

ESTABLISHING TROPHIC ECOLOGY AND MIGRATORY CONNECTIONS OF
WATERFOWL USING STABLE ISOTOPES AND MERCURY

A Thesis Submitted to the College of

Graduate Studies and Research

In Partial Fulfillment of the Requirements

For the Degree of Master of Environment and Sustainability

University of Saskatchewan

Saskatoon, Saskatchewan, Canada

By

Christian Konadu Asante

© Copyright Christian Konadu Asante, November 2015. All rights reserved.

PERMISSION TO USE

In presenting this thesis/dissertation in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis/dissertation in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis/dissertation work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis/dissertation or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis/dissertation. Disclaimer Reference in this thesis/dissertation to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favoring by the University of Saskatchewan. The views and opinions of the author expressed herein do not state or reflect those of the University of Saskatchewan, and shall not be used for advertising or product endorsement purposes. Requests for permission to copy or to make other uses of materials in this thesis/dissertation in whole or part should be addressed to: Director of School of Environment and Sustainability University of Saskatchewan, 117 Science Place, Saskatoon, SK S7N 5C8 Canada OR Dean of College of Graduate Studies and Research University of Saskatchewan, 107 Administration Place, Saskatoon, SK S7N 5A2 Canada 1

Permission to Use and Disclaimer taken from:
<http://www.usask.ca/cgsr/downloads/etd/SamplePermissionToUseDisclaimer.pdf>

ABSTRACT

The Saskatchewan River Delta (SRD) in central Canada, North America's largest inland delta, is an important spring and fall stopover site for waterfowl with thousands flocking there annually to stage. However there is very little information on their origins prior to arrival and their feeding ecology while in the Delta. To date, band recoveries are largely from birds banded south of the SRD, mostly due to limited banding activity in productive waterfowl habitats to the north such as the Peace-Athabasca Delta and the broader boreal forest. There is also very little information on the importance of the SRD as an overall recruitment area for the North American waterfowl population. No studies have used stable isotopes to infer the origins and diets of these birds. I first used stable isotopes of hydrogen ($\delta^2\text{H}$) and sulfur ($\delta^{34}\text{S}$) to infer migratory origins and specifically evaluate the contribution of local and non-local birds to the staging population in the SRD during fall migration. Based on $\delta^2\text{H}$, I found that few birds (34%) originated in the SRD despite its known role as breeding habitat; instead, most birds (56%) were migrants from the north of the SRD and a small fraction (10%) came from south of the SRD. Stable sulfur isotope data proved a useful tool in further delineation of birds into prairie and forest regions, respectively. Secondly, I used stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes and mercury concentrations in liver tissue ([Hg]) to trace nutrient sources of these waterfowl using the SRD prior to fall migration, and tested for differences in diets among species, sexes and age groups within species. I demonstrated the importance of macrophytes as a source of food, particularly among the American Widgeon and Northern Pintail (70% of the diet). However, there was some level of partitioning of resources at the species level, as Blue-winged Teal and Green-winged Teal used invertebrate sources, as did a distinct group of Mallards. This is likely a result of birds minimizing competition for resources during the short staging period in the SRD when

waterfowl densities are high. Finally, I found that $\delta^{15}\text{N}$ values in liver, a known indicator of trophic position, can be confounded by variation in basal sources; hence, there is the need to use other isotopes or tracers such as [Hg] for verification. Overall, my results suggest an important role for northern ecosystems in central Canada in contributing to the waterfowl breeding population in the Central Flyway, and a key role for the SRD in providing fuel for waterfowl during fall migration.

ACKNOWLEDGEMENTS

First and foremost, I will like to thank Dr. Tim Jardine for giving me the opportunity to study in Canada and also for being supportive throughout the thesis. This meant a lot to me. My co-supervisor Dr. Keith Hobson for his guidance, support and encouragement. My Committee members Dr. Ken Belcher and Dr. Alex Bond for their contributions and insights. Renee and Solomon Carriere for hosting, boating services and sumptuous meals. Michaela Carriere for sampling of wings and invertebrates. Keith Warner for teaching and assisting with wing identification. My lab mates Brett Mackinnon for sampling and invertebrate identification and Will Schenn for aquatic macrophytes identification. Chantel Gryba helped with isotope submission, Steve Van Wilgenburg for his inputs on assignment, Alex Bond for food webs and research design and Geoff Koehler for water isotope analysis. Stuart Slattery, Shaun Geer, Josef Schmutz for existing records and band recovery data. NSERC and new faculty funding from the University of Saskatchewan for financial support. Friends home and abroad, graduate students in SENS, my family especially my mother who paid for my undergraduate education and all my mentors back home in Ghana.

TABLE OF CONTENTS

PERMISSION TO USE.....**Error! Bookmark not defined.**

ABSTRACT.....**Error! Bookmark not defined.**

ACKNOWLEDGEMENTS.....**Error! Bookmark not defined.**

LIST OF TABLES..... **Error! Bookmark not defined.i**

LIST OF FIGURES **Error! Bookmark not defined.i**

ABBREVIATIONS.....X

1.0 GENERAL INTRODUCTION.....**Error! Bookmark not defined.**

 1.1 Wetlands and Waterfowl.....**Error! Bookmark not defined.**

 1.2 Saskatchewan River Delta.....**Error! Bookmark not defined.**

 1.3 Origins of waterfowl in the Saskatchewan River Delta 4

 1.4 Tracing nutrient sources of waterfowl in the Saskatchewan River delta ..**Error! Bookmark not defined.**

 1.5 Stable isotopes..... 6

 1.5.1 Hydrogen isotopes**Error! Bookmark not defined.**

 1.5.2 Carbon isotopes**Error! Bookmark not defined.**

 1.5.3 Nitrogen Isotopes.....**Error! Bookmark not defined.**

 1.5.4 Mixing Models10

 1.6 Study objectives**Error! Bookmark not defined.1**

 1.7 General Methods and Materials**Error! Bookmark not defined.1**

 1.7.1 Ethics statement.....**Error! Bookmark not defined.1**

 1.7.2 Wing Collection and preparation.....**Error! Bookmark not defined.**

 1.8 References**Error! Bookmark not defined.3**

2.0 THE IMPORTANCE OF THE SASKATCHEWAN RIVER DELTA AS A BREEDING, MOULTING AND STOPOVER HABITAT FOR WATERFOWL INFERRED FROM STABLE HYDROGEN ISOTOPE ANALYSIS OF FEATHERS.....**Error! Bookmark not defined.1**

 2.1 Introduction**Error! Bookmark not defined.1**

 2.2 Materials and Methods**Error! Bookmark not defined.4**

 2.2.1 Study sites.....**Error! Bookmark not defined.4**

 2.2.2 Water and Wing Collection.....**Error! Bookmark not defined.5**

2.2.3 Wing Preparation and Identification.....	Error! Bookmark not defined.	6
2.2.4 Stable hydrogen isotope analysis.....	Error! Bookmark not defined.	6
2.2.5 Statistical analyses.....	Error! Bookmark not defined.	7
2.2.6 Assignment to Origin.....	Error! Bookmark not defined.	7
2.3 Results.....	Error! Bookmark not defined.	0
2.3.1 Threshold Assignment.....	Error! Bookmark not defined.	3
2.4 Discussion.....	Error! Bookmark not defined.	4
2.5 References.....		39
3.0 TRACING NUTRIENT SOURCES OF STAGING WATERFOWL IN A LARGE RIVER DELTA USING STABLE ISOTOPES (CARBON AND NITROGEN) AND MERCURY.		
.....	Error! Bookmark not defined.	7
3.1 Introduction.....	Error! Bookmark not defined.	7
3.2 Materials and Methods.....		49
3.2.1 Liver Tissue Collection and Preparation.....		49
3.2.2 Consumer Tissue.....		50
3.2.3 Dietary Sources.....		51
3.2.4 Stable Carbon ($\delta^{13}\text{C}$) and Nitrogen ($\delta^{15}\text{N}$) Isotope Analysis.....		52
3.2.5 Total Mercury (THg) Analysis.....		52
3.2.6 Statistical Analysis.....	Error! Bookmark not defined.	3
3.2.7 Mixing Models.....	Error! Bookmark not defined.	3
3.3 Results.....	Error! Bookmark not defined.	4
3.4 Discussion.....	Error! Bookmark not defined.	2
3.5 References.....	Error! Bookmark not defined.	6
4.0 Synthesis.....	Error! Bookmark not defined.	4
4.1 Objectives.....	Error! Bookmark not defined.	4
4.2 Main Findings.....	Error! Bookmark not defined.	4
4.3 Strength and Limitations.....	Error! Bookmark not defined.	6
4.4 Future Research.....	Error! Bookmark not defined.	7
4.5 Conclusions/Significance.....	Error! Bookmark not defined.	7
4.6 References.....	Error! Bookmark not defined.	8
5.0 Appendix A.....		80
5.1 The Range of Nitrogen Isotopes for Mallards.....		80

5.2 Mixing Model output for various species.....	81
--	----

LIST OF TABLES

Table 2.1: Pairwise comparisons (p-values) of hydrogen isotope values in feathers for each species.....	32
Table 3.1: Pairwise comparisons (p-values) of $\delta^{13}\text{C}$ in waterfowl livers for each species.....	56
Table 3.2: Pairwise comparison (p-values) of $\delta^{15}\text{N}$ in waterfowl livers for each species.....	58

LIST OF FIGURES

Figure 1.1: Geographic Range of Mallards, American Widgeon, and Blue winged Teals in North America.....	12
Figure 1.2: Geographic Range of Green-winged Teals and Northern Pintails in North America	13
Figure 2.1: The Upper Saskatchewan River Delta, Saskatchewan, where waterfowl were harvested for stable isotope analysis of feathers (53, 75° N 102, 443° W, 1: Grassy Point, 2: Muskeg Lake, 3: Dumbell Lake, 4: Bens Lake, 5: Big Eddy).....	24
Figure 2.2: A map showing various geographic areas of assignment.....	28
Figure 2.3: Stable hydrogen isotope ratios in waterfowl feathers ($\delta^2\text{H}_f$) versus date of sampling in 2013 in the Saskatchewan River Delta.....	30
Figure 2.4: Stable hydrogen isotope ratios in waterfowl feathers ($\delta^2\text{H}_f$) versus date of sampling in 2014 in the Saskatchewan River Delta.....	31
Figure 2.5: Stable hydrogen isotopes ($\delta^2\text{H}$) in feathers from four waterfowl species in the Saskatchewan River Delta, according to age and sex.....	31
Figure 2.6: Origins (percent of total) assigned to each species based on $\delta^2\text{H}$ and $\delta^{34}\text{S}$ of feather.....	34
Figure 3.1: The Upper Saskatchewan River Delta, Saskatchewan, where sampling was done (53, 75° N 102, 443° W, 1: Grassy Point).....	50
Figure 3.2: Mean $\delta^{13}\text{C}$ values in liver tissue of waterfowl from the Saskatchewan River Delta, separated by age and sex for each species.....	55

Figure 3.3: Mean $\delta^{15}\text{N}$ values in liver tissue of waterfowl from the Saskatchewan River Delta, separated by age and sex for each species.....57

Figure 3.4: Mean Log Hg concentrations in liver tissue of waterfowl from the Saskatchewan River Delta, separated by age and sex for each species.....59

Figure 3.5: Isotope bi-plots showing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for dietary sources (black circles) for Green-winged Teals (green circles) and Blue-winged Teals (blue circles).....60

Figure 3.6: Isotope bi-plots showing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for dietary sources (Black circles) for American Widgeon (orange circles) and Northern Pintails (blue circles).....60

Figure 3.7: Isotope bi-plots showing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for dietary sources (black circles) for Mallards L (blue circles) and Mallards H (green circles).....61

Figure A1: Frequency histogram showing the distribution $\delta^{15}\text{N}$ of values in Mallard livers from Grassy Point in the Saskatchewan River Delta.....80

Figure A2: MIXSIAR output of American Widgeon dietary source proportion.....81

Figure A3: MIXSIAR output of Northern Pintail dietary source proportion.....82

Figure A4: MIXSIAR output of Blue winged Teal dietary source proportion.....83

Figure A5: MIXSIAR output of Green winged Teal dietary source proportion.....84

Figure A6: MIXSIAR output of Mallard dietary source proportion.....85

Figure A7: MIXSIAR output of Mallard H dietary source proportion.....86

ABBREVIATIONS

‰	Per mil
$\delta^{13}\text{C}$	Carbon 13 isotope
$\delta^{15}\text{N}$	Nitrogen 15 isotope
AIR	Atmospheric nitrogen
ANOVA	Analysis of Variance
BWB	Bowhead Whale Baleen
C ₃	Plants using the Rubisco enzyme to fix carbon dioxide
C ₄	Plants using phosphoenolpyruvate carboxylase to fix carbon dioxide
CF-IRMS	Continuous-Flow Isotope Ratio Mass Spectrometry
CFS	Chicken Feather Standard
CHS	Cow Hoof Standard
CI	Confidence Interval
CWS	Canadian Wildlife Service
DUC	Ducks Unlimited Limited
GLM	General Linear Model

MeHg	Methylmercury
MixSIAR	Mixing Model for Stable Isotope Analysis in R
NAWMP	North America Waterfowl Management Program
PHJV	Prairie Habitat Joint Ventures
THg	Total mercury
TMS	Trophic magnification factor
VCDT	Vienna Canyon Diablo Troilite
USFWS	United States Fish and Wildlife Service

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Wetlands and Waterfowl

Wetlands provide important ecosystem services that are critical to the livelihoods of human beings. They serve as water purification systems, they stabilise shorelines, and are important agents in flood control (RAMSAR 2011). Wetlands also store carbon and as such, are useful in mitigating increasing atmospheric CO₂ levels linked to climate change (Zedler & Kercher 2005).

Despite the many benefits derived from wetlands, their management is wrought with several challenges (RAMSAR 2011). For instance, wetlands are often seen as wastelands and have been drained or filled for other purposes (Higgins et al. 2002). Pumping of water from wetlands for irrigation and building purposes are also major threats to wetland ecosystems (Zedler & Kercher 2005). Industrialization and the application of pesticides threaten the ecological integrity of wetlands. All of these human influences can degrade wetlands, compromising the habitat requirements of waterfowl.

Wetlands are used by waterfowl throughout their annual cycle, and contain aquatic vegetation, invertebrates, small fish and animal matter that act as important food sources for birds during breeding, staging or wintering. Wetlands also provide a refuge from predators such as racoons (*Procyon lotor*) and mink (*Neovision vision*) that prey on young birds (Stewart 2014).

The importance of wetlands to waterfowl recruitment into the general population has been documented by the North American Waterfowl Management Plan (NAWMP 2012) whose

habitat joint ventures have improved yearly recruitment of waterfowl into the adult population. The higher abundance of wetlands per square mile in the prairies led to an increase in Mallard (*Anas platyrhynchos*) densities and propagation (Bellrose 1977). Wetland habitat on the North American Great Plains has been declining and the decline is expanding westward (Higgins et al. 2002). About 70 % of wetlands in urban areas in Canada have been lost since the 1800's (Dugan 1990). Significant additional losses of wetlands since the 1970's are coincident with declines in the population of Northern Pintails (*Anas acuta*) and American Widgeon (*Anas americana*) in Saskatchewan (PHJV 2008). Only about 25% of original wetlands remain in southwest Manitoba (Environment Canada 2010). Wetland losses in addition to drought will likely reduce waterfowl populations. In the face of this habitat loss and drought, abundant wetland habitats in areas such as the Saskatchewan River Delta (SRD) will be a refuge for displaced birds from the prairies (DUC 2014).

1.2 Saskatchewan River Delta

The SRD is North America's largest inland delta covering an area of 950,000 hectares on the borders of Saskatchewan and Manitoba in central Canada (Morrison 2012). It comprises active river channels, lakes and wetlands (81%) that support high biological diversity (Morrison 2012). The Cumberland Marshes is a large tract of wetlands in the upper SRD that contains several control structures, and has been designated as an Important Bird Area (Schmutz 2001). The Delta receives water from the Saskatchewan River, which supports upstream activities such as hydroelectric dams, irrigation, and mining (Morrison 2012). The construction of the E.B. Campbell hydroelectric dam upstream of the SRD has led to reduced sediment and nutrient flows to downstream wetlands (MacKinnon et al. in press). In addition, Lake Diefenbaker, a reservoir

created by Gardiner Dam further upstream, has altered the hydrology of the SRD, mainly through a decrease in peak summer flows (Morrison 2012, Sagin et al. 2015).

The SRD wetlands support some of the highest populations of waterfowl in Saskatchewan (Slattery 2008) and it is predicted that the recent surge in habitat losses in the prairie pothole region will increase the number of birds moving to breed and stage in the delta (DUC 2014). Prior surveys recorded approximately 72,000 breeding Ring-necked Ducks (*Aythya collaris*; Bellrose & Kortwright 1976) and the highest numbers of Lesser Scaup (*Aythya affinis*) in Saskatchewan (Smith 1996). The Canadian Wildlife Service recorded about 20,000 moulting ducks of different species in the summer (Canadian Wildlife Service 1990). This high concentration of breeding and molting waterfowl means that the SRD is a critical area for conservation used by waterfowl.

During fall migration, birds choose productive habitats to accumulate fuel reserves because migration is an energetically demanding process (Miller et al. 2000, Callicutt et al. 2011). Determining the migratory origins of waterfowl, together with data on diet will evaluate the contribution of the delta as both rearing and staging habitat to inform conservation efforts. While many birds banded in the southern Great Plains have been recaptured in the SRD (Slattery 2008), because banding activity in areas north of the delta has been limited, we know little about the use of the delta by birds produced in the north. My work will therefore shed light on the use of the delta by birds coming from areas of known high productivity such as the Peace – Athabasca Delta in northern Alberta.

1.3 Origins of waterfowl in the Saskatchewan River Delta

In addition to the SRD serving as breeding (Bellrose & Kortwright 1976) and moulting habitat (Canadian Wildlife Service 1990), it is also an important stopover site for waterfowl prior to fall migration. More than 200,000 mallards stop in the delta prior to fall migration, suggesting a mix of locally produced birds and recent arrivals (Bellrose & Kortwright 1976). No studies have documented the origins of these birds, even though such information will contribute to understanding of the delta's role in sustaining North American waterfowl populations (Nichols et al. 1995). Information on origins of waterfowl in the delta is also important because threats to populations may occur outside the bird's breeding or wintering grounds (Hobson 2011); this information is useful in the allocation of quotas for hunting, for understanding disease dynamics of waterfowl (Gunnarsson et al. 2012) and for forming partnerships for conservation.

Researchers typically rely on bands placed on birds to track their movements (Nichols et al. 1995). However the challenge with this method is that it involves recovery and re-sighting of the individuals and for many species, recovery rates are very low (Hobson 2008). There are also biases associated with recovery probabilities because of spatial and temporal variation associated with bird's movements (Schwarz & Seber 1999). Recent advances in technology have led to the development of tools such as light-sensitive geolocators. However, environmental factors that influence the natural light intensity pattern affect the calculation of the movement of birds using this technology (Lisovski et al. 2012), and these tags must also be retrieved to obtain data.

The use of intrinsic markers such as stable isotopes provides a complementary way to trace geographic origins of birds (Hobson 1999, Bowen et al. 2005). The advantage of stable isotopes is that there is no need for banding or tagging, as every capture potentially provides

information on origins (Hobson 2008, Coulton et al. 2010). Water isotope values vary widely and systematically across the continents and these are incorporated into local food webs (Hobson 1999, Bowen et al. 2005). As birds forage, these values are incorporated into tissues like feathers which are metabolically inert once formed. Based on this phenomenon, we can trace back where a bird has been the previous season based on isotope values of its feathers. However isotopes are best used if there is understanding of the biology of the species (Hobson 1999). Tissues selected for isotope analyses must reflect the period of growth under investigation (Hobson 1999, Bowen et al. 2005).

1.4 Tracing nutrient sources of waterfowl in the Saskatchewan River delta

Existing knowledge of trophic interactions among and dietary sources to waterfowl in the SRD is based on local observational data and studies conducted elsewhere; there has not been any scientific enquiry and documentation of nutrient flow in the delta food webs, hence understanding the forage base that supports migratory waterfowl prior to fall migration is important for their conservation.

While the productive nature of the SRD has been recognized (Bellrose & Kortwright 1976, Smith 1996, Schmutz 2001) no studies have evaluated the dietary contribution of various energy pathways to the food webs supporting waterfowl during their time in the SRD. Developing a system to trace energy flow to waterfowl is therefore important. Carbon and nitrogen stable isotopes in animal tissues have provided insight on dietary sources and energy flow in aquatic ecosystems (Hobson 1999, Kelly 2000, Post 2002, Inger & Bearhop 2008).

1.5 Stable isotopes

Many chemical elements are present in two or more stable forms known as stable isotopes (Fry 2006). Isotopes of elements have the same number of protons but different numbers of neutrons (Fry 2006). The lighter isotope is typically more common in nature (Hobson 1999), and the differences found in the relative abundance of stable isotopes results from tiny mass differences that cause the isotopes to react differently in biogeochemical processes. The ratio of the heavier isotope to the lighter isotope in a sample is expressed as a delta (δ) value in per parts per thousand or ‘permil’ relative to an international standard, calculated with the following equation (Hobson 2008):

$$\delta X = (R_u/R_s - 1) \times 1000$$

Where X is the heavier isotope, R is the ratio of the heavier isotope to the lighter isotope (e.g., $^{13}\text{C}/^{12}\text{C}$) in an unknown sample (R_u) or standard (R_s).

Stable isotopes used commonly in ecology include carbon ($^{13}\text{C}/^{12}\text{C}$ or $\delta^{13}\text{C}$), nitrogen ($^{15}\text{N}/^{14}\text{N}$ or $\delta^{15}\text{N}$), hydrogen ($^2\text{H}/^1\text{H}$ or $\delta^2\text{H}$) and sulphur $^{34}\text{S}/^{32}\text{S}$ (Hobson 1999). International standards for reporting these isotope values are Vienna Peedee Belemnite (VPDB) for carbon, atmospheric nitrogen (AIR) for nitrogen, Vienna Canyon Diablo Troilite (VCDT) for sulphur, and Vienna Standard Mean Ocean Water (VSMOW) for hydrogen.

Stable isotopes operate on the principle of “you are what you eat” (Fry 2006). In other words, stable isotope ratios differ among food webs and are incorporated into animal tissue via diet. It is then possible to track the movement of mobile animals such as birds as they move between food webs (Rubenstein & Hobson 2004). When birds move and use different food

webs, the isotopic values are incorporated into different tissues as a function of their metabolic activity (Tieszen et al. 1983, Hobson & Clark 1992a, 1992b, Hobson 1999, Boecklen et al. 2011). Tissues such as feathers are metabolically inert once grown and therefore incorporate isotopic values from the diet permanently (Hobson & Clark 1992a, Hobson 1999). $\delta^2\text{H}$ in particular has brought interesting revelations (Chamberlain et al. 1997, Hobson 1999) in tracing origins of birds (Hobson 1999, Bowen et al. 2005, Inger & Bearhop 2008).

