creek	10U 0680970 E	09-Sep-09	264	264	250	194	220	176	240	257	-
		6177181 N		276	276	264	202	240	188	248	261	
Zoal-id082	Dawson Creek	10U 0680970 E	09-Sep-09	268	280	258	182	232	180	244	249	-
		6177181 N		276	294	262	194	232	184	252	265	
Zoal-id083	Dawson Creek	10U 0680970 E	09-Sep-09	272	280	246	178	232	188	266	249	-
		6177181 N		284	294	258	182	244	196	272	257	
Zoal-id084	Dawson Creek	10U 0680970 E	09-Sep-09	272	276	262	178	232	192	250	245	-
		6177181 N		272	288	274	182	232	196	284	269	
Zoal-id085	Dawson Creek	10U 0680970 E	09-Sep-09	256	232	254	194	232	184	252	241	-
		6177181 N		272	268	266	198	256	184	260	265	
Zoal-id086	Dawson Creek	10U 0680970 E	10-Sep-09	272	280	256	?	224	180	252	261	-
		6177181 N		276	294	266	?	232	188	266	265	
Zoal-id087	Dawson Creek	10U 0680970 E	10-Sep-09	276	272	250	176	220	192	202	249	-
		6177181 N		280	280	270	196	228	198	202	257	
Zoal-id088	Dawson Creek	10U 0680970 E	10-Sep-09	260	268	250	178	220	180	232	249	-
		6177181 N		272	300	254	194	230	192	242	257	
Zoal-id089	Dawson Creek	10U 0680970 E	10-Sep-09	272	294	250	182	228	180	228	253	-
		6177181 N		272	294	254	198	240	192	248	257	
Zoal-id090	Dawson Creek	10U 0680970 E	10-Sep-09	272	284	254	182	232	180	236	261	-
		6177181 N		276	288	266	194	232	190	260	265	

Table A1.2 Continued

Sample ID	Location	UTM	Collection Date	Microsatellite								Stable Isotopes
				A08	B01	B03	A02	C06	C11	C07	F11	Tail δD_f (‰)
Zoal-id091	Dawson Creek	10U 0680970 E	10-Sep-09	276	276	258	182	220	184	214	245	-
		6177181 N		276	288	266	190	228	188	224	253	
Zoal-id092	Dawson Creek	10U 0680970 E	10-Sep-09	272	276	250	178	232	176	216	245	-
		6177181 N		276	298	254	182	236	192	272	265	
Zoal-id093	Dawson Creek	10U 0680970 E	10-Sep-09	272	284	254	186	220	180	214	253	-
		6177181 N		272	294	254	186	228	188	214	261	
Zoal-id095	Dawson Creek	10U 0680970 E	10-Sep-09	272	268	254	174	232	192	228	249	-
		6177181 N		280	268	254	182	236	192	228	265	
Zoal-id096	Dawson Creek	10U 0680970 E	10-Sep-09	272	276	246	178	216	192	212	249	-
		6177181 N		272	288	260	194	224	196	264	253	
Zoal-id126	Dawson Creek	10U 0680970 E	07-Sep-09	272	284	262	182	224	180	234	253	-
		6177181 N		284	294	262	186	236	192	236	257	
Zoal-id127	Dawson Creek	10U 0680970 E	07-Sep-09	268	260	250	178	224	176	236	249	-
		6177181 N		284	290	258	186	240	188	248	253	
Zoal-id128	Dawson Creek	10U 0680970 E	07-Sep-09	272	276	246	178	216	192	212	249	-
		6177181 N		272	288	260	194	224	196	266	253	
Zoal-id129	Dawson Creek	10U 0680970 E	07-Sep-09	272	268	256	178	220	192	236	249	-
		6177181 N		280	288	260	186	224	192	260	269	
Zoal-id130	Dawson Creek	10U 0680970 E	07-Sep-09	272	280	254	186	232	180	224	245	-
		6177181 N		276	294	262	190	232	192	252	249	
Zoal-id131	Dawson Creek	10U 0680970 E	07-Sep-09	264	284	254	178	224	188	200	241	-
		6177181 N		268	294	266	182	228	192	260	249	
Zoal-id132	Dawson Creek	10U 0680970 E	07-Sep-09	266	272	266	190	228	188	?	257	-
		6177181 N		276	276	274	198	240	188	?	265	
Zoal-id133	Dawson Creek	10U 0680970 E	07-Sep-09	?	276	250	186	224	188	248	253	-
		6177181 N		?	276	254	206	228	196	242	257	
Zoal-id135	Dawson Creek	10U 0680970 E	07-Sep-09	276	268	254	182	220	180	224	249	-
		6177181 N		276	294	258	190	220	192	260	253	