1.5.1 Hydrogen isotopes

The use of $\delta^2\text{H}$ has been successful in a number of migration studies involving wildlife, particularly birds. The first studies done with birds were in the 1990s and used pronounced differences in latitudinal gradients in the $\delta^2\text{H}$ of precipitation across North America (Chamberlain et al. 1997, Hobson 1999). Stable isotope values in North America exhibit a continent-wide pattern such that the $\delta^2\text{H}$ values in the southeast are enriched in ^2H and more depleted in ^2H in the northwest (Sheppard et al. 1969, Taylor 1974). Generally, increasing latitude produces more negative hydrogen isotope values. These gradients are a result of isotopic fractionation that happens during phase changes (evaporation and condensation). Hydrogen isotope values also decrease with increasing distance from the ocean as well as increasing elevation and precipitation (Clark & Fritz 1997). In a low latitude region (tropical and dry climate) where seasonality is less pronounced, and at mid-latitudes in the summer, the volume of precipitation becomes essential in determining the hydrogen values of precipitation (Dansgaard 1964). There are three principles of using isotopes to track migration in birds. First and foremost, the isotopic territory in which the bird moves must be known (Hobson 2011). This is necessary to generate a feather isoscape to which birds can be assigned (Hobson et al. 2009, Van Wilgenburg & Hobson 2011). Secondly, because of offsets from water to tissue due to isotopic

discrimination, it is important that discrimination factors are considered for the tissue used in the study (Hobson & Clark 1992b). Finally, tissue turnover rates for tissues should be taken into consideration (Hobson & Clark 1992a). Tissues such as feathers are metabolically inert and so record information on isotopic origin at the time of moult or initial feather growth (Hobson 2011, Hobson 1999).

There have been few applications of stable isotopes to determine natal origins and molt origins of waterfowl populations in western Canada. As an example, Hebert & Wassenaar (2005) assessed pintail production in three ecoregions: Northwest Territories, boreal forest and prairie pothole. They observed that feather $\delta^2\text{H}$ values decreased with latitude, consistent with $\delta^2\text{H}$ values in growing-season precipitation, and that wetland abundance influenced pintail production. In dry years, pintail production decreased in the prairies but increased in the north (Hebert & Wassenaar 2005).

1.5.2 Carbon isotopes

Carbon is a principal source of energy in food webs. Due to differences in various biogeochemical processes, food sources can have a distinct isotopic signature (Peterson & Fry 1987). These distinct isotopic values are incorporated into the food webs and because consumer tissues reflect the diet, $\delta^{13}\text{C}$ can be used as a tracer of dietary sources (Deniro & Epstein 1978). In tracing dietary sources of waterfowl, it is important to consider the photosynthetic pathways (C_3 and C_4) of plants because many species directly consume these plants (Peterson & Fry 1987). The C_3 photosynthetic pathways found in plants in most temperate areas lead to $\delta^{13}\text{C}$ values close to -27‰ while the C_4 pathway used by grasses found in warmer and drier conditions causes enriched ^{13}C values ranging from -14‰ to -9‰ (Peterson & Fry 1987). In tracing energy flow

in aquatic ecosystems another important consideration is the differences in isotopic values of phytoplankton (suspended algae), periphyton (attached algae) and macrophytes (aquatic plants) caused by spatial and temporal differences in the dissolved carbon pool (Peterson & Fry 1987). Benthic sources (algae and invertebrates) tend to be enriched in ^{13}C compared to pelagic sources because the boundary layer in benthic algal assemblages limits the supply of dissolved carbon needed for photosynthesis, leading to reduced isotopic fractionation (Hecky & Hesslein 1995). These carbon isotope values from benthic sources are transferred to organisms at higher trophic positions (Vander Zanden & Rasmussen 1999) such as insectivorous and piscivorous aquatic birds. Based on the limited change in $\delta^{13}\text{C}$ during these transfers, we can elucidate how underlying food sources, including phytoplankton, periphyton, macrophytes and terrestrial detritus support organisms (Hobson 1999).

1.5.3 Nitrogen Isotopes

Basal isotopic values of nitrogen ($^{15}\text{N}/^{14}\text{N}$) are distinct as a result of different biogeochemical processes and anthropogenic activities. However, nitrogen is particularly useful in elucidating trophic level (Deniro & Epstein 1981). This is because during protein amination and de-amination the light ^{14}N is preferentially lost to excretion of nitrogenous wastes making the consumer tissues enriched in ^{15}N (Deniro & Epstein 1981). As a result, diet-tissue discrimination factors normally range from +2 ‰ to +4 ‰ (Post 2002). The combination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ has yielded insights into waterfowl diets and energy pathways. For instance, Paszkowski et al. (2004) used carbon and nitrogen isotopes to trace the diet of Red-necked Grebes (*Podiceps grisegana*) in small wetlands and concluded these birds rely heavily on invertebrates.

1.5.4 Mixing Models

In the application of stable isotopes to trophic ecology, it has become necessary to quantify the relative contribution and importance of different food sources to the diet of consumers, and such mixing models have evolved considerably over time. Early approaches used a single isotope and a two-source linear model (France 1996, Boecklen et al. 2011) without accounting for error in isotope values of sources or consumers. Phillips & Gregg (2001) improved these models by accounting for error around consumer sources, mixtures and isotopic discrimination, including a three-source, two isotope model. Although Phillips & Gregg's (2001) multi-source model was an advancement over previous models it still did not provide a distinct resolution of food sources when the number of sources exceeds the number of isotopes by more than 1 (Boecklen et al. 2011). A Bayesian model incorporates a probability distribution of source contributions, the ability to include prior information, and variation in discrimination factors (Parnell et al. 2010, 2013).

1.6 Study objectives

The overall objective of this thesis was to trace the origins of waterfowl and nutrient sources supporting them in the SRD. This included:

1. Tracing the moult or natal origins of waterfowl in the SRD prior to fall migration.
2. Determining the nutrient sources sustaining birds in the SRD by testing for isotopic differences among species, sexes and ages within species and calculating dietary source proportions using mixing models.

1.7 General Methods and Materials

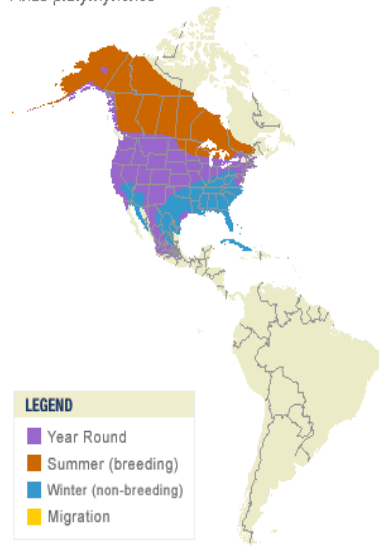
1.7.1 Ethics statement

The study was conducted in accordance with regulations stipulated by the Canadian Council for Animal Care as reviewed by the University of Saskatchewan.

1.7.2 Wing Collection and preparation

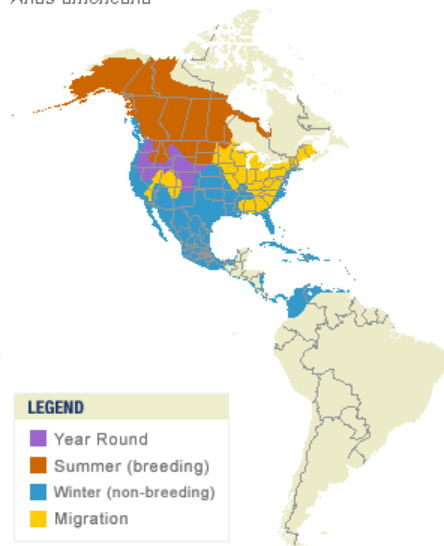
A total of 234 hunter-killed birds from nine different species (Redhead (*Aythya americana*), Ring-necked Duck, Northern Shoveler (*Anas clapaeta*), Canvasback, Mallard, Green-winged Teal (*Anas carolinensis*), Blue-winged Teal (*Anas discors*), Northern Pintail and American Widgeon) were collected from various locations in the SRD in the fall of 2013 and 2014. For the purpose of this study, five species (Mallard, Blue-winged Teal, Green-winged Teal, Northern Pintail, American Widgeon) were used in the analysis because these are the most hunted species that are important to the livelihoods of people and consequently make up the majority of the sample. Breeding ranges for each of the species are provided in the maps below (Figures 1.1 and 1.2) from the Cornell lab of Ornithology (<http://www.allaboutbirds.org>). Samples were prepared for stable isotope analysis (carbon, nitrogen, sulfur and hydrogen) and mercury concentrations. Details of the methodology are found in Chapters 2 and 3.

Mallard
Anas platyrhynchos



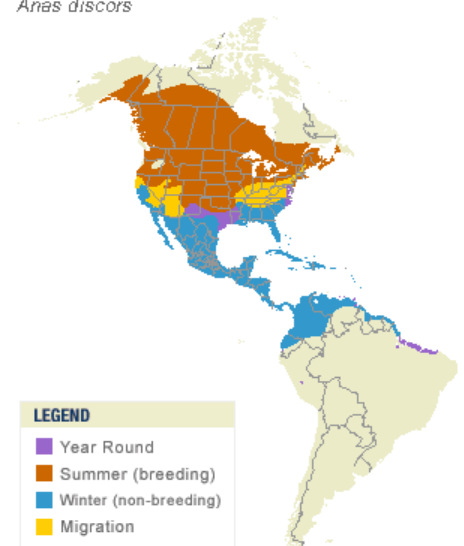
Map by Cornell Lab of Ornithology
Range data by NatureServe

American Wigeon
Anas americana



Map by Cornell Lab of Ornithology
Range data by NatureServe

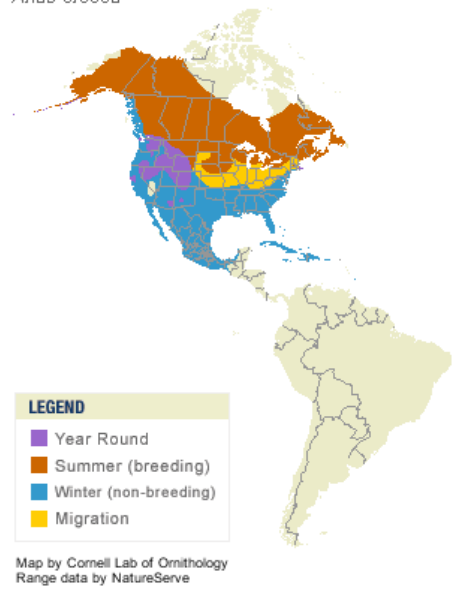
Blue-winged Teal
Anas discors



Map by Cornell Lab of Ornithology
Range data by NatureServe

Figure 1.1: Geographic Range of Mallards, American Widgeon, and Blue -winged Teal in North America (Cornell lab of Ornithology 2014).

Green-winged Teal
Anas crecca



Northern Pintail
Anas acuta

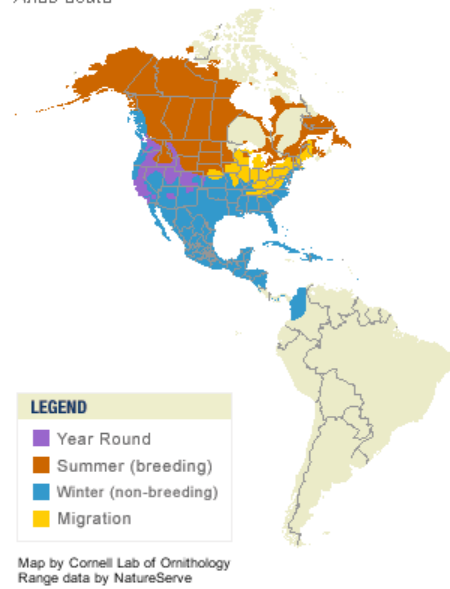


Figure 1.2: Geographic Range of Green-winged Teal and Northern Pintail in North America (Cornell lab of Ornithology 2014).

1.8 References

- Inger, R., & Bearhop, S. (2008). Applications of stable isotope analyses to avian ecology. *Ibis*, *150* (3), 447-461.
- Bellrose, F.C. (1977). Species distribution, habitats, and characteristics of breeding dabbling ducks in North America. In Bookhout, T.A., *Waterfowl and Wetlands: An integrated review: Proceedings of a symposium held at the 39th Midwest Fish and Wildlife Conference, Madison, Wis., La Crosse Printing Co., Inc. 152pp.*
- Bellrose, F.C., & Kortwright, F.H. (1976). Ducks, geese and swans of North America. Wildlife Management Institute, Stackpole Books, Harrisburg, PA, U.S.A.
- Boecklen, W. J., Yarnes, C. T., Cook, B. A., & James, A. C. (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution, and Systematics*, *42* (1), 411-440.
- Bowen, G. J., Wassenaar, L. I., & Hobson, K. A. (2005). Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia*, *143* (3), 337-348.
- Callicutt, J. T., Hagy, H. M., & Schummer, M. L. (2011). The food preference paradigm: a review of autumn-winter food use by North American dabbling ducks (1900-2009). *Journal of Fish and Wildlife Management*, *2*(1), 29-40.
- Canadian Wildlife Service. (1990). Priority migratory bird habitats of Canadian prairies, Environment Canada. Edmonton, AB.

- Chamberlain, C. P., Blum, J. D., Holmes, R. T., Feng, X., Sherry, T. W., & Graves, G. R. (1997). The use of isotope tracers for identifying populations of migratory birds. *Oecologia*, *109*(1), 132-141.
- Clark, I. D., & Fritz, P. (1997). *Environmental isotopes in hydrogeology*. CRC press.
- Coulton, D. W., Clark, R. G., & Hebert, C. E. (2010). Determining natal origins of birds using stable isotopes ($\delta^{34}\text{S}$, δD , $\delta^{15}\text{N}$, $\delta^{13}\text{C}$): model validation and spatial resolution for mid-continent Mallards. *Waterbirds*, *33* (1), 10-21.
- Cornell Lab of Ornithology (2014). Retrieved June 7, 2014 from <http://www.allaboutbirds.org>.
- Dansgaard, W. (1964). Stable isotopes in precipitation. *Tellus*, *16* (4), 436-468.
- DeNiro, M.J., & Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, *42*(5), 495-506.
- DeNiro, M.J., & Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, *45*(3), 341-351.
- Ducks Unlimited Canada. (2014). Retrieved April 2, 2014 from <http://www.ducks.ca/learn-about-wetlands/wildlife/>.
- Ducks Unlimited Canada. (2011). Habitat report, Saskatchewan.
- Dugan, P.J. 1990. Wetlands conservation: a review of current issues and required action. IUCN. Gland, Switzerland. 94 p
- Environment Canada. (2010). Wetlands. Retrieved March 10, 2014, from <http://www.ec.gc.ca/eau-water/default.asp?lang=En&n=27147C37-1>.

- France, R. (1996). Ontogenetic shift in crayfish $\delta^{13}\text{C}$ as a measure of land-water ecotonal coupling. *Oecologia*, *107*(2), 239-242.
- Fry, B. (2006). *Stable Isotopes in Ecology*. Springer-Verlag.
- Gunnarsson, G., Latorre-Margalef, N., Hobson, K.A., Van Wilgenburg, S.L, Elmberg, J., Olsen, B., Fouchier, R. A. M., & Waldenstrom, J. (2012). Disease dynamics and bird migration—linking Mallards *Anas platyrhynchos* and subtype diversity of the Influenza A virus in time and space. *PloS one*, *7*(4), e35679.
- Hebert, C., & Wassenaar, L. (2005). Stable isotopes provide evidence for poor northern pintail production on the Canadian prairies. *Journal of Wildlife Management*, *69* (1), 101–109.
- Hecky, R. E., & Hesslein, R. H. (1995). Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *Journal of the North American Benthological Society*, *14*(4), 631-653.
- Higgins, K., Naugle, D., & Forman, K. (2002). A case study of changing land use practices in the Northern Great Plains, USA: an uncertain future for waterbird conservation. *Waterbirds*, *25*(2002), 42–50.
- Hobson, K. A., & Clark, R. G. (1992a). Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. *Condor*, *94*(1), 181-188.
- Hobson, K. A., & Clark, R. G. (1992b). Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. *Condor*, *94*(1), 189-197.
- Hobson, K. A. (1999). Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, *120* (3), 314-326.

- Hobson K.A. (2008). Applying isotopic methods to tracking animal migration. In Hobson K.A, Wassenaar, L.I (eds) Tracking animal migration with stable isotopes. Academic press, London, pp 45-78.
- Hobson, K.A., Wunder, M.B., Van Wilgenburg, S.L., Clark, R.G., & Wassenaar, L.I., (2009). A method for investigating population declines of migratory birds using stable Isotopes: origins of harvested Lesser Scaup in North America. *PloS one*, 4(11), e7915.
- Hobson, K. A. (2011). Isotopic ornithology: a perspective. *Journal of Ornithology*, 152 (1), 49-66.
- Kelly, J.F. (2000). Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology*, 78 (1), 1-27.
- Lisovski, S., Hewson, C.M., Klaassen, R.H.G., Korner-Nievergelt, F., Kristensen, M.W., & Hahn, S. (2012). Geolocation by light: accuracy and precision affected by environmental factors. *Methods in Ecology and Evolution*, 3 (3), 603-612.
- Miller, O. D., Wilson, J. A., Ditchkoff, S. S., & Lochmiller, R. L. (2000). Consumption of agricultural and natural foods by waterfowl migrating through central Oklahoma. *Proceedings of the Oklahoma Academy of Science*, 80, 25-31.
- MacKinnon, B. D., Sagin, J., Baulch, H. M., Lindenschmidt, K. E., & Jardine, T. D. (2015). Influence of hydrological connectivity on winter limnology in floodplain lakes of the Saskatchewan River Delta, Saskatchewan. *Canadian Journal of Fisheries and Aquatic Sciences*, 73(999), 1-13.

- Morrison, A. (2012). Opaskwayak Cree Nation wetland ethnoecology: land, identity and well-being in a flooded landscape. Master's thesis, University of Manitoba.
- Nichols, J. D., Johnson, F. A., & Williams, B. K. (1995). Managing North American waterfowl in the face of uncertainty. *Annual Review of Ecology and Systematics*, 26, 177-199.
- North American Waterfowl Management Plan. (2012). Retrieved from <http://nawmp.wetlandnetwork.ca/nawmp-revision-2012/>.
- Parnell, A. C., Inger, R., Bearhop, S., & Jackson, A. L. (2010). Source partitioning using stable isotopes: coping with too much variation. *PloS one*, 5(3), e9672.
- Parnell, A.C. Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W., Jackson, A.L., Grey, J., Kelly D.J., & Inger, R. (2013). Bayesian stable isotope mixing models. *Environmetrics*, 24 (6), 387-399.
- Paszkowski, C. A., Gingras, B. A., Wilcox, K., Klatt, P. H., & Tonn, W. M. (2004). Trophic relations of the red-necked grebe on lakes in the western boreal forest: a stable-isotope analysis. *The Condor*, 106 (3), 638-651.
- Peterson, B., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Reviews in Ecological Systems*, 18, 293-320.
- Phillips, D.L & Gregg, J.W. (2003). Uncertainty in Source Partitioning using Stable Isotopes *Oecologia*, 136 (2), 261-269.
- Prairie Habitat Joint-Venture. (2008). Prairie Habitat Joint-Venture Implémentation Plan 2007-2012. Report of the Prairie Habitat Joint-Venture. Environment Canada, Edmonton, AB. 34 pp. (Revised May 2009).

- Post, D.M. (2002). Using Stable Isotopes to estimate trophic position: Models, methods, assumptions. *Ecology*, 83 (3), 703-718.
- Ramsar Convention on Wetlands. (2011). Wetland Ecosystem Services. Retrieved March 15, 2014, from http://www.ramsar.org/cda/en/ramsar-pubs-info-ecosystem-services/main/ramsar/1-30-103^24258_4000_0.
- Rubenstein, D. R., & Hobson, K. A. (2004). From birds to butterflies: animal movement patterns and stable isotopes. *Trends in Ecology & Evolution*, 19 (5), 256-263.
- Sagin, J., Sizo, A., Wheeler, H., Jardine, T. D., & Lindenschmidt, K. E. (2015). A water coverage extraction approach to track inundation in the Saskatchewan River Delta, Canada. *International Journal of Remote Sensing*, 36(3), 764-781.
- Schmutz, J. K. (2001). Cumberland Marshes Important Bird Areas. Retrieved from <http://www.ibacanada.ca/conservationplans/skcumberlandmarshes.pdf>.
- Schwarz, C. J., & Seber, G. A. (1999). Estimating animal abundance: review III. *Statistical Science*, 14(4), 427-456.
- Sheppard, S. M., Nielsen, R. L., & Taylor, H. P. (1969). Oxygen and hydrogen isotope ratios of clay minerals from porphyry copper deposits. *Economic Geology*, 64(7), 755-777.
- Slattery, S. (2008). Status of waterfowl in the Saskatchewan River Delta. Symposium proceedings of the Saskatchewan River delta. pp 1-9.
- Smith, A.R. (1996). Atlas of Saskatchewan birds. Nature Saskatchewan special publication No: 22, Regina.

- Stewart, R. E. Jr. (2014). Technical Aspects of Wetlands as Bird Habitat. National Water Summary on Wetland Resources. United States Geological Survey Water Supply Paper 2425.
- Taylor, H.P Jr. (1974). An application of oxygen and hydrogen iso-tope studies to problems of hydrothermal alteration and ore deposition. *Economic Geology*, 69 (6), 843-883.
- Tieszen, L. L., Boutton, T. W., Tesdahl, K. G., & Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia*, 57(1-2), 32-37.
- Vander Zanden, M. J., & Rasmussen, J. B. (1999). Primary consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the trophic position of aquatic consumers. *Ecology*, 80 (4), 1395-1404.
- Van Wilgenburg, S. L., & Hobson, K. A. (2011). Combining stable-isotope (δD) and band recovery data to improve probabilistic assignment of migratory birds to origin. *Ecological Applications*, 21(4), 1340-1351.
- Zedler, J. B., & Kercher, S. (2005). Wetland resources: status, trends, ecosystem services, and restorability. *Annual Review of Environment and Resources*, 30 (1), 39–74.

CHAPTER 2

2.0 THE IMPORTANCE OF THE SASKATCHEWAN RIVER DELTA AS A BREEDING, MOULTING AND STOPOVER HABITAT FOR WATERFOWL INFERRED FROM STABLE HYDROGEN ISOTOPE ANALYSIS OF FEATHERS

2.1 Introduction

Knowing the connections between breeding, stopover and wintering sites used by migratory birds is important for their conservation (Webster et al. 2002). For example, information on migratory connectivity enables the identification of critical rearing areas that generate recruits for the adult population and stopover sites that are important for fueling during migration (Szymanski et al. 2007, Hobson et al. 2009). Such information also facilitates decisions about the allocation of harvest quotas (Alisauskas et al. 1998, Hobson et al. 2009), provides information on where an infection was acquired when there is a disease epidemic (Gunnarson et al. 2012) and protects habitats that are important to long-term population viability (Webster & Marra 2005).

In North America, ~50 million waterfowl (USFWS 2015) breed in northern regions then migrate to Mexico, the Caribbean, Central America and South America for the winter (Baldassarre 2014). These waterfowl, many of which are harvested, follow four migratory flyways that are used to connect to different habitats at different times of the year (Johnsgard 2012). The Central Flyway, encapsulating the Great Plains, accounts for roughly half of the North American waterfowl population, with approximately 12.3 million waterfowl breeding in Canadian portions of this flyway and another 12.2 million breeding in the north-central United States in 2013 (DUC 2013). Understanding the origins of these birds is critical for their

management, as population trends for several species differ among regions within this flyway (Boere & Stroud 2006).

Various tools have been employed over time to study migratory connectivity; principal among them is the use of extrinsic markers such as bands (Hobson & Norris 2008). Bands have increased our knowledge and understanding about migratory connectivity between sites used throughout the annual cycle. For instance band returns for Canada Geese (*Branta canadensis*) have been very successful (Hobson 2005). However for species groups such as songbirds, band recovery rates are low (Nichols et al. 1995), biased to the region of sampling (Korner – Nievergelt et al. 2010) and banding young-of-the-year proves difficult unless the birds are colonial (Hobson & Wassenaar 2008). Satellite transmitters can be affixed to birds to track their movement. For instance, Dieter & Anderson (2009) used telemetry to track molt migration of Canada Geese in Eastern South Dakota; however, these transmitters are expensive and not readily available to most researchers. Geolocators have also been employed to track migrant birds but this method comes with the challenge that tags have to be recovered to obtain data (Bridge et al. 2011).

In response to the limitations of extrinsic markers, indirect methods such as stable isotopes have been used to study migratory origins and connectivity of species to avoid these inherent biases. The application of stable isotopes to study migratory connectivity is based on animal tissues recording isotopic information of the food webs where the tissues were grown. Hydrogen isotopes ($^2\text{H}/^1\text{H}$, measured as $\delta^2\text{H}$) in particular have proven useful due to predictable patterns in food webs across the continents. Hydrogen isotope ratios are therefore useful in discriminating between residents and migrants in a mixed population. For instance the production of prairie wetland breeding Northern Pintails (*Anas Acuta*) inferred from $\delta^2\text{H}$ in

feathers from juvenile birds was lower than expected based on breeding population data in western Canada (Hebert & Wassenaar 2005a). Another study by Hobson et al. (2006) used $\delta^2\text{H}$ in feathers to delineate origins of harvested Sandhill Crane (*Grus Canadensis*) in the Central Flyway. They found that adult birds molted throughout the breeding range but the majority of young were produced in more restricted regions. Hobson et al. (2009) observed that the proportion of young Lesser Scaup (*Aythya affinis*) produced in the north versus south of the boreal forest and the Prairie Parkland regions was inversely related to the proportions of adults breeding in these regions.

The Saskatchewan River Delta (SRD) in the heart of the Central Flyway is North America's largest inland delta and an Important Bird Area comprised of approximately 80% wetlands (Baschuk et al. 2012, Schmutz 2001). This delta has been impacted by the construction of E.B. Campbell, Gardiner and Francois Finlay dams upstream that were completed in 1963, 1967 and 1985, respectively (Gober & Wheeler 2013). As a result, the Saskatchewan River has reduced summer peak floods and total wetland area (Baschuk et al. 2012, Sagin et al. 2015). Despite these changes, the SRD still remains an important breeding and stopover site for waterfowl (Bellrose & Kortwright 1976, Schmutz 2001). More than 200,000 staging Mallards (*Anas platyrhynchos*) have been recorded in the SRD. Despite the importance of this delta as both rearing and staging habitat, there is little information on the origins of the birds that throng there every year prior to fall migration. Band recoveries are largely from birds banded to the south (i.e. the central United States). This is primarily due to limited banding activity in productive waterfowl habitats to the north such as the Peace-Athabasca and MacKenzie River Deltas. There is also little information on the importance of the SRD as an overall recruitment area for the North American waterfowl population.

I used $\delta^2\text{H}$ values from feathers to evaluate the contribution of local and non-local birds to the staging population in the SRD during fall migration. In so doing, my goal was to fill a major gap in our understanding of the migratory origins of waterfowl in this important river delta. I hypothesized that the delta would be the origin of the majority of birds because of its known role as breeding habitat (Baschuk et al. 2012, DUC 2014) with additional birds coming from north of this important stop over site (Bellrose & Kortwright 1976) and few birds originating from south of the delta.

2.2 Materials and Methods

2.2.1 Study sites

Sampling was conducted at five shallow lakes and wetlands (Figure 2.1) in the upper SRD in the fall of 2013 (September 5 - October 22) and 2014 (September 17 - October 10).

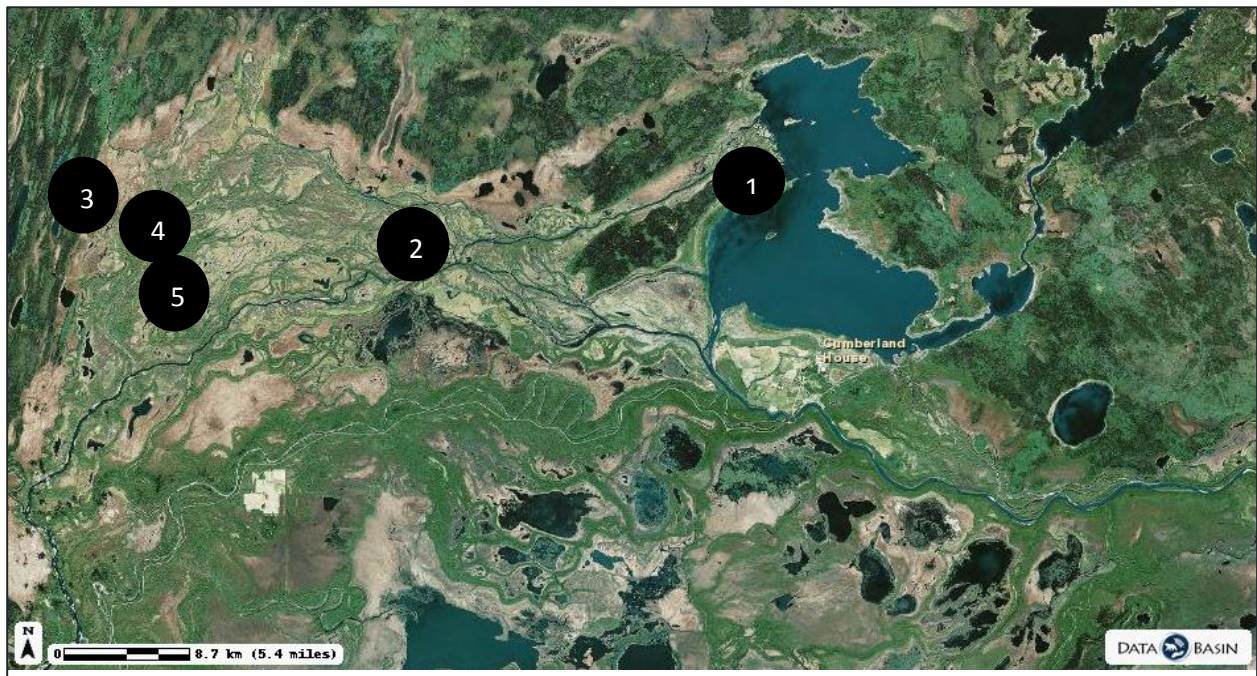


Figure 2.1: The Upper Saskatchewan River Delta, Saskatchewan, where waterfowl were

harvested for stable isotope analysis of feathers (53, 75° N 102, 443° W, 1: Grassy Point, 2: Muskeg Lake, 3: Dumbell Lake, 4: Bens Lake, 5: Big Eddy).

In 2013, there was extensive flooding in the SRD with spring and summer river discharge greater compared to the long term recorded average (spring peak discharge = 1690m³/s; summer peak discharge = 3640m³/s; compared to average spring and summer values of 650 m³/s and 870 m³/s, respectively; Water Survey of Canada gauge station 05KD003). There was similar spring flooding but reduced summer flooding in 2014 (spring peak discharge = 1750m³/s; summer peak discharge = 1730m³/s). At the time of sample collection, the wetlands and lakes were open water with vegetation interspersed at the periphery. Aquatic macrophytes such as sedges (*Carex* spp.), reed grass (*Phragmites australis*), and cattails (*Typha* spp.) dominated the emergent zone of the wetlands and shallow lakes.

2.2.2 Wing and Water Collection

A total of 236 hunter-killed birds were sampled comprising Mallard (n = 93), Northern Pintail (*Anas acuta*, n = 36), American Widgeon (*Anas americana*, n = 20), Green-winged Teal (*Anas crecca*, n = 35) and Blue-winged Teal (*Anas discors* n = 52). With the exception of Mallards, all species were collected in both years, with the majority of collections occurring in 2013 (202 of 236 birds). Wing samples from each bird were kept on ice in the field, frozen and transported to the aquatic food webs laboratory at the Toxicology Centre, University of Saskatchewan comprising adult females (n=59), adult males (n=44), juvenile females (n=80) and juvenile males (n=53).

To compare with modeled amount-weighted mean annual $\delta^2\text{H}$ in precipitation ($\delta^2\text{H}_p$), surface water samples (n = 7) were collected at the five shallow lakes and wetlands where birds

were harvested. These water samples were kept at room temperature in the dark until they were analysed for $\delta^2\text{H}$.

2.2.3 Wing Preparation and Identification

Wings were allowed to thaw and then were sexed and aged based on a wing identification manual by the U.S. Fish and Wildlife Service (<http://www.npwrc.usgs.gov/resource/birds/idguide/index.htm>, Version 18SEP97) with assistance from a trained technician at the Canadian Wildlife Service, Saskatoon, Canada. The first primary feather (P1) from each wing was cleaned of surface oils and dirt using a 2:1 chloroform: methanol solution by soaking for 24 hours and drying under a fume hood for 48 hours. Feather vane material was then clipped from the distal portion of the feather vane and weighed into silver cups (~0.35mg) for stable isotope analysis.

2.2.4 Stable hydrogen isotope analysis

Both water and feather samples were analysed for $\delta^2\text{H}$ at the National Hydrology Research Center of Environment Canada in Saskatoon, Saskatchewan. Water $\delta^2\text{H}$ values were measured with a LGR DLT-100 OA-ICOS liquid water isotope analyzer coupled to a LC-PAL auto sampler. Each of the samples was injected six times and the results of the first three injections were discarded to eliminate memory effect between samples. Two reference waters that isotopically bracketed the sample values were included in each sample analysis. These references were previously calibrated with Standard Light Antarctic Precipitation (SLAP) and Vienna Standard Mean Ocean Water (VSMOW). Results were estimated based on a rolling calibration so that each sample is calibrated by the three standards analysed closest in time to that of the sample.

Feather samples were analyzed by high temperature (1350 °C) flash pyrolysis in a Euro vector Elemental Analyzer (Milan, Italy) interfaced with an Elementar Isoprime™ (Isoprime, Manchester, UK) Continuous Flow Isotope Mass Spectrometer (CFIRMS). Analysis of $\delta^2\text{H}$ was conducted using the comparative equilibration method of Wassenaar & Hobson (2003) with three calibrated keratin reference materials (CBS, $\delta^2\text{H} = -197\text{‰}$; KHS, $\delta^2\text{H} = -54.1\text{‰}$; SPK, $\delta^2\text{H} = -121.6\text{‰}$) corrected for linear instrumental drift. Based on within-run analyses ($n = 5$) of the keratin standards, measurement error was assumed to be $\text{ca} \pm 2\text{‰}$ for hydrogen isotopes in feather ($\delta^2\text{H}_f$).

2.2.5 Statistical analyses

All statistical analyses were conducted in SPSS version 22.0 (IBM, Armonk NY). First, ordinary least squares regression was used to test for the effects of timing of collection on $\delta^2\text{H}_f$ and a general linear model (GLM) was used to test for a year effect. The year effect was tested using only four of the five species because Mallards were not collected in 2014. A GLM was then used to evaluate whether observed $\delta^2\text{H}_f$ differed according to age (adult and juveniles), sex (male and female) and species. A Wald Chi-Square post hoc test was used to make comparisons of marginal means for each species. Differences were considered significant at $\alpha = 0.05$.

2.2.6 Assignment to Origin

Most studies rely on annual or growing season $\delta^2\text{H}_p$ to estimate expected $\delta^2\text{H}_f$ at a given location (Bowen et al. 2005). However, in my case, waterfowl habitat in the SRD was clearly dominated by flows from the Saskatchewan River (Sagin et al. 2015), ~75% of which originates from the Rocky Mountains in Alberta where $\delta^2\text{H}_p$ tends to be very negative [-179‰ to -126‰, $n = 10$, queried in Online Isotopes in Precipitation Calculator (OIPC) (Bowen & Revenaugh 2003)

for locations west of the cities of Calgary and Edmonton, -114.78° W 52.19° N to -116.66° W 52.21° N]. Evaporation of this water during transit along the basin further complicates the choice of $\delta^2\text{H}_p$. Thus, I used my summer-collected surface water samples (mean = -126.1‰ , S.D = 13.2‰ , range = -140‰ to -100‰ , $n = 7$) as a best estimate of water available for feather growth of local birds. This approach was further supported by average winter water isotope values from wetlands in the same region (mean = -124.5‰ , SD = 10.6 range = -145‰ to -112‰ , $n = 26$ sites, MacKinnon et al. in press).

Using a threshold approach and the average of $\delta^2\text{H}$ in SRD surface water, the expected feather value for the Delta ($\delta^2\text{H}_{fd}$) was estimated using a re-scaling function (Wunder 2010). This function was generated from the regression of $\delta^2\text{H}_f$ of known-origin Lesser Scaup (*Aythya affinis*) against $\delta^2\text{H}_p$ (Clark et al. 2006). Birds were assigned to the following categories: Delta, North of Delta, and South of Delta (Figure 2.2).

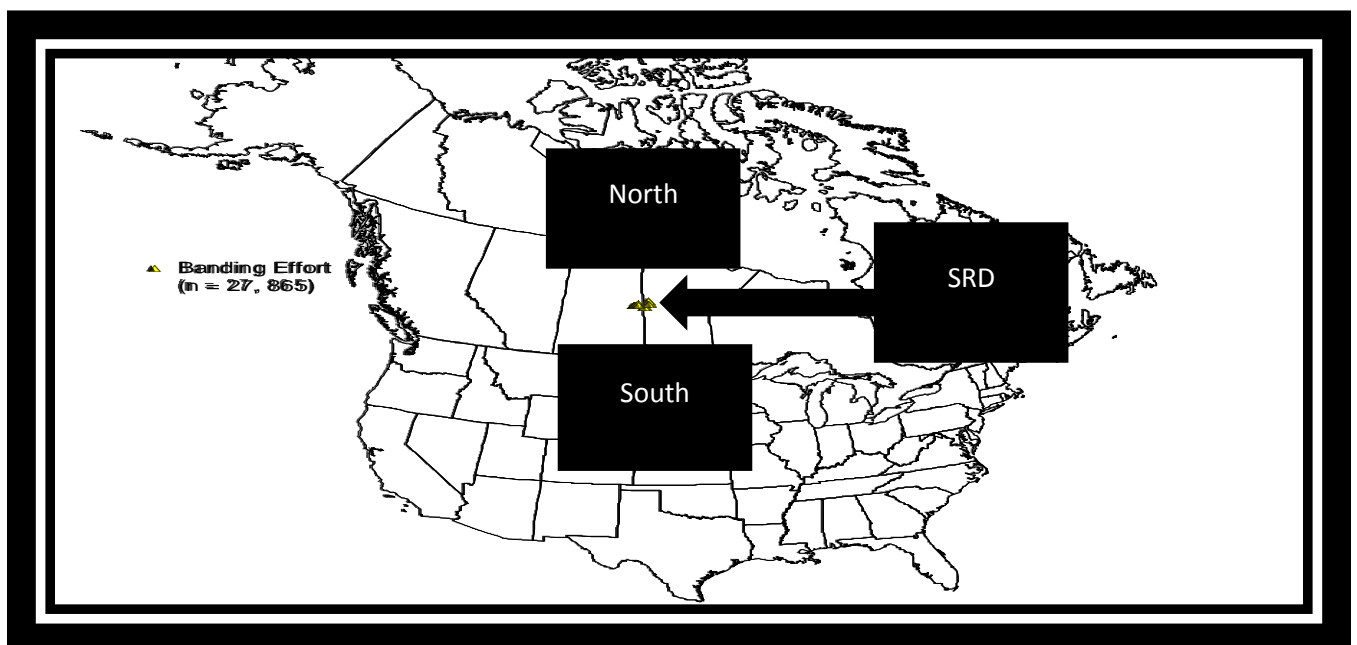


Figure 2.2: Broad geographic areas of assignment categorized using $\delta^2\text{H}_f$.

I applied a standard deviation (SD) of ± 12 ‰ around the expected value for $\delta^2\text{H}_{fd}$, thereby encompassing 67% of the distribution or a 2:1 odds ratio (Chabot et al. 2012). Thus, birds less than $\delta^2\text{H}_{fd} - 12$ ‰ were classed as Northern birds, those higher than $\delta^2\text{H}_{fd} + 12$ ‰ were classified as Southern birds, and birds that fell between these thresholds were classed as Delta birds (local birds). The SD of 12 ‰ was based on the residual in the rescaling function (Clark et al. 2006, 2009) and it is justified because Lesser Scaup is also a wetland species with a similar natural history to the species used in this study. Similarly, Hobson et al. (2012) revealed a residual variation of 12.9‰ for Neotropical migrant songbirds of known origin, and, though the residual variation of these birds varied by guild and foraging substrate, there was no significant inter-annual variation in the regression residuals from the long-term isoscape (Hobson et al. 2012).

After assignment to the three categories, those birds classed as “Delta” and “South of the Delta” were then analysed for stable sulfur isotopes ($^{34}\text{S}/^{32}\text{S}$ or $\delta^{34}\text{S}$) to further segregate their origins into major biomes. Agricultural activity, including the application of sulfate fertilizers in the Prairie pothole region, imparts a distinct $\delta^{34}\text{S}$ relative to forested areas (Hebert & Wassenaar 2005b) such as the SRD. Hence, I expected birds that molted in those Prairie wetlands to have more negative sulfur isotope values (< -6 ‰) based on Hebert & Wassenaar’s (2005b) values for juvenile mallards. This threshold was further evaluated by analyzing $\delta^{34}\text{S}$ in invertebrate and fish samples collected from SRD wetlands in August 2013. Because there is little to no fractionation of $\delta^{34}\text{S}$ in food webs (McCutchan et al. 2003), I assumed that local (delta) birds would have the same $\delta^{34}\text{S}$ as invertebrates and fishes collected there.

Feather, invertebrate and fish samples were weighed (~3.5mg) into tin capsules for analysis by CFIRMS and calibrated using secondary organic isotopic reference materials (BWB; $\delta^{34}\text{S} = 17.5\text{‰}$ n=5, and CFS; $\delta^{34}\text{S} = -3.8\text{‰}$, n=5). The instrument used was a Thermo Finnigan Delta V stable isotope ratio mass spectrometer coupled to a Costech model 4010 elemental analyser via an open split. Results were expressed as isotopic ratios in units of per mil and normalized by Canyon Diablo Triolite. Based on within-run analyses of standards I assumed measurement error to be ca $\pm 0.3\text{‰}$ for $\delta^{34}\text{S}$.

2.3 Results

The range of $\delta^2\text{H}_f$ values observed for all 236 samples was from -230‰ to -60‰. However, the majority of the data were clustered between -180‰ and -140‰ and there was no significant variation with respect to collection date ($p > 0.05$) in 2013 (Figure 2.3) or 2014 (Figure 2.4) and there was no difference between collection years ($p = 0.212$). Therefore, data were grouped into a single analysis for sex, age and species effects.

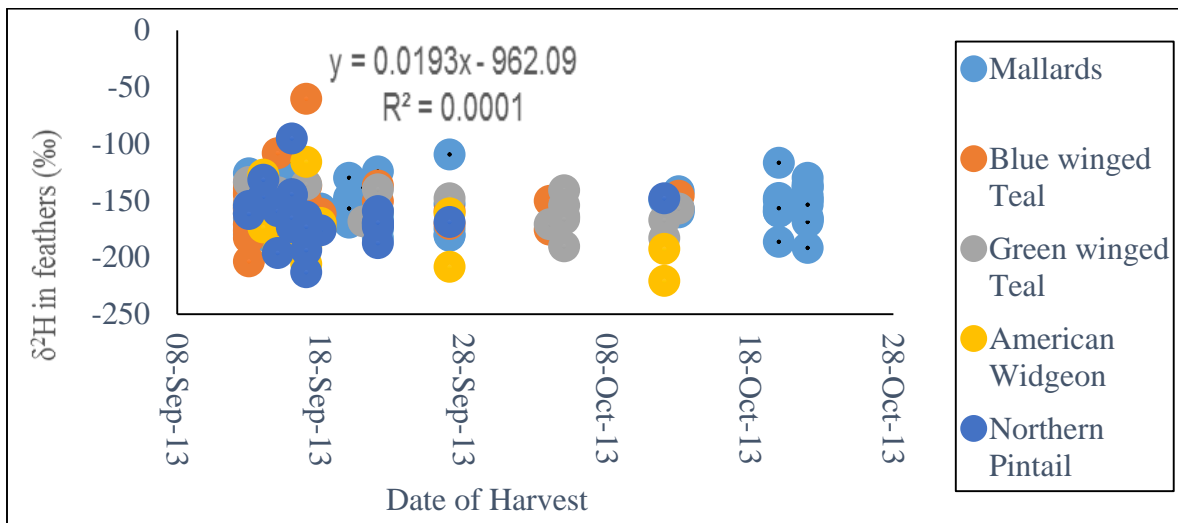


Figure 2.3: Stable hydrogen isotope ratios in waterfowl feathers ($\delta^2\text{H}_f$) versus date of sampling in 2013 in the Saskatchewan River Delta.

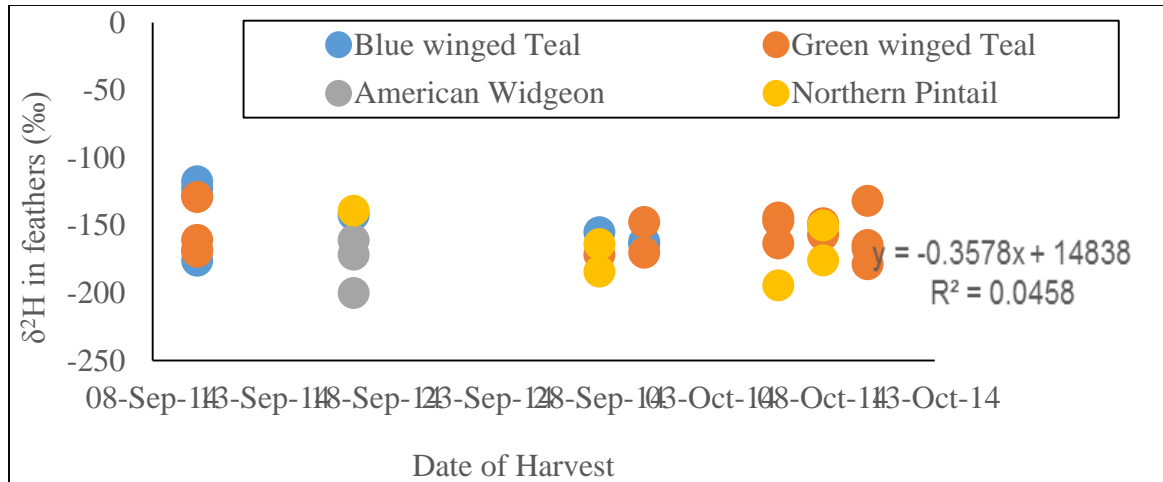


Figure 2.4: Stable hydrogen isotope ratios in waterfowl feathers (δ^2H_f) versus date of sampling in 2014 in the Saskatchewan River Delta.

Neither sex ($p = 0.127$) nor age ($p = 0.708$) had significant effects on observed δ^2H_f , but there were significant differences among species ($p = 0.001$) (Figure 2.5).