APPENDIX 2 MITOCHONDRIAL DNA HAPLOTYPES





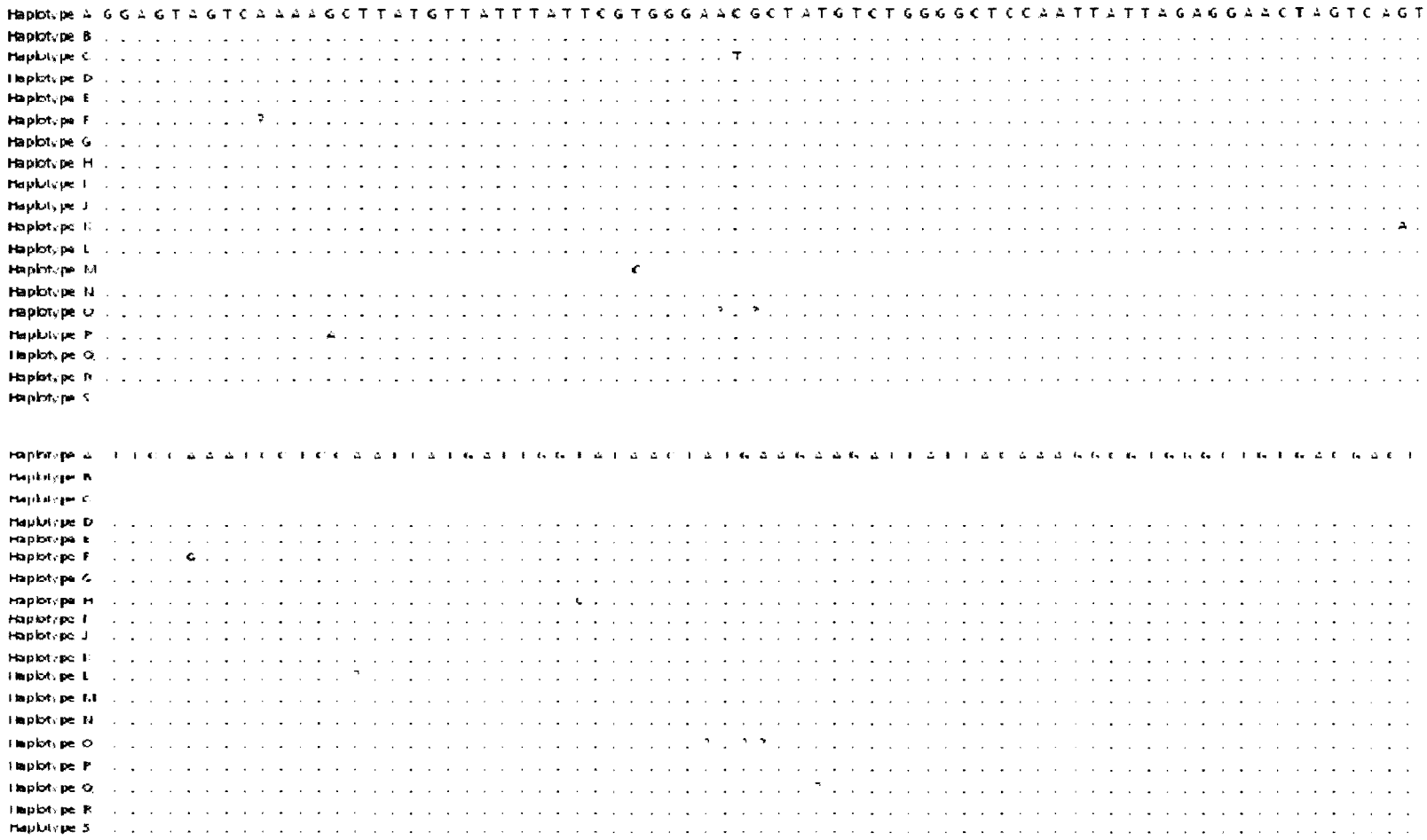


Figure A2.1 *Cytochrome Oxidase I (COI)* fragment of the 19 different haplotypes of White-throated Sparrow sequences found during the present study at the breeding territories of Western Canada and Ontario.