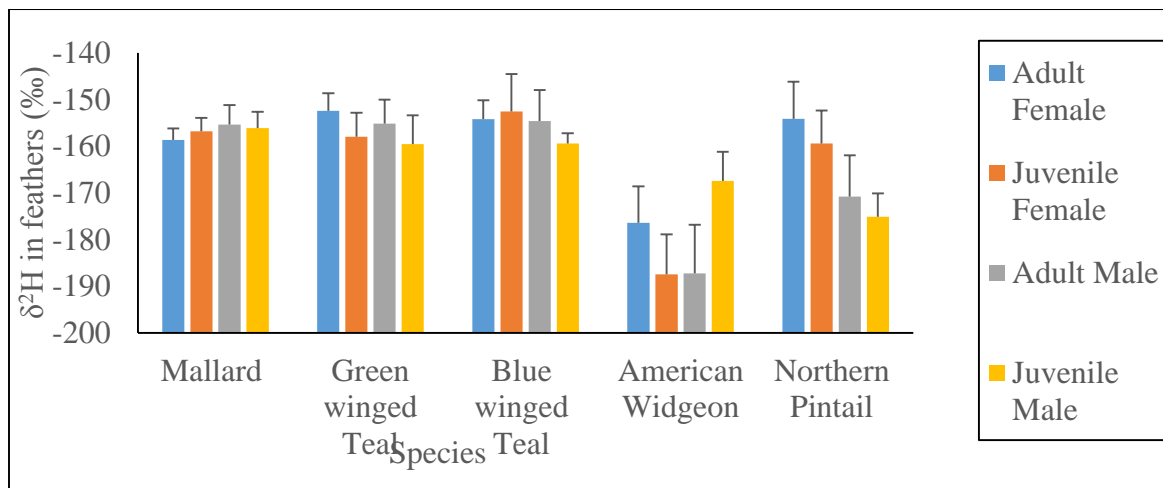


Figure 2.5: Stable hydrogen isotopes (δ^2H) in feathers from five waterfowl species in the Saskatchewan River Delta, according to age and sex.

American Widgeon had the lowest δ^2H_f mean and standard deviation ($-180.5 \pm 4.7\text{‰}$) that was different from all other species ($p \leq 0.001$ for all pairwise comparisons, Table 2.2).

Mallards ($-156.5 \pm 1.7\text{‰}$) differed significantly from Blue winged Teal ($-154.8 \pm 3.6\text{‰}$) but not from Northern Pintail ($-164.8 \pm 3.8\text{‰}$) and Green winged Teal ($-158.6 \pm 2.5\text{‰}$). Both Green winged Teal and Blue winged Teal were significantly different than Northern Pintails but were not significantly different from each other. All comparisons not involving American widgeon were not significant after a Bonferroni adjustment to probabilities ($p < 0.005$) for multiple comparisons ($\alpha/10$).

Table 2.1: Pairwise comparisons (p-values) of hydrogen isotope ratios in feathers for each species.

(I) Species	(J) Species	P value
Blue winged Teal	Green winged Teal	0.871
	Mallard	0.048
	Northern Pintail	0.010
	American Widgeon	0.001
Green winged Teal	Blue winged Teal	0.871
	Mallard	0.167
	Northern Pintail	0.049
	American Widgeon	0.001
Mallard	Blue winged Teal	0.048
	Green winged Teal	0.167
	Northern Pintail	0.453
	American Widgeon	0.001
Northern Pintail	Blue winged Teal	0.010
	Green winged Teal	0.049
	Mallard	0.453
	American Widgeon	0.001
American Widgeon	Blue winged Teal	0.001
	Green winged Teal	0.001
	Mallard	0.001
	Northern Pintail	0.001

2.3.1 Threshold Assignment

Using $\delta^2\text{H}_f$ and the defined thresholds for the three regions (Delta $\delta^2\text{H}_f > -158\text{‰}$ and $< -134\text{‰}$, South of Delta $\delta^2\text{H}_f > -134\text{‰}$, North of Delta $\delta^2\text{H}_f < -158\text{‰}$), I classified feathers from 80 birds as having been grown in the Delta, representing 34% of the total. Only 24 birds were classified as South of Delta representing 10% while the majority, 131 birds or 56% were classified as North of Delta.

The $\delta^{34}\text{S}_f$ values for Delta and Southern birds ranged from -19‰ to $+12\text{‰}$. A re-assignment of these birds based on $\delta^{34}\text{S}$ yielded 80% of that cohort re-classified as Prairie ($\delta^{34}\text{S}_f < -6\text{‰}$), 15% classified as coming from forested areas ($\delta^{34}\text{S}_f > +1\text{‰}$) that typically occur north of the delta and only 5% within the expected range for Delta birds (-6‰ to $+1\text{‰}$) based on values for delta invertebrates ($\delta^{34}\text{S}$: $-3.2 \pm 1.8\text{‰}$, range = -5.8‰ to -1.2‰ , $n = 5$) and fishes ($\delta^{34}\text{S}$: $-1.9 \pm 0.8\text{‰}$, range = -3.2‰ to -1.2‰ , $n = 31$). All subsequent assignment reported for each species takes into consideration both $\delta^2\text{H}$ and $\delta^{34}\text{S}$.

At the species level, 39% of mallards ($n = 35$) were classified as Prairie birds, 50% ($n = 46$) were assigned to the North and 12% ($n = 11$) were assigned to Delta origins (Figure 2.5). The two teal species had similar distributions as the mallards. Blue-winged Teal had almost equal numbers from the Prairie (42%, $n = 22$) and the North (46%, $n = 25$), and 12% ($n = 6$) from the Delta, and Green-winged Teal had 17 birds (49%) assigned as northern birds, 15 birds (43%) assigned to the south and Prairies and only three of 35 birds (9%) assigned as Delta birds. In contrast, the vast majority of the American Widgeons (95%) were assigned to the North, with only 5% assigned to southern or Prairie origins and none to the Delta. The Northern Pintails also exhibited a similar trend with the majority (25 of 36 birds, 69%) assigned to the North. Only

eight birds (22%) were classified as southern or Prairie birds and only three birds were assigned to the Delta (8%).

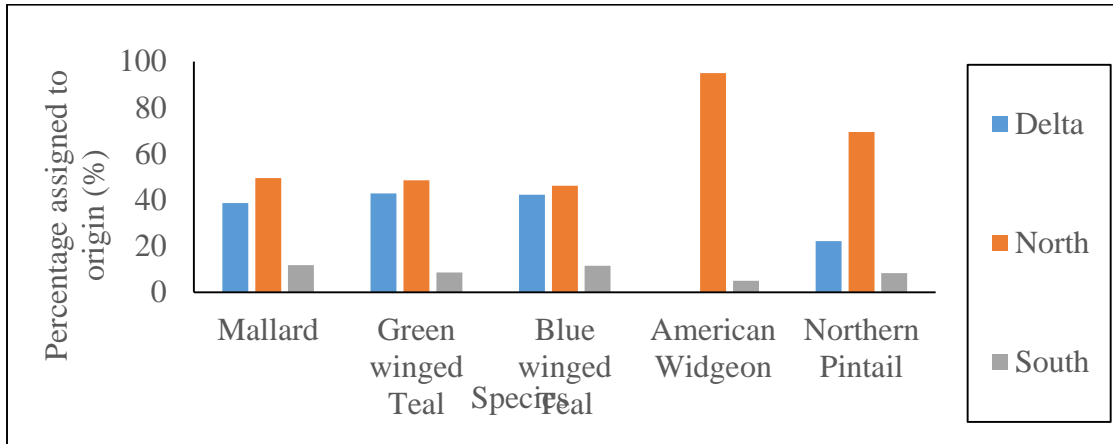


Figure 2.6: Origins (percent of total) assigned to each species based on $\delta^2\text{H}$ and $\delta^{34}\text{S}$ of feathers.

2.4 Discussion

By measuring $\delta^2\text{H}_f$ and $\delta^{34}\text{S}_f$ values in feathers of hunter killed birds, my results show the importance of northern ecosystems, including the boreal forest, as important breeding areas for waterfowl in the Central Flyway. These results also confirm the importance of the SRD as a stopover site for migratory birds prior to fall migration. The assignment of birds to various origins based on the isotope data complements existing understanding from band recovery data and the literature. Sulfur isotope data proved to be a useful tool in further delineation of birds into prairie and forest regions, respectively, and the dual isotope approach improved assignment, Hebert and Wassenaar (2005a, 2005b).

The lack of variation in $\delta^2\text{H}_f$ according to age and sex suggests no differential migration between sexes and ages. This is in agreement with Guillemain et al. (2009a, 2009b) and Legagneux et al. (2012) on Eurasian Teals (*Anas crecca*). However, my observations contrast

with studies done by Moisan et al. (1967) and Jahn & Hunt (1964) where Green-winged Teal and Blue-winged Teal exhibited inter-individual variation in migration. American Widgeon and Northern Pintail individuals also demonstrate this behavior (Sowls 1955, Jahn & Hunt 1964) as do Mallards (Boyd 1961). My observations do not support the hypothesis that males travel to specific sites to moult far from the breeding grounds (Bellrose & Kortwright 1976, Munro 1949) which would have produced variation in $\delta^2\text{H}_f$ between males and females. While males and females could still be moving to different places with similar isotopic values after breeding, these movements are likely relatively restricted, with adult males molting at locations within the same isotopic range of the females and juveniles. The non-significant differences between sexes could also be a result of the relatively small sample size for these species.

It is important to acknowledge that assignment based on the re-scaling function has several sources of error caused by within-population variation in $\delta^2\text{H}_f$, and errors associated with the iso-scape calculated from local precipitation ($\delta^2\text{H}_p$) can influence the assignment of birds to their origins using thresholds. For this reason most researchers prefer spatial, probabilistic assignment. Spatial interpolation error can also arise when data from points are interpolated over a given geographic area using precipitation models (Wunder & Norris 2008). To account for this error, I used $\delta^2\text{H}$ of river water in the years of study to create a threshold or possible range for the SRD and then calibrated the existing feather iso-scape using known-origin samples (Clark et al. 2006).

Other sources of error include documented isotopic inconsistencies in feathers of adults and juveniles grown at the same location (Coulton et al. 2010, Langin et al. 2007) as well as a large overall range in values within sites for known-origin birds (Clark et al. 2006). This was accounted for by using the standard deviation of the residuals to develop the thresholds. Also,

because the slope of the re-scaling function (Clark et al. 2006) does not equal one, the fixed intercept derived from expected $\delta^2\text{H}$ values in feathers and precipitation will affect assignment to origins (Hobson 2008, Wunder & Norris 2008). Band recovery data and information from the literature can provide prior information on movements of birds. I used this information qualitatively to help interpret biologically meaningful areas to assign the birds, but banding information can also be incorporated formally as prior information in Bayesian assignment approaches (Van Wilgenburg & Hobson 2011). However there are constraints to the banding approach, for example, how many banded birds are available for recapture (Hobson 2008)

The assignment of a large proportion of birds to the North suggests that the few band recoveries taken in the SRD from northern areas (only four of 625 total recoveries) reflects a lack of banding effort in those areas (Slattery 2008), rather than limited production, especially considering that the period under investigation was a wet year. Prairie wetland abundance correlates positively with the amount of waterfowl produced (PHJV 2008). Fewer birds produced and molted in the SRD than expected may be a result of extensive flooding in summer 2013; nests and moulting individuals were likely displaced as water levels were abnormally high (C. Asante, personal observation). Timoney (2013) made a similar observation for reduced waterfowl breeding in the Peace-Athabasca Delta.

My results suggest that birds flock into the SRD from the boreal forest and large wetland complexes such as the Peace-Athabasca Delta (theoretical $\delta^2\text{H}_p$ range for that area = -130‰ to -139‰, expected $\delta^2\text{H}_f \approx$ -169‰ to -145‰) during fall migration and most of these birds are produced or have molted in the north. This is supported by $\delta^{34}\text{S}$ data for feathers that further classified supposed SRD birds as coming from forested areas north of the SRD, as suggested by prior studies (Herbert & Wassenaar 2005a, 2005b). An additional challenge with hydrogen

isotopes is that in North America, there are similarities in $\delta^2\text{H}_p$ between southwest and central Canada, northern Quebec/Labrador and Alaska (Wunder & Norris 2008, Hobson 2005), meaning incorrect assignment can occur if birds cross flyways. Band recovery data (USFWS) suggests that a small proportion of birds that were banded in Alaska have been recovered in the SRD and the Prairies (18 recoveries over the past 50 years). Nevertheless, applying band recovery data to constrain assignments requires further research and development (Van Wilgenburg & Hobson 2011).

The presence of Prairie birds and Southern birds in the SRD with $\delta^{34}\text{S} < -6 \text{‰}$, however few, is surprising. Since Prairie pond habitats dry up as the summer progresses, the occurrence of these birds might be because foraging waterfowl must balance declining habitat and food availability with likely inter- and intra-specific competition in the many SRD wetlands (Pyke et al. 1977, Caraco et al. 1980, Stephens et al. 2007). The former could be the reason for the exodus of those birds to the SRD during fall migration. Generally the large number of birds assigned to the prairies indicated that these birds were either produced or molted there, likely because 2013 was a wet year (annual precipitation 7% above average, Environment Canada 2014) with many available wetlands, as wetland abundance directly correlates with Mallard population size (Johnson et al. 2005).

Mallards are the most abundant waterfowl species in North America and also have the largest breeding range (DUC 2014). Assignment for this species was consistent with knowledge of its breeding range and $\delta^2\text{H}_f$ of Southern birds ($\delta^2\text{H}_f > -134\text{‰}$) was consistent with a previous study by Szymanski et al. (2007) who report values between -120‰ and -80‰ for flightless Mallards in North Dakota. Most of the birds in the SRD that were later classified as Prairie were Mallards. These birds may be avoiding conspecific competition and choosing to forage with

other species, partitioning resources through interspecific differences in phenotype such as body size and bill morphology (Chesson 2000, Gurd 2008). Mallards feeding in an SRD lake exhibited a high diversity in the types of prey with some foraging on macrophytes and another group foraging on invertebrates (Chapter 3), further suggesting dietary plasticity in this species.

The Green-winged Teal had a similar proportion of birds assigned to the north as its counterpart the Blue-winged Teal, despite expected differences in their breeding ranges. The Blue-winged Teal is noted to breed and molt in southern areas such as the prairie pothole region and the St. Denis Wildlife Area (DUC 2015). The above observations are not entirely consistent with the species geographic range (Cornell Lab of Ornithology, Johnsgard 2012).

The overwhelming assignment of the American Widgeon and Northern Pintail to Northern geographical areas is consistent with these species' range maps (Cornell Lab of Ornithology, DUC 2014); notably, for Northern Pintails there have been isotope studies connecting the species to breeding areas as far north as Alaska (Yerkes et al. 2008). It is also important to note that about 64% of the North American population of American Widgeon breed in the Boreal Forest of Canada (BSI 2015) and the Northern Pintail have one of the most northern migrations (DUC 2014). Isotopic studies of this species based on $\delta^2\text{H}$ and $\delta^{34}\text{S}$ showed that the isotopes provided reliable estimates of origins of individual birds at broad spatial scales, and that Pintails breeding in the north are underestimated because of lack of regular surveys (Herbert & Wassenaar 2005a, 2005b). The Northern Pintail is of conservation significance because of a reduction in populations (NAWMP 2014, Miller & Duncan 1999). Increases in wetland density have not improved populations suggesting wetland quality is an issue (Podruzny et al. 2002). The decrease in numbers may be attributed to agricultural activity in the prairies leading to a decline in habitat availability.

Because waterfowl survey and banding efforts are very limited in northern Canada, the potential of northern habitats to contribute to waterfowl is highly under-represented. Most waterfowl surveys have been in the Prairies (Herbert & Wassenaar 2005a). The implications of this study are that the SRD will become an extremely important refuge for waterfowl. With climate change, drought and land use impacting prairie wetlands, the SRD will be a refuge for displaced birds in the prairies (DUC 2014). This study provides information on the importance of northern breeding areas to waterfowl in the Central Flyway, and may provide a tool that could complement the limited banding efforts in the region.

2.6 References

- Alisauskas, R. T., Klaas, E. E., Hobson, K. A., & Ankney, C. D. (1998). Stable-carbon isotopes support use of adventitious color to discern winter origins of Lesser Snow Geese. *Journal of Field Ornithology*, 69 (2), 262-268.
- Baldassarre, G. (2014). *Ducks, Geese, and Swans of North America* (Vol. 1). JHU Press.
- Baschuk, M. S., Koper, N., Wrubleski, D. A., & Goldsborough, G. (2012). Effects of water depth, cover and food resources on habitat use of marsh birds and waterfowl in boreal wetlands of Manitoba, Canada. *Waterbirds*, 35(1), 44-55.
- Bellrose, F. C., & Kortright, F. H. (1976). *Ducks, geese & swans of North America*. Stackpole Books.
- Boere, G.C. & Stroud, D.A. 2006. The flyway concept: what it is and what it isn't. In: G.C. Boere, C.A. Galbraith & D.A. Stroud (eds). *Waterbirds around the world*. The Stationery Office, Edinburgh, UK. pp. 40-47.

- Boreal Songbird Initiative. (2015). Retrieved from <http://www.borealbirds.org/>.
- Bowen, G. J., & Revenaugh, J. (2003). Interpolating the isotopic composition of modern meteoric precipitation. *Water Resources Research*, *39* (10), 1299.
- Bowen, G. J., Wassenaar, L. I., & Hobson, K. A. (2005). Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia*, *143* (3), 337-348.
- Boyd, H. (1961). The flightless period of the Mallard in England. *Wildfowl*, *12* (12), 140-143.
- Bridge, E. S., Thorup, K., Bowlin, M. S., Chilson, P. B., Diehl, R. H., Fléron, R. W., & Wikelski, M. (2011). Technology on the move: recent and forthcoming innovations for tracking migratory birds. *BioScience*, *61*(9), 689-698.
- Caraco, T., Martindale, S., & Whittam, T. S. (1980). An empirical demonstration of risk-sensitive foraging preferences. *Animal Behaviour*, *28* (3), 820-830.
- Chabot, A.A., Hobson, K.A., Van Wilgenburg, S.L., McQuat, G.J., & Loughheed, S.C. (2012). Advances in linking wintering migrant birds to their breeding-ground origins using combined analyses of genetic and stable isotope markers. *PLoS One*, *7*(8), e43627.
- Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, *31*(1), 343-366.
- Clark, R. G., Hobson, K. A., & Wassenaar, L. I. (2009). Corrigendum- Geographic variation in the isotopic (δD , $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) composition of feathers and claws from lesser scaup and northern pintail: implications for studies of migratory connectivity. *Canadian Journal of Zoology*, *87*(6), 553-554.

- Clark, R. G., Hobson, K. A., & Wassenaar, L. I. (2006). Geographic variation in isotopic (δD , $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$) composition of feathers and claws from lesser scaup and northern pintail: implications for studies of migratory connectivity. *Canadian Journal of Zoology*, *84* (10), 1395-1401.
- Coulton, D. W., Clark, R. G., & Hebert, C. E. (2010). Determining natal origins of birds using stable isotopes ($\delta^{34}\text{S}$, δD , $\delta^{15}\text{N}$, $\delta^{13}\text{C}$): model validation and spatial resolution for mid-continent Mallards. *Waterbirds*, *33*(1), 10-21.
- Dieter, C. D., & Anderson, B. J. (2009). Reproductive success and brood movements of giant Canada geese in eastern South Dakota. *American Midland Naturalist*, *162* (2), 373-381.
- Ducks Unlimited Canada (2013). Habitat Report. Retrieved May, 2015 from <http://www.ducks.ca/learn-about-wetlands/wildlife/>.
- Ducks Unlimited Canada. (2014). Retrieved April 2, 2014 from <http://www.ducks.ca/learn-about-wetlands/wildlife/>.
- Gunnarsson, G., Neus, L.M., Hobson, K.A., Wilgenburg, S., Elmberg, J., Olsen, B., Fouchier, R.A.M & Waldenström, J. (2012). Disease dynamics and bird migration—linking mallards *Anas platyrhynchos* and subtype diversity of the influenza A virus in time and space. *PLoS One* *4*(7), e35679.
- Guillemain, M., Hearn, R., King, R., Gauthier-Clerc, M., Simon, G., & Caizergues, A. (2009a). Comparing migration of Teal from two main wintering areas of Western Europe: a long term study from Essex, England, and Camargue, France. *Ringing and Migration* *24*, 273–276.

- Guillemain, M., Hearn, R., King R, Gauthier-Clerc, M., Simon, G., & Caizergues, A. (2009b) Differential migration of the sexes cannot be explained by the body size hypothesis in Teal. *Journal of Ornithology*, 150 (1), 685–689.
- Gurd, D. B. (2008). Mechanistic analysis of interspecific competition using foraging trade-offs: Implications for duck assemblages. *Ecology*, 89 (2), 495-505.
- Gober, P., & Wheeler, H. S. (2013). Socio-hydrology and the science-policy interface: a case study of the Saskatchewan River Basin. *Hydrology and Earth System Sciences Discussions*, 10 (5), 6669-6693.
- Hebert, C. E., & Wassenaar, L. I. (2005a). Feather stable isotopes in western North American waterfowl: spatial patterns, underlying factors, and management applications. *Wildlife Society Bulletin*, 33 (1), 92-102.
- Hebert, C., & Wassenaar, L. (2005b). Stable isotopes provide evidence for poor northern pintail production on the Canadian prairies. *Journal of Wildlife Management*, 69 (1), 101–109.
- Hobson, K. A. (2005). Stable isotopes and the determination of avian migratory connectivity and seasonal interactions. *Auk*, 122 (4), 1037-1048.
- Hobson, K. A., Van Wilgenburg, S., Wassenaar, L. I., Hands, H., Johnson, W., O’Melia, M., & Taylor, P. (2006). Using stable-hydrogen isotopes to delineate origins of Sandhill Cranes harvested in the Central Flyway of North America. *Waterbirds*, 29 (2), 137-147.
- Hobson, K. A., & Wassenaar, L. I. (2008). *Tracking animal migration with stable isotopes*. Academic Press.

- Hobson, K. A., & Norris, D. R. (2008). Animal migration: a context for using new techniques and approaches. *Terrestrial Ecology*, 2, 1-19.
- Hobson K.A. (2008). Applying isotopic methods to tracking animal migration. In Hobson K.A., Wassenaar, L.I (eds) *Tracking animal migration with stable isotopes*. Academic press, London, pp 45-78.
- Hobson, K. A., Wunder, M. B., Van Wilgenburg, S. L., Clark, R. G., & Wassenaar, L. I. (2009). A method for investigating population declines of migratory birds using stable isotopes: origins of harvested lesser scaup in North America. *PLoS One*, 4 (11), e7915.
- Hobson, K. A., Van Wilgenburg, S. L., Wassenaar, L. I., & Larson, K. (2012). Linking hydrogen ($\delta^2\text{H}$) isotopes in feathers and precipitation: sources of variance and consequences for assignment to isoscapes. *PLoS One*, 7(4), e35137.
- Jahn, L. R., & Hunt, R. A. (1964). *Duck and coot ecology and management in Wisconsin* (No. 33-39). Wisconsin State Conservation Department.
- Johnson, W.C., Millett, B.V., Gilmanov, T., Voldseth, R.A., Guntenspergen, G.R., & Naugle, D.E. (2005). Vulnerability of northern prairie wetlands to climate change. *BioScience*, 55(10), 863-872.
- Korner-Nievergelt, F., Sauter, A., Atkinson, P. W., Guélat, J., Kania, W., Kéry, M., & Van Noordwijk, A. J. (2010). Improving the analysis of movement data from marked individuals through explicit estimation of observer heterogeneity. *Journal of Avian Biology*, 41 (1), 8-17

- Langin, K. M., Reudink, M. W., Marra, P. P., Norris, D. R., Kyser, T. K., & Ratcliffe, L. M. (2007). Hydrogen isotopic variation in migratory bird tissues of known origin: implications for geographic assignment. *Oecologia*, *152* (3), 449-457.
- Legagneux, P., Clark, R. G., Guillemain, M., Eraud, C., Théry, M., & Bretagnolle, V. (2012). Large-scale geographic variation in iridescent structural ornaments of a long-distance migratory bird. *Journal of Avian Biology*, *43* (4), 355-361.
- MacKinnon, B. D., Sagin, J., Baulch, H. M., Lindenschmidt, K. E., & Jardine, T. D. (2015). Influence of hydrological connectivity on winter limnology in floodplain lakes of the Saskatchewan River Delta, Saskatchewan. *Canadian Journal of Fisheries and Aquatic Sciences*, *73* (999), 1-13.
- McCutchan, J. H., Lewis, W. M., Kendall, C., & McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, *102* (2), 378-390.
- Miller, M. R., & Duncan, D. C. (1999). The northern pintail in North America: status and conservation needs of a struggling population. *Wildlife Society Bulletin*, *27* (3), 788-800.
- Moisan, G., Smith, R.I., and Martinson, R. K. (1967). *The green-winged teal: its distribution, migration, and population dynamics*. U.S. Dep. Interior, Fish and Wildlife Service, Special Science Report 100.
- Munro, J. A. (1949). Studies of waterfowl in British Columbia: baldpate. *Canadian Journal of Research*, *27*(5), 289-307.
- Nichols, J. D., Johnson, F. A., & Williams, B. K. (1995). Managing North American waterfowl in the face of uncertainty. *Annual Review of Ecology and Systematics*, *26*, 177-199.

- North American Waterfowl Management Plan. (2012). Retrieved from <http://nawmp.wetlandnetwork.ca/nawmp-revision-2012/>
- Podruzny, K. M., Devries, J. H., Armstrong, L. M., & Rotella, J. J. (2002). Long-term response of northern pintails to changes in wetlands and agriculture in the Canadian Prairie Pothole Region. *The Journal of wildlife management*, 66 (4), 993-1010.
- Prairie Habitat Joint-Venture. (2008). Prairie Habitat Joint-Venture Implementation Plan 2007-2012. Report of the Prairie Habitat Joint-Venture. Environment Canada, Edmonton, AB. 34 pp. (Revised May 2009).
- Pyke, G. H., Pulliam, H. R., & Charnov, E. L. (1977). Optimal foraging: a selective review of theory and tests. *Quarterly Review of Biology*, 52 (2), 137-154.
- Sagin, J., Sizo, A., Wheeler, H., Jardine, T. D., & Lindenschmidt, K. E. (2015). A water coverage extraction approach to track inundation in the Saskatchewan River Delta, Canada. *International Journal of Remote Sensing*, 36(3), 764-781.
- Schmutz, J. K. (2001). Cumberland Marshes Important Bird Areas. Retrieved from <http://www.ibacanada.ca/conservationplans/skcumberlandmarshes.pdf>
- Slattery, S. (2008). Status of waterfowl in the Saskatchewan River Delta. *Symposium proceedings of the Saskatchewan River delta*, pp. 1-9.
- Sowls, L. K., (1955). *Prairie Ducks: A Study of Their Behavior, Ecology and Management*. Wildlife Management Institute, Stackpole Co, Harrisburg.
- Stephens, D. W., Brown, J. S., & Ydenberg, R. C. (2007). *Foraging: behavior and ecology*. University of Chicago Press, pp.15.

- Szymanski, M. L., Afton, A. D., & Hobson, K. A. (2007). Use of stable isotope methodology to determine natal origins of mallards at a fine scale within the upper Midwest. *Journal of Wildlife Management*, 71 (4), 1317-1324.
- Timoney, K. P. (2013). *The Peace-Athabasca Delta: Portrait of a Dynamic Ecosystem*. University of Alberta Press, pp 8.
- U.S. Fish and Wildlife Service. (2014) Duck identification guide for hunters. Northern Prairie Wildlife Research Center Online. U.S. Fish and Wildlife Service, Washington, D.C.
- U.S. Fish and Wildlife Service. (2015). Waterfowl Status Report. Retrieved from http://flyways.us/sites/default/files/uploads/statusreport2015_final_7-23-15.pdf
- Van Wilgenburg, S.L. & Hobson, K.A. (2011). Combining stable isotope (δD) and band recovery data to improve probabilistic assignment of migratory birds to origin. *Ecological Applications*, 21(4), 1340-1351.
- Wassenaar, L.I., & Hobson, K.A. (2003). Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. *Isotopes in Environmental and Health Studies*, 39 (3), 211-217.
- Webster, M. S., Marra, P. P., Haig, S. M., Bensch, S., & Holmes, R. T. (2002). Links between worlds: unraveling migratory connectivity. *Trends in Ecology & Evolution*, 17 (2), 76-83.
- Webster, M. S., & Marra, P. P. (2005). The importance of understanding migratory connectivity and seasonal interactions. In: *Birds of two worlds: the ecology and evolution of migration*. Johns Hopkins University Press, Baltimore, pp. 199-209.

Wunder, M.B. (2010). Using isoscapes to model probability surfaces for determining geographic origins. In: *Isoscapes: Understanding movement, pattern and process on Earth through isotope mapping*. (J.B. West et al., eds.). Springer, New York, pp. 251-270.

Wunder, M. B., & Norris, D. R. (2008). Improved estimates of certainty in stable-isotope-based methods for tracking migratory animals. *Ecological Applications*, 18 (2), 549-559.

Yerkes, T., Hobson, K. A., Wassenaar, L. I., Macleod, R., & Coluccy, J. M. (2008). Stable isotopes (δD , $\delta^{13}\text{C}$, $\delta^{15}\text{N}$) reveal associations among geographic location and condition of Alaskan Northern Pintails. *Journal of Wildlife Management*, 72 (3), 715-725.

CHAPTER 3

3.0 TRACING NUTRIENT SOURCES OF STAGING WATERFOWL IN A LARGE RIVER DELTA USING STABLE ISOTOPES (CARBON AND NITROGEN) AND MERCURY.

3.1 Introduction

Animal migration confers considerable physiological demands such as the provisioning of fuel through lipid synthesis and protein catabolism, processes well demonstrated by migrating waterfowl (Davidson & Evans 1986, Klaassen et al. 2007, 2012). During fall migration, many waterfowl stage in productive habitats, such as wetlands, where they build lipid reserves to fuel their subsequent journey. Wetland quality, as characterized by the abundance, composition and spatiotemporal dynamics of food sources, is an important factor influencing waterfowl populations (Davis & Smith 1998, Taft & Haig 2005, Hartke et al. 2009). Understanding waterfowl dietary needs during stopover or refueling periods, including which habitats best provide these requirements, is critical for successful waterfowl conservation and management (Havera 1999).

Submerged and emergent aquatic macrophytes and invertebrates are common foods for waterfowl during migration (Martin & Uhler 1939, Tidwell et al. 2013). Seeds of submerged macrophytes can be one of the most important sources of plant carbohydrates used in this period sustaining thousands of individuals (Chura 1961, Hay 1974). Seeds and fruits of macrophytes provide the bulk of carbohydrates for lipid production, and invertebrates provide protein (Brochet et al. 2012). Tracking the flow of energy in stopover sites used by migratory birds is necessary because populations of waterfowl in an entire flyway may be affected by the

availability of food (Myers 1983, Arzel et al. 2009) and habitat quality may also affect the timing of migration (Schneider & Harrington 1981). High quality foraging habitats are therefore important to maximize body condition for successful migration (Drobney & Fredrickson 1979, Reid et al. 1989).

Conventional approaches for investigating consumers' nutrient sources, such as gut content analysis or visual observation, have limitations. First, they provide only information on ingested materials that may not be assimilated (Hobson 1999). Second, soft-bodied invertebrates that are an essential part of the diet may be difficult to trace because they are digested more rapidly (Hobson 1999). Stable-isotope analysis of animal tissues and potential dietary sources has provided a complementary understanding of trophic ecology and nutrient sources (Hobson 1999, Post 2002). This method tracks nutrient sources over longer periods and gives information on assimilated nutrients. Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values have been used primarily as tracers. The amount of isotopic change or discrimination as source diet is converted to proteins in consumer tissue is low for $\delta^{13}\text{C}$ ($< 1\%$), making it an important ecological tracer of diet and connections to primary productivity (Hobson 1999, Inger & Bearhop 2008). Nitrogen undergoes isotopic discrimination at a rate of 2-5% from diet to consumer (Hobson 1999, Post 2002), making it a good indicator of trophic position. Ecosystem baseline differences in $\delta^{15}\text{N}$, however, can confound their interpretation. For instance, amphipods that are considered primary consumers can have highly variable $\delta^{15}\text{N}$ values (Hobson et al. 2005). Use of other elements such as mercury (Hg), which is known to bio-magnify in food webs (Lavoie et al. 2013), may also be useful as an indicator of trophic levels in complex aquatic habitats where baseline $\delta^{15}\text{N}$ may vary, but to date [Hg] measurements have rarely been used to infer trophic position or to

delineate food web structure (Zhang et al. 2012) and dietary source proportions using mixing models.

The Saskatchewan River Delta (SRD) in central Canada, North America's largest inland delta, is an important stopover site for waterfowl with thousands flocking annually there to stage (Bellrose & Kortwright 1976, Baschuk et al. 2012). Because many of these birds originate from elsewhere (Chapter 2, Slattery 2008) and densities are high, there is potential for diet segregation associated with differences in morphology and physiology (Gurd 2008, Brochet et al. 2012). Past information on nutrient sources used by these birds is based on gut content analysis and observational methods (Dirschl 1969). Here, I studied nutrient flow in this important waterfowl habitat using $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and [Hg] to unravel information that will be important to wetland conservation and management efforts in the SRD.

I aimed to determine general dietary sources to and trophic position of waterfowl using the SRD during their fall migration. I was specifically interested in the relative contributions of detritus- and algal-based pathways to the diet of waterfowl as mediated through consumption of invertebrates, and how diets differ among species, sexes and age groups within species. I predicted that aquatic macrophytes would dominate waterfowl diet as recorded in other systems such as Heron Lake (Wersal et al. 2005) and because of known seasonal shifts to this source in the fall (Dirschl 1969, Brochet et al. 2012). I anticipated that different species would use different resources, with American Widgeon having the most herbivorous diet (Knapton & Pauls 1994), and expected more variation among species than within species. These comparisons would provide insights into how birds maximize energy gains during short periods when potential competition for resources is high, and test theory that foraging of animals evolves to maximize their net energy returns (Staddon 2013).

3.2 Materials and Methods

3.2.1 Liver Tissue Collection and Preparation

3.2.2 Consumer Tissue

I collected a total of 118 hunter-killed birds comprising five species from Grassy Point in the SRD (53.75° N 102.443° W, Figure 3.1) in September and October of 2013. Focal species were Mallard (n = 56), Northern Pintail (*Anas acuta*, n = 25), American Widgeon (n = 13), Green-winged Teal (*Anas crecca*, n = 4) and Blue-winged Teal (*Anas discors*, n = 20).



Figure 3.1: The Upper Saskatchewan River Delta, Saskatchewan, where sampling was done (53, 75° N 102, 443° W, 1: Grassy Point).

Birds were sexed and aged based on a wing identification manual by the U.S. Fish and Wildlife Service (<http://www.npwrc.usgs.gov/resource/birds/idguide/index.htm>, Version 18SEP97) with assistance from a trained technician at the Canadian Wildlife Service in

Saskatoon, Canada. At the time of collection the wetlands were open water with vegetation interspersed at the periphery. Aquatic macrophytes such as sedges (*Carex* spp.), reed grass (*Phragmites australis*), and cattails (*Typha* spp.) dominated the emergent zone of the wetlands. Following dissection in the field, liver tissue was removed, stored in Ziploc bags and transported frozen to the Aquatic Food Webs Lab in the Toxicology Center at the University of Saskatchewan.

To assess the contribution of the different nutrient sources using stable isotopes, liver tissue was selected because it has a rapid turnover rate and short half-life. Liver half-lives likely vary as a function of body size, as noted for avian blood (Carleton & Martinez Del Rio 2005) and for birds the size of the waterfowl I examined an average half-life of 7 days (Boecklen et al. 2011) was estimated. This tissue therefore provides information on the most recent diet (Hobson & Clark 1992a, 1992b, Hobson 1999) which is important in this study system because, based on evidence from stable hydrogen isotope ratios ($\delta^2\text{H}$) of feathers (Chapter 2), many of the birds were not long-term residents of the delta but were staging there. In the laboratory, liver samples were washed of blood and debris with de-ionized water prior to preparation for isotope analysis. Samples were then oven dried and powdered. Using a 2:1 chloroform: methanol solution, lipid extraction was also done on a subset of samples ($n = 8$) to assess the utility of lipid correction equations in estimating lipid-free $\delta^{13}\text{C}$. This was done because variations in tissue lipid content can affect bulk tissue $\delta^{13}\text{C}$ and can be wrongly interpreted as dietary or habitat shifts (Logan et al. 2008). Powdered samples were soaked in chloroform: methanol solution for one week after which they were dried under a fume hood and re-analyzed. Un-extracted samples had C: N ratios, a proxy for lipid content, which ranged from 4-6. Upon extraction, $\delta^{13}\text{C}$ did not differ ($P = 0.48$) from values obtained using a correction equation ($\delta^{13}\text{C}_{\text{normalized}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times$

C: N) described by Post et al. (2007). Therefore, the correction equation was applied to the remainder of the un-extracted samples.

3.2.3 Dietary sources

Potential diet items for waterfowl were collected from the site where the birds were harvested, and other nearby locations in the SRD where birds are known to congregate in groups to feed. Since waterfowl are highly mobile, they might feed in areas far from where they were harvested. Three replicate samples of invertebrates were collected with a consistent sweep of 40 seconds using a dip net (150 μm mesh), coarsely live-sorted and stored in plastic vials. Aquatic macrophytes were handpicked and stored in Ziploc bags. All samples were transported on ice to the laboratory and stored frozen. In the laboratory, samples were thawed, counted and identified. Invertebrates were identified to the family level (Merritt & Cummins 2008) and individuals of the same family were grouped together for isotope analysis. Macrophyte samples were thawed, counted and identified to the species level based on Lahring (2003).

3.2.4 Stable Carbon ($\delta^{13}\text{C}$) and Nitrogen ($\delta^{15}\text{N}$) Isotope Analysis

All samples were dried in an oven for 72 hours at 50°C, weighed into tin capsules (0.9-1.1 mg for animals and 3.5-4.5 mg for plants) and shipped to the Stable Isotope Facility at the University of California Davis for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. Samples were analyzed using a PDZ Europa ANCA-GSL Elemental Analyzer interfaced to a PDZ Europa (20-20) isotope ratio mass spectrometer (Sercon Ltd, Cheshire UK). During analysis, at least two laboratory standards were included; Glutamic acid ($\delta^{13}\text{C} = -28.8 \pm 0.1\text{‰}$, $\delta^{15}\text{N} = -4.3 \pm 0.2\text{‰}$, $n = 20$) and Bovine Liver ($\delta^{13}\text{C} = -21.7 \pm 0.1\text{‰}$, $\delta^{15}\text{N} = 7.7 \pm 0.2\text{‰}$, $n=20$). The standards were calibrated against NIST standard reference materials (IAEA N1, USGS-40). The isotope values were expressed relative

to international standards V-PDB (Vienna PeeDee Belemnite) and atmospheric nitrogen for carbon and nitrogen respectively.

3.2.5 Total Mercury (THg) Analysis

Total mercury (THg) liver tissue, invertebrates and aquatic macrophytes were determined by Direct Mercury Analysis on a DMA-80 (Milestone, Inc., Shelton, CT). TORT-3 (lobster hepatopancreas) and DORM-4 (dogfish muscle) were used as Certified Reference Materials (CRMs). Mean recovery of THg in the TORT-3 CRM was $107 \pm 10 \%$ ($n = 57$) and in the DORM-4 CRM was $98 \pm 6 \%$ ($n = 18$). Certified concentrations of TORT-3 and DORM-4 are $0.29 \mu\text{g/g}$ and $0.41 \mu\text{g/g}$, respectively.

3.2.6 Statistical Analysis

A general linear model (GLM) in SPSS version 22.0 (IBM, Armonk NY) was used to evaluate whether observed $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ values and log-transformed [Hg] values in liver tissue differed according to age (adult or juvenile), sex (male or female) and species. Before this was done, homogeneity of variances was tested using Bartlett's test in R Version 3.1.3 (R Core Team 2014). This test indicated that there were differences in variance with respect to age ($p = 0.006$) and species ($p = 0.001$) but not sex ($p = 0.895$) for $\delta^{13}\text{C}$. For $\delta^{15}\text{N}$, age and sex had homogenous variances ($p = 0.421$, $p = 0.751$) but it was not the same for species ($p = 0.001$). Mercury concentrations, the third tracer, had homogenous variances for sex and species ($p = 0.142$ and $p = 0.160$) but not for age ($p = 0.020$). Interactions among the three explanatory variables were also tested using the GLM by first running the full model with all terms, including three-way interaction, then removing terms if they were not significant. Differences were considered significant at $\alpha = 0.05$.

3.2.7 Mixing Models

I used the mixing model MIXSIAR programmed in R 3.1.3 (2013) (Phillips et al 2014) to estimate the relative contribution of each of four food sources: macrophytes and invertebrates classified into three categories (Herbivores, Carnivores and Omnivores based on Merritt and Cummins 2008). Herbivores were composed of the families Corixidae, Physidae and Docielinotidae, omnivores were comprised of the families Cambaridae and Gammaridae, and carnivores were composed of the families Notonectidae, Coenagrionidae, and Aeshnidae.

Using the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ data and [Hg] and a concentration-dependent model, I estimated the contribution of the four sources to waterfowl diet. A concentration- dependent model was used because the species used in the study are considered omnivorous (Baschuk et al. 2012, Tidwell et al. 2013) and there are large differences in %C and %N among sources. I used discrimination factors for liver summarized from the literature (Ogden et al. 2004) to back-calculate from the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the consumer of interest to the underlying source: $4.0 \pm 0.2\text{‰}$ SD for $\delta^{15}\text{N}$ and $1.1 \pm 0.2\text{‰}$ SD for $\delta^{13}\text{C}$. Discrimination factors for Hg were calculated based on the knowledge that THg increases by 4.7 ± 4.7 SD times per trophic level (Lavoie et al. 2013). Therefore, I multiplied the [Hg] of each of the sources by 4.7, and then subtracted the source Hg concentration from the resulting mixture [Hg] to obtain source-specific discrimination factors ($\text{Hg}_{\text{consumer}} - \text{Hg}_{\text{source}}$).

The MIXSIAR model parameterization included three chains, a chain length of 50000, burn in of 25000, and thin of 25, and results are reported as 95% credible intervals (CrIs). For analysis in the mixing model, because of the bimodality in the $\delta^{15}\text{N}$ values of Mallards, this species was split into two groups [Mallards (L) with $\delta^{15}\text{N} < 11\text{‰}$ and Mallards (H) with $\delta^{15}\text{N} >$

11‰]. 95% CrIs were examined among species because of limited differences among ages and sexes.

3.3 Results

Food sources for the birds examined were highly variable but macrophytes differed isotopically and in [Hg] relative to invertebrates ($p < 0.001$). The mean $\delta^{13}\text{C}$ for macrophytes was -25.5 ± 3.7 ‰ SD ($n = 8$), mean $\delta^{15}\text{N}$ was $+2.6 \pm 5.0$ ‰ SD, and mean Log Hg was -1.5 ± 2.0 $\mu\text{g/g}$ dry weight SD. Invertebrates were ^{15}N -enriched and ^{13}C -depleted, and had higher Hg concentrations compared to macrophytes. Omnivorous invertebrates ($\delta^{13}\text{C} = -27.9 \pm 2.7$ ‰ SD, $\delta^{15}\text{N} = +11.8 \pm 0.9$ ‰ SD, Log Hg = -0.9 ± 0.6 $\mu\text{g/g}$ dry weight SD, $n = 3$), had higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than carnivorous invertebrates ($\delta^{13}\text{C} = -28.6 \pm 2.3$ ‰ SD, $\delta^{15}\text{N} = +7.8 \pm 3.0$ ‰ SD, Hg = -0.7 ± 0.5 $\mu\text{g/g}$ dry weight SD, $n = 8$) and herbivorous invertebrates ($\delta^{13}\text{C} = -32.0 \pm 1.6$ ‰ SD, $\delta^{15}\text{N} = +7.2 \pm 2.3$ ‰ SD, Hg = -0.9 ± 0.1 $\mu\text{g/g}$ dry weight SD, $n = 5$).

For $\delta^{13}\text{C}$, there were no significant interactions ($p > 0.05$), including three-way interaction (age*sex*species). While sex did not significantly affect $\delta^{13}\text{C}$ values ($p = 0.851$), there were significant differences among species ($p = 0.001$) and with respect to age ($p = 0.027$) (Fig. 3.2). For Mallards, $\delta^{13}\text{C}$ values (-27.9 ± 1.7 ‰) differed significantly from all other species ($p < 0.05$ for all comparisons, Table 1). There were no significant differences between Green-winged Teal ($\delta^{13}\text{C} = -29.0 \pm 1.3$ ‰) and Blue-winged Teal ($\delta^{13}\text{C} = -28.5 \pm 1.6$ ‰; $p = 0.151$) but these species differed from all others ($p < 0.05$, Table 1). American Widgeon ($\delta^{13}\text{C} = -25.7 \pm 1.4$ ‰) was not significantly different from Northern Pintails ($\delta^{13}\text{C} = -26.6 \pm 2.2$ ‰; $p = 0.159$) but these two species also differed from all others ($p < 0.05$, Table 3.1).

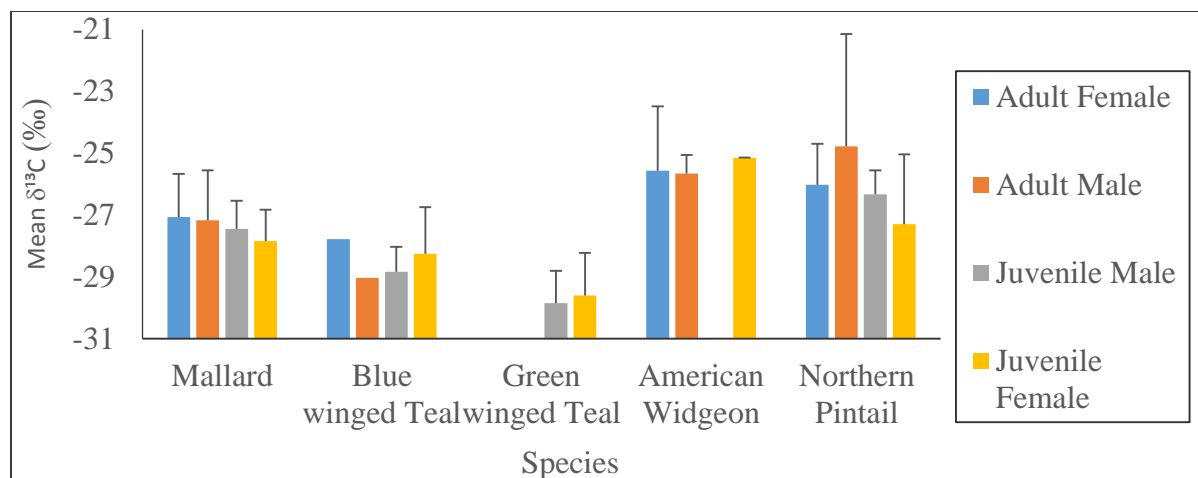


Figure 3.2: Average $\delta^{13}\text{C}$ values in liver tissue of waterfowl from the Saskatchewan River Delta, separated by age and sex for each species.

Table 3.1: Pairwise comparisons (p-values) of $\delta^{13}\text{C}$ in waterfowl livers for each species.

Species	Species	p-value
Mallard	Green-winged Teal	0.007
	Blue-winged Teal	0.016
	Northern Pintail	0.003
	American Widgeon	0.001
Green-winged Teal	Mallard	0.007
	Blue-winged Teal	0.151
	Northern Pintail	0.001
	American Widgeon	0.001
Blue-winged Teal	Mallard	0.016
	Green-winged Teal	0.151
	Northern Pintail	0.001
	American Widgeon	0.001
Northern Pintail	Mallard	0.003
	Green-winged Teal	0.001
	Blue-winged Teal	0.001
	American Widgeon	0.159
American Widgeon	Mallard	0.001
	Green-winged Teal	0.001
	Blue-winged Teal	0.001
	Northern Pintail	0.159

For $\delta^{15}\text{N}$, the various interactions were not significant ($p > 0.05$) and there were no significant differences between ages or sexes ($p = 0.138$ and $p = 0.871$; Fig. 3.3). There were significant effects with respect to species ($p = 0.001$) but differences were few and small. Mallards ($9.6 \pm 2.6 \text{ ‰}$) did not differ significantly from any other species ($p > 0.05$) except Blue-winged Teal ($11.6 \pm 2.2 \text{ ‰}$; $p = 0.009$). Green-winged Teal ($8.4 \pm 2.0 \text{ ‰}$) did not differ from any other species ($p > 0.05$), nor did American Widgeon ($9.9 \pm 2.8 \text{ ‰}$; $p > 0.05$); however, Northern Pintails ($9.6 \pm 1.6 \text{ ‰}$) differed significantly from Blue-winged Teal ($p = 0.001$; Table 3.2).

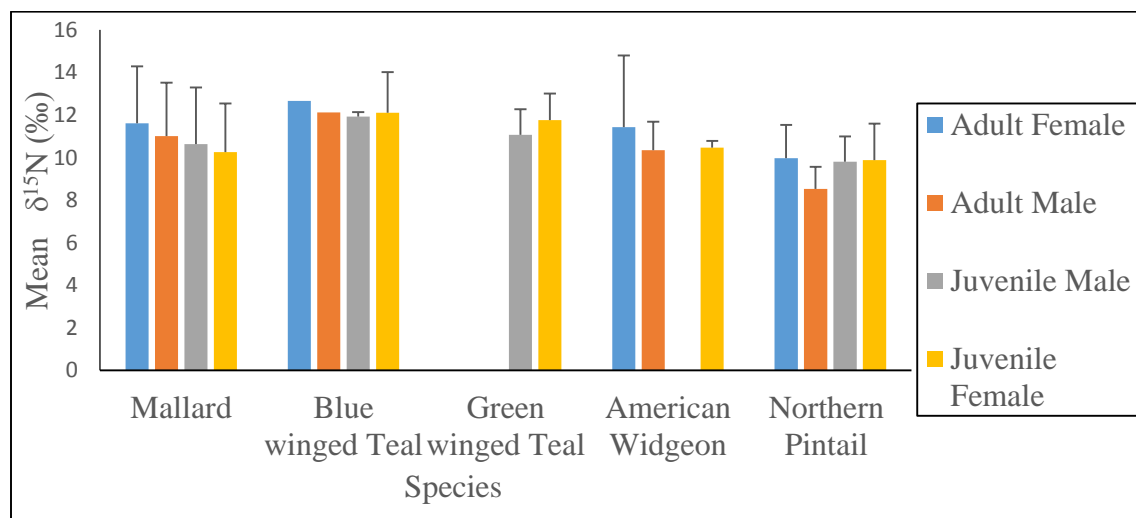


Figure 3.3: Mean $\delta^{15}\text{N}$ values in liver tissue of waterfowl from the Saskatchewan River Delta, separated by age and sex for each species.

Table 3.2: Pairwise comparison (p-values) of $\delta^{15}\text{N}$ in waterfowl livers for each species.

Species	Species	P-value
Mallard	Green-winged Teal	0.532
	Blue-winged Teal	0.009
	Northern Pintail	0.076
	American Widgeon	0.772
Green-winged Teal	Mallard	0.532
	Blue-winged Teal	0.505
	Northern Pintail	0.166
	American Widgeon	0.682
Blue-winged Teal	Mallard	0.009
	Green-winged Teal	0.505
	Northern Pintail	0.001
	American Widgeon	0.094
Northern Pintail	Mallard	0.076
	Green-winged Teal	0.166
	Blue-winged Teal	0.001
	American Widgeon	0.133
American Widgeon	Mallard	0.772
	Green-winged Teal	0.682
	Blue-winged Teal	0.094
	Northern Pintail	0.133

Although Blue-winged Teal clearly had the highest Hg concentrations, the three-way interaction age* sex * species was significant ($p = 0.002$; Fig. 3.4). Therefore, I proceeded with testing for species effects using each age-sex combination. All results below are reported as log [Hg] in $\mu\text{g/g}$ dry weight.

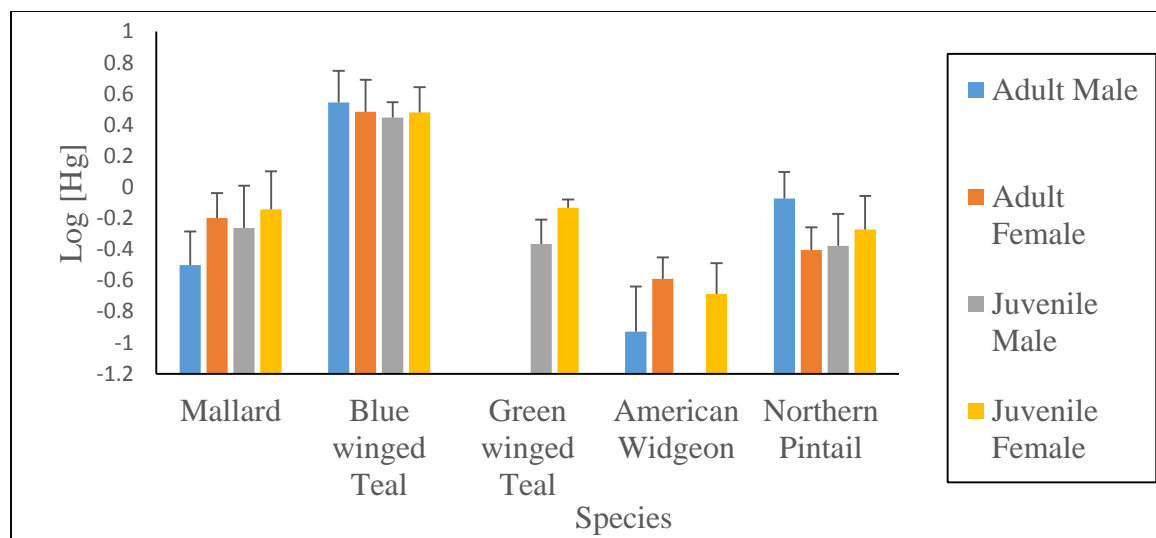


Figure 3.4: Mean Hg values in liver tissue of waterfowl from the Saskatchewan River Delta, separated by age and sex for each species.

Among the adult males, all species [American Widgeon (-0.9 ± 0.3), Blue-winged Teal (0.5 ± 0.2), Mallard (-0.5 ± 0.2), and Northern Pintail (-0.1 ± 0.2)] differed significantly from each other ($p < 0.05$). A similar trend was observed for adult females. The American Widgeon (-0.6 ± 0.1), Blue-winged Teal (0.5 ± 0.2), Mallard (-0.2 ± 0.2), and Northern Pintail (-0.4 ± 0.2) differed significantly from each other ($p < 0.05$). However among the juvenile females, a different observation was made. While Blue-winged Teal again had the highest concentrations (0.5 ± 0.2) and differed from all other species ($p < 0.05$), the Green-winged Teal (-0.1 ± 0.1) was not significantly different from Mallards (-0.1 ± 0.2 , $p = 0.946$) and Northern Pintails (-0.3 ± 0.2 , $p = 0.363$) but was different from American Widgeon (-0.7 ± 0.2 , $p = 0.005$). Juvenile female Mallards differed significantly from American Widgeon ($p < 0.05$) but not from Northern Pintails ($p = 0.088$). The Northern Pintails were also different from American Widgeon. Among the juvenile males, Blue-winged Teal (0.5 ± 0.1) differed from all other species ($p < 0.05$) but Green-winged Teal (-0.4 ± 0.2), Mallards (-0.3 ± 0.3) and Northern Pintail (-0.4 ± 0.2) were not

significantly different from each other ($p > 0.05$). Taken together, different species exhibited different diets as exhibited by their spread in dual isotope space (Fig. 3.5, 3.6, 3.7) and based on MIXSIAR outputs for each source.

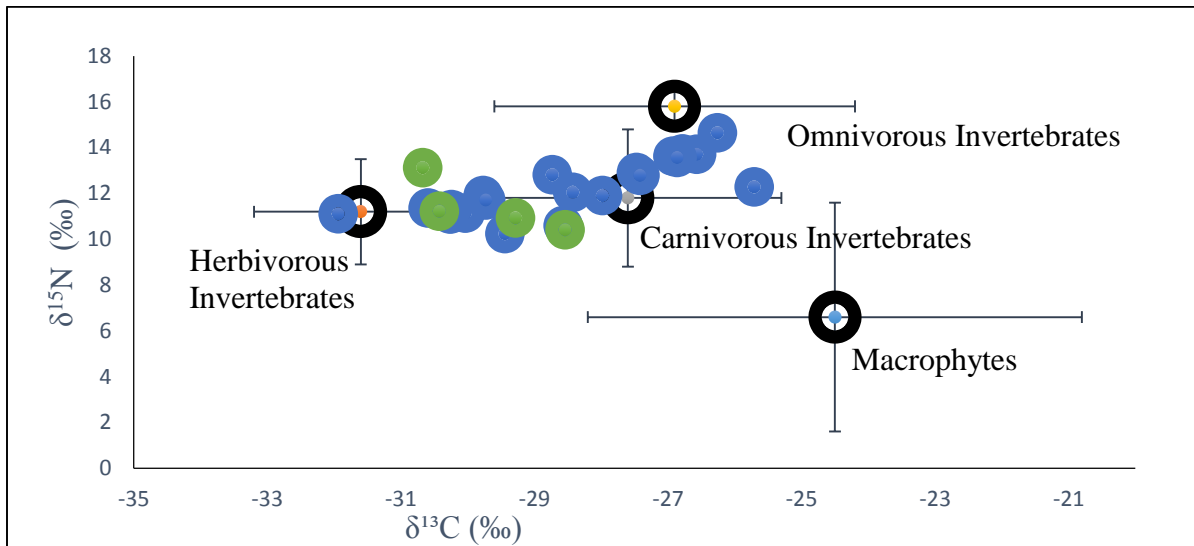


Figure 3.5: Isotope bi-plots showing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for dietary sources (black circles) for Green-winged Teals (green circles) and Blue-winged Teals (blue circles)

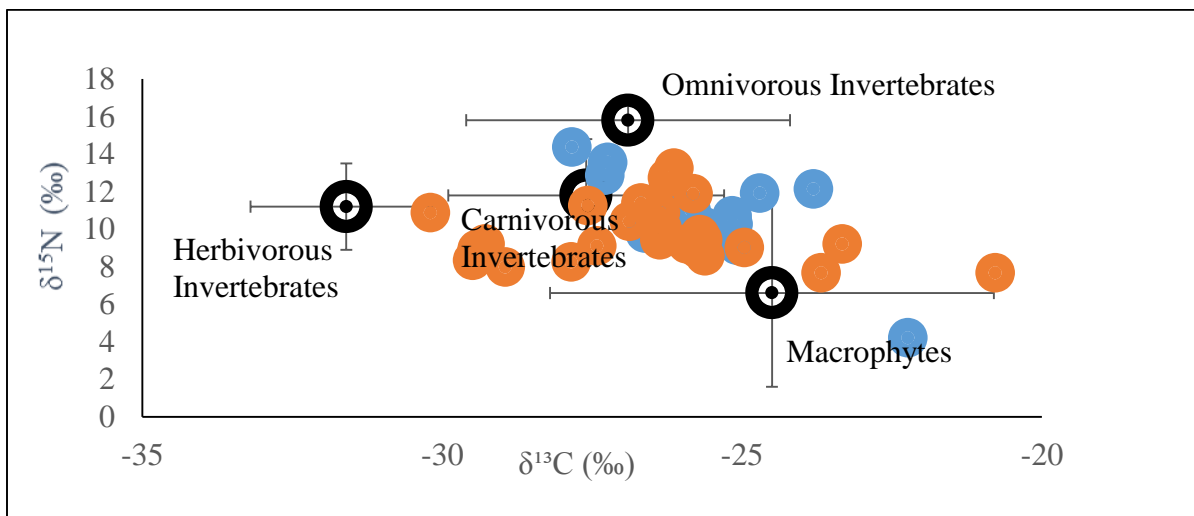


Figure 3.6: Isotope bi-plots showing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for dietary sources (Black circles) for American Widgeon (orange circles) and Northern Pintails (blue circles).

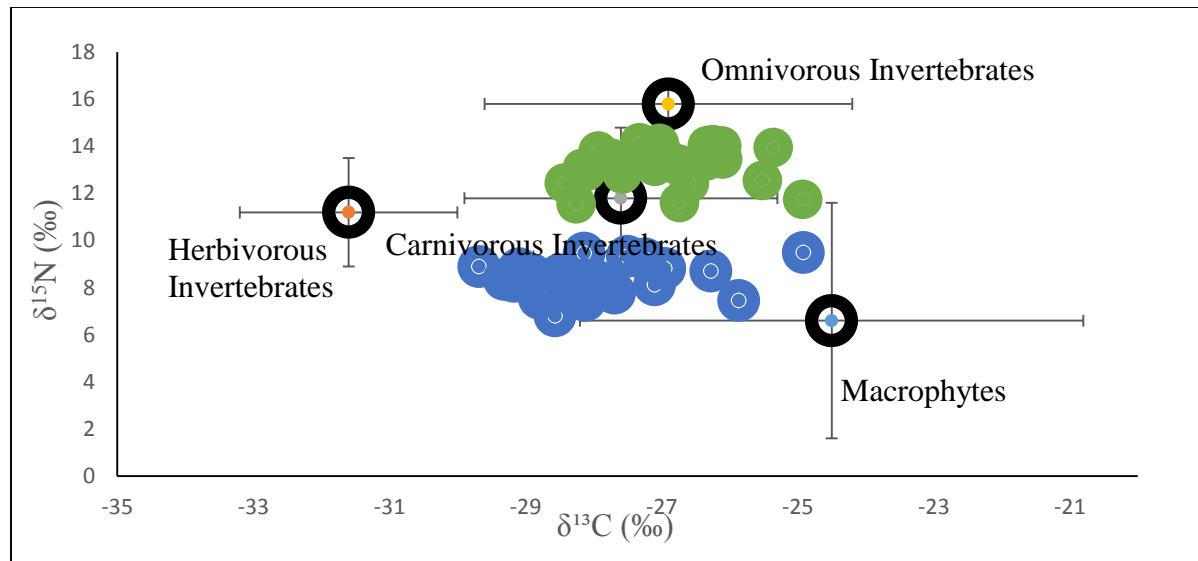


Figure 3.7: Isotope bi-plots showing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for dietary sources (black circles) for Mallards L (blue circles) and Mallards H (green circles).

Macrophytes contributed the most to the diet of American Widgeon (CrI = 0.26-0.67). Carnivorous invertebrates contributed 0.18-0.56 while herbivorous and omnivorous invertebrates contributed 0.07-0.27 and 0.01-0.04, respectively, in this species. A similar trend was observed for the Northern Pintail with 0.11-0.47, 0.07-0.40, and 0.01-0.08 CrIs for carnivorous, herbivorous and omnivorous invertebrates, respectively, and 0.30-0.64 for macrophytes. Blue-winged Teal and Green-winged Teal appear to forage at a higher trophic level than the other birds supported by higher [Hg] and $\delta^{15}\text{N}$ values, particularly for Blue-winged Teal. Blue-winged Teal recorded 0.02-0.34, 0.10-0.52, and 0.21-0.59 dietary contributions for carnivorous, herbivorous and omnivorous invertebrates, respectively, and only 0.03-0.27 for macrophytes. In the Green-winged Teal, macrophytes (0.02-0.26) also made a small contribution; instead, herbivorous invertebrates dominated the diet at 0.14-0.53, followed by 0.07-0.34 for carnivorous invertebrates, and < 0.01-0.07 for omnivorous invertebrates. Mallards (L) recorded a fairly equal representation of herbivorous invertebrates (0.37-0.69) and macrophytes (0.22-0.53), and lower

contributions from carnivorous invertebrates (0.02-0.21) and omnivorous invertebrates (< 0.01-0.05). The second group, Mallards (H) recorded an overwhelming 0.51-0.86 for carnivorous invertebrates and only 0.02-0.27, 0.02-0.11 and 0.03-0.26 for herbivorous and omnivorous invertebrates and macrophytes, respectively.

3.4 Discussion

By estimating dietary composition of 5 species of waterfowl in the SRD using stable isotopes, my results demonstrate the importance of macrophytes prior to fall migration. However there was some level of partitioning of resources at the species level (Chesson 2000). This likely resulted from birds maximizing the energy gains during short periods when the competition for resources was high and supports the theory that foraging of animals evolves to maximize their net energy returns (Staddon 2013). While source tracing with stable isotopes, especially $\delta^{15}\text{N}$, can be confounded by variation in isotopic baselines, this study was the first to use Hg concentrations as a tracer in Bayesian Mixing Models, allowing further interrogation of dietary and trophic level patterns evident in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data.

The significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among species resulted from feeding on different dietary sources ($\delta^{13}\text{C}$) and at different trophic levels ($\delta^{15}\text{N}$), demonstrating resource partitioning in this waterfowl assemblage composed of surface feeders (Bellrose & Kortwright 1976, Johnsgard 2012, Wersal et al. 2005). This was in agreement with previous studies where high waterfowl densities led to resource partitioning among sympatric species such as Mallards, Pintails and Teals (Guillemain & Fritz 2002). Nudds et al. (2000) also demonstrated the effects of habitat structure resulting in diet partitioning among ducks, *Anas* spp. However, other researchers have argued that phenotypic characters such as bill morphology (Nudds et al. 1994)

are responsible for dietary segregation and resource partitioning among waterfowl, specifically the *Anas* spp. which were used in this study.

Since all species in this study foraged at the same location at high densities (C. Asante, personal observation) in a shallow lake, it is likely that some species opted to eat other foods instead of competing with conspecifics for the same food resource, which would be consistent with optimal foraging theory. This theory states that animals make foraging decisions to enhance their survival by foraging on a resource they would not have otherwise consumed in the absence of competitors (Pyke et al. 1977, Sinervo 1997, Sih & Christensen 2001).

Variation in the fundamental niche of an organism can affect population stability, among-species competition and the fitness of the entire population (Bolnick et al. 2002). I observed variation in diet amongst individuals within a species in Mallards, with two very clear groups separated by liver $\delta^{15}\text{N}$ values. Birds with $\delta^{15}\text{N}$ values ranging from +10-14 ‰ could be assigned to a higher trophic position than birds averaging +7-9 ‰ because of known $\delta^{15}\text{N}$ discrimination (+3.4 ‰ per trophic level, Post, 2002). While MIXSIAR outputs suggested these two groups were feeding on different sources, Hg concentrations were similar, suggesting differences in $\delta^{15}\text{N}$ values could simply be attributed to differences in isotopic baseline (Hobson et al. 2005) rather than true differences in trophic position.

American Widgeon and Northern Pintail had the most herbivorous diets, consistent with the literature (DUC 2014, Miller 1987) while the two teal species fed at a higher trophic level. The exceptionally high Hg concentrations in Blue-winged Teal (mean = 2.9 $\mu\text{g/g}$ dry weight, range = 0.2 to 7.1) can be explained partly by feeding on omnivorous invertebrates that had high Hg concentrations and also higher $\delta^{15}\text{N}$ values than the other sources ($11.8 \pm 0.9\text{‰}$). A subset of

Blue-winged Teal muscle samples analyzed (n = 10) did not, however, have high Hg concentrations (mean = 0.7 $\mu\text{g/g}$ dry weight, C. Asante, unpublished data). This was also observed by Gerstenberger (2004) who recorded higher Hg concentrations in liver than in muscle of waterfowl, consistent with the role of liver as a de-toxifying organ. Overall, the slope of the relationship between Log [Hg] and $\delta^{15}\text{N}$ (known as the Trophic Magnification Slope, TMS) in liver for the entire waterfowl assemblage was 0.06. This is low compared to global averages for food webs (TMS of 0.16 ± 0.11 ranging from -0.19 to 0.48, Lavoie et al. 2013) and likely stems in part from considerable $\delta^{15}\text{N}$ baseline variation.

Apart from significant differences with respect to species for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, there were also significant differences in $\delta^{13}\text{C}$ with respect to age. This may result from adults and juveniles feeding on different sources, or different rates of metabolism as demonstrated by Williams et al. (2007) and Sears et al. (2009). The lack of significant differences for sexes for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ implies that in my study males and females did not make different dietary choices nor occupy different trophic positions. Measures of availability of different dietary sources would help clarify how much choice was responsible for the observed results.

My study predicted aquatic macrophytes as the dominant food source for waterfowl used in the study (Johnsgard 2012, Wersal et al. 2005, Knapton & Pauls 1994). This was expected because aquatic macrophytes contain carbohydrates that supply energy needed for the fall migration. Invertebrates are primarily a protein source which is used mostly in building and repairing muscle, although in dire situations, such as extended periods of fasting protein can also provide calories. These two food sources therefore have different macronutrient composition but may also vary in macronutrient isotopic values and these macromolecules are known to be routed to different tissues (Martinez del Rio & Wolf 2005). Voigt et al. (2008) observed carbohydrates

are used preferentially in powering metabolism, while proteins are more likely to be used in tissue synthesis (Tieszen & Fagre 1993). Liver plays a role in both metabolism that mainly involves carbohydrates and anabolism and allocation to tissues and organs that mainly involves proteins, which is why it was selected as an indicator of bulk diet.

Because my study species are omnivorous and tend to switch diets seasonally (Bellrose & Kortwright 1976), caution should be taken in interpreting the mixing model. It is important to understand the foraging choices by consumers in order to better characterise the spatio-temporal variations in consumers and their prey items (Bastille-Rousseau et al. 2011), and because the birds used in this study are omnivorous, their dietary choices can influence food web dynamics as they could alternately exert top-down control on various resources (Polis & Strong 1996).

My results underline the importance of the SRD as a key stop over site for migratory waterfowl prior to fall migration and how these birds partition resources. The heavy dependence on macrophytes as an energy source points to wetland vegetation as vital for fuel during fall migration. Anecdotal evidence for increased dominance of the invasive common reed (*Phragmites australis*) in SRD wetlands points to possible long-term changes in the forage base for waterfowl (Cross and Fleming 1989). Understanding the forage base that supports these birds prior to fall migration is therefore important for their conservation. While the productive nature of the SRD has long been recognized (Bellrose & Kortwright 1976, Smith 1996, Schmutz 2001) this study adds important detail on the dietary contribution of various energy pathways to the food webs supporting waterfowl during their time in the SRD. Therefore, developing a system to trace nutrient flow in this important waterfowl habitat using $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and [Hg] will be important to wetland research, conservation and management efforts in the SRD.

3.5 References

- Arzel, C., Elmberg, J., Guillemain, M., Lepley, M., Bosca, F., Legagneux, P., & Nogues, J. B. (2009). A flyway perspective on food resource abundance in a long-distance migrant, the Eurasian teal (*Anas crecca*). *Journal of Ornithology*, *150* (1), 61-73.
- Baschuk, M. S., Koper, N., Wrubleski, D. A., & Goldsborough, G. (2012). Effects of water depth, cover and food resources on habitat use of marsh birds and waterfowl in boreal wetlands of Manitoba, Canada. *Waterbirds*, *35* (1), 44-55.
- Bastille-Rousseau, G., Fortin, D., Dussault, C., Courtois, R., & Ouellet, J. P. (2011). Foraging strategies by omnivores: are black bears actively searching for ungulate neonates or are they simply opportunistic predators? *Ecography*, *34* (4), 588-596.
- Bellrose, F.C., & Kortwright, F.H. (1976). Ducks, geese and swans of North America. Wildlife Management Institute, Stackpole Books, Harrisburg, PA, U.S.A.
- Boecklen, W. J., Yarnes, C. T., Cook, B. A., & James, A. C. (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution, and Systematics*, *42* (1), 411-440.
- Bolnick, D. I., Yang, L. H., Fordyce, J. A., Davis, J. M., & Svanbäck, R. (2002). Measuring individual-level resource specialization. *Ecology*, *83* (10), 2936-2941.
- Brochet, A. L., Mouronval, J. B., Aubry, P., Gauthier-Clerc, M., Green, A. J., Fritz, H., & Guillemain, M. (2012). Diet and Feeding Habitats of Camargue Dabbling Ducks: What Has Changed Since the 1960s? *Waterbirds*, *35* (4), 555-576.

- Carleton, S.A., & Martinez del Rio, C. (2005). The effect of cold-induced increased metabolic rate on the rate of ¹³C and ¹⁵N incorporation in house sparrows (*Passer domesticus*). *Oecologia*, *144* (2), 226-232.
- Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, *31*(1), 343-366.
- Chura, N. J. (1961). Food availability and preferences of juvenile mallards. *Transactions of the North American Wildlife and Natural Resources Conference*, *26*, 121-134
- Cross, D.H., & Fleming, K.L. (1989). Control of Phragmites or common reed. Waterfowl Management Handbook, Fish and Wildlife Leaflet. US Department of the Interior, Washington, DC.
- Davidson, N. C., & Evans, P. R. (1986). The role and potential of man-made and man-modified wetlands in the enhancement of the survival of overwintering shorebirds. *Colonial Waterbirds*, *9* (2), 176-188.
- Davis, C. A., & Smith, L. M. (1998). Ecology and management of migrant shorebirds in the Playa Lakes Region of Texas. *Wildlife Monographs*, *140*, 3-45.
- Dirschl, H. J. (1969). Foods of lesser scaup and blue-winged teal in the Saskatchewan River Delta. *Journal of Wildlife Management*, *33*, 77-87.
- Drobney, R. D., & Fredrickson, L. H. (1979). Food selection by wood ducks in relation to breeding status. *Journal of Wildlife Management*, *49* (1), 109-120.
- Ducks Unlimited Canada. (2014). Retrieved April 2, 2014 from <http://www.ducks.ca/learn-about-wetlands/wildlife/>.

- Gerstenberger, S. L. (2004). Mercury concentrations in migratory waterfowl harvested from southern Nevada wildlife management areas, USA. *Environmental Toxicology*, 19 (1), 35-44.
- Guillemain, M. & Fritz, H. (2002). Temporal variation in feeding tactics: exploring the role of competition and predators in wintering dabbling ducks. *Wildlife Biology*, 8 (2), 81–90.
- Gurd, D. B. (2008). Mechanistic analysis of interspecific competition using foraging trade-offs: implications for duck assemblages. *Ecology*, 89 (2), 495-505.
- Hartke, K. M., Kriegel, K. H., Nelson, G. M., & Merendino, M. T. (2009). Abundance of wigeongrass during winter and use by herbivorous waterbirds in a Texas coastal marsh. *Wetlands*, 29 (1), 288-293.
- Havera, S. P. (1999). Waterfowl of Illinois. *Illinois Natural History Survey Special Publication*. Volume 21, pp.436.
- Hay, R.L. (1974). Moulting biology of male gadwalls at Delta, Manitoba. M.S. thesis, University of Wisconsin, Madison, Wisconsin. 20 pp.
- Hobson, K. A., & Clark, R. G. (1992a). Assessing avian diets using stable isotopes I: turnover of ¹³C in tissues. *Condor*, 94 (1), 181-188.
- Hobson, K. A., & Clark, R. G. (1992b). Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. *Condor*, 94 (1), 189-197.
- Hobson, K. A. (1999). Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, 120 (3), 314-326.
- Hobson, K. A., Thompson, J.E., Evans, M.R., & Boyd, S. (2005). Tracing nutrient allocation to reproduction in Barrow's goldeneye. *Journal of Wildlife Management*, 69(3), 1221-1228.

- Inger, R., & Bearhop, S. (2008). Applications of stable isotope analyses to avian ecology. *Ibis*, *150*(3), 447-461.
- Johnsgard, P. (2012). *Wings over the Great Plains: Bird Migrations in the Central Flyway*. Zebra E-books. Book 13.
- Klaassen, M., & Nolet, B. A. (2007). The role of herbivorous water birds in aquatic systems through interactions with aquatic macrophytes, with special reference to the Bewick's Swan—Fennel Pondweed system. In *Shallow Lakes in a Changing World* (pp. 205-213). Springer Netherlands.
- Klaassen, M., Hoye, B. J., Nolet, B. A., & Buttemer, W. A. (2012). Ecophysiology of avian migration in the face of current global hazards. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367* (1596), 1719-1732.
- Knapton, R. W., & Pauls, K. (1994). Fall food habits of American wigeon at Long Point, Lake Erie, Ontario. *Journal of Great Lakes Research*, *20* (1), 271-276.
- Lahring, H. (2003). *Water and wetland plants of the Prairie Provinces* (Vol. 44). University of Regina Press. Regina, Saskatchewan.
- Lavoie, R. A., Jardine, T. D., Chumchal, M. M., Kidd, K. A., & Campbell, L. M. (2013). Biomagnification of mercury in aquatic food webs: a worldwide meta-analysis. *Environmental Science & Technology*, *47* (23), 13385-13394.
- Logan, J. M., Jardine, T. D., Miller, T. J., Bunn, S. E., Cunjak, R. A., & Lutcavage, M. E. (2008). Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *Journal of Animal Ecology*, *77* (4), 838-846.

- Martin, A. C., & Uhler, F. M. (1939). *Food of game ducks in the United States and Canada* (No. 634). US Dept. of Agriculture.
- Martinez del Rio, C. M., & Wolf, B. O. (2005). Mass-balance models for animal isotopic ecology. *Physiological and Ecological Adaptations to Feeding in Vertebrates. Science, Enfield, NH*, pp.141-174.
- Merritt, R. W., & Cummins, K. W. (Eds.). (2008). *An introduction to the aquatic insects of North America*. Kendall/Hunt Publishing Company
- Miller, M. R. (1987). Fall and winter foods of northern pintails in the Sacramento Valley, California. *The Journal of wildlife management*, 51(2), 405-414.
- Myers, J. P. (1983). Conservation of migrating shorebirds: staging areas, geographic bottlenecks, and regional movements. *American Birds*, 37(1), 23-25.
- Nudds, T.D, Sjöberg, K., & Lundberg, P. (1994). Ecomorphological relationships among Palearctic dabbling ducks on Baltic coastal wetlands and a comparison with the Nearctic. *Oikos*, 69(2), 295-303.
- Nudds, T.D., Elmberg, J., Sjöberg, K., Pöysä, H. & Nummi, P. (2000). Eco- morphology in breeding Holartic dabbling ducks: the importance of lamellar density and body length varies with habitat type. *Oikos* 91 (3), 583–588.
- Ogden, L. J. E., Hobson, K. A., & Lank, D. B. (2004). Blood isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) turnover and diet-tissue fractionation factors in captive dunlin. *Auk*, 121 (1), 170-177.

- Phillips, D.L., Inger, R., Bearhop, S., Jackson, A.L., Moore, J.W., Parnell, A.C., Semmens, B.X., & Ward, E. J. (2014). Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology* 92 (10), 823-835.
- Polis, G. A., & Strong, D. R. (1996). Food web complexity and community dynamics. *American Naturalist*, 147 (5), 813-846.
- Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83 (3), 703-718.
- Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., & Montana, C. G. (2007). Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, 152 (1), 179-189.
- Pyke, G. H., Pulliam, H. R., & Charnov, E. L. (1977). Optimal foraging: a selective review of theory and tests. *Quarterly Review of Biology*, 52 (2), 137-154.
- Reid, F. A., J. R. Kelly Jr., T. S. Taylor, and L. H. Fredrickson. (1989). Upper Mississippi Valley wetlands—refuges and moist-soil impoundments, p. 181–202. *In*: L. M. Smith, R. L. Pederson and R. M. Kaminski (eds.). Habitat management for migrating and wintering waterfowl in North America. Texas Tech University Press.
- Schmutz, J. K. (2001). Cumberland Marshes Important Bird Areas. Retrieved from <http://www.ibacanada.ca/conservationplans/skcumberlandmarshes.pdf>
- Schneider, D.C., & Harrington, B.A. (1981). Timing of shorebird migration in relation to prey depletion. *Auk*, 98, 801-811.

- Sears, J., Hatch, S. A., & O'Brien, D. M. (2009). Disentangling effects of growth and nutritional status on seabird stable isotope ratios. *Oecologia*, *159* (1), 41-48.
- Sih, A., & Christensen, B. (2001). Optimal diet theory: when does it work, and when and why does it fail? *Animal Behaviour*, *61* (2), 379-390.
- Sinervo, B. (1997). Optimal Foraging Theory: Constraints and Cognitive Processes. *University of Southern California Santa Cruz*, 2006.
- Slattery, S. (2008). Status of waterfowl in the Saskatchewan River Delta. *Symposium proceedings of the Saskatchewan River delta*, pp. 1-9.
- Smith, A.R. (1996). Atlas of Saskatchewan birds. Nature Saskatchewan special publication No: 22, Regina.
- Staddon, J. E. R. (Ed.). (2013). *Limits to action: The allocation of individual behavior*. Academic Press, pp.14.
- Taft, O. W., & Haig, S. M. (2005). The value of agricultural wetlands as invertebrate resources for wintering shorebirds. *Agriculture, Ecosystems & Environment*, *110* (3), 249-256.
- Tidwell, P. R., Webb, E. B., Vrtiska, M. P., & Bishop, A. A. (2013). Diets and food selection of female mallards and blue-winged teal during spring migration. *Journal of Fish and Wildlife Management*, *4* (1), 63-74.
- Tieszen, L. L., & Fagre, T. (1993). Effect of diet quality and composition on the isotopic composition of respiratory CO₂, bone collagen, bio apatite, and soft tissues. In *Prehistoric Human Bone* (pp. 121-155). Springer Berlin Heidelberg.

- Voigt, C. C., Rex, K., Michener, R. H., & Speakman, J. R. (2008). Nutrient routing in omnivorous animals tracked by stable carbon isotopes in tissue and exhaled breath. *Oecologia*, *157* (1), 31-40.
- Wersal, R. M., McMillan, B. R., & Madsen, J. D. (2005). Food habits of dabbling ducks during fall migration in a prairie pothole system, Heron Lake, Minnesota. *Canadian Field-Naturalist*, *119* (4), 546-550.
- Williams, C. T., Buck, C. L., Sears, J., & Kitaysky, A. S. (2007). Effects of nutritional restriction on nitrogen and carbon stable isotopes in growing seabirds. *Oecologia*, *153* (1), 11-18.
- Zhang, L., Campbell, L. M., & Johnson, T. B. (2012). Seasonal variation in mercury and food web biomagnification in Lake Ontario, Canada. *Environmental Pollution*, *161*, 178-184.

CHAPTER 4

4.0 Synthesis

4.1 Objectives

An estimated 50% of global wetlands have been lost (IUCN 2007). In Canada, > 90% of wetlands have been lost in some regions. DUC estimates that in southern Saskatchewan and Southern Manitoba approximately 350,000 ha of wetlands have been drained over the last 40-60 years. Wetland losses in addition to drought have negative implications for waterfowl populations. In the face of this habitat loss and drought, abundant wetland habitats in areas such as the SRD will be a refuge for displaced birds in the prairies (DUC 2014). This is because the SRD historically is known to be a breeding and staging site for waterfowl as documented by Bellrose & Kortwright (1976), Canadian Wildlife Service (1990), and Schmutz (2001).

Using stable isotope analysis, I determined the migratory origins of waterfowl, together with subsequent dietary investigations, to help quantify the contribution of the SRD as both rearing and staging habitat to inform conservation efforts. Because banding activity in areas north of the delta has been limited, we knew little about the use of the SRD by birds produced in the north. I chose the fall season to sample birds for two reasons: 1) it presented an opportunity to test hypotheses about the breeding origins of these birds and 2) it was an opportunity to investigate the dietary sources that dominate the diet of these birds as they build fuel reserves for the energetically demanding migration to the wintering ground (Dingle 2014).

4.2 Main Findings

I observed that of the three main potential origins of birds in the SRD (Delta/Local, North of Delta and South of Delta), North of Delta had the highest numbers of birds (56% based on

$\delta^2\text{H}_f$). I therefore rejected the hypothesis that most of the birds gathering in the SRD in the fall would be locally-produced birds. This supports the assertion that because there is little banding activity in northern Canada, previous research underestimated the importance of the Delta as a staging ground for migrants from the north (Slattery 2008). Another point of contention was whether the birds classified as Delta based on $\delta^2\text{H}$ (34% of total) were indeed from the SRD, or from the Prairie, where $\delta^2\text{H}_p$ and hence $\delta^2\text{H}_f$ may have similar values as the SRD (Coulton et al. 2009). Sulfur isotopes provided a means to further sort the birds into Prairie and Delta categories because of known effects of agricultural activity on $\delta^{34}\text{S}_f$ (Hebert & Wassenaar 2005). In so doing, 23 presumed Delta birds were re-assigned to non-Delta categories, meaning very few of the 232 waterfowl shot in the SRD had moulted or hatched there. At the species level, American Widgeon and Northern Pintail were classified as the most Northern birds. The Pintails are of importance because of recorded declines in population (PHJV 2008). Mallards, Green winged Teal and Blue winged Teal had birds assigned to all categories, consistent somewhat with their geographic range (Cornell Lab of Ornithology 2014).

In chapter 3, I examined the food webs of waterfowl feeding in a lake in the SRD. Macrophytes were the most important dietary source for waterfowl prior to fall migration for the species used in this study (Figuerola & Green 2002). For instance, prior to fall migration, Knapton & Pauls (1994) observed that American Widgeon diet consisted mainly of macrophytes. Wersal et al. (2005) also observed that Mallards feed on macrophytes on Heron Lake, a Prairie pothole wetland. This is because of carbohydrates and the energy it provides to fuel long distance migration. I hypothesized that dietary overlap may lead to partitioning of resources (Chesson 2000), and my observations supported that hypothesis. American Widgeon and Northern Pintail diets were dominated by macrophytes whereas the Green winged and Blue

winged Teals were dominated by invertebrates. Even among the mallards, two groups emerged based on the diet. One group denoted as Mallard L consisted largely of macrophytes and the other group's diet was mainly invertebrates. The species used in the study all belong to the family Anatidae which are documented omnivorous species (Johnsgard 2012) hence their ability to partition diet when faced with competition from conspecifics or other species.

4.3 Strength and Limitations

The major strengths of the study include the multi-tracer and multi-species approaches. For instance in the assignment to origin, the use of $\delta^{34}\text{S}$ in addition to $\delta^2\text{H}$ helped to make a more accurate assignment, separating Delta and Prairie birds. The qualitative use of band recoveries validated information about movement of birds to the SRD. Secondly, the use of mercury as a tracer helped in the resolution of trophic level, and it holds potential as a relatively low-cost addition to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as dietary source tracers. By studying five different, but related, species I gained insight into multiple trophic levels and ecological interactions that would not have been observed had I focused on a single species. Finally, two years of field sampling increased sample sizes and also allowed a comparison among years.

One major limitation of the study is the small sample size for species such as American Widgeon and Northern Pintail. Although my observations of their assignment to mostly northern origins is consistent with the literature, larger sample sizes may have revealed additional individuals from the South, particularly for the Northern Pintails whose band recoveries from the USFWS reveals in addition to using northern habitats, they occupy habitats in southern Saskatchewan.

4.4 Future Research

The study should be extended to other species such as the Lesser Scaup which are in decline (Hobson et al. 2009) as well as other waterfowl species known to be in high numbers in the Cumberland Marshes of the SRD, such as Tundra Swan (*Cygnus columbianus*), Ring-necked Duck (*Aythya collaris*), Common Goldeneye (*Bucephala clangula*), Redhead (*Aythya americana*), Canvasback (*Aythya valisneria*) and Gadwall (*Anas strepera*) (Schmutz 2001). For the species already examined here, carbon and nitrogen isotopes should be run on feathers to further refine the spatial assignment, and finally, mercury accumulation with respect to migration should be investigated, particularly when some of the species such as Blue winged Teal had mercury concentrations higher than expected. Because of the importance of macrophytes in the diets of many of these species, future research could examine changes in the vegetation community associated with management activities such as water level manipulation (Baschuk et al. 2012).

4.5 Conclusions/Significance

My analysis revealed that the SRD is not only an important breeding ground but also an important staging ground for birds from the Prairies and the boreal forest. Secondly my observations support the literature that macrophytes are an important diet source prior to fall migration. However in the face of competition, there is partitioning of resources. My approach for evaluating migratory origins and diet upon arrival to the SRD synthesizes all available data and exploits the advantages of isotopes that link birds to the landscape. The conservation significance of this study is that conservationists and policy makers should focus and increase banding efforts in Northern ecosystems in the Central Flyway. Secondly, wildlife managers should continue with wetland protection and banding efforts in the SRD. Finally, management

of E.B. Campbell Dam to provide summer floods that replenish wetlands, would also assist in providing habitat for birds during fall migration.

4.6 References

- Baschuk, M. S., Koper, N., Wrubleski, D. A., & Goldsborough, G. (2012). Effects of water depth, cover and food resources on habitat use of marsh birds and waterfowl in boreal wetlands of Manitoba, Canada. *Waterbirds*, 35(1), 44-55.
- Bellrose, F.C., & Kortwright, F.H. (1976). Ducks, geese and swans of North America. Wildlife Management Institute, Stackpole Books, Harrisburg, PA, U.S.A.
- Canadian Wildlife Service. (1990). Priority migratory bird habitats of Canadian prairies, Environment Canada. Edmonton, AB.
- Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual review of Ecology and Systematics*, 31(3), 343-366.
- Cornell Lab of Ornithology (2014). Retrieved June 7, 2014 from <http://www.allaboutbirds.org>.
- Coulton, D. W., Clark, R. G., Hobson, K. A., Wassenaar, L. I., & Hebert, C. E. (2009). Temporal sources of deuterium (δD) variability in waterfowl feathers across a prairie-to-boreal gradient. *The Condor*, 111(2), 255-265.
- Dingle, H. (2014). *Migration: the biology of life on the move*. Oxford University Press.
- Ducks Unlimited Canada. (2014). Retrieved April 2, 2014 from <http://www.ducks.ca/learn-about-wetlands/wildlife/>.
- Figuerola, J., & Green, A. J. (2002). Dispersal of aquatic organisms by waterbirds: a review of past research and priorities for future studies. *Freshwater biology*, 47(3), 483-494.

- Hebert, C., & Wassenaar, L. (2005). Stable isotopes provide evidence for poor northern pintail production on the Canadian prairies. *Journal of Wildlife Management*, 69 (1), 101–109.
- Hobson, K. A., Wunder, M. B., Van Wilgenburg, S. L., Clark, R. G., & Wassenaar, L. I. (2009). A method for investigating population declines of migratory birds using stable isotopes: origins of harvested lesser scaup in North America. *PLoS One*, 4(11), e7915.
- IUCN. (2007). Water and Wetlands. Retrieved May 25, from www.iucn.org/about/union/secretariat/offices/iucnmed/iucn_med_programme/terrestrial_ecosystems___livelihoods/goods_and_services/water_and_wetlands/.
- Johnsgard, P. (2012). *Wings over the Great Plains: Bird Migrations in the Central Flyway*. Zebra E-books. Book 13.
- Knapton, R. W., & Pauls, K. (1994). Fall food habits of American wigeon at Long Point, Lake Erie, Ontario. *Journal of Great Lakes Research*, 20 (1), 271-276.
- Prairie Habitat Joint-Venture. (2008). Prairie Habitat Joint-Venture Implementation Plan 2007-2012. Report of the Prairie Habitat Joint-Venture. Environment Canada, Edmonton, AB. 34 pp. (Revised May 2009).
- Schmutz, J. K. (2001). Cumberland Marshes Important Bird Areas. Retrieved from <http://www.ibacanada.ca/conservationplans/skcumberlandmarshes.pdf>
- Slattery, S. (2008). Status of waterfowl in the Saskatchewan River Delta. Symposium proceedings of the Saskatchewan River delta. pp 1-9.

Wersal, R. M., McMillan, B. R., & Madsen, J. D. (2005). Food habits of dabbling ducks during fall migration in a prairie pothole system, Heron Lake, Minnesota. *The Canadian Field-Naturalist*, 119 (4), 546-550.

5.0 APPENDICES

5.1 The Range of Nitrogen Isotopes for Mallards

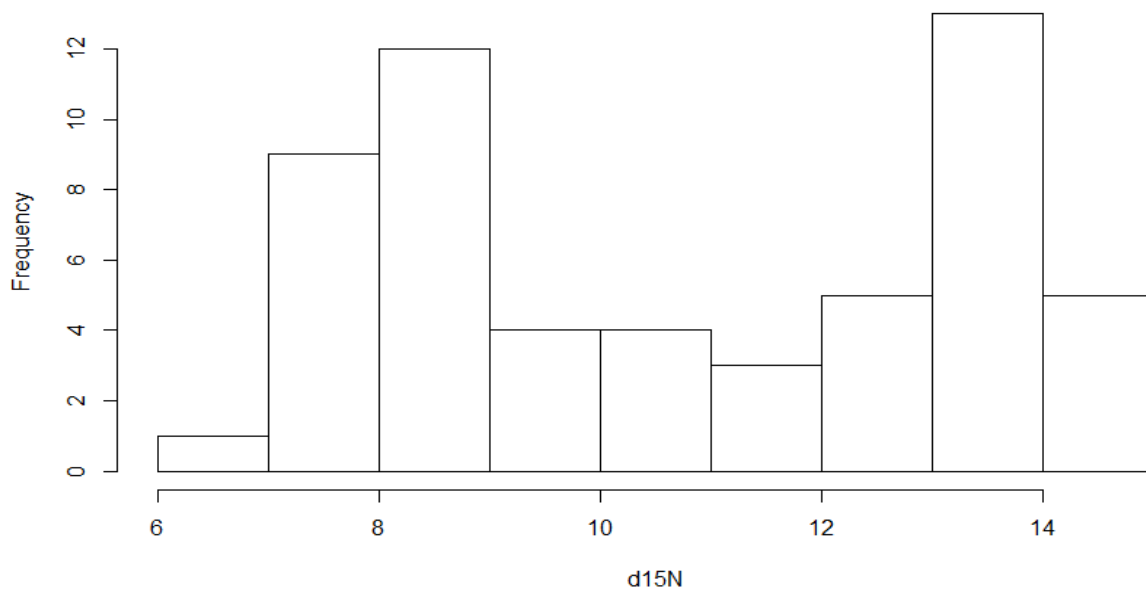


Figure A1: Frequency histogram showing the distribution of $\delta^{15}\text{N}$ values in Mallard livers from Grassy Point in the Saskatchewan River Delta.

5.2: Mixing Model output for various species

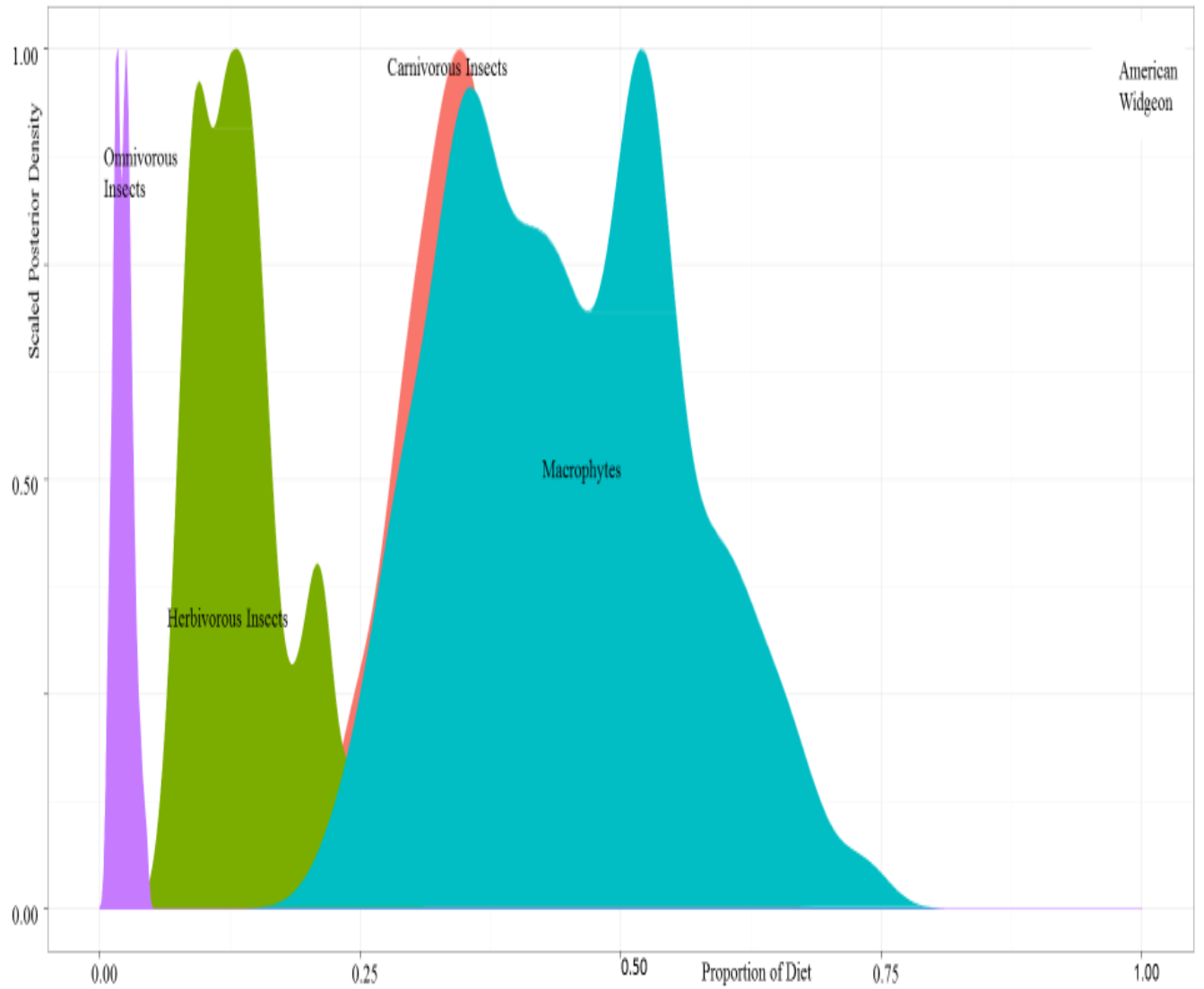


Figure A2: MIXSIAR output of American Widgeon dietary source proportions.

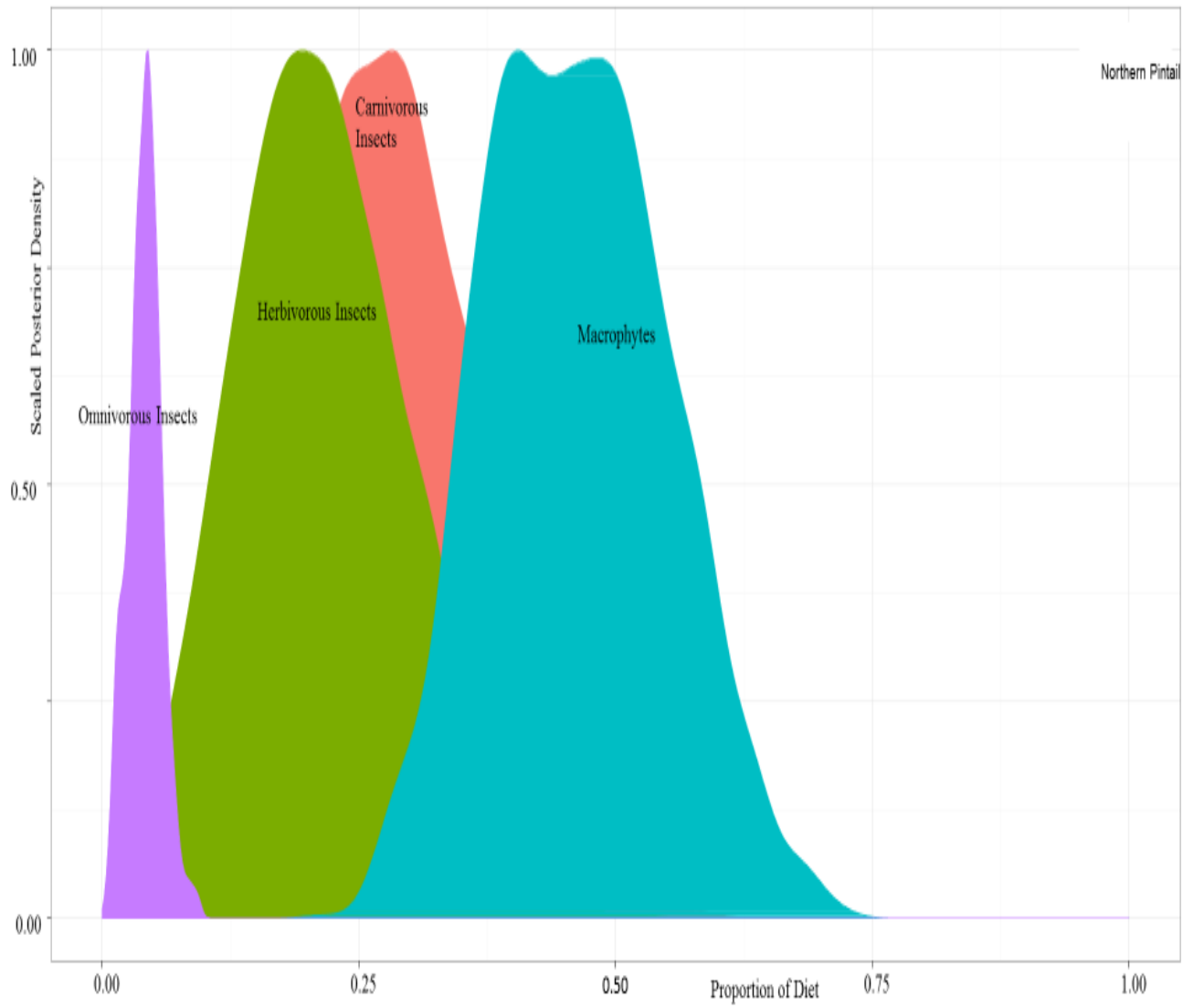


Figure A3: MIXSIAR output of Northern Pintail dietary source proportions.

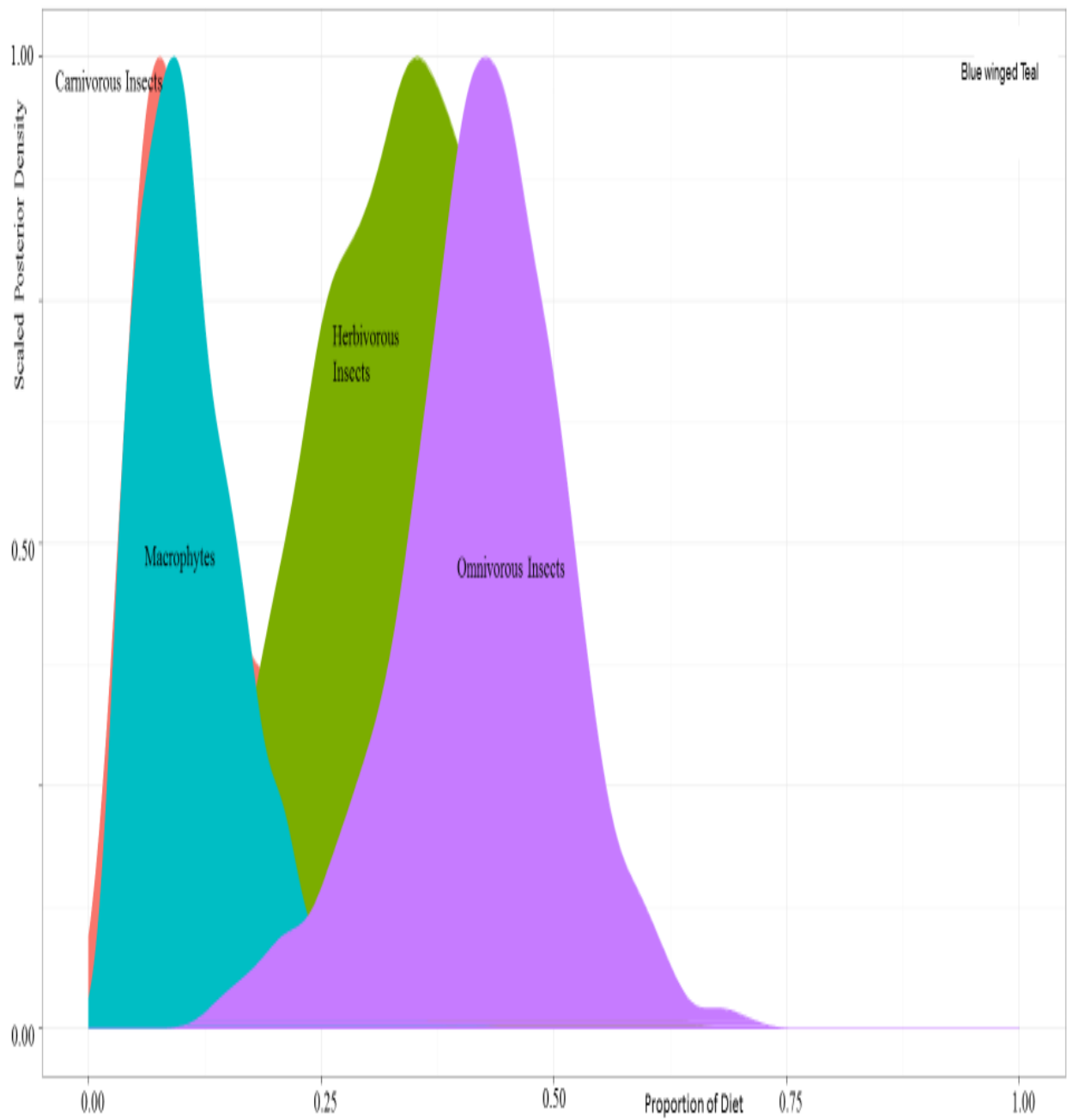


Figure A4: MIXSIAR output of Blue winged Teal dietary source proportions.

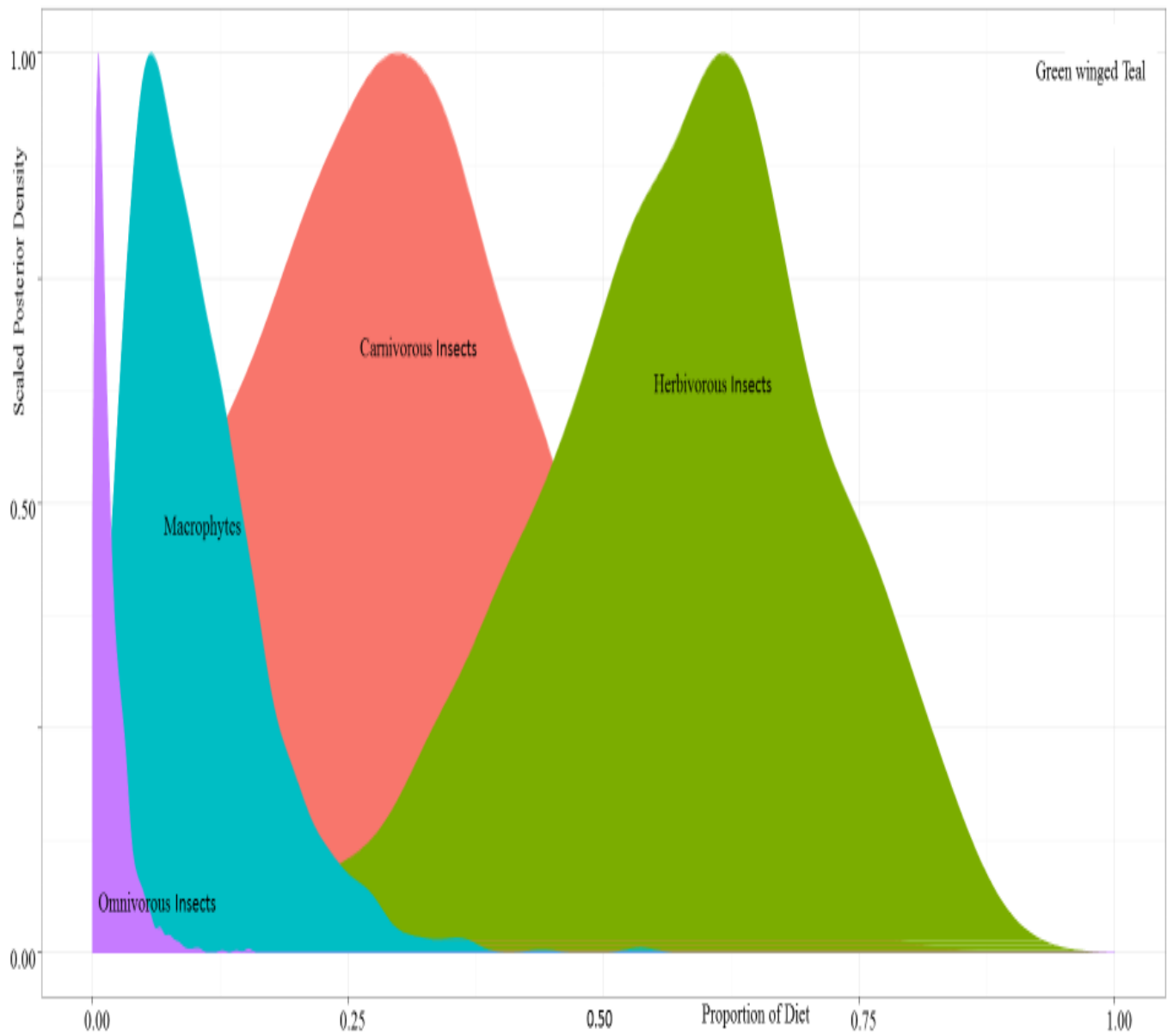


Figure A5: MIXSIAR output of Green winged Teal dietary source proportions.

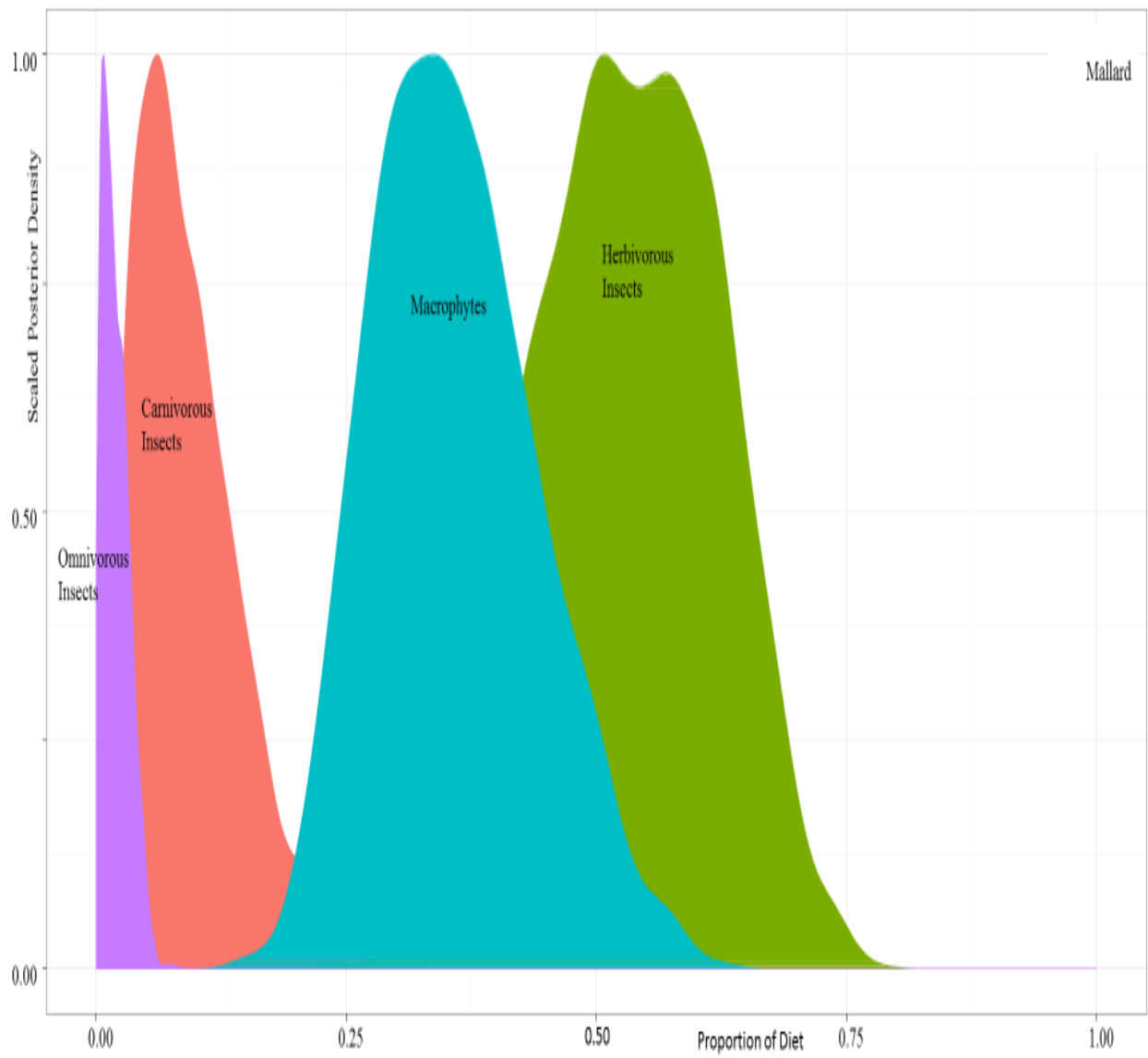


Figure A6: MIXSIAR output of Mallard L dietary source proportions.

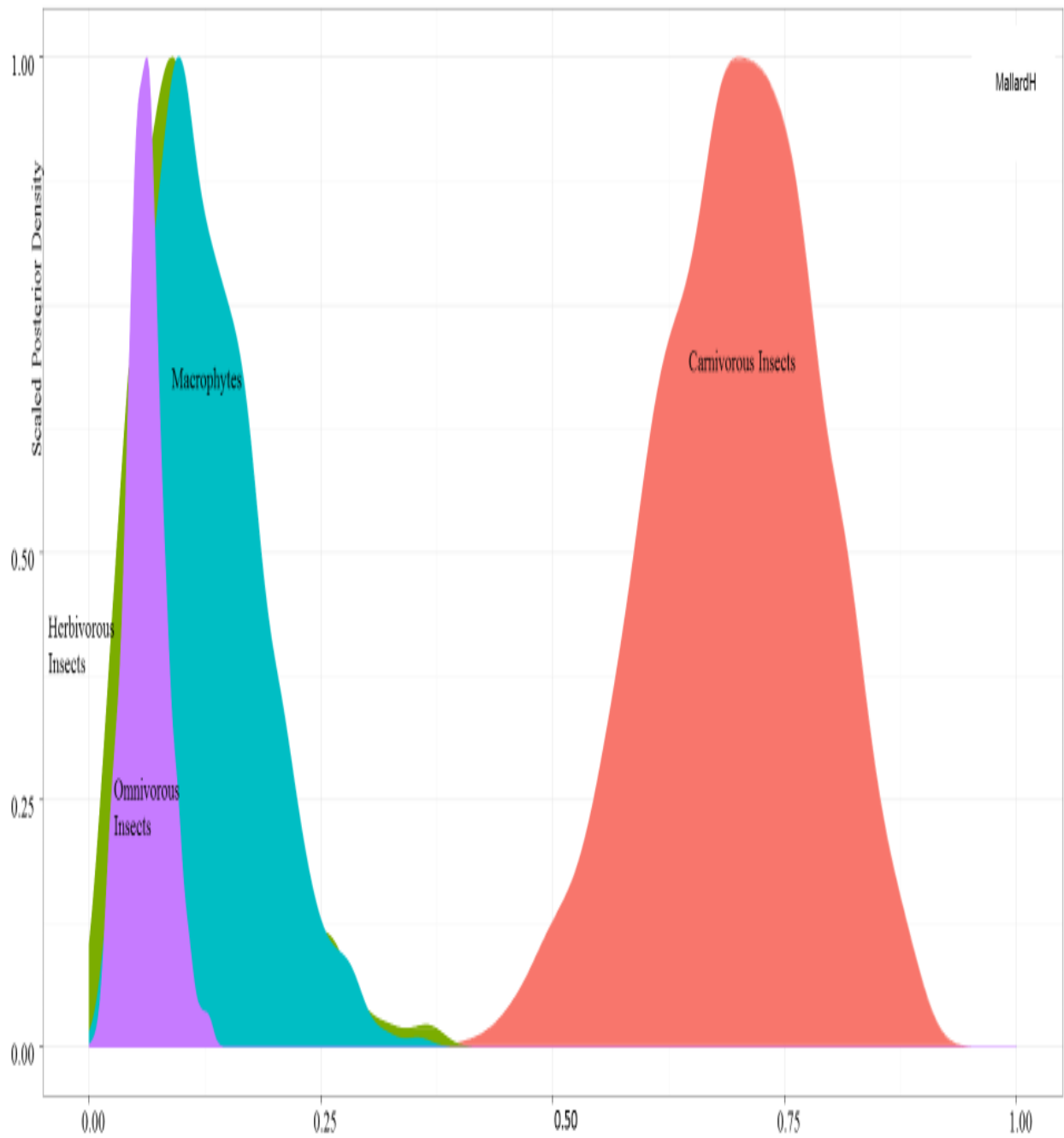


Figure A7: MIXSIAR output of Mallard H dietary source proportions.